NORADRENERGIC MODULATION OF STRESS-INDUCED DEFICITS IN FEAR EXTINCTION

A Dissertation

by

THOMAS F. GIUSTINO

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

DOCTOR OF PHILOSOPHY

Chair of Committee,	Stephen Maren
Committee Members,	James Grau
	Mark Packard
	Michael Smotherman
Head of Department,	Michael Smotherman

August 2019

Major Subject: Neuroscience

ABSTRACT

Early intervention strategies (e.g., psychological debriefing and exposure therapy) are thought to rely on extinction-like mechanisms to reduce pathological fear. These therapies attempt to reduce or prevent the development of stress- and trauma-related disorders such as posttraumatic stress disorder (PTSD). Despite this, disorders of fear and anxiety are prevalent in our society, due in part to a lack of empirically-driven treatment options. For example, both animal models and human data suggest early interventions after a traumatic event may actually undermine long-term recovery. In the laboratory, Pavlovian fear conditioning procedures in rats have provided fundamental knowledge regarding the brain circuits mediating learned fear. However, less is known about the mechanisms of extinction learning, a process intended to reduce conditional fear, which is highly sensitive to stress. We aimed to examine how stress, and the stress hormone norepinephrine, act to impair extinction learning. We show that fear conditioning produces rapid and sustained changes in both the medial prefrontal cortex (mPFC) and the basolateral complex of the amygdala (BLA) which impinges upon extinction learning when extinction training occurs soon after conditioning, a phenomenon called the immediate extinction deficit (IED). We demonstrate that this stress-induced suppression of mPFC and simultaneous excitation in the BLA soon after conditioning can be normalized with systemic propranolol, a beta-blocker. In addition, we further show that local application of propranolol into the BLA, but not the mPFC, enables extinction learning under stress, where it normally fails. We point to a role for the locus coeruleus norepinephrine system (LC-NE) using cell-specific manipulations to alter NE release. LC excitation paired with a weak footshocks mimics

stronger footshock activation of BLA activity, which may lead to extinction deficits. Lastly, we demonstrate that the LC-NE system is also involved in fear relapse. That is, LC excitation resulted in elevated conditioned fear responses to a previously extinguished cue. Overall, the data suggest a complex circuit in which LC-NE modulates both the mPFC and BLA to toggle high and low fear states. The LC-NE system represents a promising therapeutic target for individuals suffering from stressor-and trauma-related disorders.

ACKNOWLEDGEMENTS

I would like to begin by thanking my committee chair, Dr. Maren, and my committee members Dr. Grau, Dr. Packard, and Dr. Smotherman for their guidance and support throughout my time in graduate school. Admittedly, I can be stubborn and opinionated about my science – though this has resulted in a productive working relationship. Dr. Maren has shown an ability to shape his mentoring style to each individual member in the laboratory, a skill I greatly admire and hope to one day mimic. Dr. Maren has been a caring and thoughtful mentor and has helped mold my way of thinking as a scientist. His insight on experimental design, data processing, and visualization has transformed my approach to science. Perhaps above anything else, Dr. Maren has provided me with endless opportunity to pursue my line of interest and for that I will always be grateful.

I would next like to thank Dr. Paul Fitzgerald. When I joined the laboratory, Paul took me under his wing and was instrumental in training me as a scientist. He taught me to appreciate the little victories along the way in science, and has been highly influential in shaping my research interests, career goals, and experimental designs. In graduate school and in science you often face many failures, but regardless of how an experiment turned out, Paul always used to say, "Well Tom, we're still learning something." This seemingly simple statement has had a profound impact on my line of thinking, and I often times find myself repeating it to younger graduate students when they are unsatisfied with results in the laboratory. This mantra has served a pivotal role in my success as a graduate student because it taught me that perseverance and showing up to continue your work is the only way to accomplish your goals, despite the many bumps along the way.

I would also like to thank all the individuals who have been part of a support team along the way. Being part of “original” Maren Lab crew at Texas A&M University has truly been a blessing. Travis Goode, Gillian Acca, Angie Wang, Jingji Jin, Karthik Ramanathan, Reed Ressler, Michael Totty, Olivia Miles, Martin Payne, and Cecily Oleksiak have all made transplanting myself from Connecticut to Texas a seamless transition. I have made many life-long friends along this six-year journey and it excites me to see their continued success.

Lastly, I would like to thank my friends and family for their endless support. I’m sure my parents weren’t thrilled to hear I was moving 1700 miles from home, but they never wavered in their support. My brothers, Rocco and Cheech, along with James Ineson have always made a concerted effort to visit at least once a year. Our treks to and from Dallas to unfortunately watch the Giants lose nearly every time are some of my fondest memories from my time in Texas. Evan Hart has been the best friend I could ask for, and I thank him for helping spark my interest in science while we were roommates as undergraduates at UConn. And last, but certainly not least, I want to thank my loving fiancée, Stephanie Calora. She has continued to love and support me despite our career paths keeping us physically apart for many years. Steph is always engaged and listens to my endless complaining during tough times throughout graduate school. She is also always attentive when I seemingly can’t “turn it off” and keep talking about my experiments and ideas for hours. In fact, she often uses this “trick” to keep us on the phone when I otherwise would be heading to bed because she knows how important my work is to me, despite my suspicions that she probably has no idea what I’m rambling about half

the time. I can’t begin to thank my friends and family for their support. If not for all those mentioned above, I certainly would not be the person and scientist I am today.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor Stephen Maren [advisor], Professor James Grau and Professor Mark Packard of the Department of Psychological and Brain Sciences and Institute for Neuroscience, and Professor Michael Smotherman [Department of Biology and Institute for Neuroscience].

Funding Sources

This work was made possible in part by the National Institutes of Mental Health [R01MH065961 (SM), R01MH117852 (SM), and F31MH112208 (TFG)]. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

NOMENCLATURE

ANOVA	Analysis of Variance
AR	Adrenoceptor
BL	Basolateral Nucleus
BLA	Basolateral Complex of the Amygdala
BM	Basomedial Nucleus
CeA	Central Amygdala
CeL	Lateral Nucelus of the Central Amygdala
CeM	Medial Nucleus of the Central Amygdala
CNO	Clozapine *N*-oxide
CR	Conditioned Response
CS	Conditioned Stimulus
DREADDs	Designer Receptors Exclusively Activated by Designer Drugs
HPC	Hippocampus
IED	Immediate Extinction Deficit
IL	Infralimbic Cortex
ITCs	Intercalated Cells
LA	Lateral Amygdala
LC	Locus Coeruleus
mPFC	Medial Prefrontal Cortex
NE	Norepinephrine
PL	Prelimbic Cortex

PTSD	Posttraumatic Stress Disorder
US	Unconditioned Stimulus

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

1. INTRODUCTION*

1.1 Overview

Pavlovian fear conditioning is a form of learning that serves as a robust model to explore the neurobiological underpinnings of disorders of fear and anxiety, including post-traumatic stress disorder (PTSD). In a typical rodent experiment, an innocuous conditioned stimulus (CS; e.g., an auditory tone) is paired with an aversive unconditioned stimulus (US; e.g., a mild electric footshock). After one or more conditioning trials, presentation of the CS alone comes to elicit a conditioned fear response (CR) that includes freezing behavior (i.e., immobility except that necessary for respiration), changes in heart rate and respiration, and potentiated acoustic startle (Davis, 1992; LeDoux, 2000; Maren, 2001). Importantly, these fear CRs can be extinguished by repeated presentations of the CS in the absence of the US. In rodents and humans alike, CRs to an extinguished CS tend to return under a number of conditions including the passage of time (spontaneous recovery), when the CS is presented outside the extinction context (renewal), or with exposure to an unsignaled US (reinstatement) (Bouton, 2000, 2002; Goode and Maren, 2014; Hermans et al., 2006; Maren et al., 2013; Vervliet et al., 2013). These recovery or relapse phenomena suggest that extinction does not erase fear memories, but generates a new safety memory that inhibits the expression of fear. In addition, extinction learning itself is a fragile process, dependent on many factors including timing relative to

* Parts of this chapter have been reprinted with permission from Frontiers in Behavioral Neuroscience Giustino TF and Maren, S (2015). The role of the medial prefrontal cortex in the conditioning and extinction of fear. ***Front. Behav. Neurosc****i*, *9*, 298. http://doi.org/10.3389/fnbeh.2015.00298 Giustino TF, and Maren S. (2018). Noradrenergic modulation of fear conditioning and extinction. ***Front. Behav. Neurosc****i.* 12, 43. doi:10.3389/fnbeh.2018.00043.

conditioning (Maren, 2014; Maren and Chang, 2006; Myers et al., 2006) and stress (Maren and Holmes, 2016; Giustino and Maren, 2018; Giustino et al., 2016b).

While learned fear serves an adaptive purpose aiding survival, pathological fear states are thought to underlie various stress and trauma-related disorders such as PTSD, which has a lifetime prevalence of nearly 8% in the general population (Kessler et al., 2005, 1995). Not surprisingly, this number increases to as high as 30% in combat-exposed veterans (Koenen et al., 2008), amplifying the need for more effective therapies. PTSD has been described as the only mental health disorder with a known cause (i.e., a traumatic experience) (Pitman et al., 2012) and is characterized by heightened arousal and resistance to extinction learning (Rauch et al., 2006). Many have argued that PTSD may, at least in part, be a disorder of the fear circuitry (Shin and Handwerger, 2009) and an enhanced understanding of learned fear is relevant to the psychological processes underlying this disorder (Liberzon and Sripada, 2008; VanElzakker et al., 2014). It is possible that PTSD patients exhibit exaggerated fear conditioning, resistance to extinction, or both; ultimately, they exhibit persistent fear CRs (Pitman, 1988).

Due to the prevalence and debilitating nature of stress and trauma-related disorders, there has been a surge in interest in understanding the neural processes subserving learned fear and its subsequent extinction (Maren et al., 2013; Milad and Quirk, 2012; Quirk and Mueller, 2008). A triad of brain regions, including the amygdala, hippocampus and medial prefrontal cortex (mPFC) has been heavily studied in relation to fear (Dejean et al., 2015; Herry et al., 2010; Maren and Quirk, 2004). Importantly, individuals with PTSD also present with elevated levels of the stress neurotransmitter,

norepinephrine (NE) (Kosten et al., 1987; Geracioti et al., 2001; Pervanidou and Chrousos, 2010; Yehuda et al., 1992; Naegeli et al., 2017; Southwick et al., 1999c). The vast majority of central norepinephrine is produced by and released from the locus coeruleus (LC) and NE has profound effects on the amygdala, prefrontal cortex, and hippocampus (Jodo et al., 1998; Sabban et al., 2018; Giustino et al., 2017, 2016b; Fitzgerald et al., 2015; Giustino and Maren, 2018; Arnsten and Li, 2005; Arnsten, 2009; Ramos and Arnsten, 2007; Robbins and Arnsten, 2009). Below I discuss past work on the fear circuit and the locus coeruleus norepinephrine system which has largely informed the direction of my doctoral work.

1.2 The fear circuit

It is well established that both the acquisition and extinction of fear memories requires synaptic plasticity within the amygdala (Duvarci and Pare, 2014; Fanselow and LeDoux, 1999; Herry et al., 2010; LeDoux, 2003; Lee et al., 2013; Maren and Quirk, 2004; Pape and Pare, 2010). The amygdala is a node of highly interconnected nuclei; the basolateral complex of the amygdala (BLA; consisting of the lateral, basal and basomedial nuclei) and the central nucleus of the amygdala (CeA; consisting of lateral and medial components) play critical roles in the acquisition of both fear and extinction memories. It has been suggested that inhibitory interneurons within the amygdala play a role in regulating fear output. These include 1) the intercalated cell masses (ITCs) positioned between the BLA and CeA (Duvarci and Pare, 2014; Lee et al., 2013; McDonald and Augustine, 1993; Nitecka and Ben-Ari, 1987; Paré and Smith, 1993; Royer et al., 1999), 2) local inhibitory interneurons within the BLA (Spampanato et al., 2011; Wolff et al.,

2014), and 3) inhibitory interneurons in CeL that project to CeM (Haubensak et al., 2010; Ciocchi et al., 2010).

How one structure supports the formation and storage of opposing memories is not fully understood, although it appears that distinct cell populations within the BLA may preferentially encode low and high fear states (Goosens et al., 2003; Hobin et al., 2003; Herry et al., 2008; Senn et al., 2014). For example, lesions of the lateral amygdala (LA), a locus for CS and US convergence, or the CeA disrupt fear conditioning (Goosens and Maren, 2001; LeDoux et al., 1990; Wilensky et al., 2006). Similarly, reversible inactivation of the BLA prevents the acquisition and expression of conditioned fear (Helmstetter and Bellgowan, 1994; Muller et al., 1997), suggesting a large degree of overlap between the subnuclei of the amygdala. Studies using overtraining procedures have demonstrated that amygdala lesions disrupt fear memories, not the ability of animals to emit conditioned fear responses (Maren, 1998, 1999). Single-unit recordings have demonstrated learning-related changes in short-latency (less than 15 ms) CS-evoked responses in the LA after fear conditioning, suggesting that these changes are mediated by direct thalamo-amygdala projections (Maren, 2000; Quirk et al., 1995). Moreover, these conditioning-induced changes in spike firing are specifically related to the associative nature of the CS, indicating that the LA is a crucial site of plasticity for fear memories independent of freezing behavior (Goosens et al., 2003). In contrast, the CeA is primarily thought of as an output station, relaying information to the brain stem, hypothalamus and periaqueductal gray to initiate fear responses such as freezing (Paré et al., 2004). Whereas the CeL is necessary for fear acquisition, CRs are mediated by CeM output (Ciocchi et al.,

2010; Haubensak et al., 2010). Curiously, while the LA encodes CS-US information, there are no direct connections between the LA and CeA to directly mediate fear output, suggesting that the BL or BM or both may act as an interface (Amano et al., 2011). Interestingly, post-conditioning lesions of the basal nuclei block fear expression while leaving learning intact (Anglada-Figueroa and Quirk, 2005; Amano et al., 2011). Selective inactivation of either BM or BL alone was not sufficient to mimic this effect, whereas inactivation of both BM and BL was sufficient. This implies that some level of functional overlap exists between these two regions (Amano et al., 2011).

Additionally, several studies have shown that BLA synaptic plasticity is crucial for the acquisition of extinction (Falls et al., 1992; Herry et al., 2006, 2008; Kim et al., 2007; Lu et al., 2001; Sotres-Bayon et al., 2007). Upon extinction learning, LA neurons typically show a reduction in CS-evoked neural activity (Quirk et al., 1995; Repa et al., 2001). However, a distinct population of LA cells maintain CS-evoked responding throughout extinction learning (Repa et al., 2001). Interestingly, after extinction, patterns of CS-evoked neural activity in LA are mediated by the context and reflect the level of freezing (i.e., larger responses occur when fear renews) (Hobin et al., 2003). In summary, there is compelling evidence to support the notion that the amygdala is a crucial locus for the acquisition and extinction of learned fear with both 'fear' and 'extinction' neurons existing within the same subnuclei whose CS-evoked activity strongly correlates with the level of fear expression (Goosens et al., 2003; Herry et al., 2008; Quirk et al., 1995; Repa et al., 2001; Senn et al., 2014).

The hippocampus has also been identified as a key mediator of learned fear. Given the role of the hippocampus in encoding contextual and spatial information it is not surprising this region plays a substantial role in the fear circuit. Numerous studies have shown that hippocampal lesions dampen fear to a context previously associated with a shock US (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Selden et al., 1991). Importantly, hippocampal lesions produce larger deficits when made soon after context conditioning, suggesting that recent memories rely more heavily on the integrity of the hippocampus (Anagnostaras et al., 1999; Maren et al., 1997). Interestingly, hippocampal lesions do not necessarily interfere with context conditioning when damage is made prior to training (Maren et al., 1997; Frankland et al., 1998), although deficits in the acquisition of contextual fear can be obtained with single-trial procedures (Wiltgen et al., 2006). Collectively, these results suggest that the hippocampus is required for forming and storing memories of the context, but not necessarily context-US associations (Young et al., 1994). These findings support the notion that hippocampus plays a key role in both the acquisition and expression of conditioned fear to a particular context.

As mentioned above, the extinction of fear is highly context-dependent, that is, fear returns or "renews" when the CS is presented outside the extinction context. Considerable evidence indicates that the renewal of fear is mediated by the hippocampus (Bouton, 2000, 2002; Bouton et al., 2006; Goode and Maren, 2014; Hermans et al., 2006; Maren et al., 2013; Vervliet et al., 2013). For example, many studies have shown that hippocampal inactivation dampens fear renewal when the CS is presented outside of the extinction context (Corcoran and Maren, 2001; Hobin et al., 2006; Holt and Maren, 1999;

Marek et al., 2018a; Maren and Hobin, 2007; Zelikowsky et al., 2012). In addition, disconnections of the hippocampus from the amygdala or prefrontal cortex impair renewal (Orsini et al., 2011; Marek et al., 2018a), amygdala neurons engaged during fear renewal receive hippocampal and prelimbic input (Knapska et al., 2012) and individual hippocampal neurons expressing Fos after fear renewal preferentially project to both the amygdala and prefrontal cortex (Jin and Maren, 2015a). These data suggest that the hippocampus integrates contextual information during conditioning and likely regulates the context dependent recall of fear after extinction learning.

Fear regulation must be tightly controlled and this is thought to depend on the mPFC. Two subdivisions of mPFC in rodents, and their human homologs, have been identified as having distinct roles within the fear circuit. The prelimbic cortex (PL) is thought to regulate fear expression, whereas the infralimbic cortex (IL) mediates fear suppression (Milad and Quirk, 2012; Quirk and Beer, 2006; Riga et al., 2014; Sotres-Bayon and Quirk, 2010). A similar division of labor has been proposed in humans, indicating that the neural mechanisms of extinction learning may be conserved across species (Milad and Quirk, 2012; Linnman et al., 2012; Phelps et al., 2004; Schiller et al., 2008; Sehlmeyer et al., 2009; Vervliet et al., 2013). The prefrontal cortex is thought to be responsible for "higher order" executive functions; however, cortical activity is highly sensitive to stress and elevated levels of the stress neurotransmitter, norepinephrine, which may play a critical role in regulating fear memories and their extinction (Giustino and Maren, 2015; Mueller et al., 2008; Giustino and Maren, 2018; Raio et al., 2014; Arnsten and Li, 2005; Arnsten, 2009; Ramos and Arnsten, 2007).

1.3 The locus coeruleus norepinephrine system

The locus-coeruleus norepinephrine (LC-NE) system has numerous functions including regulating the sleep-wake cycle, arousal, respiration, motivation, cognition, and learning and memory. In particular, NE plays a broad role in the formation and retrieval of emotional memories. As such, NE is a candidate molecule for the treatment of trauma- and stressor-related disorders and a number of studies suggest that the NE system may be dysregulated in posttraumatic stress disorder (PTSD); (Southwick et al., 1999c; Morilak et al., 2005; Yehuda et al., 1992; Southwick et al., 1999a, 1997; Bremner et al., 1996; Southwick et al., 1999b; Giustino et al., 2016b; Arnsten, 2009, 2015; Arnsten et al., 2015; Kroes et al., 2016a; Raio and Phelps, 2015; Rodrigues et al., 2009). Despite the extensive research on this topic, the use of NE-altering drugs for PTSD treatment remains controversial.

1.3.1 The LC-NE system anatomy and physiology

The LC is a bilateral brainstem nucleus located adjacent to the fourth ventricle. While the LC is small in terms of cell count (~1,500 in rats and ~15,000 in humans) it has been implicated in a range of behavioral phenomenon (Sara, 2015, 2009; Arnsten, 2009; Robbins and Arnsten, 2009; Arnsten, 2015; Aston-Jones and Waterhouse, 2016; Aston-Jones and Cohen, 2005). The LC was first described in detail by Dahlstrom and Fuxe in 1964 (Dahlström and Fuxe, 1964). This discovery led to a surge of interest into this small nucleus in the subsequent decades. Below we review the broad afferent and efferent connectivity of the LC and briefly describe the different receptor subtypes of the NE system.

1.3.2 Efferents

The LC projects broadly throughout the brain and is largely thought to be the sole source of cortical NE. Due to its vast projections, it is not surprising that NE has an important role in many aspects of behavior and cognition. LC neurons fire at low basal rates (~1-3 Hz) and can fire in two modes: phasic states of firing (i.e., bursts of activity) occur in response to relevant environmental stimuli whereas the LC fires tonically during periods of stress. Increased tonic firing rates are associated with less phasic activity (Aston-Jones et al., 1999; Aston-Jones and Cohen, 2005). How these distinct firing modes affect aversive learning and memory is not well characterized. The LC consists of at least two cell types with the smaller fusiform cells being found in more dorsal portions of the nucleus whereas larger multipolar cells tend to be located more ventrally (Swanson, 1976; Grzanna and Molliver, 1980). Because this review is largely focused on fear conditioning and extinction, we restrict our focus to projections to the medial prefrontal cortex (mPFC), the basolateral amygdala (BLA, encompassing all nuclei), the central amygdala (CeA), and the hippocampus (HPC) all of which are heavily innervated by the LC (Swanson and Hartman, 1975; Swanson, 1976; Amaral and Sinnamon, 1977; Foote et al., 1983; Segal and Landis, 1974; Pickel et al., 1974; Gerfen and Clavier, 1979; Descarries and Lapierre, 1973; Lapierre et al., 1973; Descarries et al., 1977; Morrison et al., 1979; Jones and Moore, 1977; Fallon et al., 1978; Loughlin et al., 1982; Moore and Bloom, 1979). Recent work has led to an increasingly complex view of LC function based on the discovery of target-specific subpopulations within this small nucleus. I will discuss the contribution of distinct LC efferents in more detail as it relates to aversive learning and memory.

1.3.3 Homogeneous or heterogeneous output?

Upon the initial discovery of the LC it was determined that all neurons within this nucleus were noradrenergic (Dahlström and Fuxe, 1964). Further anatomical work on the extensive projections of the LC promoted the idea that this nucleus was largely homogenous, serving to distribute NE throughout the forebrain to coordinate global brain states. For example, several tracing studies describe collateralization of LC projections (Swanson and Hartman, 1975; Steindler, 1981; Jones and Yang, 1985; Nagai et al., 1981; Room et al., 1981). In addition, physiological evidence supported this idea -- LC firing properties were found to be topographically homogeneous, phasic activity was synchronized amongst neurons, and local field potentials also displayed high synchronization (Aston-Jones and Bloom, 1981a; Ishimatsu and Williams, 1996). How could the LC-NE system dynamically modulate so many different aspects of behavior and cognition which depend on distinct brain regions/systems if its effects are global, rather than task and target specific?

Indeed, others have argued that the LC is comprised of distinct target-specific subpopulations. Several tracing studies have shown the LC consists of largely non-overlapping populations of neurons that can be defined based on their downstream target (Loughlin et al., 1986, 1982; Uematsu et al., 2015, 2017; Waterhouse and Chandler, 2016; Chandler et al., 2014; Chandler and Waterhouse, 2012; Chandler et al., 2013; Waterhouse et al., 1983; Agster et al., 2013; Hirschberg et al., 2017). Waterhouse and colleagues have provided extensive anatomical and physiological evidence to suggest that the LC does not simply distribute NE equally to its many targets. For example, in a series of studies using

retrograde tracers infused into different cortical regions, they have shown strong evidence for separate populations of cells within the LC (Waterhouse and Chandler, 2016; Chandler et al., 2014; Chandler and Waterhouse, 2012; Chandler et al., 2013; Agster et al., 2013; Waterhouse et al., 1983). Moreover, they have recorded from these distinct LC populations and demonstrated that LC neurons projecting to the mPFC (compared to motor cortex) show different molecular properties that promote increased excitability. The cellular properties of target-specific LC populations may be related to the functional needs of their unique downstream targets (Waterhouse and Chandler, 2016; Chandler et al., 2014). A recent study has further confirmed the LC has highly divergent projections (i.e., it is not completely homogeneous) and suggests that small subpopulations may be selective for target regions, but propose that the LC may still serve to dictate brain-wide states (Schwarz and Luo, 2015; Schwarz et al., 2015). Moving forward, it will be important to examine the target specificity of the LC as well as how phasic vs tonic firing in these discrete populations affect their downstream target to influence learning and memory.

1.3.4 Synaptic or volume transmission?

A second issue regarding how the LC influences both brain-wide states, as well as distinct target regions, revolves around the potential mechanism of NE transmission. How the LC releases NE has been an area of debate with two possible mechanisms receiving attention. Traditional synaptic release of NE being one possibility and the other being volume transmission, or nonsynaptic release. Some evidence suggests that LC terminals release NE at traditional synapses (Papadopoulos et al., 1987, 1989; Papadopoulos and Parnavelas, 1990). In contrast, others have proposed that LC-NE functions primarily via

volume transmission (i.e., nonsynaptic or extrasynaptic release) (Descarries and Lapierre, 1973; Lapierre et al., 1973; Descarries et al., 1977; Agster et al., 2013). It is likely that the LC-NE system supports both synaptic and nonsynaptic release and this may be area specific (Olschowka et al., 1981; Farb et al., 2010). It is possible that volume transmission preferentially influences brain-wide states/NE-tone whereas synaptic release is dependent upon local needs of specific target regions, though these ideas remain to be tested.

1.3.5 Afferents

Complementing its widespread projections throughout the forebrain, the LC receives dense reciprocal feedback from many of its targets. Indeed the LC is highly interconnected with the mPFC, BLA, CeA, and HPC (Arnsten and Goldman-Rakic, 1984; Cedarbaum and Aghajanian, 1978; Jodo et al., 1998; Sara and Hervé-Minvielle, 1995; Schwarz and Luo, 2015; Schwarz et al., 2015; Valentino and Van Bockstaele, 2008; Van Bockstaele et al., 1998; Aston-Jones et al., 1986). The LC expresses several peptides including, but not limited to, vasopressin, somatostatin, neuropeptide y, enkephalin, neurotensin, corticotropin releasing hormone, galanin, glutamate, acetycholine, and serotonin (Aston-Jones et al., 2004; Berridge and Waterhouse, 2003; Schwarz and Luo, 2015; Schwarz et al., 2015). These observations suggest that the LC is highly responsive to numerous transmitter and peptide systems and likely integrates information from several incoming sources. A recent study has shown that the LC receives direct projections from 111 brain regions (Schwarz et al., 2015). How the LC integrates this information is a subject of great interest. The LC has extensive dendritic arborization extending into the periocoerulear region which receives widespread non-NE synaptic contact (Shipley et al., 1996) and

nearby GABAergic cells within this dendritic zone likely serve to regulate LC function (Aston-Jones et al., 2004). Understanding how the LC reciprocal network affects both LC signaling and target regions remains an important question.

1.3.6 Receptor subtypes

NE exerts its function via action at three G-protein coupled receptor subtypes with the α2-adrenoceptors (ARs; A, B, and C subtypes) having the highest affinity, followed by α1-ARs (A, B, and D), and the lowest affinity β-ARs (1, 2, and 3); (Ramos and Arnsten, 2007; Berridge and Waterhouse, 2003). The heterogeneous distribution, distinct subtypes, and differing affinities of each class of receptor provide yet another mechanism by which NE may exert target-specific effects. The α2-ARs are G_i-coupled leading to the inhibition of cAMP and thereby reducing neuronal excitability and primarily serve as presynaptic autoreceptors, although they are also expressed postsynaptically (Ramos and Arnsten, 2007; Ramos et al., 2006; MacDonald et al., 1997). Several studies have demonstrated strong expression patterns in the mPFC, HPC, and amygdala using in situ hybridization (McCune et al., 1993; Nicholas et al., 1993b; Scheinin et al., 1994; Wang et al., 1996; Zeng and Lynch, 1991), radioligand binding (Boyajian et al., 1987; Unnerstall et al., 1984), and immunohistochemical techniques (Rosin et al., 1996a, 1996b; Aoki et al., 1994).

The α1-ARs are generally thought to be excitatory in nature and are G_q-coupled. Activation of these receptors acts via phospholipase C and phosphatidyl inositol intracellular signaling mechanisms, activating protein kinase C and subsequent release of intracellular calcium (Ramos and Arnsten, 2007; Birnbaum et al., 2004; Johnson and

Minneman, 1985; Marshall et al., 1999). This class of ARs can also be found throughout the cortex, HPC, and amygdala (McCune et al., 1993; Pieribone et al., 1994; Day et al., 1997; Domyancic and Morilak, 1997; Rainbow and Biegon, 1983; Young and Kuhar, 1980; Jones et al., 1985; Palacios et al., 1987); however, α2-ARs tend to be more widespread than α1-ARs (McCune et al., 1993). This may serve as a mechanism for target regions to regulate NE action to reduce signaling by having densely expressed, high-affinity autoreceptors.

Lastly, the lowest-affinity β-ARs are G_s-coupled to adenylyl cyclase resulting in increased cAMP and enhanced cellular excitability (Ordway et al., 1987; Ferry et al., 1999a, 1999b; Zhang et al., 2005). β-ARs show high expression levels throughout the brain, particularly in the HPC, mPFC, and amygdala (Nicholas et al., 1993a; Summers et al., 1995; Booze et al., 1993; Rainbow et al., 1984; Milner et al., 2000). Interestingly, β-ARs are also expressed on astrocytes which may indirectly influence neural signaling (Milner et al., 2000). Signaling via α1- and β-ARs has been proposed to have opposing effects on the mPFC and BLA. High levels of NE may bias instinctive and reflexive responses mediated by NE action at α1- and β-ARs in the BLA and whereas activation of these receptors may impair mPFC function. This has important implications for aversive learning and memory (Arnsten, 2009, 2015; Arnsten et al., 2015).

1.4 Stress, the LC-NE system, and the fear circuit

The LC responds to both appetitive and aversive stimuli (Sara and Segal, 1991; Aston-Jones and Bloom, 1981b; Ventura et al., 2008; Bouret and Sara, 2004; Aston-Jones and Waterhouse, 2016; Aston-Jones and Cohen, 2005; Aston-Jones et al., 1999), however the

focus of this section will be to examine how NE affects key nodes in the fear circuit. Footshock serves as the US in the majority of Pavlovian fear conditioning experiments, and it is well document that footshock and other acute stressors increase LC activity (Passerin et al., 2000; Pezzone et al., 1993; Smith et al., 1992; Thierry et al., 1968; Chen and Sara, 2007; George et al., 2013; Uematsu et al., 2017; Sara and Segal, 1991; Sved et al., 2002). Below we discuss how LC activity and NE affects the fear circuit.

1.4.1 NE and the amygdala

The BLA plays a crucial role in the formation and retrieval of fear conditioning and extinction memories (Maren and Quirk, 2004; Dejean et al., 2015; Johansen et al., 2011; Herry and Johansen, 2014; Maren, 2011, 2001; LeDoux, 2000; Myers and Davis, 2007). NE signaling in the amygdala appears to be critical for most aspects of Pavlovian fear conditioning and extinction (see below). Increased LC activity in response to acute stressors (including footshock) produces robust increases in amygdalar NE content (Galvez et al., 1996; Quirarte et al., 1998; Morilak et al., 2005; Arnsten, 2009, 2015; Ramos and Arnsten, 2007; McGaugh, 2004, 2000). How increased NE affects BLA signaling is therefore a fundamental question when studying emotional learning and memory. It has been proposed that heightened NE levels in the amygdala promote instinctive and reflexive responses to environmental stimuli (which would presumably bias responses for emotional events and memories); (Arnsten, 2009; Southwick et al., 1999a; Arnsten, 2015; Arnsten et al., 2015; Ramos and Arnsten, 2007). For example, one study found that footshock-induced increases in LC and BLA Fos were significantly reduced by LC inhibition prior to footshock (using the $GABA_A$ antagonist muscimol).

Moreover, drugs that increase NE efflux (such as the α2 autoreceptor antagonist yohimbine) produce robust increases in BLA Fos expression (Singewald et al., 2003). This suggests that heightened noradrenergic activity in the BLA promotes excitability which would likely strengthen fear memories. However, a pair of studies has demonstrated that footshock, LC stimulation, or iontophoresis of NE (or NE-increasing drugs) into the BLA produced heterogeneous responses in BLA single-unit activity, although the BLA was generally suppressed in response to increased NE (Buffalari and Grace, 2007; Chen and Sara, 2007). This is perhaps counterintuitive if increased amygdalar excitability is associated with fear memory formation and recall, but NE did increase the spontaneous firing rate of a smaller subpopulation of BLA neurons (Buffalari and Grace, 2007). Interestingly, the suppression of BLA firing is dependent upon α2-AR signaling insofar as iontophoresis of clonidine mimicked the effects of NE and these inhibitory effects are potentiated with the systemic administration of propranolol (Buffalari and Grace, 2007). It is possible that the smaller population of BLA cells that showed excitation is sufficient for memory formation or that higher levels of NE (that engaged the lower affinity receptors) would result in more excitation.

The central amygdala (CeA) is viewed as the output region of the amygdala that drives fear expression, although mounting data indicate it too plays a role in the acquisition of fear conditioning (Yu et al., 2017; Goosens and Maren, 2003). That said, CeA microcircuits and projections to downstream targets such as the periaqueductal grey are particularly important for generating freezing behavior (Ciocchi et al., 2010; Haubensak et al., 2010; Fadok et al., 2017). Importantly, the CeA is reciprocally connected with the

LC (Valentino and Van Bockstaele, 2008; Van Bockstaele et al., 1998). Under stress, the CeA activates the LC via corticotropin-releasing hormone (McCall et al., 2015; Van Bockstaele et al., 1998; Prouty et al., 2017) which may act as a positive feedforward mechanism to maintain high levels of LC activity and NE transmission, particularly in the amygdala. This circuit could provide a way to generate sustained fear responses, particularly in the aftermath of conditioning.

1.4.2 NE and the hippocampus

The hippocampus is critical for integrating and processing spatial information which is important in context fear conditioning among other types of learning and memory (Chen et al., 2017; Maren et al., 2013; Bouton et al., 2006; Hansen, 2017; Jin and Maren, 2015b) and LC input to the hippocampus has been shown to impact learning about a novel context (Wagatsuma et al., 2018). Indeed, NE has a major influence on hippocampal function and LC stimulation, footshock, and other acute stressors increase hippocampal NE levels (Hajós-Korcsok et al., 2003; Abercrombie et al., 1988; Yavich et al., 2005). In addition, drugs that increase NE levels, such as yohimbine, amplify this effect whereas drugs that reduce NE levels, such as clonidine, blunt stress-induced hippocampal NE release (Abercrombie et al., 1988). Moreover, a number of studies suggest that NE enhances hippocampal long-term potentiation (LTP), particularly in the dentate gyrus and CA1, which is dependent upon both α1 and β-AR mechanisms (Bliss et al., 1983; Neuman and Harley, 1983; Lacaille and Harley, 1985; Chaulk and Harley, 1998; Segal et al., 1991; Harley, 2007; Stanton and Sarvey, 1985b, 1985a, 1987; Dahl and Sarvey, 1989; Hopkins and Johnston, 1988; Izumi and Zorumski, 1999; Katsuki et al., 1997; Dunwiddie et al.,

1992; Yang et al., 2002). For example, NE applied either directly to the dentate gyrus or applied to the perforant pathway increases excitatory postsynaptic potentials, decreases spike onset latency, and increases the population spike amplitude; these effects promote LTP induction and may be important for memory formation (Neuman and Harley, 1983; Lacaille and Harley, 1985; Harley, 2007). However, NE effects on LTP may depend on stimulation parameters and the areas being stimulated which suggests that NE can dynamically modulate HPC function (Dahl and Sarvey, 1989; Harley, 2007). In line with this idea, restraint stress and tail shock (which would presumably increase hippocampal NE) have been shown to impair hippocampal LTP (Foy et al., 1987). It may be that stress-induced increases in HPC-NE are beyond optimal levels and exceed the levels used in many of the recording studies showing that NE enhances HPC-LTP. Despite these possibilities, it appears NE generally enhances hippocampal synaptic efficacy which may function to enhance emotional learning and memory. Of course, this may be sensitive to the prevailing level of NE and the subregions being examined, allowing NE to bidirectionally modulate HPC function.

1.4.3 NE and the mPFC

The prelimbic (PL) and infralimbic (IL) subdivisions of the mPFC are thought to regulate the expression and suppression of fear, respectively (Giustino and Maren, 2015; Quirk and Mueller, 2008; Milad and Quirk, 2012; Dejean et al., 2015; Herry et al., 2010; Knapska and Maren, 2009). Several studies have examined the effects of NE and stress on prefrontal function. Footshock and other acute stressors increase NE levels in the mPFC (Korf et al., 1973; Hatfield et al., 1999; Gresch et al., 1994; Ishizuka et al., 2000; Finlay

et al., 1995; Morilak et al., 2005; Girotti et al., 2017). Similar to other brain regions, NE effects on PFC function are highly dependent upon the prevailing level of NE and the task requirements. Lower levels of NE (engaging postsynaptic α2-ARs) appear to promote cortical function such as cognitive flexibility and working memory, whereas high levels impair prefrontal signaling via α1- and β-AR dependent mechanisms (Arnsten, 2009, 2015; Ramos and Arnsten, 2007; Ramos et al., 2006; Arnsten and Li, 2005). Interestingly, the PFC has more dopamine-β hydroxylase (DβH) varicosities relative to sensory cortical areas (Agster et al., 2013). This raises the possibility that prefrontal regions might be subjected to greater release of NE (via volume transmission in addition to synaptic transmission), which may help explain why the PFC is highly sensitive to stress. An important topic for future research will be to address differences in LC projections to PL and IL and how these affect fear expression.

1.5 Stress and NE are key components regulating extinction deficits

Overall, there is substantial evidence that suggests NE plays a prominent role in stressor- and trauma-related disorders, including PTSD. My doctoral work has focused on understanding the neurobiology of the immediate extinction deficit (IED). As described above, the IED is a phenomenon in which animals and humans alike fail to show a successful reduction in fear when extinction occurs soon after (minutes to hours) after trauma exposure (Maren, 2014; Maren and Chang, 2006; Merz et al., 2016). My doctoral work has hypothesized that this extinction deficit is due to high levels of stress (and NE) in the wake of trauma which may subsequently impede mPFC activity, while promoting BLA firing, a neural state that we believe to promote high fear. We combine sophisiticated

behavioral approaches with systemic and intracranial pharmacology, chemogeneitcs, immunohistochemistry, and *in vivo* recordings in anesthetized and freely moving rats to examine how altered NE signaling affects the mPFC and BLA in relation to the IED.

2. NORADRENERGIC BLOCKADE STABILIZES PREFRONTAL ACITIVTY AND ENABLES FEAR EXTINCTION UNDER STRESS*

2.1 Introduction

Individuals exposed to extreme psychological stress, such as combat-related trauma or sexual abuse, are at risk for developing anxiety and trauma disorders, including post-traumatic stress disorder (PTSD). Although the etiology of PTSD is complex, it is widely believed that associative learning processes, including Pavlovian fear conditioning, contribute to its genesis. Moreover, an inability to suppress or extinguish fear memories may sustain pathologically high levels of fear in patients with PTSD years after the trauma (Kessler et al., 1995; Weston, 2014; Guthrie and Bryant, 2006; Milad et al., 2009; Blechert et al., 2007; Jovanovic et al., 2010). A variety of clinical interventions to facilitate fear extinction in patients with PTSD are currently being explored, although effective treatment for many afflicted individuals remains elusive (Fitzgerald et al., 2014a; Oznur et al., 2014).

One promising therapeutic target for facilitating extinction in PTSD patients is the noradrenergic system. Norepinephrine (NE) not only plays an important role in mood and arousal, but also in the encoding, retrieval, and reconsolidation of emotional memories (McGaugh, 2004; Debiec and Ledoux, 2004; Soeter and Kindt, 2011b; van Stegeren et al., 2010; Bos et al., 2012). Endogenous NE signaling is elevated in PTSD and drugs that block NE receptors are already being used with some success to either prevent or treat

* Reprinted with permission from PNAS Fitzgerald PJ, Giustino TF, Seemann JR, and Maren S (2015). Noradrenergic blockade stabilizes prefrontal activity and enables fear extinction under stress. ***Proc. Natl. Acad. Sci.***, 201500682. doi:10.1073/pnas.1500682112.

PTSD, including its symptoms of hyperarousal and nightmares (Southwick et al., 1997, 1999a; Pervanidou and Chrousos, 2010; Brunet et al., 2008, 2014). Clinically effective noradrenergic drugs include the α1-adrenoceptor antagonist, prazosin, and the β1/β2-adrenoceptor antagonist, propranolol (Brunet et al., 2008; Koola et al., 2014; Writer et al., 2014; Vaiva et al., 2003). The neural mechanisms underlying the efficacy of these drugs remain poorly understood.

It has previously been suggested that stress-induced changes in prefrontal cortical structure and function observed in animal models may contribute to the extinction deficits observed in patients with PTSD (Farrell et al., 2013; Chang et al., 2010; Fitzgerald et al., 2014b). Given the abundant literature implicating NE signaling in prefrontal cortical function (Arnsten, 2009; Robbins and Arnsten, 2009), it is conceivable that stress-induced elevations in prefrontal NE release (Finlay et al., 1995; Dazzi et al., 2005; Gresch et al., 1994) contribute to extinction impairments associated with PTSD. The mPFC, comprising the prelimbic (PL) and infralimbic (IL) subdivisions in rodents, plays a key role in the regulation of emotional behavior in both humans and rats (Farrell et al., 2010; Rive et al., 2013). Previous studies have suggested that PL plays an important role in fear expression, whereas IL is preferentially involved in fear extinction (Vidal-Gonzalez et al., 2006; Corcoran and Quirk, 2007; Burgos-Robles et al., 2009). Stress-induced alterations in the balance of neuronal activity in PL and IL as a consequence of noradrenergic hyperarousal might therefore contribute to extinction impairments and the maintenance of PTSD. To address this question, we combine *in vivo* microelectrode recording in freely moving rats with behavioral and pharmacological manipulations to determine whether β-

noradrenergic receptors mediate stress-induced alterations in mPFC neuronal activity. Further, we examine whether systemic propranolol treatment, given immediately after an aversive experience, rescues stress-induced impairments in fear extinction.

2.2 Results

2.2.1 Propranolol stabilizes medial prefrontal activity after footshock stress

To investigate the effect of systemic noradrenergic blockade on stress-induced changes in the mPFC, we performed single-unit recordings in freely moving rats (see Materials and Methods). Animals were first surgically implanted with a 16-channel microelectrode array (Innovative Neurophysiology) that spanned both PL and IL (8 wires in each) in the right hemisphere of each animal. Array placements in each rat are shown in Figure 2.1a. The recording sites varied somewhat in their mediolateral (i.e., laminar) or dorsoventral position within PL and IL across rats. However, there were no significant differences in single-unit firing or bursting as a function of mediolateral or dorsoventral position within these brain regions. Representative single-unit waveforms and their corresponding clusters from PL and IL are shown in Figure 2.1b.

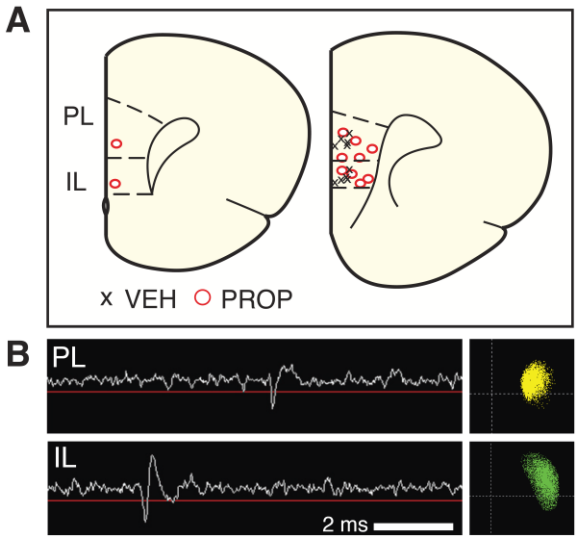

Figure 2.1 *In vivo* mPFC recordings in freely moving rats. a) Histological localization of the center of each electrode array in the mPFC; each array targeted both PL (8 wires) and IL (8 wires). Right hemisphere, coronal sections represent (left to right) coordinates +3.2 and +2.7 relative to bregma in the anteroposterior plane. Six rats received propranolol (PROP) treatment, and 5 received vehicle (VEH) treatment. In one of the propranolol rats, recordings were obtained only from PL. b) Example voltage trace from an electrode in PL (top panel) and IL (bottom panel), showing an action potential and its corresponding principal component scatter plot.

After a one-week recovery period, rats were transported to the recording chamber for the first of two recording sessions. During the first recording session, the animals received a standard Pavlovian fear conditioning procedure after systemic administration of either vehicle or propranolol (10 mg/kg, i.p.); the second session served as a retention test for conditioned fear. For both recording sessions, rats were connected with a flexible headstage cable to a multichannel OmniPlex recording system (Plexon), and their freezing behavior was monitored inside a standard conditioning chamber using a load-cell transducer and amplifier (Maren, 1998). We recorded from a total of 220 mPFC neurons on Day 1 [vehicle (VEH)-PL, n = 52; VEH-IL, n = 34; propranolol (PROP)-PL, n = 85;

PROP-IL, n = 49] and 185 neurons on Day 2 [VEH-PL, n = 47; VEH-IL, n = 34; PROP-PL, n = 63; PROP-IL, n = 41]. Although some of the units recorded on Day 2 may have been the same as those recorded on Day 1, we did not assume that they were and treated them as a separate population of neurons. The baseline firing rates of the units recorded on Day 1 (mean ± SEM; 3-min pre-drug baseline) were: VEH-PL = 5.15 ± 0.78 Hz (range: 0.26 -26.21 Hz); VEH-IL = 5.59 ± 0.88 Hz (range: 1.39 - 21.13 Hz); PROP-PL = 7.52 ± 0.75 Hz (range: 0.28 - 41.09 Hz); PROP-IL = 8.60 ± 1.41 Hz (range: 0.46 - 55.90 Hz). Although 23 cells had firing rates (>15 Hz; 10% of the sample) typical of those observed in inhibitory interneurons, it was not clear that these cells reflected a different population when various rate and waveform parameters were examined and they were consequently included in all analyses.

Drug administration prior to Pavlovian fear conditioning on Day 1 (Figure 2.2a) did not significantly alter the spontaneous firing rate of PL or IL neurons. Although firing rates decreased slightly in all animals over the pre-conditioning recording period, this decrease was similar in vehicle- and propranolol-treated rats [main effect of time, $F(2,432) = 15.57$, $p < 0.01$; time x drug interaction *ns*; time x drug x brain region interaction *ns*]; this contrasts with a previous report that found a significant decrease in PL firing with propranolol (Rodriguez-Romaguera et al., 2009). Average firing rates in the final 3-min block of the post-injection period were: VEH-PL = 4.73 ± 0.71 Hz; VEH-IL = 4.79 ± 0.92 Hz; PROP-PL = 7.17 ± 0.75 Hz; PROP-IL = 7.36 ± 1.14 Hz.

Eighteen minutes after drug administration, the rats received 5 pairings of an innocuous auditory conditioned stimulus (CS; 2 sec, 2 kHz, 80dB) with an aversive

footshock unconditioned stimulus (US; 0.5 sec, 1.0 mA); trials were separated by a 1-min inter-trial interval (ITI). Fear conditioning was followed by a 60-min stimulus-free period during which the neural and behavioral effects of conditioning were recorded. Not surprisingly, fear conditioning yielded robust increases in freezing behavior in vehicle-treated rats. Interestingly, post-shock freezing was significantly blunted by propranolol treatment [main effect of drug, $F(1,9) = 22.97$, $p < 0.01$] (Figure 2.2b). Correspondingly, fear conditioning produced dramatic changes in the spontaneous firing rate of PL and IL neurons and these conditioning-induced changes in firing rate were dampened in propranolol-treated rats. Figure 2.2c shows firing rate histograms for representative single-units in PL and IL from vehicle- and propranolol-treated rats. In vehicle-treated rats, fear conditioning produced substantial changes in the firing rate in both PL and IL; these changes were minimal in single-units from propranolol-treated rats. This is particularly evident in heat maps illustrating the normalized firing rate in the entire population of single-units recorded on Day 1 (Figure 2.2d). A much greater proportion of neurons recorded in vehicle-treated rats exhibited either markedly enhanced (light orange) or suppressed (light blue) firing rates soon after fear conditioning ($t > 0$) relative to units recorded in propranolol-treated rats.

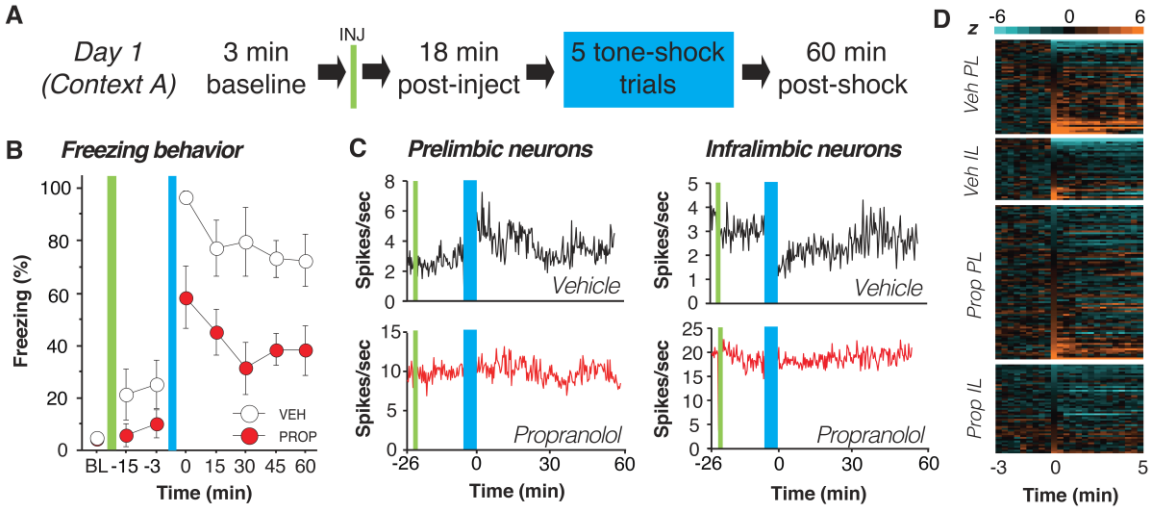

Figure 2.2 Propranolol stabilizes single-unit firing in mPFC neurons after footshock stress. a) Day 1 experimental design. b) Propranolol-treated rats (red circles) exhibited reduced freezing throughout the Day 1 recording session relative to vehicle-treated controls (white circles). c) Four representative histograms (20-sec bins) showing spontaneous firing rate from neurons recorded in PL (left panels) and IL (right panels). Fear conditioning (blue bar) altered the firing rate of the PL and IL neurons obtained from vehicle-treated rats (black traces, top panels) and propranolol administration mitigated this effect (red traces, bottom panels). d) Normalized firing rate heat maps showing post-conditioning increases (light orange) and decreases (light blue) relative to baseline (pre-conditioning) firing rate (black) for all of the units recorded in each group and brain region. Only the 3 minutes prior to conditioning and the first 5 minutes after conditioning are shown for clarity. In both PL and IL, single-units in vehicle-treated rats exhibited increases or decreases in firing rate after conditioning, and propranolol treatment mitigated these effects. Injection (INJ) is denoted by green vertical bar; conditioning (tone-shock pairings) is denoted by blue vertical bar. Data during the conditioning period were not recorded. All values are means ± SEM for freezing.

To assess group differences in spontaneous firing rate, we generated average firing rate histograms for all of the units recorded in each area and treatment condition (Figure 2.3). In vehicle-treated rats, fear conditioning massively, but transiently, increased the spontaneous firing rate among mPFC neurons in the minutes following fear conditioning. These firing rate changes were mitigated by propranolol-treatment in both PL [Figure

2.3a; drug x time interaction, $F(178,24030) = 4.36$, $p < 0.01$] and IL [Figure 2.3b; drug x time interaction, $F(178,14418) = 2.82$, $p < 0.01$]. For example, propranolol significantly attenuated conditioning-related increases in PL in the first immediate post-shock period [Figure 2.3a inset, $t(135) = 2.26$, $p < 0.05$] and IL [Figure 2.3b inset, $t(81) = 1.98$, $p = 0.05$] firing.

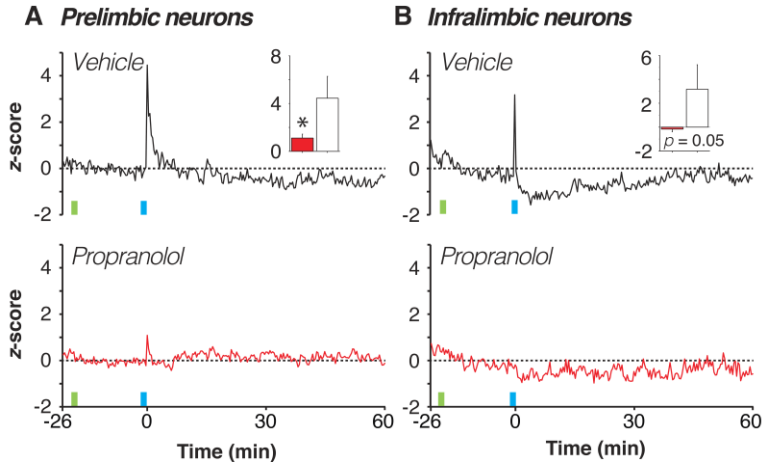

Figure 2.3 Propranolol stabilizes single-unit firing in the population of mPFC neurons. Spontaneous firing rates were averaged across all neurons and normalized to the pre-conditioning baseline for each brain region and treatment group. Fear conditioning (blue arrow) induced a dramatic increase in average spontaneous firing rate in PL neurons from vehicle-treated rats [a, black trace; inset shows first 20-sec post-shock bin, comparing vehicle (white bar) with drug (red bar)] that was mitigated by propranolol treatment (a, red trace). Conditioning induced a weaker post-shock increase in spontaneous firing in IL neurons from vehicle-treated rats (b, black trace; inset shows first 20-sec post-shock bin), and produced an enduring suppression of this activity. Propranolol treatment (b, red trace) counteracted both types of firing rate changes in IL. Injection (INJ) is denoted by green vertical bar; conditioning (tone-shock pairings) is denoted by blue vertical bar. Data during the conditioning period were not recorded. *$p < 0.05$ versus vehicle. All values are means (± SEM for insets).

In addition to the rapid increases in IL and PL firing rate after conditioning, IL neurons exhibited a sustained decrease in spontaneous firing that persisted (on average) for roughly 30 minutes after conditioning. This corresponds to a time window within which rats are resistant to extinction (Chang et al., 2010), and suggests that shock-induced depression of IL activity may, at least in part, account for this stress-induced "immediate extinction deficit" (IED).

Bursting of mPFC neurons has been implicated in both extinction learning (Chang et al., 2010; Robbins and Arnsten, 2009) and the IED (Chang et al., 2010). We therefore examined whether burst firing in PL and IL was modulated by footshock stress and noradrenergic blockade. Although normalized burst firing mirrored the patterns observed for overall firing rate (Figure 2.4), propranolol treatment did not reliably alter shock-induced changes in burst firing in the immediate post-shock period in PL neurons. This does not however, rule out the possibility that shock-induced changes in spontaneous firing in mPFC are responsible for the IED.

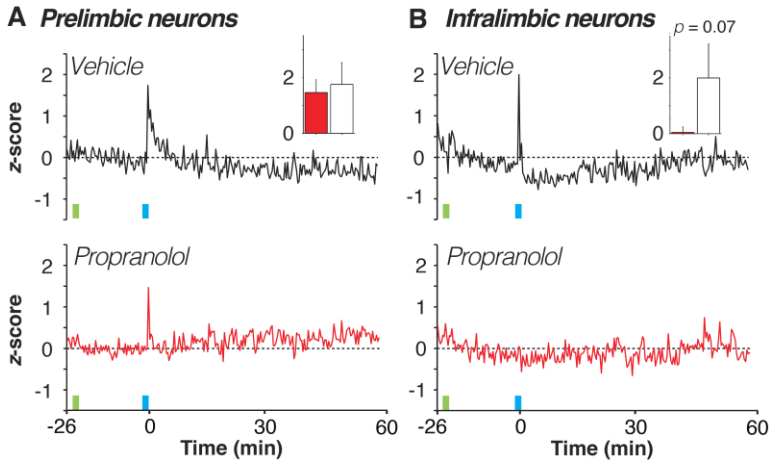

Figure 2.4 Propranolol stabilizes single-unit bursting in the mPFC after footshock stress. a, b) Spontaneous bursting rates averaged across all neurons and normalized to the pre-conditioning baseline for each brain region and treatment group. Fear conditioning (blue bar) induced an increase in average spontaneous bursting rate in PL neurons from vehicle-treated rats (a, black trace; inset shows first 20-sec post-shock bin in vehicle- and propranolol-treated rats) that was mitigated by propranolol treatment (a, red trace). The inset graph reveals no drug-induced difference in PL bursting, unlike for Day 1 firing rate; red bar indicates propranolol and white bar indicates vehicle. Conditioning also induced a post-shock increase in spontaneous bursting in IL neurons from vehicle-treated rats (b, black trace; inset shows first 20-sec post-shock bin comparing vehicle and drug), and produced an enduring suppression of this activity. Propranolol treatment (b, red trace) mitigated this effect. Injection (INJ) is denoted by green vertical bar; conditioning (tone-shock pairings) is denoted by blue vertical bar. Data during the conditioning period were not recorded. All values are means (± SEM in insets).

2.2.2 Propranolol mitigates shock-induced increases and decreases in mPFC firing rate

Fear conditioning induced robust changes in the average spontaneous firing rate of mPFC neurons. Nonetheless, individual single-units exhibited considerable diversity in their firing rate after fear conditioning (Figure 2.2d). We were interested in whether propranolol altered the proportion of neurons that increased or decreased their firing rate after conditioning, and whether the magnitude of the firing rate changes in these

populations differed in the treatment conditions. We therefore categorized units in both PL and IL according to their response bias in the immediate post-shock interval (i.e., the 20-sec period after the final tone-shock pairing), as well as during a 20-sec period at the end of the 60-min post-shock recording session.

There were no significant differences in the proportion of neurons increasing or decreasing their firing rates in vehicle- compared to propranolol-treated rats at either the immediate (Figure 2.5a) or remote time points (Figure 2.6). Yet despite increasing their firing rates after conditioning, the majority of single-units in both PL and IL exhibited a suppression of firing 60-min after conditioning (Figure 2.6). This increase in the proportion of neurons with suppressed firing rates across the recording session was observed in both the PL [$X^2(1) = 3.90$, $p < 0.05$] and IL [$X^2(1) = 4.98$, $p < 0.05$] of vehicle-treated rats. In propranolol-treated rats, this effect was only observed in PL neurons [$X^2(1) = 10.38$, $p < 0.01$], insofar as IL neurons were already more likely to be suppressed immediately after conditioning. Overall, these data indicate that the tendency for many neurons to show a transient increase in firing rate in the immediate post-shock period gave way to suppression in rate by the end of the 60-min post-shock period.

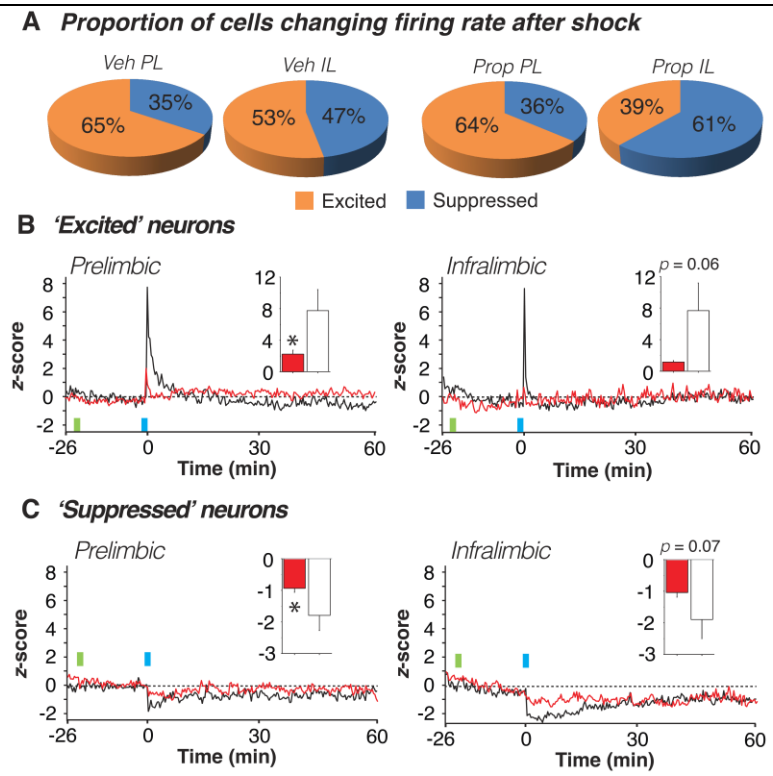

Figure 2.5 Propranolol stabilizes both increases and decreases in mPFC firing rate. a) Proportion of neurons in each of the four groups exhibiting immediate post-conditioning (first 20-sec bin) increases ($z > 0$, 'excited') or decreases ($z < 0$, 'suppressed') in spontaneous firing rate. Regardless of drug treatment, PL neurons tended to increase their firing rate after shock more so than IL neurons. b, c) Normalized firing rate histograms for PL (left panels) and IL (right panels) neurons that were 'excited' (b) or 'suppressed' c) in the immediate post-shock period in vehicle- (black traces) and propranolol-treated (red traces) rats. Inset graphs show the values for the first 20-sec post-shock bin in vehicle- (white bars) and propranolol-treated (red bars) rats. Injection (INJ) is denoted by green vertical bar; conditioning (tone-shock pairings) is denoted by blue vertical bar. Data during the conditioning period were not recorded. *$p < 0.05$ versus vehicle. All values are means (± SEM in insets).

Although the proportion of neurons showing immediate post-shock changes in firing was similar in vehicle- and propranolol-treated rats, there were considerable differences in the firing rate of these neurons (Figure 2.5b, c). "Excited" PL neurons in vehicle-treated rats that exhibited immediate post-shock increases in firing rate showed a

large, but transient increase in firing in the post-conditioning period; this effect was counteracted by propranolol treatment [drug x time interaction, $F(178,15308) = 5.68$, $p < 0.01$] (Figure 2.5b, left panel). Indeed, shock-induced increases in firing in the first immediate post-shock bin were significantly attenuated by propranolol treatment [$t(86) = 2.56$, $p < 0.05$] (Figure 2.5b, left inset). Similarly, "excited" IL neurons exhibited a transient increase in firing in the post-conditioning period that was attenuated in propranolol-treated rats [drug x time interaction, $F(178,6230) = 2.71$, $p < 0.01$] (Figure 2.5b, right panel). However, this effect only approached statistical significance in the first post-shock bin [$t(35)=1.92$, $p = 0.06$] (Figure 2.5b, right inset).

Conditioning-induced decreases in firing were also sensitive to propranolol treatment (Figure 2.5c). In vehicle-treated rats, "suppressed" PL neurons that exhibited immediate post-shock decreases in rate showed a decrease in firing in the post-conditioning period that was mitigated in propranolol-treated rats [drug x time interaction, $F(178,8366) = 1.33$, $p < 0.01$] (Figure 2.5c, left panel). Firing during the immediate post-shock bin was significantly greater in propranolol-treated rats [$t(47) = 2.19$, $p < 0.05$] (Figure 2.5c, left inset). Similarly, propranolol treatment limited the magnitude of firing rate suppression in IL neurons [drug x time interaction, $F(178,7832) = 2.90$, $p < 0.01$] (Figure 2.5c, right panel), although this only approached significance in the first immediate post-shock bin [$t(44) = 1.84$, $p = 0.07$] (Figure 2.5c, right inset). In summary, noradrenergic blockade stabilizes fear conditioning-induced changes in mPFC firing rate by limiting both increases and decreases in spontaneous firing rate.

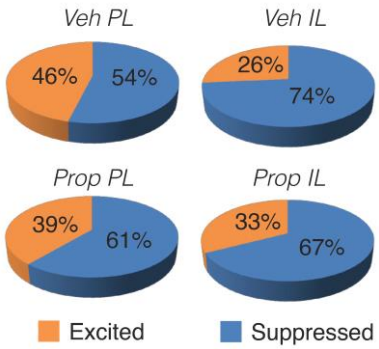

Figure 2.6 Single-unit firing rates tend to be suppressed after 60 minutes. At the 60 min time point of Day 1, the proportion of neurons with suppressed firing was significantly increased relative to the immediate post-shock period in all of the regions and conditions, except in IL neurons from propranolol-treated rats.

2.2.3 Expression of conditional freezing is not sufficient to increase mPFC firing rate

Twenty-four hours after the first recording session, a second session (Day 2) was conducted to examine whether the neural changes observed immediately after aversive conditioning also occurred during the expression of fear to the CS. To this end, rats were returned to the recording chamber, which was modified to create a context that was distinct from that used for conditioning (Figure 2.7; see Materials and Methods for details). After a 3-min baseline period, rats received five CS-alone trials (i.e., without footshocks; 1-min ITI), and a subsequent 60-min stimulus-free period. Both groups of rats exhibited high levels of conditioned freezing in the 3-min period immediately after presentation of the tones, indicating that systemic administration of propranolol prior to fear conditioning the previous day did not prevent the acquisition of conditioned fear (Figure 2.7). There was

no significant effect of pre-conditioning drug treatment on Day 1 on conditioned freezing behavior on Day 2 (Fs < 1).

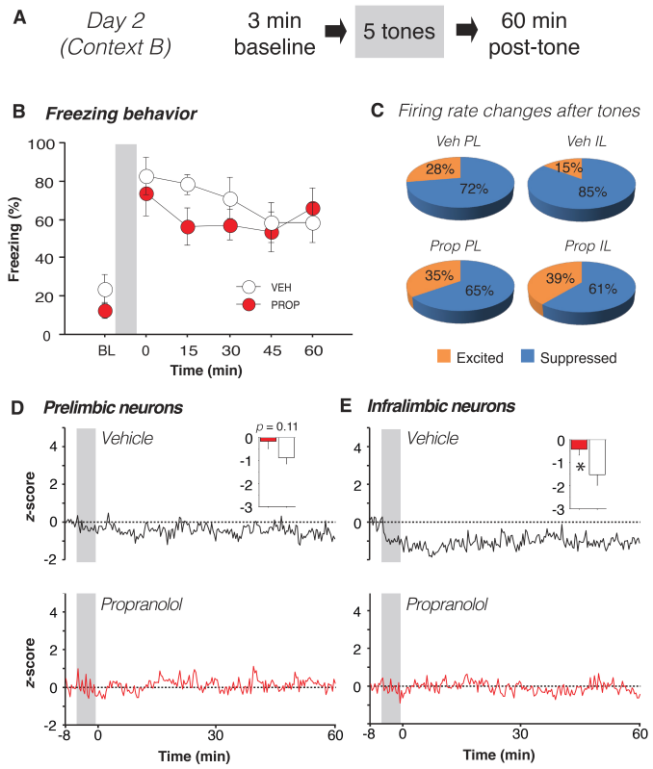

Figure 2.7 Propranolol stabilizes single-unit firing in IL after CS delivery. a) Day 2 experimental design. b) Propranolol-treated rats (red circles) did not exhibit altered freezing relative to vehicle-treated rats (white circles) when averaged across the entire session. c) After CS presentation, the majority of mPFC neurons in vehicle-treated rats showed decreases in firing rate, and this was most pronounced in IL neurons. A significantly greater proportion of IL neurons was suppressed in the vehicle- compared to propranolol-treated animals. (d, e) Unlike Day 1, the normalized firing rate of PL neurons in both vehicle- and propranolol-treated rats exhibited little change after presentation of the aversive CS. Interestingly, IL firing rate was depressed by CS presentations and this effect was mitigated by propranolol. Insets show normalized firing in the first 20-sec bin after the last CS for neurons from vehicle- (white bars) and propranolol-treated rats (red bars). Gray bars denote the CS period. *p < 0.05 versus vehicle. All error bars indicate mean ± SEM.

Importantly, CS presentations on Day 2 produced qualitatively different changes in spontaneous firing rate relative to Day 1 despite yielding high levels of conditioned freezing behavior (Figure 2.7). After CS presentation, the majority of mPFC neurons in rats that had been treated with vehicle the previous day showed decreases in firing rate (Figure 2.7c), and this was most pronounced in IL neurons. A significantly greater proportion of IL neurons was suppressed in the vehicle- compared to propranolol-treated animals [$X^2(1) = 5.45$, $p < 0.05$]. This pattern was also reflected in the normalized firing rates across the recording session (Figure 2.7d,e). Spontaneous firing tended to decrease in both PL and IL after CS presentation, and propranolol administration prior to fear conditioning on Day 1 reliably dampened this effect in IL [drug x time interaction, $F(179,13067) = 2.25$, $p < 0.01$]. This latter effect was particularly robust in the 20-sec period immediately after delivery of the last tone [$t(73) = 2.14$, $p < 0.05$] (Figure 2.7, inset). Similar to Day 1, neuronal bursting largely mirrored the spontaneous firing rate data. In addition to influencing spontaneous firing rate on Day 2, propranolol administration prior to conditioning on Day 1 significantly affected CS-evoked firing during the tone presentations themselves (Figure 2.8).

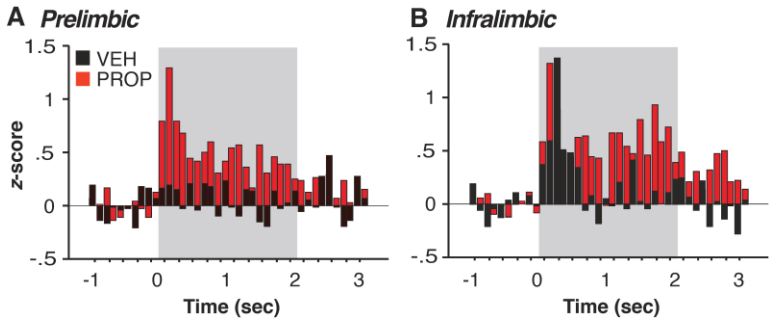

Figure 2.8 CS-evoked responses in the mPFC are modulated by propranolol. On Day 2, CS-evoked activity was significantly different between vehicle- and propranolol-treated rats. Peri-event time histograms (100-msec bins) computed on average normalized firing rate (post-CS firing normalized to 1-sec pre-CS baseline) across the five CS presentations revealed that both PL (a) and IL (b) neurons exhibited increased firing due to prior propranolol treatment [PL, drug x time interaction, $F(29,2958) = 2.52$, $p < 0.01$; IL, $F(29,2117) = 1.67$, $p < 0.05$].

The fact that CS presentations on Day 2 evoked robust freezing behavior, but minimally altered spontaneous firing in mPFC suggests that a transition from a low fear state to a high fear state is not responsible for the firing rate changes observed on Day 1. Figure 2.9 illustrates this observation by plotting freezing behavior and normalized firing rate across the two recording sessions. Although the levels of freezing were similar immediately after either CS-US (Day 1) or CS-alone (Day 2) trials on each day, changes in neuronal activity were markedly different. On Day 1, vehicle-treated rats showed a post-shock increase in spontaneous firing rate relative to propranolol-treated animals [main effect of drug, $F(1,216) = 8.41$, $p < 0.01$]. This contrasted with Day 2, where vehicle rats showed *suppression* of firing that was counteracted by propranolol [main effect of drug, $F(1,181) = 7.18$, $p < 0.01$]. Thus, on both days, propranolol treatment mitigated changes in post-CS firing that were evident in vehicle-treated rats.

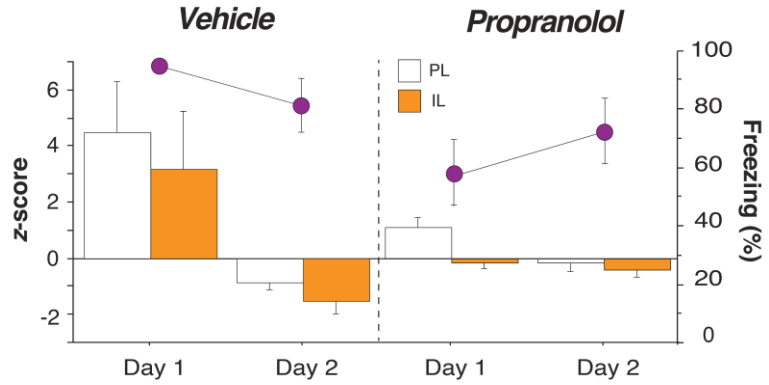

Figure 2.9 Freezing behavior does not alter mPFC firing rate. Freezing behavior in both vehicle-treated (**left panel**) and propranolol-treated (**right panel**) rats was not markedly different during the immediate post-shock period on Day 1 compared with the immediate post-CS period on Day 2 [values represent the average freezing immediately following the last US (Day 1) or CS (Day 2)]. Despite similarities in Day 1 and Day 2 freezing, normalized firing rate during the first 20 sec following the last US (Day 1) or CS (day 2) was dramatically elevated in both IL and PL on Day 1 relative to Day 2 in vehicle-treated rats (**left panel**). This effect was mitigated by propranolol treatment (**right panel**). Hence, marked differences in firing rate in the mPFC cannot be attributed to freezing behavior *per se*, because both post-trial periods (on Day 1 and Day 2) yielded similar and high levels of freezing. All values are means ± SEM.

2.2.4 Propranolol facilitates extinction under stress

We have previously established that extinction fails when given soon after footshock, when levels of acute psychological stress are high; that is, rats exhibit an "immediate extinction deficit" (IED) when extinction trials are delivered soon after fear conditioning (Chang et al., 2010; Maren and Chang, 2006; Chang and Maren, 2009; Maren, 2014) but also see (Myers et al., 2006; Norrholm et al., 2008). It is notable that we now show that fear conditioning is followed by a lasting suppression of IL firing (Figure 3b), a time at which CS-alone trials are ineffective at supporting long-term extinction (Maren and Chang, 2006; Chang and Maren, 2009; Maren, 2014). We have previously suggested that

the IED results from a high state of fear that interferes with mPFC function. Given that systemic propranolol reduces shock-induced freezing and stabilizes mPFC neural activity, we tested whether it would mitigate the IED.

In this experiment, rats were systemically administered either vehicle (n = 7) or propranolol (n = 7) immediately after fear conditioning; an immediate extinction session consisting of 45 presentations of the CS (1-min ITI) was conducted 30 min after conditioning. All rats were then given a retrieval test 48 hours after extinction using identical procedures to those used during the extinction session (i.e., 45 CS-alone "test" trials). Both groups acquired fear conditioning and there were no differences in conditioned freezing prior to drug treatment [Figure 2.10a; $F(1,12) < 1$]. However, propranolol treatment prior to the immediate extinction session significantly reduced both pre-trial (BL) and within-session freezing during the extinction trials [Figure 2.10b; main effect of drug, $F(1,12) = 8.40$, $p < 0.05$] of the immediate extinction session. Importantly, propranolol facilitated the acquisition of long-term extinction; freezing in propranolol-treated rats was significantly lower during the first 9-trial block of the retention test [Figure 2.10c, drug x time interaction, $F(5,60) = 3.27$, $p < 0.05$]. Thus, propranolol treatment before the immediate extinction procedure limited the spontaneous recovery of fear that characterizes the IED in vehicle-treated rats and promoted the retention of extinction under conditions of high psychological stress that normally impair extinction learning.

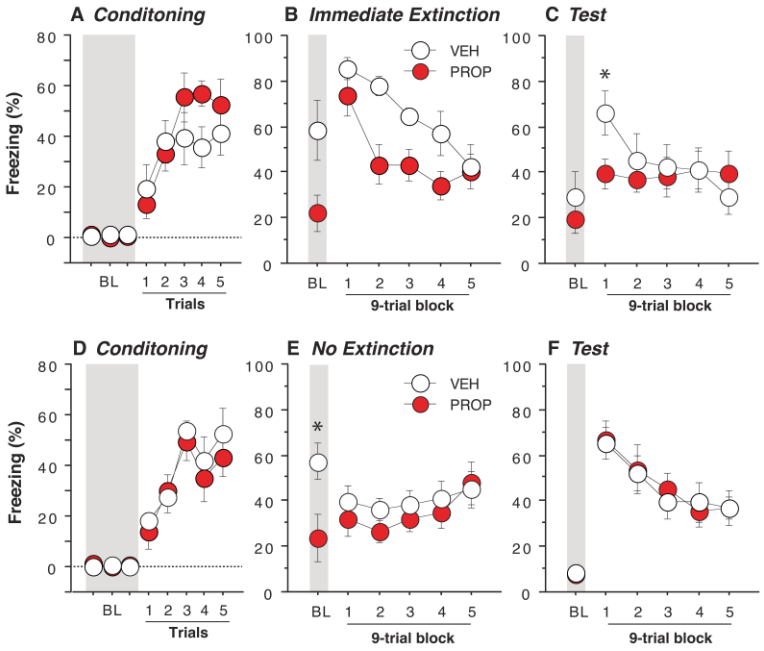

Figure 2.10 Propranolol mitigates the immediate extinction deficit. Propranolol administration immediately after fear conditioning (a) reduced both baseline (BL) and freezing during the immediate extinction session (b, 30 min after conditioning). Propranolol facilitated the recall of extinction during a retention test conducted 48 hours after extinction (vehicle, *n*=7; propranolol, *n*=7) (c). This effect on freezing was not due to impaired consolidation of the fear conditioning memory. Rats that were administered propranolol immediately after conditioning (d), but not extinguished exhibited reductions in freezing early in the no-extinction session (e, context exposure only) and high levels of freezing during the retention test that did not differ from vehicle-treated controls (vehicle, *n*=8; propranolol, *n*=8) (f). Gray bars denote the 3-min baseline (BL) period prior to delivery of conditioning or extinction trials. $*p < 0.05$ versus propranolol. All values are means ± SEM.

Of course, in testing the effects of propranolol on the IED, it is possible that propranolol administered immediately after fear conditioning reduced conditioned freezing by impairing the consolidation of the fear memory, rather than facilitating extinction. To address this possibility, we conducted an additional experiment in which rats received vehicle (n = 8) or propranolol (n = 8) immediately after conditioning, but did

not receive extinction trials (Figure 2.10d-f). As before, both groups acquired fear conditioning and exhibited similar levels of conditioned freezing prior to drug treatment [Figure 2.10d; main effect of group, $F(1,14) < 1$]. Thirty minutes after conditioning, the rats were returned the conditioning chambers (context B), but no extinction trials were delivered. Similar to the previous IED experiment, propranolol-treated rats exhibited reduced freezing early in the session [Figure 2.10e; drug x time interaction, $F(5,70) = 2.88$, $p < 0.05$]. Importantly, during the fear recall test 48 hrs after conditioning, there was no difference in conditioned freezing between the groups [Figure 2.10f; Fs < 1]. This indicates that post-conditioning propranolol did not impair consolidation of the fear memory, an effect that is consistent with a previous report (Debiec and Ledoux, 2004). These data support the view that post-conditioning propranolol facilitates immediate extinction by reducing post-shock fear and stabilizing mPFC firing.

If propranolol facilitates extinction learning under conditions of high noradrenergic arousal, how might it affect learning when the psychological stress level is presumably lower? To address this question, we conduced another experiment in which rats received vehicle or propranolol ($n = 8$ per group) prior to delayed extinction (24 hours after conditioning). As shown in Figure 11, freezing behavior prior to the first CS trial during the extinction session was low (Figure 2.11b) in both vehicle- and propranolol-treated rats. This indicates that delayed extinction limits the high levels of sensitized fear observed with the immediate extinction procedure (Figure 2.10b, e). In addition, propranolol administration did not influence either the expression of fear or within-session extinction [Figure 2.11b; Fs < 2], consistent with the suggestion that basal noradrenergic

arousal must be high in order for propranolol to limit freezing. However, propranolol-treated rats exhibited *impairments* in extinction recall during the retention test place 24 hrs after extinction. Specifically, rats in the propranolol group exhibited greater levels of freezing during the first block of 9 trials of the session relative to vehicle-treated rats [Figure 2.11c; drug x time interaction, $F(5,70) = 2.84$, $p < 0.05$]. Thus, propranolol given before delayed extinction impaired, rather than enhanced, learning during this putatively lower state of psychological stress.

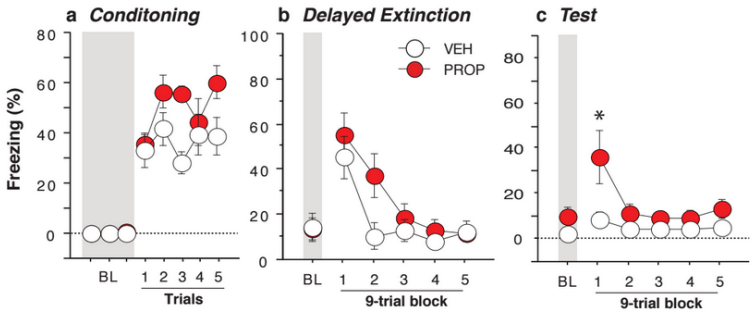

Figure 2.11 Propranolol impairs delayed extinction. In contrast to its effects on immediate extinction, propranolol given 30 min before a delayed extinction session, which took place 24 hrs after conditioning (a) did not significantly alter within-session extinction (b). Moreover, propranolol-treated rats exhibited a deficit in extinction recall 24 hrs after extinction (c) (vehicle, *n*=8; propranolol, *n*=8). Gray bars denote the 3-min baseline (BL) period prior to delivery of conditioning or extinction trials. *$p < 0.05$ versus vehicle. All values are means ± SEM.

2.3 Discussion

Stress-induced extinction deficits, including the IED, have been posited to arise from dysregulation of mPFC function (Chang et al., 2010; Maren, 2014; Izquierdo et al., 2006). In support of this hypothesis, the present experiments reveal that footshock stress

accompanying fear conditioning produces dramatic changes in both the firing rate and bursting profile of single-units recorded in PL and IL. Systemic beta-adrenergic blockade by propranolol counteracts these effects by maintaining a relative "balance" in PL and IL neural activity after the footshock stressor. In addition, propranolol administration rescued the IED, suggesting that noradrenergic stabilization of mPFC activity buffers against the deleterious effects of stress on extinction learning. This novel finding has important implications for understanding how beta-noradrenergic interventions minimize the deleterious effects of marked psychological stress or trauma and improve psychotherapeutic outcomes (Brunet et al., 2008, 2014). Moreover, these data suggest that propranolol may be particularly effective in facilitating fear reduction when prevailing stress at the onset of extinction training is high. Thus, the timing of propranolol administration may be critical to maximizing its therapeutic efficacy in the treatment of stress- or trauma-related disorders such as PTSD.

It has previously been suggested that PL and IL have opposing roles in the regulation of fear (Chang et al., 2010; Vidal-Gonzalez et al., 2006; Corcoran and Quirk, 2007; Burgos-Robles et al., 2009). The present data lend some support to this view insofar as the expression of freezing behavior after conditioning was associated with distinct patterns of firing among simultaneously recorded neurons in PL and IL. Immediately after the last fear conditioning trial on Day 1, PL neurons (on average) exhibited a massive, but transient increase in spontaneous firing rate. In contrast, IL neurons (on average) exhibited a weaker increase in firing rate, followed by a sustained decrease in firing that persisted for much of the recording session. Hence, a shift in the balance of PL and IL activity

accompanied both the induction and maintenance of freezing behavior in the aftermath of conditioning. However, it is important to note that sustained decreases in IL firing, rather than sustained increases in PL firing, were associated with the maintenance of freezing behavior. This observation suggests that regulation of IL-mediated inhibition of amygdala excitability, for example, is not only involved in the expression of extinction (Quirk et al., 2003), but also the expression of conditioned fear. Consistent with the sustained changes in firing rate we observed after footshock, a study of restraint stress in rats found that a population of mPFC neurons showed an increase in firing rate that persisted for over 2 hours after the stressor (Jackson and Moghaddam, 2006).

Surprisingly, fear-related changes in mPFC firing were qualitatively different after fear conditioning than after the presentation of fear CSs. Neurons in both IL and PL exhibited much more dramatic changes in spontaneous firing immediately after CS-US pairings on Day 1 than after presentation of the CS alone on Day 2, despite similar (and nearly asymptotic) levels of freezing behavior in each session, particularly in vehicle-treated rats. These results indicate that it is not high levels of fear *per se* that correlate with changes in mPFC neuronal firing (Chang et al., 2010; Fitzgerald et al., 2014b; Vidal-Gonzalez et al., 2006; Corcoran and Quirk, 2007; Burgos-Robles et al., 2009), but rather the emotional context in which that fear is experienced (Goosens et al., 2003; Hobin et al., 2003). Specifically, our results suggest that mPFC firing is particularly sensitive to the acute effects of the footshock US, possibly reflecting unconditioned components of fear in the immediate aftermath of shock exposure. Experiments examining the consequences of footshock delivery immediately upon placement in the recording chamber (i.e., an

immediate shock procedure that yields little freezing) would help to resolve this issue (Fanselow, 1980; Lattal and Abel, 2001). Collectively, our results reveal that whereas recent exposure to footshock strongly modulates neuronal firing in mPFC, exposure to the CS alone does not. It should also be noted that within-session spontaneous firing rates in PL and IL did not correlate with ongoing freezing behavior (and by inference, fear state). For example, firing rates in both PL and IL largely returned to baseline by the end of the first recording session, despite the fact that freezing behavior remained markedly elevated relative to the pre-shock baseline. This suggests that circuits other than mPFC mediate the sustained freezing behavior we observed, although PL and IL may initiate or otherwise contribute to this effect.

Because beta-adrenergic receptors have previously been implicated in stress-induced modulation of prefrontal function (Ramos and Arnsten, 2007), we next examined whether systemic propranolol administration affected shock-induced changes in PL and IL firing. We found that propranolol administration prior to fear conditioning stabilized spontaneous activity in PL and IL after footshock, dampening the magnitude of shock-induced spike firing changes observed among single-units in each area. Specifically, propranolol both attenuated the immediate post-shock increases in firing rate in PL, as well as the decreases in IL firing that accompanied the expression of fear during the remainder of the session. This suggests that propranolol may reduce fear after conditioning, at least in part, by stabilizing neuronal firing in PL and IL in the aftermath of footshock.

Indeed, the stabilizing effect of propranolol on PL and IL spike firing may underlie the facilitation of extinction that we observed behaviorally when this drug was given prior to immediate extinction. That is, propranolol administered immediately after fear conditioning reduced the expression of freezing behavior during the immediate extinction session and facilitated lasting extinction. This effect was not due to an effect of propranolol on fear memory consolidation (see also (Debiec and Ledoux, 2004)) as the drug had no effect on conditioned freezing in animals that did not undergo extinction. Similar to our results, Quirk and colleagues observed decreases in freezing behavior after systemic propranolol administered before an extinction session that was conducted twenty-four hours after conditioning (Rodriguez-Romaguera et al., 2009). Interestingly, however, they found no lasting effect of propranolol on extinction under these conditions (Rodriguez-Romaguera et al., 2009) and, in a related study, they reported extinction impairments after intra-IL propranolol infusion (Mueller et al., 2008). We suggest that the disparities in these results are related to the timing of extinction and propranolol administration relative to fear conditioning. Specifically, propranolol administration soon after conditioning facilitates immediate extinction by dampening shock-induced noradrenergic arousal (Gresch et al., 1994; Finlay et al., 1995; Dazzi et al., 2005; Galvez et al., 1996), whereas propranolol administration long after conditioning, when noradrenergic arousal is low, impairs extinction learning by reducing adrenergic transmission below optimal levels (Arnsten, 2009). This latter hypothesis is consistent with the present data showing that propranolol administered before delayed extinction

actually impairs learning. Collectively, our data suggest that propranolol administered during stress stabilizes PL and IL activity and facilitates extinction learning.

Of course, a critical question is whether the beta-adrenergic receptors mediating the effects of systemic propranolol are located in the mPFC or in other brain regions that regulate the mPFC including the locus coeruleus (LC) and basolateral amygdala (BLA) (McIntyre et al., 2012). Consistent with the former possibility, IL infusion of propranolol has been reported to influence extinction recall (Mueller et al., 2008); however, it is not known whether this manipulation facilitates immediate extinction. Alternatively, noradrenergic modulation of BLA excitability (McIntyre et al., 2012) may influence mPFC firing to regulate extinction. Consistent with this possibility, it has been found that induction of inflammatory pain decreases mPFC firing, a change that was mediated by hyperexcitability in the BLA (Ji et al., 2010). Indeed, other stressors have also been reported to modulate mPFC through the amygdala (Maroun and Richter-Levin, 2003), and the BLA regulates fear and extinction through its long-range projections to mPFC (Senn et al., 2014). Ultimately, stress-induced NE release from LC terminals, which has been broadly implicated in the regulation of memory and emotion (McIntyre et al., 2012), may influence mPFC spike firing either directly or through indirect modulatory circuits.

The present experiments have critical implications for developing pharmacotherapeutic interventions for anxiety- and trauma-related disorders in humans. For example, a commonly used, albeit controversial approach to prevent PTSD is so-called psychological debriefing, in which behavioral therapy is given soon after exposure to a traumatic event (Deahl, 2000; Mansdorf, 2008; Forneris et al., 2013). Administration of

noradrenergic pharmacological agents, such as propranolol, soon after trauma could enhance the effectiveness of debriefing or other early interventions by modulating prefrontal cortical activity as we have described here. Consistent with this, it has been reported that propranolol treatment within days of trauma in humans reduces the incidence of PTSD (Vaiva et al., 2003). Moreover, propranolol administered after trauma reactivation in patients with PTSD has a therapeutic effect on physiological responding to traumatic imagery weeks after the pharmacological intervention (Brunet et al., 2014, 2008). Together, these studies suggest that propranolol administration may be particularly effective when trauma-related arousal is high (i.e., soon after trauma and after trauma reactivation). The present data suggest that the efficacy of propranolol under these conditions would be greatly enhanced by concurrent exposure therapy.

In summary, exposure to footshock stress initiates pronounced signaling changes in mPFC and freezing behavior, and β-noradrenergic blockade by propranolol mitigates these effects. Collectively, these findings shed light on prefrontal executive control of fear-related behavior, while also suggesting that propranolol treatment may enhance behavioral debriefing aimed at preventing PTSD development after recent exposure to trauma.

2.4 Materials and Methods

2.4.1 Subjects

Adult male Long-Evans Blue Spruce rats (weighing 200-224 g; 50-57 days old) were obtained from a commercial supplier (Harlan Sprague-Dawley, Indianapolis, IN). Upon arrival and throughout the experiments, these experimentally naïve rats were individually

housed in cages within a temperature- and humidity-controlled vivarium, and kept on a 14:10 hr light/dark cycle (lights on at 7 am) with ad libitum access to food and water. All experiments took place in the daytime during the light phase. Rats were handled for ~30 seconds a day for 5 days before any behavioral testing or surgical procedures were carried out to habituate them to the experimenter. The number of rats used in each experiment is stated in the figure legends. All experiments were conducted at Texas A&M University with full approval from its Animal Care and Use Committee.

2.4.2 Drugs

D,L-propranolol hydrochloride was obtained from a commercial supplier (Sigma-Aldrich, St. Louis, MO). The drug was dissolved in distilled water (5 mg/ml) and injected systemically (10 mg/kg, i.p.) in a volume of 2 ml/kg.

2.4.3 *In vivo* electrophysiology

Twelve rats (vehicle, n = 6; propranolol, n = 6) were used for the electrophysiological experiments; one rat in the vehicle group died prior to completing the experiment leaving five rats in that group. Rats were assigned to each drug condition such that each condition was alternated across the experiment. For implantation of the recording array, rats were anesthetized with isoflurane (5% induction, 2% maintenance) and secured in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). The scalp was incised and retracted; three burr holes were drilled for anchor and ground screws. A portion of the skull overlying the medial prefrontal cortex (mPFC) was removed to allow for microelectrode implantation. The rat was implanted with a 16-channel microelectrode array (Innovative Neurophysiology, Durham, NC) targeting both the prelimbic (PL; 8 wires) and infralimbic

(IL; 8 wires) subdivisions of the mPFC in the right hemisphere. The 2x8 wire microarray was constructed from two rows of 50 µm diameter tungsten wires of two different lengths (PL, 6.9 mm; IL, 8.0 mm); wires in each row and the rows themselves were spaced 200 µm apart. The array was positioned with its long axis parallel to the anterior-posterior plane. The coordinates for the centermost wires of the array was: PL, +2.7 mm AP, +0.55 mm ML, -4.0 mm DV and IL, +2.7 mm AP, +0.35 mm ML, -5.1 mm DV (relative to bregma at skull surface). The mediolateral offset (200 µm) between the PL and IL electrode rows minimized damage to the overlying cortex during array implantation. Also, the slightly more medial coordinate of the IL wires, relative to the PL ones, accommodates the slightly thinner IL cortex, allowing recordings in similar layers in the two brain areas within a given rat. The array was secured to the skull with dental acrylic and one week was allowed for recovery before *in vivo* recordings began.

A standard rodent conditioning chamber (30x24x21 cm, Med Associates, St. Albans, VT) housed in a sound-attenuating cabinet was modified to allow for electrophysiological recordings. The chamber consisted of two aluminum sides, a Plexiglas rear wall, and a hinged Plexiglas door. The grid floor contained 19 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center-to-center). Rods were connected to a shock source and solid-state grid scrambler (Med Associates) for the delivery of footshocks. A loudspeaker mounted on the outside of a grating in one aluminum wall was used to play auditory tones. Locomotor activity was transduced by a load-cell under the floor of the chamber, and the output of the load-cell was recorded by an OmniPlex

recording system (Plexon, Dallas, TX). The experimenters were not blind to drug treatment group, but all behavioral and neural activity was recorded automatically.

Single-unit recordings occurred over two days in two distinct contexts. On Day 1, the rats were transported to the recording room in a black box, connected to the headstage cable, and placed in the recording chamber. The chamber was cleaned with 1% ammonium hydroxide to provide a distinct olfactory cue, a black pan containing a thin layer of the same solution was placed under the grid floor, and the room was illuminated with ambient red lights (context A). After a 3-min stimulus-free baseline period, the rat was briefly removed from the chamber and injected with either propranolol (10 mg/kg, i.p) or vehicle (distilled water) and then immediately returned to the recording chamber. Neural and behavioral data were not recorded during injection (~1 min) due to the electrical noise associated with handling the rat. Twenty minutes after the injection (Rodriguez-Romaguera et al., 2009), five tone (2 sec, 80 dB, 2 kHz)-footshock (0.5 sec, 1 mA) trials were delivered (shock onset occurred at tone offset) with a 1-min inter-trial interval (ITI). Behavioral and neuronal data were not recorded during the conditioning period due to the electrical noise associated with shock delivery; recordings commenced immediately after the last footshock. The recording session continued for 60-min after the last footshock, after which the rat was returned to its home cage.

On Day 2, the transport and recording contexts were altered to reduce generalization of fear from the conditioning session to the test session. The rat was transported in a white box. The recording chamber was cleaned with 1% acetic acid to provide a distinct olfactory cue, a white pan containing a thin layer of the same solution

was placed under the grid floor, the grid floor was covered with a transparent rubber mat, the back wall was covered with an alternating black and white stripes, and the room was illuminated with ambient fluorescent lights (context B). After a 3-min stimulus-free baseline period, the rat was presented with five tone-alone trials (1-min ITI; all tone parameters were the same as on Day 1); the rat remained in the chamber for 60-min after the final tone and behavioral and neuronal data were recorded throughout the session.

Extracellular single-unit activity was recorded using a multichannel neurophysiological recording system (OmniPlex, Plexon, Dallas, TX). Wideband signals recorded on each channel were referenced to one of the recording wires (resulting in 15 channels of activity per rat), amplified (8,000x), digitized (40 kHz), and saved on a PC for offline sorting and analysis. The recording reference wire was located in PL, and was randomly selected to optimize the quality of the recordings. After high-pass filtering the signal at 600 Hz, waveforms were sorted manually using 2-dimensional principal component analysis (Offline Sorter, Plexon). Only well-isolated units were used in the analysis. If two units with similar waveforms and identical time stamps for their action potentials appeared on adjacent electrodes, only one unit was used. Sorted waveforms and their timestamps were then imported to NeuroExplorer (Nex Technologies, Madison, AL) for analysis.

The analysis of neuronal activity focused on spontaneous single-unit firing and bursting during each recording session; CS-evoked activity was also analyzed on day 2. To compute firing rate histograms, spike rates were binned (20 sec) and normalized (*z*-scores) to control for differences in baseline firing rate. On day 1, firing rate was

normalized to the entire pre-conditioning period (3-min pre-injection and 20-min post-injection periods). On day 2, the data were normalized to the 3-min baseline period prior to CS presentations. For the burst analyses, a burst was defined as two spikes with an inter-spike interval of <25 msec followed by a third spike within 50 msec of the second spike (Burgos-Robles et al., 2007); bursts could continue if additional spikes occurred within 50-msec intervals of one another.

2.4.4 Immediate extinction deficit (IED) and delayed extinction experiments

Sixteen adult Long-Evans rats served as subjects. All behavior training was conducted in two adjacent rooms, each containing eight identical conditioning chambers (same dimensions as for the *in vivo* recordings). Video cameras mounted above the behavioral chambers were used to monitor the animals during each session. Each chamber rested on a load-cell platform that transduced locomotor activity (Med Associates). Load-cell activity was digitized (Threshold Activity Software, Med Associates) and transformed as previously described to measure freezing behavior. Rats received three phases of training. They first received fear conditioning (context A, room 1) followed 30 minutes later by an "immediate" extinction session (context B, room 2). An extinction retrieval test (Context B, room 2) was conducted 48 hours after extinction. Contexts had distinct olfactory and visual cues, similar to those described above.

Fear conditioning consisted of a 3-min stimulus-free baseline period, followed by 5 tone (10 sec, 80 dB, 2 kHz)-shock (2 sec, 1 mA) pairings (1 min ITI); the rats remained in the chambers 3 minutes after the last trial. Immediately after conditioning, half the rats received systemic administration of propranolol (n = 8, 10 mg/kg, i.p.) and the other half

received vehicle (n = 8, distilled water); after injection, they were returned to their home cages in the vivarium. Thirty minutes after fear conditioning, the rats were returned to a novel room and context (context B) and presented with 45 tone-alone trials (1-min ITI; same tone parameters as during fear conditioning) after a 3-min baseline. All rats were given a subsequent extinction retrieval session (retention test; context B; same tone parameters as before) 48 hours after conditioning to assess long-term extinction memory. One rat from each group exhibited levels of conditioned freezing during the retention test that was ±2 standard deviations from the group mean; these statistical outliers were excluded from the analysis.

In a second behavioral experiment ("no extinction") with 16 adult Long-Evans rats, we examined whether post-conditioning propranolol treatment interferes with fear memory consolidation. The experiment was identical to that described in preceding IED experiment, except that CS-alone trials were not delivered 30 minutes after conditioning, although rats were still placed in the extinction context. Rats did receive a retention test 48 hrs later, in which the CSs were administered.

A third experiment examined delayed extinction (16 adult Long-Evans rats), in which the extinction session took place 24 hrs after fear conditioning and propranolol or vehicle was given 30 min before extinction. A retention test was then given 24 hrs after extinction.

2.4.5 Histology

After the completion of experiments, recording rats were overdosed with pentobarbital, and electrolytic lesions (80 µA, 10 sec; A365 stimulus isolator, World Precision

Instruments, Sarasota, FL) were generated through six of the recording wires to mark the location of the recording array in the medial prefrontal cortex. The rats were then perfused transcardially with 0.9% saline followed by 10% formalin. Brains were extracted from the skull and post-fixed in a 10% formalin solution for 24 hours followed by 10% formalin/30% sucrose solution where they remained for a minimum of 48 hours. After the post-fix period, brains were sectioned (50 μm) on a cryostat (-20^{o} C), mounted on subbed microscope slides, and stained with thionin (0.25%) to visualize electrode placements.

2.4.6 Statistics

Data were analyzed with conventional parametric statistics (StatView, SAS Institute). Two-way analysis of variance (ANOVA) and repeated-measures ANOVA were used to assess general main effects and interactions ($\alpha = 0.05$). Unpaired student's two-tailed *t*-tests were also used for pairwise comparisons of means. Results are shown as means ± SEMs.

3. BETA-ADRENOCEPTOR BLOCKADE IN THE BASOLATERAL AMYGDALA, BUT NOT THE MEDIAL PREFRONTAL CORTEX, RESCUES THE IMMEDIATE EXTINCTION DEFICIT*

3.1 Introduction

Early interventions (e.g., exposure therapy) after psychological trauma are aimed at reducing the development of stressor- and trauma-related disorders such as posttraumatic stress disorder (PTSD). The timing of these therapeutic interventions relative to trauma may be a key factor in their long-term success. For example, both human and animal research suggest that early interventions may actually worsen symptoms (Bryant, 2002; Maren and Chang, 2006) relative to delayed interventions. Using Pavlovian fear conditioning and extinction procedures in rats, we and others have similarly found that administering extinction trials soon (minutes to several hours) after conditioning yields little long-term extinction (Kim et al., 2010; MacPherson et al., 2013; Maren, 2014; Maren and Chang, 2006; Stafford et al., 2013). This "immediate extinction deficit" (IED) may be caused by stress-induced impairments of extinction learning and recall (Maren and Holmes, 2016), because it is not observed with weak conditioning procedures (Maren and Chang, 2006). Thus, "extinction-like" therapies in humans may fail when administered soon after trauma (when victims are still under duress) and lead to fear relapse (Bouton, 2000; Goode and Maren, 2014).

* Reprinted with permission from Neuropsychopharmacology Giustino TF, Seemann JR, Acca GM, Goode TD, Fitzgerald PJ, Maren S. (2017). β-Adrenoceptor Blockade in the Basolateral Amygdala, but not the Medial Prefrontal Cortex, Rescues the Immediate Extinction Deficit ***Neuropsychopharmacology*** doi:10.1038/npp.2017.89.

Considerable evidence indicates that stress-induced impairments in extinction learning are mediated by the medial prefrontal cortex (mPFC) (Holmes and Wellman, 2009; Maren and Holmes, 2016), a brain area that is critical for extinction learning (Giustino and Maren, 2015; Milad and Quirk, 2012). In particular, activity in the infralimbic (IL) subdivision of the mPFC is thought to underlie extinction learning (Fitzgerald et al., 2014b; Do-Monte et al., 2015; Bukalo et al., 2015). One possibility is that stress-induced elevations in noradrenergic signaling dysregulate mPFC function and impair mPFC-dependent psychological processes, including extinction. Consistent with this possibility, it has long been appreciated that norepinephrine (NE) is chronically elevated in patients with PTSD (Geracioti et al., 2001; Southwick et al., 1999a), and animal research has shown that stressors, including footshock, result in elevated prefrontal NE release (Hugues et al., 2007). Norepinephrine may also indirectly influence the mPFC by modulating its inputs (Arnsten et al., 2015), including the basolateral amygdala (BLA), another brain area implicated in stress-impaired extinction learning (Maren and Holmes, 2016).

In support of this model, we have recently shown that systemic administration of D,L propranolol, a non-selective β-adrenoceptor antagonist, immediately after fear conditioning (and just prior to immediate extinction) facilitated extinction retention and "rescued" the immediate extinction deficit (Fitzgerald et al., 2015). Propranolol also mitigated conditioning-induced suppression of IL spontaneous single-unit activity, which may have facilitated extinction learning under stress. In contrast, systemic propranolol administered prior to delayed extinction (24 hrs after conditioning) impaired extinction

retention (Fitzgerald et al., 2015). Interestingly, recent work indicates that oral propranolol administration in humans also facilitates extinction learning under some conditions (Kroes et al., 2016b). Little is known, however, concerning the brain areas mediating the effects of systemic propranolol on extinction learning. Indeed, there is some evidence that propranolol administered directly to the mPFC impairs delayed extinction (Mueller et al., 2008), but the brain regions mediating the effects of propranolol on immediate extinction are not known. Here we examine the contribution of β-adrenoceptor activity in either IL or BLA during immediate and delayed extinction, using bilateral intracranial infusions of propranolol.

3.2 Results

3.2.1 Intra-BLA Propranolol Rescues the IED

This experiment sought to determine whether antagonizing β-adrenoceptors in the infralimbic division of the mPFC or BLA would facilitate extinction learning soon after conditioning. Rats were first conditioned with 5 tone-shock pairings in Context A. Immediately after conditioning, rats received either intra-mPFC or intra-BLA propranolol (or vehicle) followed (~20 min) by extinction trials in a new context (Context B). Forty-eight hours later, the animals were returned to Context B for an extinction retrieval session. There were no differences in the behavior of control rats receiving VEH infusions into the IL or BLA, so these groups were combined to form a single control group. Represenative histology and schematic placement of cannula tips are shown in Figure 3.1.

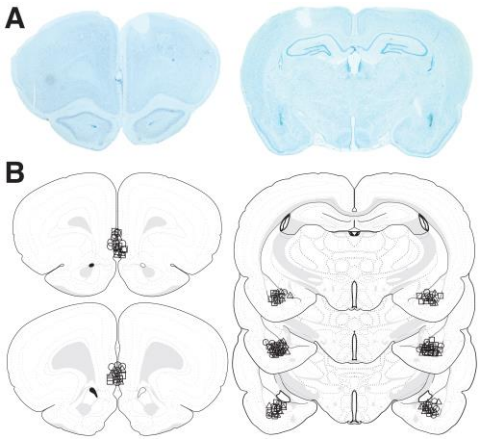

Figure 3.1 Histology. A) Photomicrographs of representative thionin-stained coronal sections depicting cannula placements in the mPFC (left) or BLA (right). B) Cannula placements for all the animals are depicted in schematic coronal sections. For ease of illustration, we plot placements across the three extinction manipulations (immediate extinction = triangles, no-extinction = squares, and delayed extinction = circles); the distribution of placements was similar across experiments and drug groups.

As shown in Figure 3.2a, rats exhibited low levels of freezing behavior during the baseline period prior to the first conditioning trial, and increased their freezing behavior across conditioning trials; there were no differences between the groups (the animals were drug-free during conditioning). An ANOVA with a between-subjects variable of group and a within-subjects variable of trial revealed only a significant effect of trial [$F(2,5) = 32.9$, $p < 0.01$]. During the extinction session (Figure 3.2b), intra-mPFC or intra-BLA propranolol infusions also did not affect freezing behavior. Rats in each group showed modest levels of freezing during the pre-CS baseline period, which is typical of recently shocked animals (Maren and Chang, 2006). Presentation of CS-alone trials augmented freezing in all of the animals, and this decreased similarly between groups over the course

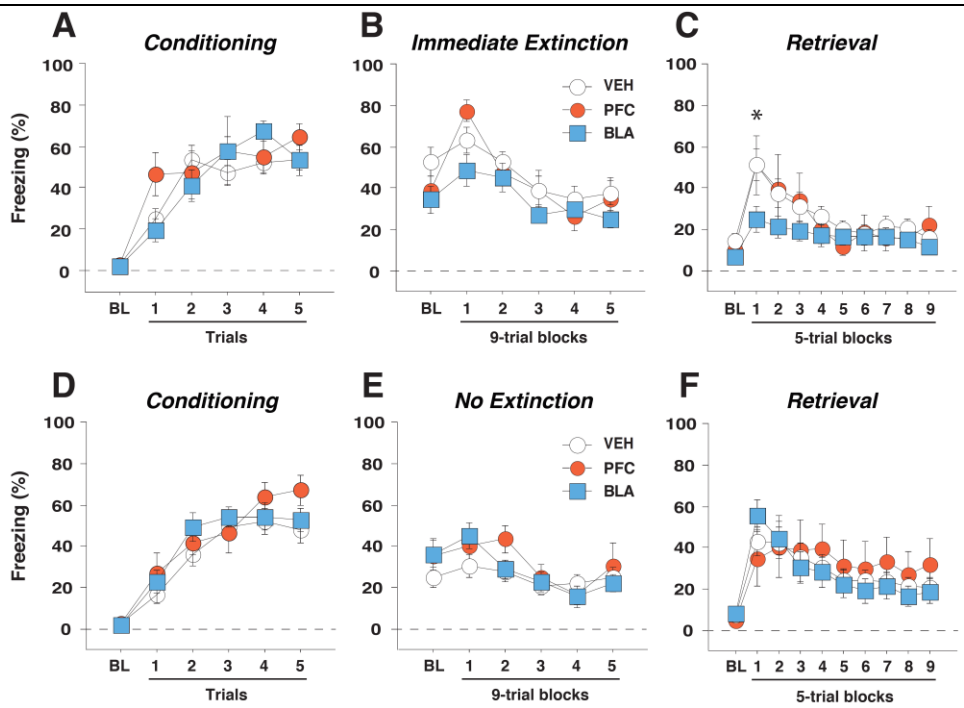

Figure 3.2 Intra-BLA propranolol rescues the immediate extinction deficit. A) Percentage of freezing behavior (means ± SEMs) during fear conditioning in animals that underwent immediate extinction (VEH, *n* = 18; mPFC, *n* = 5; BLA *n* = 14). Intra-cranial injections were made immediately after fear conditioning. B) Percentage of freezing behavior (means ± SEMs) during the extinction session, which was conducted 20 minutes after fear conditioning. Intra-cranial infusions of propranolol into the BLA or PFC did not affect freezing behavior during the extinction session. C) Percentage of freezing behavior (means ± SEMs) during a second drug-free extinction session, which served as an index of extinction retention. Rats that received intra-BLA propranolol immediately after fear conditioning exhibited lower levels of freezing than those receiving mPFC propranolol or vehicle infusions. This suggests that intra-BLA propranolol facilitated extinction retention. D) Percentage of freezing behavior (means ± SEMs) during fear conditioning in control animals that received post-training intracranial drug injections, but did not undergo extinction (VEH, *n* = 19; mPFC, *n* = 6; BLA *n* = 14). Intra-cranial injections were made immediately after fear conditioning. E) Percentage of freezing behavior (means ± SEMs) during the context exposure session, which was conducted 20 minutes after fear conditioning; tones were not delivered during this session. Intra-cranial infusions of propranolol into the BLA or mPFC did not affect freezing behavior during the exposure session. F) Percentage of freezing behavior (means ± SEMs) during a drug-free extinction session, which served as an index of the retention of conditioned fear. Post-conditioning intra-cranial propranolol infusions did not affect freezing behavior during the retention test; propranolol facilitated extinction retention (C) rather than disrupting the consolidation of the conditioning memory.

of extinction training. These impressions were confirmed in an ANOVA which revealed only a main effect of trials [$F(2,5) = 14.72$, $p < 0.01$].

Forty-eight hours later the rats received a drug-free retention test consisting of a second extinction session (i.e., 45 tone-alone trials in Context B). All groups showed fairly low levels of baseline freezing (Figure 3.2c). CS presentations increased freezing in each group, and this decreased throughout the session [main effect of trials, $F(2,9) = 10.06$, $p < 0.01$]. Importantly, planned comparisons made on the first 5-trial block revealed that rats receiving intra-BLA, but not intra-mPFC, propranolol infusions prior to the immediate extinction session exhibited lower levels of freezing than rats in the other groups. Indeed, an ANOVA with a between-subjects variable of group run on the first 5-trial block revealed a main effect of group [Figure 3.2c, $F(2,34) = 3.40$, $p < 0.05$]. In other words, noradrenergic antagonism in the BLA attenuated the immediate extinction deficit and promoted long-term extinction retention. This is in agreement with an earlier report in which systemic propranolol facilitated extinction retention in the initial trials of the retrieval test (Fitzgerald et al., 2015).

Of course, it is possible that post-conditioning propranolol infusions into the BLA simply interfered with consolidation of the conditioning memory. To examine this possibility, another group of animals underwent a protocol identical to that described above except that no extinction trials were delivered after drug infusion (i.e., "no-extinction"; context exposure only). As expected, all groups displayed similar increases in freezing behavior across conditioning. An ANOVA revealed only a main effect of trials [Figure 3.2d, $F(2,5) = 45.43$, $p < 0.01$]. During the context exposure session, all groups

exhibited modest levels of freezing that decreased throughout the session. These observations were confirmed by an ANOVA which revealed only a main effect of trials [Figure 3.2e, $F(2,5) = 7.8$, $p < 0.01$]. Forty-eight hours later, rats received a retrieval session consisting of 45 CS-alone trials. After the baseline period, all groups showed high levels of freezing to the CS which decreased throughout the session at a similar rate. These observations were confirmed by ANOVA which revealed only a main effect of trials [Figure 3.2f, $F(2,9) = 17.4$, $p < 0.01$]. Hence, post-conditioning propranolol in the absence of extinction training did not affect the consolidation of fear (Debiec and Ledoux, 2004; Fitzgerald et al., 2015; Schiff et al., 2017).

3.2.2 Intra-BLA or Intra-mPFC Propranolol does not Affect Delayed Extinction

Previous experiments have revealed that systemic (Fitzgerald et al., 2015) or intra-mPFC (Mueller et al., 2008) propranolol impairs extinction using a standard delayed protocol. Here we sought to compare the effects of intra-BLA and intra-mPFC propranolol using a delayed extinction procedure. Animals underwent a behavioral protocol similar to that in Experiment 1 except that drug infusion (and extinction) occurred 24 hours after fear conditioning (delayed extinction). Similar to the experiments above, all groups exhibited low levels of freezing during the pre-conditioning baseline period, and increased freezing over the course of conditioning. This was confirmed by an ANOVA which revealed only a main effect of trials [Figure 3.3a, $F(2,5) = 37.34$, $p < 0.01$]. Twenty-four hours after conditioning, the rats received intracranial infusions prior to delayed extinction training. Intra-cranial propranolol administration did not affect freezing during the extinction session [Figure 3.3b, $F(2,5) = 28.94$, $p < 0.01$], nor did it affect the retention of extinction

the following day (Figure 3.3c). A repeated measures ANOVA revealed no effect of drug on extinction performance, and all groups showed a small increase in CS-elicited freezing across the test session [Figure 3.3c, main effect of trials, $F(2,90 = 7.85, p < 0.01]$.

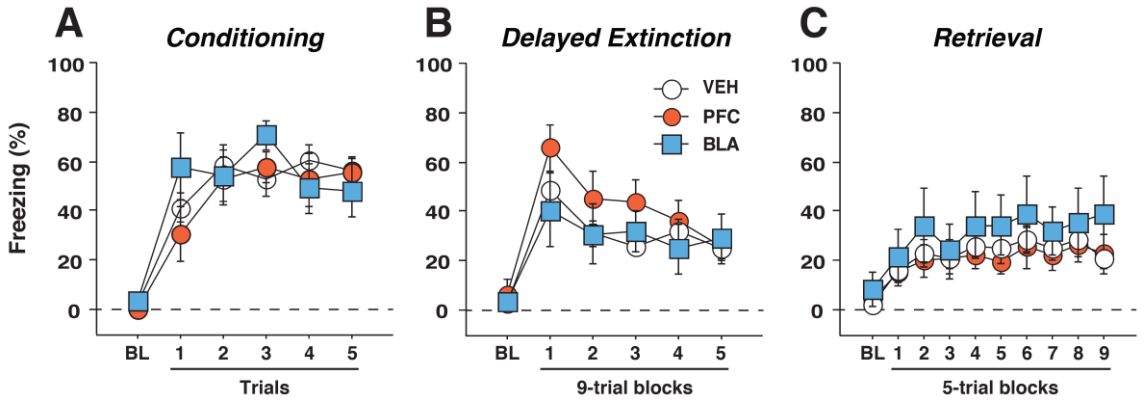

Figure 3.3 Intra-BLA or Intra-mPFC propranolol does not affect delayed extinction. A) Percent of freezing behavior (means ± SEMs) during fear conditioning (VEH n = 1 , mPFC n = 7, BLA n = 6). B) Percentage of freezing behavior (means ± SEMs) during a delayed extinction session that occurred 24 hrs after conditioning (and immediately after intra-cranial infusions). Drug infusion did not alter freezing behavior between groups. C) Percentage of freezing behavior (means ± SEMs) during a drug-free extinction retrieval test that took place 24 hrs following extinction. Prior drug did not impact extinction retrieval.

To compare the behavioral outcomes across the experiments, we analyzed the first 5-trial block of the extinction retrieval test for each experimental condition. As shown in Figure 3.4, rats that received VEH or propranolol infusions into the mPFC exhibited an immediate extinction deficit; they exhibited freezing that was no different from non-extinguished controls (and substantially higher than that after delayed extinction). In contrast, rats that received intra-BLA propranolol infusions did not exhibit an IED, and showed low levels of freezing under both extinction conditions. These impressions were

confirmed in an ANOVA with variables of group and extinction condition which revealed a main effect of extinction condition [$F(2,2) = 5.54$, $p < 0.01$]. This supports the idea that the timing of extinction relative to conditioning is a key factor determining the long-term retention of extinction (i.e., immediate extinction is impaired relative to delayed). In addition, we observed a significant group x extinction condition interaction [$F(2, 94) = 2.59$, $p < 0.05$], which reveals the differential effect of intra-BLA propranolol on freezing in the IED relative to the other groups. Interestingly, we did not find that intra-mPFC propranolol impairs delayed extinction, as has been previously reported (Mueller et al., 2008).

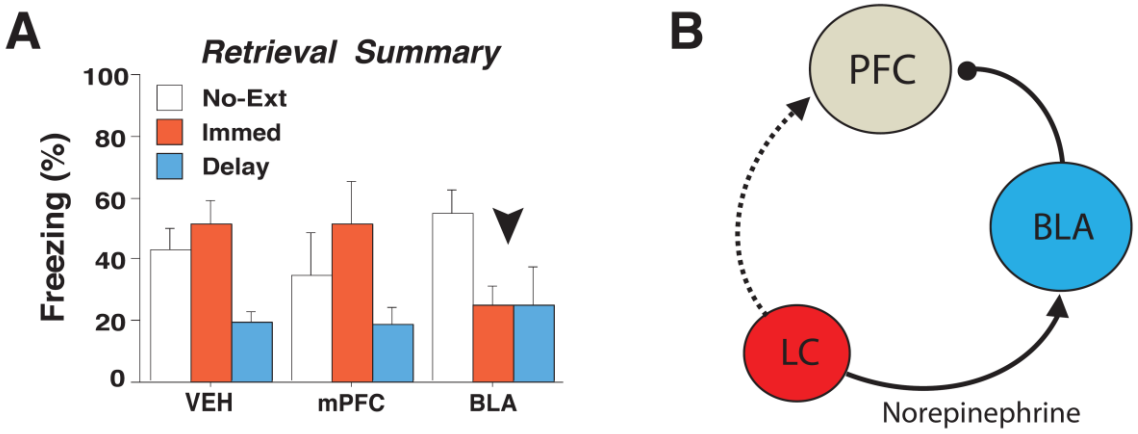

Figure 3.4 Summary and circuit model. A) Percentage of freezing behavior (means ± SEMs) plotting the average freezing for the first five trials (CS+ITI) during the retrieval test for all groups from each behavioral condition. All vehicle groups showed high freezing, characteristic of the immediate extinction deficit, which did not differ from no-extinction controls. Rats that underwent immediate extinction following intra-BLA propranolol did not exhibit the IED, showing similarly low levels of freezing to animals that underwent delayed extinction. B) We propose a potential circuit underlying the IED where locus coeruleus (LC) norepinephrine preferentially increases BLA activity, which ultimately dampens mPFC output resulting in impaired extinction.

3.3 Discussion

Fear extinction deficits, including the IED, are thought to reflect impaired mPFC function (Chang et al., 2010; Fitzgerald et al., 2015; Fucich et al., 2016; Giustino et al., 2016b; Kim et al., 2010; Maren, 2014) and this may relate to elevated noradrenergic signaling (Fitzgerald et al., 2015; Giustino et al., 2016b). We demonstrate here that selectively blocking β-adrenoceptors within the BLA enables extinction where it normally fails. This effect was not observed when propranolol was infused into the mPFC. Importantly, neither intra-mPFC nor intra-BLA propranolol altered fear memory consolidation or delayed extinction learning. These data suggest that heightened noradrenergic signaling in the BLA may be a particularly important component underlying stress-induced extinction deficits.

We and others have demonstrated that extinction learning is impaired when administered soon after conditioning (Hollis et al., 2016; Kim et al., 2010; MacPherson et al., 2013; Maren, 2014; Maren and Chang, 2006; Merz et al., 2016). The IL is thought to underlie successful extinction learning (Bukalo et al., 2015; Do-Monte et al., 2015). Therefore, it has been suggested that aberrant mPFC activity may result in extinction deficits (Fucich et al., 2016; Giustino and Maren, 2015; Maren, 2014; Milad and Quirk, 2012). Because immediate extinction takes place soon after conditioning, during a state of high psychological stress, we hypothesized that elevated NE (presumably released from the locus coeruleus) may subserve extinction deficits. Previous work suggests that NE levels are elevated during conditioning and delayed extinction in the mPFC and BLA (Galvez et al., 1996; Hugues et al., 2007; Ishizuka et al., 2000). We have recently shown

that systemic propranolol rescues the IED and that this seemed to be due to stabilizing prefrontal activity soon after conditioning (Fitzgerald et al., 2015).

Importantly, the effects of systemic propranolol on the IED do not appear to be due to antagonism of β-adrenoceptors in the mPFC. We show that intra-mPFC propranolol has no effect on the three conditions tested: immediate extinction, fear memory consolidation, and delayed extinction. During delayed extinction, animals that received intra-mPFC propranolol tended to show moderately elevated levels of freezing; however, this was not significantly different from vehicle controls or rats that received intra-BLA propranolol prior to delayed extinction. Our results contrast with others who have observed that intra-mPFC propranolol impairs delayed extinction learning (Mueller et al., 2008). However, there are several reasons that might explain this disparity. First, Mueller and colleagues (2008) trained rats to lever press for food in the conditioning chambers, and then measured freezing coincident with response suppression during the CS. Response suppression might engage mPFC to a greater extent than the standard fear conditioning procedure used here. Second, Mueller and colleagues (2008) used much shorter and weaker footshocks than those used in the present study. It is conceivable that these procedures resulted in relatively lower levels of fear-induced NE release during extinction, thereby rendering it more sensitive to intra-mPFC propranolol. Lastly, it is also possible that strain differences in the contribution of the mPFC to extinction learning accounted for the disparity in these reports (Chang and Maren, 2010).

An important finding in the present experiment is that post-conditioning propranolol alone did not affect the retention of fear conditioning. These data are in

agreement with our systemic propranolol findings (Fitzgerald et al., 2015) and reveal that propranolol did not attenuate the IED by impairing the consolidation of the conditioning memory. This is in line with work that has demonstrated that β-adrenoceptor activity is critical for the acquisition, but not consolidation, of conditioned fear (Bush et al., 2010; Debiec and Ledoux, 2004; Roozendaal et al., 2006b; Schiff et al., 2017). Here, we confirm and extend these results. Our data suggest that while elevated noradrenergic activity may not be directly involved in the consolidation of the CS-US memory, heightened noradrenergic activity soon after conditioning appears to interfere with the successful acquisition and retention of a new and competing extinction memory. Indeed, the IED may be due to a deficit in the consolidation of the extinction memory insofar as within-session extinction proceeds normally during immediate extinction and is unaffected by intra-BLA propranolol infusions. Nonetheless, systemic propranolol influences prefrontal cortical neuronal activity soon after fear conditioning, and this might influence encoding of long-term extinction memories.

Interestingly, the present results reveal that β-adrenoceptors in the BLA mediate the effects of systemic propranolol on the IED and possibly shock-induced changes in mPFC spike firing (Fitzgerald et al., 2015). While our previous work suggested that the mPFC may be a key locus of action for propranolol, it is conceivable that shock-induced changes in mPFC activity are regulated by mPFC afferents (including the BLA). Indeed, heightened β-adrenoceptor activation promotes BLA excitability during conditioning (Skelly et al., 2017). This may modulate mPFC activity (Arnsten, 2009; Arnsten et al., 2015; Ji et al., 2010), leading to the IED. Consistent with this idea, (Lin et al., 2016)

showed that extinction deficits (caused by single-prolonged stress) are associated with increased NE in both the mPFC and amygdala. In addition, it has recently been shown that propranolol infusions in the BLA facilitate the induction of hippocampal-prefrontal synaptic plasticity (Lim et al., 2017), which has previously been implicated in extinction learning (Peters et al., 2010; Stafford et al., 2012; Deschaux et al., 2011). Ultimately, the BLA is well positioned to modulate the contribution of the mPFC to extinction learning. Importantly, our data show that intra-BLA propranolol prior to delayed extinction had no effect on extinction retrieval. This time-dependent enhancement of extinction suggests NE levels may be beyond "optimal" soon after conditioning, impairing prefrontal processing and resulting in the IED. However, stress (and NE) may be relatively lower at the onset of delayed extinction, leading to decreased recruitment of low affinity β-adrenoceptors in either the mPFC or BLA. Considerable data reveal that stress increases the activity of locus coeruleus (LC) neurons (Bangasser et al., 2016; Valentino and Van Bockstaele, 2008), and this is associated with increased noradrenergic release in the BLA and mPFC, which may play a role in learning and memory (Uematsu et al., 2015; Berridge and Waterhouse, 2003; Sara, 2015). We propose that LC-driven increases in BLA excitability through activation of β-adrenoceptors might ultimately suppress mPFC activity, thereby undermining successful extinction when it occurs soon after conditioning (Figure 3.4b). Indeed, it is possible that different populations of BLA- and mPFC-projecting neurons in the LC are engaged during immediate and delayed extinction.

Overall, the present data contribute to a growing literature suggesting dissociable roles for key nodes in the fear extinction circuit depending on the timing of extinction

relative to conditioning. Our data suggest that elevated noradrenergic activity in the BLA, but not the mPFC, underlies extinction deficits during high psychological stress. Further work is required to explore this circuitry, but one possibility is that LC-NE enhances BLA excitability which ultimately suppresses mPFC activity, resulting in the IED. Propranolol may therefore be a useful adjunct to behavioral therapeutic interventions in recently traumatized individuals who are at risk for developing trauma-related disorders (Giustino et al., 2016b; Kroes et al., 2016b).

3.4 Materials and Methods

3.4.1 Subjects

One hundred and twenty-eight experimentally naïve adult male Long-Evans rats (Blue-Spruce strain; weighing 200-224 g; 50-57 days old) were obtained from a commercial supplier (Envigo, Indianapolis, IN). The rats were individually housed in cages within a temperature- and humidity-controlled vivarium, and kept on a 14:10 hr light/dark cycle (lights on at 7am) with ad libitum access to food and water. All experiments took place during the light phase of the cycle. Rats were handled for ~30 seconds a day for 5 days to habituate them to the experimenter before any behavioral testing or surgical procedures were carried out. All experiments were conducted at Texas A&M University with full approval from its Animal Care and Use Committee.

3.4.2 Surgical Procedures

Rats were randomly assigned to experimental groups prior to surgical procedures. One week before behavioral testing took place, rats were anesthetized with isoflurane (5% induction, ~2% maintenance) and secured in a stereotaxic apparatus (Kopf Instruments,

Tujunga, CA). The scalp was incised and retracted; three burr holes were drilled for anchor screws; additional holes were drilled in the skull overlying the medial prefrontal cortex or amygdala to allow for cannula implantation. The infralimbic cortex was targeted by a single cannula (8 mm, 26 gauge; Plastics One) implanted on the midline [AP: +2.7, ML: +1.0 (insertion point), DV: -4.9 at an 11-degree angle; all coordinates relative to bregma at skull surface]. This procedure has been used by other laboratories to pharmacologically manipulate the IL in both hemispheres of the brain (Mueller et al., 2008). For the BLA, guide cannulae (10 mm, 26 gauge) were implanted bilaterally (AP: -2.9, ML: +/- 4.8, DV: -8.55). Three jeweler's screws were affixed to the skull, and the skull surface was covered with dental cement to secure the cannulae to the skull. Stainless steel dummy cannulae (30 gauge) were inserted into the guide cannulae (extending 1 mm beyond the end of the guide). Rats were allowed to recover on a warmed heating pad prior to returning to the vivarium. Dummy cannulae were replaced twice in the week following surgery (prior to behavior) and again after the infusion session.

3.4.3 Drug Infusions

Intracranial infusions were made as previously described (Acca et al., 2017). Briefly, rats were transported to an infusion room (either from the conditioning room for immediate and no-extinction procedures or from the vivarium for delayed extinction). Dummies were then removed, and stainless steel injectors (33 gauge) connected to Hamilton syringes mounted in an infusion pump were inserted into the guide cannulae for intracranial infusions. All infusions were made approximately 20 min prior to the extinction or no-extinction session. D,L-propranolol hydrochloride was obtained from a commercial

supplier (Sigma-Aldrich) and was dissolved in sterile saline (10 µg/µl for mPFC and 5 µg/µl for BLA). Infusions (0.5 µl/target) were made at a rate of 0.25 µl/min for 2 min and the injectors were left in place for 1 min to allow for diffusion (mPFC: 5 µg along midline; BLA: 2.5 µg/hemisphere). The propranolol dose and volume were chosen based on previous reports (Dębiec et al., 2011; Debiec and Ledoux, 2004; Mueller et al., 2008). After the infusions clean dummies were secured to the guide cannulae.

3.4.4 Behavioral Apparatus and Procedures

The behavioral procedures were conducted in 16 standard rodent conditioning chambers (30x24x21 cm, Med Associates, St. Albans, VT) housed in sound-attenuating cabinets. Each chamber consisted of two aluminum sides, a Plexiglas rear wall and top, and a hinged Plexiglas door. The grid floor contained 19 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center-to-center). Rods were connected to a shock source and solid-state grid scrambler (Med Associates) for the delivery of footshocks. A loudspeaker mounted on the outside of a grating in one aluminum wall was used to deliver auditory stimuli. Locomotor activity was transduced into an electrical signal by a load-cell under the floor of the chamber to automatically measure freezing.

Approximately one week after surgery, rats underwent fear conditioning, extinction (immediate, no-extinction, or delayed) and extinction retrieval sessions using an "ABB" design: conditioning occurred in context A and extinction training and retrieval testing occurred in context B. Rats were run in squads of eight. For conditioning (context A), rats were transported from the vivarium to the behavioral room in black plastic transport boxes. The conditioning chambers were cleaned with a 1% ammonium

hydroxide solution and a metal pan beneath the grid floor contained a thin layer of the same solution. The room had red ambient lighting and the sound attenuating cabinet doors were closed prior to beginning the session. Conditioning consisted of a 3 min stimulus-free baseline period followed by 5 tone (10 sec, 2 kHz, 80 dB)-shock (2 sec, 1 mA) pairings (shock onset occurred at tone offset) with a 1 min inter-trial interval (ITI) between each tone presentation. Rats remained in the chamber for 3 min following the last footshock.

For the extinction, no-extinction, and retrieval sessions (context B), the rats were transported in white plastic boxes. Chambers were cleaned with a 1% acetic acid solution and a metal pan beneath the grid floor contained a thin layer of the same solution. House lights and ventilation fans within each chamber were turned on and the room containing the chambers was illuminated with overhead white fluorescent lights. The doors of the sound attenuating cabinets were left open. After a 3 min baseline period, rats received 45 tone-alone trials (1 min ITI) and remained in the chamber for 3 min following the last tone. The extinction training and retrieval test sessions were identical. Rats in the "no-extinction groups" underwent an identical procedure except that no tone-alone trials were delivered during the initial extinction session (i.e., soon after conditioning and intra-cranial infusions).

In Experiment 1, we examined the influence of intra-cranial propranolol infusions on immediate extinction (BLA VEH, *n*=12; mPFC VEH=6; BLA PROP=14; mPFC PROP=5). We also included a no-extinction control group (BLA VEH=12; mPFC VEH=7; BLA PROP=14; mPFC PROP=6) to determine whether intra-cranial propranolol

affected consolidation of the conditioning memory (Fitzgerald et al., 2015). In Experiment 2, we examined the effects of intra-cranial propranolol infusions on delayed extinction (BLA VEH=6; mPFC VEH=7; BLA PROP=6; mPFC PROP=7).

3.4.5 Histology

Rats were overdosed with sodium pentobarbital (100 mg/kg) and perfused transcardially with 0.9% saline followed by 10% formalin. Brains were extracted from the skull and post-fixed in a 10% formalin solution for 24 hours followed by a 30% sucrose solution where they remained for a minimum of 48 hours. After the brains were fixed, coronal sections (40 µm thickness) were made on a cryostat (-20^{o} C), mounted on subbed microscope slides, and stained with thionin (0.25%) to visualize cannula placements (Figure 1). Twenty-six rats with cannula placements that were not located within the target region were excluded from the analyses.

3.4.6 Data Analysis

Data were analyzed with conventional parametric statistics (StatView, SAS Institute). Freezing for each trial was determined for each 70-sec interval, which includes both the CS (10-sec) and ITI (60-sec). Freezing during the CS+ITI period is highly correlated with freezing to the CS itself, but is less susceptible to competition by the CS-elicited orienting response (that represents 10-20% of the CS duration). Two-way analysis of variance (ANOVA) and repeated-measures ANOVA were used to assess general main effects and interactions ($\alpha = 0.05$). Results are shown as mean ± SEM.

4. LOCUS COERULEUS NOREPININEPHRINE DRIVES STRESS-INDUCED INCREASES IN BASOLATERAL AMYGDALA FIRING AND IMPAIRS EXTINCTION LEARNING

4.1 Introduction

Stress contributes to a number of psychiatric disorders and it is well known that stress influences aversive learning processes that contribute to the development and maintenance of posttraumatic stress disorder (PTSD) (Maren and Holmes, 2016; Milad et al., 2009; Raio et al., 2014; Arnsten, 2009; Morilak et al., 2005; Pervanidou and Chrousos, 2010; O'Donnell et al., 2004; Parsons and Ressler, 2013; Arnsten, 2015; Arnsten et al., 2015). For example, there are numerous studies demonstrating that either acute or chronic stress impairs the extinction of fear after Pavlovian conditioning (Raio et al., 2014; Miracle et al., 2006; MacPherson et al., 2013; Raio and Phelps, 2015; Merz et al., 2014; Izquierdo et al., 2006; Chang and Maren, 2009; Wilber et al., 2011; Maren and Holmes, 2016). Extinction learning is thought to mediate, in part, cognitive-behavioral therapies for PTSD including exposure therapy, and patients with PTSD exhibit deficits in extinction learning (Wessa and Flor, 2007; Maren and Holmes, 2016; Garfinkel et al., 2014; Giustino et al., 2016b; Pitman et al., 2012). In the laboratory, we, and others, have shown that extinction learning is impaired in humans and rodents when it occurs within minutes to hours of fear conditioning (Maren and Chang, 2006; Fitzgerald et al., 2015; Maren and Holmes, 2016; Chang et al., 2010; Kim et al., 2010; Hollis et al., 2016; Merz et al., 2016). Considerable evidence suggests that this "immediate extinction deficit" (IED) is mediated by footshock stress during fear conditioning itself (Maren and Chang, 2006; Maren and Holmes, 2016;

Chang et al., 2010; Giustino et al., 2017; Fitzgerald et al., 2015). Importantly, the IED models extinction learning impairments in both rodents and humans in the aftermath of acute trauma, as well as extinction impairments associated with symptomatic stress in patients with PTSD (Raio et al., 2014; Merz et al., 2016; Maren and Chang, 2006; Wessa and Flor, 2007; Giustino et al., 2016b; Milad et al., 2009; Rauch et al., 2006; Giustino et al., 2017; Fitzgerald et al., 2015).

Previous work indicates that stress-induced extinction deficits are mediated by forebrain norepinephrine release in the medial prefrontal cortex and amygdala (Giustino and Maren, 2018; Giustino et al., 2016b; Arnsten, 2015; Giustino et al., 2017; Kim et al., 2010; Arnsten, 2009). Indeed, individuals suffering from PTSD and related disorders present with elevated amygdala activity as well as heightened levels of neuromodulators, including norepinephrine (Milad et al., 2009; Giustino et al., 2016b; Krystal et al., 2018; Southwick et al., 1999a, 1999c). The locus coeruleus-norepinephrine (LC-NE) system heavily innervates the amygdala and is highly responsive to stress (McCall et al., 2017, 2015; Naegeli et al., 2017; Giustino and Maren, 2018; Fallon et al., 1978; Foote et al., 1980; Jodo et al., 1998; Passerin et al., 2000; Loughlin et al., 1986; Quirarte et al., 1998; Buffalari and Grace, 2007; Chen and Sara, 2007). Past work has demonstrated that LC projections to the amygdala are associated with increased fear and anxiety-like behavior (McCall et al., 2017; Uematsu et al., 2017), and noradrenergic blockade in the amygdala is sufficient to rescue stress-induced deficits in fear extinction (Giustino et al., 2017).

These data suggest the LC-NE system critically regulates amygdala activity, which may ultimately drive stress-induced extinction deficits via interactions with the mPFC

(Giustino et al., 2019). To address this possibility, we combine single-unit BLA recordings with systemic pharmacology in freely moving rats to examine whether beta-adrenoceptors mediate stress-induced changes in amygdala firing rates. We next combined single-unit amygdala recordings with LC-specific chemogenetic manipulations to determine if LC-NE drives changes in amygdala activity. Lastly, we directly examine the contribution of the LC-NE system to the immediate extinction deficit.

4.2 Results

4.2.1 Propranolol reduces footshock-induced freezing and mitigates BLA firing

Recent work suggests that intra-BLA propranolol reduces the IED and enables extinction learning under stress (Giustino et al., 2017). We sought to examine if footshock stress alters single-unit firing in the BLA and contributes to extinction deficits (Figure 1a shows the experimental design). Animals were implanted with a 16-channel microelectrode array targeting the amygdala (example histology and schematic representation of electrode placements shown in Figure 1b). Animals were transported to the recording room for fear conditioning (Context A). After a 3-min stimulus-free baseline period, animals were injected with either vehicle (VEH, n = 5) or propranolol (PROP, n = 5). The animals remained in the chamber for 20 min to allow sufficient time for drug to take effect. Animals then received 5 CS-US pairings (see methods for details) and remained in the chamber for 60 min following the last footshock. As expected, vehicle-treated animals exhibited sustained increases in freezing behavior and this was limited by propranolol treatment [Figure 2a, main effect of drug, $F(1,8) = 19.30$, $p = 0.0023$]. During this session, we recorded from a total of 280 single-units in the BLA (VEH: $n = 143$; baseline firing

rate = 2.89 ± 0.16; PROP: n = 137; baseline firing rate = 3.00 ± 0.15). Propranolol treatment prior to fear conditioning did not influence spontaneous firing rate among these neurons. However, fear conditioning produced a dramatic increase in spontaneous firing rate in the population of neurons recorded in the BLA, an effect that was attenuated by propranolol [Figure 2b, main effect of drug, $F(1,278) = 19.26$, $p < 0.0001$].

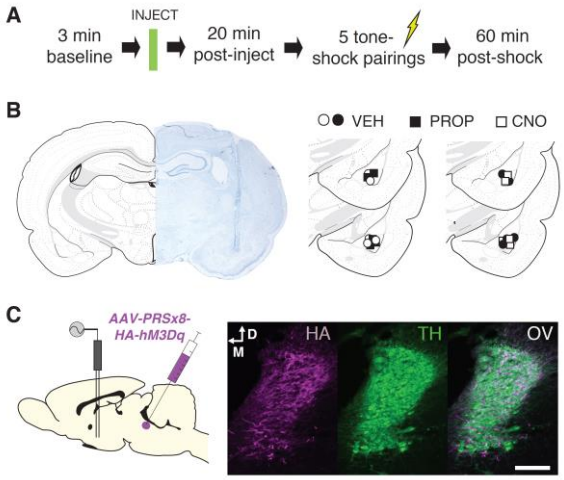

Figure 4.1 Experimental design and representative histology. A) Experimental design. B) Nissl stained tissue showing representative electrode placement in the amygdala alongside schematic histology depicting location of the center of the recording array for all rats split by recording experiment. C) Experimental approach for the recording experiment paired with LC-NE activation alongside representative LC viral histology. (HA = HA-tag immunolabeled for viral expression, TH = tyrosine hydroxylase, OV = overlay; scale bar = 100um).

Although footshock stress increased the average firing rate data of BLA neurons, there was considerable heterogeneity in the response of individual BLA neurons. We therefore divided the single-units into two populations based on the direction of their post-shock firing rate change in the first 20-sec bin after footshock; based on this criterion

neurons were classified as either "excited" ($z > 0$) or "suppressed" ($z < 0$) immediately after the last footshock. Figure 2c demonstrates that no difference was observed when comparing drug treatment in terms of the proportion of neurons showing shock-induced increases or decreases in firing rate [χ^2 (1) = 0.94, p = 0.33]. However, we did observe differences in the magnitude of both "excited" [Figure 2e, main effect of drug, F(1,133) = 13.92, p = 0.0003] and "suppressed" [Figure 2f, main effect of drug, F(1,143) = 4.92, p = 0.028] population activity based on drug treatment. These differences are further demonstrated by the heatmaps (Figure 2d) depicting each neuron across the entire session. That is, propranolol treatment limited both shock-induced increases and decreases in BLA firing rates. These data suggest that footshock-stress induces rapid and sustained changes in the magnitude of BLA spontaneous firing rates and this is regulated by the action of norepinephrine at beta-adrenoceptors.

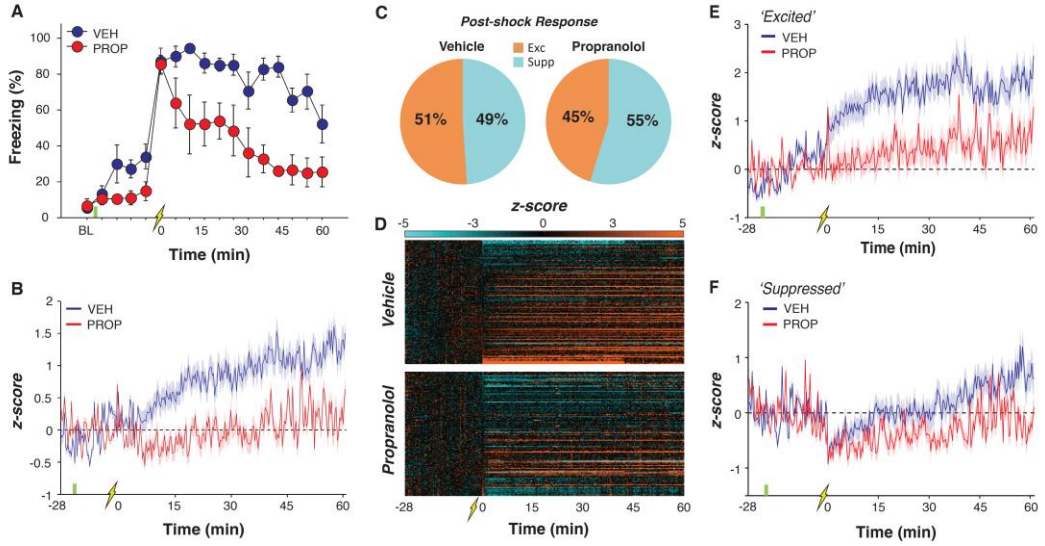

Figure 4.2 Propranolol reduces footshock-induced freezing and mitigates changes in BLA firing. A) Percentage of freezing (mean ± SEM) across the duration of the session. PROP treatment produced a reliable decrease in post-shock freezing throughout the session B) Average firing rate over the course of the session split by drug treatment (20 sec bins). Footshock produced rapid and sustained changes in amygdala firing rates which were mitigated by propranolol treatment (t = 0 is immediately after the last conditioning trial). C) Pie charts showing the percentage of neurons (split by drug) that increased or decreased in firing rate after the last conditioning trial. No difference between drug treatment was observed in the proportion of single-units showing footshock-induced changes in firing rate. D) Heatmaps depicting normalized firing rate for every neuron recorded split by drug treatment. E, F) Average firing rate over the course of the session split by drug comparing "excited" and "suppressed" neuronal populations (20 sec bins). Propranolol treatment limited both increases and decreases in amygdala activity.

4.2.2 LC-NE activation paired with weak footshocks induces sustained freezing and BLA firing

Because propranolol mitigated stress-induced alterations in BLA firing and past work has shown a role for LC projections to the amygdala in fear conditioning (Uematsu et al., 2017), we hypothesized that the LC-NE system was driving changes in BLA activity. In order to examine this possibility, we tested whether pairing LC-NE activation with a

weaker, and presumably less stressful, footshock (see methods for details) would recapitulate our previous findings. Animals received bilateral infusions of Gq coupled LC-specific DREADD (AAV9-PRSx8-hM3Dq-HA) to selectively activate LC-NE release (Figure 1c shows experimental approach and representative histology). We, along with others, have previously validated this virus *in vivo* (Giustino et al., 2019; Vazey and Aston-Jones, 2014). Animals were also implanted with a 16-channel microelectrode array targeting the BLA (Figure 1b shows placements). We used an identical protocol to the previous experiment except the shock parameters (Figure 1a). Animals were transported to the recording room and after a 3 min stimulus free baseline period, injected with either VEH (n = 5) or CNO (clozapine *N*-oxide, the DREADD ligand, n = 5). Twenty minutes was allowed for drug to enter the brain. Animals then received 5 CS-US pairings and remained in the chamber for an additional 60 min. Vehicle treated animals showed elevated freezing levels that dissipated throughout the session. However, CNO treated rats exhibited prolonged freezing behavior in the post-shock period [Figure 3a; main effect of drug, $F(1,8) = 6.27$, $p = 0.037$]. During this session, we recorded from a total of 233 single-units in the BLA (VEH: $n = 117$; baseline firing rate = 2.34 ± 0.21;CNO: $n = 116$; baseline firing rate = 2.57 ± 0.26). Differences in footshock-induced freezing corresponded with differences in BLA spontaneous firing rates. As shown in Figure 3b, single-units recorded from vehicle-treated rats showed a moderate increase in average firing rates over the course of the session and this was markedly enhanced in the CNO treated group [main effect of drug, $F(1, 231) = 12.67$, $p = 0.0005$].

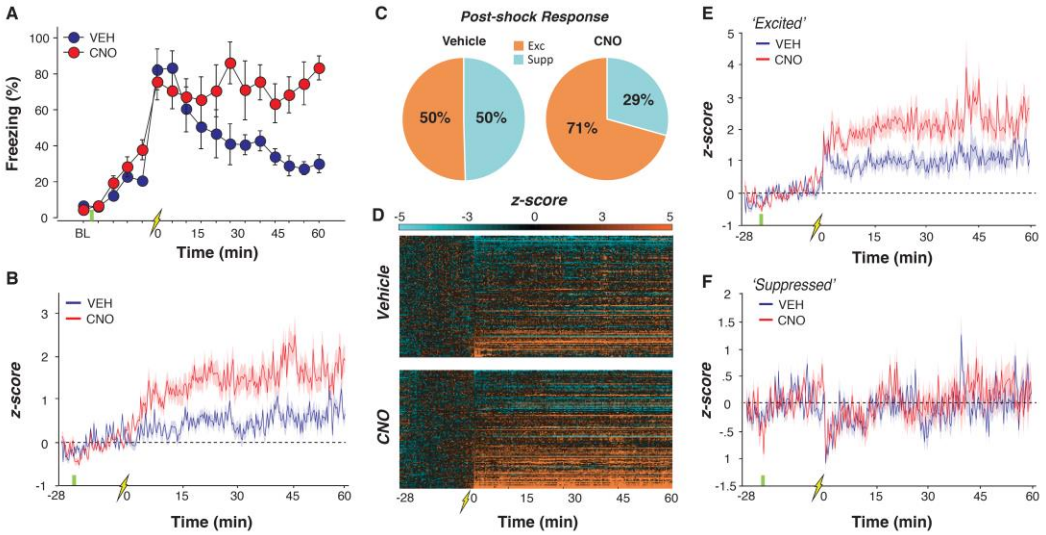

Figure 4.3 LC-NE activation paired with weak footshocks induces sustained freezing and BLA firing. A) Percentage of freezing (mean ± SEM) across the duration of the session. Animals treated with CNO (to activate LC-NE release) showed sustained freezing relative to vehicle controls. B) Average firing rate over the course of the session split by drug treatment (20 sec bins, t = 0 is immediately after the last conditioning trial). Neurons recorded from vehicle treated rats showed moderate levels of increased BLA firing whereas CNO rats showed a marked increase in firing rate for the duration of the session. C) Pie charts showing the percentage of neurons (split by drug) that increased or decreased firing rate after the last conditioning trial. CNO treatment resulted in a larger proportion of recorded units showing increased firing rate in the post-shock period. Heatmaps depicting normalized firing rate for every neuron recorded split by drug treatment. E, F) Average firing rate over time split by drug comparing "excited" and suppressed" neuronal populations. CNO treatment produced a marked increase in the magnitude of excitation, but not inhibition within the BLA.

In order to determine if LC-NE activation altered the proportion of neurons showing increased amygdala firing we classified neurons as described above. Single-units were considered to be either "excited" or "suppressed" if they showed an increase or decrease following the last footshock, respectively. A chi-square analysis revealed that CNO-mediated LC-NE activation resulted in a larger proportion of BLA neurons showing excitation after fear conditioning relative to vehicle treated animals [Figure 3c; χ^2 (1) =

10.83, $p = 0.001$]. Moreover, LC-NE activation resulted in a larger magnitude of stress-induced BLA firing in the "excited" neuronal population [Figure 3e; main effect of drug, $F(1,158) = 10.35$, $p = 0.0016$] but not the "suppressed" population (Figure 3f). The heatmaps showing each recorded neuron across the entire session further demonstrate this observation (Figure 3d). These data suggest that the LC-NE drives stress-induced increases in amygdala firing rates as well as freezing behavior.

4.2.3 LC-NE activation induces an immediate extinction deficit

While the above data show the LC-NE system critically regulates amygdala firing rates and freezing behavior, the LC has not been directly implicated in extinction deficits. We next attempted to induce an immediate extinction deficit with a conditioning protocol that would not otherwise produce extinction deficits. Animals received bilateral infusions of the Gq-coupled LC-specific DREADD. Animals were conditioned with a single, weak CS-US trial (same shock parameters as the weak shock above). Animals received either vehicle (n = 11) or CNO (n = 12, to activate LC-NE) approximately 10 min prior to conditioning (Context A). All groups exhibited similar levels of conditioning as confirmed by a repeated measures ANOVA which revealed only a main effect of time [Figure 4a; main effect of time, $F(1,21) = 92.54$, $p < 0.0001$]. Approximately 15-20 min after conditioning, animals underwent immediate extinction (45 CS alone trials) in an alternate context (Context B). Vehicle treated animals showed reduced freezing throughout the session whereas LC-NE activation resulted in sustained freezing for the duration of extinction training [Figure 4b; main effect of drug, $F(1, 21) = 93.37$, $p < 0.0001$]. Animals were returned to their home cages following the end of the session. Forty-eight hours later

animals returned to Context B for drug-free extinction retrieval testing (45 CS-alone trials). Vehicle treated animals showed little CS-evoked freezing, indicating successful extinction retrieval (i.e., no extinction deficit). However, CNO treated rats that displayed a marked increase in CS-evoked freezing. This observation was confirmed with a repeated measures ANOVA [Figure 4c; main effect of drug, $F(1, 21) = 13.67$, $p = 0.0013$].

Of course, it is possible that pre-conditioning manipulations may be altering fear memory consolidation, rather than affecting extinction learning per se. In order to test this possibility, a set of rats received identical behavioral protocols except that they underwent no-extinction procedures (i.e., context exposure only). Both groups (VEH v CNO, n = 8 per group) showed similar levels of fear conditioning which was confirmed by a repeated measures ANOVA which revealed only a main effect of time [Figure 4d; main effect of time, $F(1, 14) = 91.33$, $p < 0.0001$]. Approximately 15-20 min after conditioning, these animals underwent no extinction procedures (context B exposure only, no CS presentation). As expected, vehicle treated animals showed low levels of freezing to this distinct context. However, CNO treated rats showed a marked elevation of freezing throughout the session [Figure 4e; main effect of drug $F(1, 14) = 21.78$, $p = 0.0004$]. Forty-eight hours later these animals returned to context B for a drug-free session consisting of 45 CS-alone trials. No difference was observed in CS-evoked freezing between the two drug groups (Figure 4f), suggesting that LC-NE activation did not simply strengthen the fear memory. These data show that LC-NE stimulation is sufficient to induce an immediate extinction deficit. Because there were differences in baseline freezing (prior CNO > prior VEH) during the drug-free extinction retrieval test we

normalized the first 9-trial block by subtracting baseline freezing. Figure 5a depicts the normalized data and further confirms that LC-NE activation promoted an extinction deficit [extinction x drug interaction, $F(1,35) = 6.45$, $p = 0.016$]. Importantly, these data show that this stress-induced extinction deficit is not due to LC-NE activation simply creating a stronger fear memory insofar as both VEH and CNO NO-EXT groups show similarly elevated levels of CS-evoked freezing during the drug-free test.

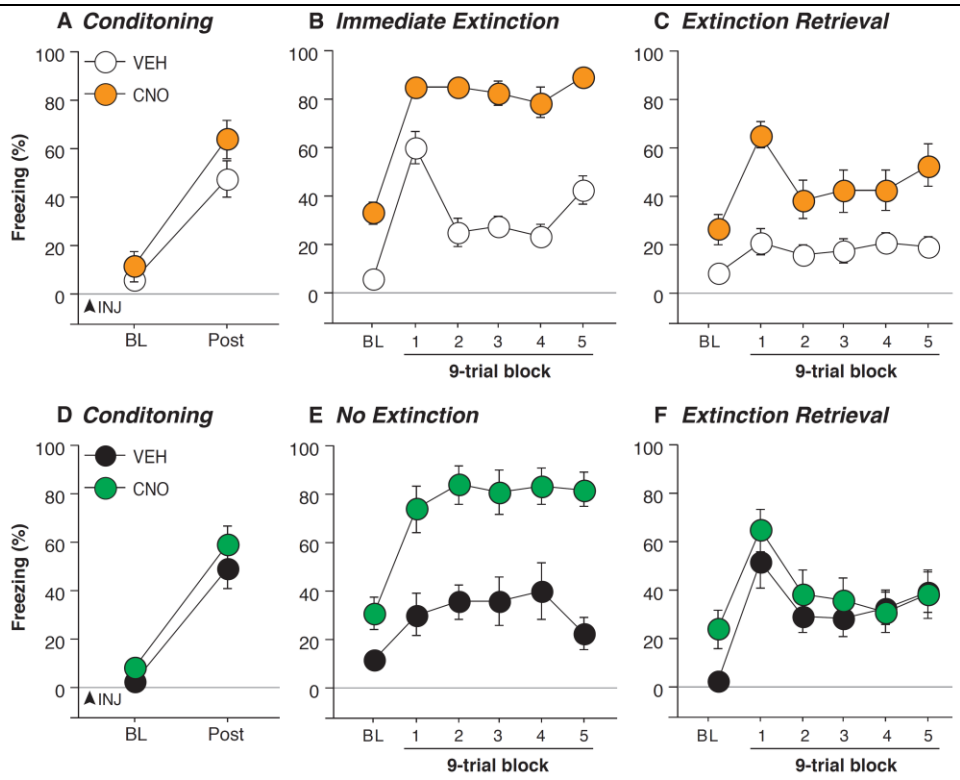

Figure 4.4 LC-NE activation induces an immediate extinction deficit. A) Percentage of freezing (mean ± SEM) is shown for all sessions. Animals were injected with VEH or CNO approximately 10 min prior to conditioning. Both groups showed similar levels of conditioned freezing. B) Freezing during immediate extinction training. CNO treated rats showed elevated freezing levels throughout the session. C) Freezing during the drug-free extinction retrieval test. VEH rats showed little CS-evoked freezing, indicative of successful extinction retrieval. However, prior CNO treatment increased levels of CS-evoked freezing, suggesting LC-NE drives extinction deficits. D) Conditioned freezing behavior for a separate set of animals that underwent identical procedures except they received no-extinction training. Both groups showed similar levels of conditioned freezing behavior. E) Freezing behavior during the no-extinction session (i.e., context exposure only, no CS presentation). VEH treated rats showed low levels of freezing behavior in this distinct context; however, CNO treated rats displayed elevated freezing levels throughout the session. F) Drug-free test session which consisted on 45 CS-alone trials. No difference was observed in CS-evoked freezing between drug groups suggesting that LC-NE activation did not simply increase the strength of the fear memory.

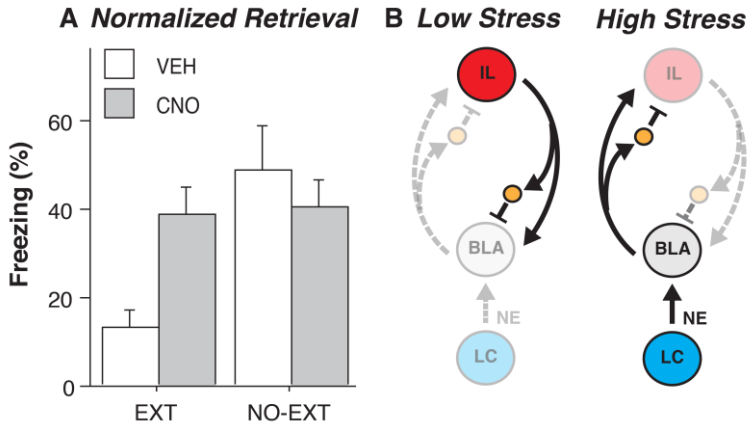

Figure 4.5 Proposed circuit schematic by which LC-NE drives extinction deficits. A) Normalized (baseline subtracted) percentage of freezing (mean ± SEM) for the first 9 trial block of the drug-free extinction retrieval session which further depicts CNO-induced LC-NE activation resulted in an extinction deficit. That is, the CNO, EXT group showed comparable CS-evoked freezing to both the no-extinction groups, indicative of an extinction deficit. B) Proposed circuit mechanism underlying stress-induced extinction deficits. Under low levels of stress, the LC-NE system is minimally engaged. This enables successful extinction learning via IL mediated feedforward inhibition of the BLA. In contrast, this circuit reverses under high levels of stress and this reversal is driven by LC-NE. Activation of the BLA results in shunted IL firing via feedforward inhibition thereby interfering with extinction learning. Abbreviations: LC – locus coeruleus, NE – norepinephrine, BLA - basolateral amygdala, IL – infralimbic cortex.

4.3 Discussion

Here we show that footshock stress induces rapid and sustained increases in the spontaneous firing rate of BLA single-units. These increases in BLA firing were blocked by systemic propranolol administration and potentiated by chemogenetic activation of LC-NE, suggesting a key role for beta-adrenoceptors. These stress-related changes in BLA activity persisted for up to an hour after footshock, a time window that corresponds with stress-induced deficits in extinction learning. Lastly, we demonstrate that LC-NE activation impairs extinction learning, presumably by exciting BLA circuits that promote

fear expression at the expense of extinction learning (see Figure 5b for a proposed circuit mechanism).

We, along with others, have suggested that stress acts to impair extinction learning by altering medial prefrontal cortex (mPFC) function, and this may be mediated by NE (Chang et al., 2010; Maren and Holmes, 2016; Kim et al., 2010; Fitzgerald et al., 2015). Activity in the infralimbic (IL) subdivision of the mPFC is thought to regulate successful extinction learning (Giustino and Maren, 2015; Maren and Quirk, 2004; Milad and Quirk, 2002). We have previously shown that noradrenergic blockade enables extinction learning under stress and initially hypothesized that this was mediated by footshock-induced changes in mPFC single-unit activity (Fitzgerald et al., 2015). Consistent with this, we observed that footshock-stress resulted in rapid and sustained decreases in IL firing rates, and this could be blocked by systemic propranolol. We surmised that this decreased IL activity was an underlying factor in the immediate extinction deficit, but intra-IL infusions of propranolol had no effect on stress-induced extinction deficits (Giustino et al., 2017). However, both the mPFC and BLA are highly sensitive to stress and it has been suggested that stress impairs prefrontal function while enhancing BLA activity, a state that may limit extinction learning (Giustino and Maren, 2018; Arnsten, 2009; Arnsten et al., 2015; Arnsten, 2015). Indeed, intra-BLA propranolol, on the other hand, rescued the immediate extinction deficit (Giustino et al., 2017). We now show that footshock-stress dramatically increases spontaneous firing rates among neurons in the BLA and this is dependent upon the action of NE at beta-adrenoceptors insofar as propranolol limits these changes.

Past work has demonstrated that BLA projections to the mPFC are involved in both the conditioning and extinction of fear (Senn et al., 2014; Klavir et al., 2017; Burgos-Robles et al., 2017). It is possible that the observed NE dependent changes in BLA firing also mediated stress-induced decreases in IL firing via these direct projections. Senn and colleagues (2014) showed differing levels of activity in BLA projections to PL and IL influence extinction retention. This is in line with our current and past work in which we show footshock-induced suppression of IL firing that outlasts changes in PL firing rates (Fitzgerald et al., 2015). Our current data now demonstrate that footshock stress produces rapid and prolonged activation of the BLA, a change in firing that is opposite to that observed in IL after footshock. These data suggest a circuit mechanism by which BLA projections to the IL may mediate extinction deficits (Fig 5B). It is possible that LC-NE drives increases and decreases in BLA and IL via direct projections, respectively. In line with this idea, a recent study has shown that LC projections to the mPFC mediate aversion and increase anxiety-like behavior, though it is not known if this was due to a suppression of mPFC activity (Hirschberg et al., 2017). Another possibility is that in addition to direct projections, LC-NE may be acting to enhance BLA firing in amygdala neurons that project to IL and synapse on inhibitory interneurons to promote feedforward inhibition, thus resulting in impaired extinction. Indeed, BLA neurons projecting to the IL have been shown to dampen IL firing via a feedforward inhibitory mechanism (McGarry and Carter, 2016) and IL projections to the BLA mediate both the acquisition and recall of extinction learning (Cho et al., 2013). Under conditions of high stress, it seems likely that these

circuits are tilted in favor of BLA mediated suppression of IL, thus promoting a high fear state while simultaneously leading to poor extinction.

Extinction deficits may be mediated, in part, by the LC-NE system prioritizing consolidation of the recent fear memory at the expense of a new extinction memory, particularly when extinction learning occurs soon after fear conditioning. Indeed, NE has long been implicated in fear memory consolidation (Giustino and Maren, 2018; McGaugh and Roozendaal, 2002; Roozendaal et al., 2006a; McIntyre et al., 2012; Cahill et al., 1994; McGaugh, 2000). Along these lines, recent work has shown that optogenetic stimulation of LC projections to the BLA mediate fear consolidation whereas inhibiting this pathway reduced the strength of fear memories (Uematsu et al., 2017). However, it is unknown how these manipulations affected single-unit and population level dynamics in both the BLA and mPFC. McCall and colleagues (2017) demonstrated that optogenetic activation of LC projections to the BLA resulted in a majority of responsive BLA neurons showing increased firing, whereas a smaller proportion of BLA neurons were suppressed (McCall et al., 2017). These data align nicely with our current findings, which show an overall excitatory population response in the BLA that is amplified by synthetic LC activation.

Interestingly, others have shown that footshock as well as infusion of adrenoceptor agonists into the BLA suppress firing rates in anesthetized animals (Buffalari and Grace, 2007; Chen and Sara, 2007). However, these authors also noted that a small subpopulation of neurons in the BLA showed increased spiking in response to stimulation of amygdala adrenoceptors. Of course, it is has been shown that anesthesia influences basal NE activity in a way that might influence pharmacological manipulations of adrenergic

receptors within the BLA (Vazey and Aston-Jones, 2014). In addition, pharmacological manipulations, whether locally infused or systemically administered, may not entirely replicate synthetic activation of the LC or its terminals in downstream brain regions, which may help explain some of these discrepant findings. Our current work shows a robust footshock-induced increase in population activity within the BLA, although a number of neurons were also suppressed. When these footshocks were then paired with LC-NE stimulation this further augmented BLA excitability in terms of the magnitude as well as the proportion of neurons showing increased firing suggesting that elevated levels of NE facilitate BLA spiking.

In humans, individuals suffering from PTSD and related disorders present with heightened amygdala activity (Milad et al., 2009; Debiec and LeDoux, 2006; Rauch et al., 2006; Giustino and Maren, 2018), elevated NE (Arnsten, 2009; Southwick et al., 1999a; Krystal et al., 2018; Yehuda et al., 1992; Giustino and Maren, 2018; Giustino et al., 2016b), and extinction impairments (Milad et al., 2009; Garfinkel et al., 2014; Giustino et al., 2016b). The current data strongly suggest that extinction deficits may result from elevated LC-NE activity that, in turn, increases BLA firing rates. Recent advantages in neuroimaging technology now allows researchers to measure activity in the human LC, an advance that will further our understanding of the role of LC-NE in PTSD (Krystal et al., 2018; Priovoulos et al., 2018; Liu et al., 2017). Although pharmacological manipulations of NE transmission have shown some promise for the treatment of PTSD, the literature is largely split on their efficacy and utility (Giustino and Maren, 2018). Our data suggest that both stress and proximity to trauma are key factors that influence how

the LC and BLA interact to influence extinction learning; this interaction is critical for designing interventions that are appropriately timed to yield the most effective clinical outcomes. Future work in humans will likely shed light on the role of the LC-NE system on an individual-to-individual basis, which may better allow us to appreciate when and why NE-altering drugs may be useful for reducing PTSD symptomatology.

Overall, we demonstrate that stress induces prolonged increases in BLA spontaneous firing rates and this is highly sensitive to manipulations of the NE system. That is, reducing the action of NE via propranolol eliminated these changes in firing rate whereas selective LC-NE activation via chemogenetics enhanced stress-induced increases in BLA activity. We also show that stress and LC-NE activation induces extinction deficits, most likely due to the observed increases in amygdala firing. These data have important clinical implications for individuals suffering from stress- and trauma-related disorders, such as PTSD and suggest the LC-NE system may be a key regulator of heightened amygdala activity which is observed in those with PTSD.

4.4 Materials and Methods

4.4.1 Subjects

Fifty-nine experimentally naïve adult male Long-Evans Blue Spruce rats (weighing 200-224 g; 50-57 days old) were obtained from a commercial supplier (Envigo, Indianapolis, IN). Upon arrival and throughout the experiments, rats were individually housed in cages within a humidity- and temperature-controlled vivarium, and kept on a 14:10 hr light/dark cycle (lights on at 7 am) with ad libitum access to food and water. All experiments were conducted in the daytime during the light phase. Rats were handled for ~30 seconds a day

for 5 days to habituate them to the experimenter before any behavioral testing or surgical procedures were carried out. All procedures were conducted at Texas A&M University and were performed in strict accordance with the guidelines and regulations set forth by the National Institutes of Health and Texas A&M University with full approval from its Animal Care and Use Committee.

4.4.2 Surgeries

Rats were anesthetized with isoflurane (5% induction, 2% maintenance) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) for implantation of microelectrode arrays targeting the amygdala and viral infusions targeting the LC. The scalp was incised and retracted. For rats receiving viral infusions of the LC-specific DREADD, the head was tilted downward at a 15 degree angle such that bregma skull surface was 2mm below intersectional lambda in the horizontal plane. The skull overlying the LC was removed in both hemispheres and were then separately infused with LC-DREADDs (AAV9-PRSx8-hM3Dq-HA). The PRSx8 promoter is a synthetic dopamine-beta hydroxlyase promoter that restricts expression to noradrenergic neurons (Vazey and Aston-Jones, 2014). Viral infusions were made with a hypodermic injector (Small Parts/Amazon, Seattle, WA) that was connected to a Legato 101 infusions pump (KD Scientific, Holliston, MA) and a 10 µl syringe (Hamilton Company, Reno, NV) using polyethylene tubing (Braintree Scientific, Braintree, MA). Virus was infused at a rate of 0.25 µl/min and the injector was removed 5 min after the infusion ended to allow for adequate diffusion. The coordinates for each infusion (relative to intersection lambda) were as follows: AP: -3.8, ML: +/- 1.4, and DV: -7.0, -6.5, and -6.0. We infused 0.5 µl at these 3 depths in each hemisphere due

to the specificity of the virus and variability from animal to animal in terms of the DV location of the LC. At least two weeks were allowed following viral infusions prior to beginning experiments.

For rats that received a microelectrode array targeting the BLA (regardless if this was preceded by LC viral infusions) the skull was leveled in the horizontal plane. Three-five burr holes were drilled for anchor screws. A portion of the skull overlying the BLA in the right hemisphere was also removed. The animal was then implanted with a 16 channel microelectrode array (Innovative Neurophysiology, Durham, NC). The 4x4 array was constructed of 16 individual 50-µm diameter tungsten wires of equal length (10.5 mm). The wires in each row and the rows themselves were spaced 200 µm apart (center-to-center). The array was positioned with its long axis parallel to the anteroposterior plane. Coordinates for the center most wires relative to bregma skull surface were as follows: AP: - 2.9, ML: + 4.8, DV: -8.55. One electrode was selected to be used as a ground to optimize recordings, resulting in a maximum of 15 channels per animal. The array was then secured to the skull with dental acrylic. Animals were given at least one week (two if viral manipulations were involved) to recover prior to behavioral and recording procedures.

4.4.3 Behavioral apparatus and *in vivo* electrophysiology in freely moving rats

A modified rodent behavioral chamber (30x24x21 cm, Med Associates, St. Albans, VT) enclosed in a sound-attenuating cabinet was used for recording experiments. This chamber was modified to allow for freely moving electrophysiological recordings as well (described later). The chamber was comprised of two aluminum side walls, a Plexiglas

rear wall, a hinged Plexiglas door, and an open top. The grid floor consisted of 19 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center-to-center). A loudspeaker attached to the outside of a grating in one aluminum wall was used to play auditory tones. Locomotor activity of the rat was transduced by a load-cell under the floor of the chamber, and the output of the load-cell was recorded by an OmniPlex recording system (Plexon, Dallas, TX). Thus, all behavioral and neural activity was recorded automatically with this system.

Each rat was individually fear conditioned in context A. In this procedure, the rat was transported to the room in a black plastic box, connected to a headstage with a flexible cable (Plexon) and placed in the behavioral chamber. The chamber had been cleaned with 1% ammonium hydroxide to provide a distinct olfactory cue, and a black pan containing a thin layer of the same solution had been placed under the grid floor. The room was illuminated with red ambient lights (Context A). After a 3-min stimulus-free baseline period, the system was briefly paused while the animal remained plugged in and was injected with either vehicle or propranolol (10 mg/kg, i.p.), or CNO (3 mg/kg, i.p. – for the LC DREADD experiment). The rat was placed back in the chamber for 20 min to allow adequate time for drug to take effect. The rat then received five auditory tone-footshock pairings. Recordings did not occur during shock presentation due to electrical noise. The tones (conditioned stimuli; CS) were 2 sec, 80 dB, 2 kHz; the shocks (unconditioned stimuli; US) were 0.5 sec and 1 mA (for the propranolol experiment), and 0.5 sec, 0.5 mA for the LC-DREADD weak-shock experiment, where shock onset occurred at tone offset. There was a 1-min inter-trial interval (ITI) between shocks. The

session continued for 60 min after the final shock, and then the rat was returned to its home cage.

Extracellular single-unit activity was recorded with a multichannel neurophysiological recording system (OmniPlex, Plexon, Dallas, TX). Wideband signals recorded on each channel were referenced to one of the recording wires (resulting in a maximum of 15 channels of activity per rat), amplified (8,000x), digitized (40 kHz sampling rate), and saved on a PC for offline sorting and analysis. The recording reference wire we chose for each session was selected to optimize the quality of the recordings. After high-pass filtering the signal at 600 Hz, we sorted waveforms manually using 2-dimensional principal component analysis (Offline Sorter, Plexon). Only well-isolated units were used in our analysis. If two units with similar waveforms and identical time stamps for their action potentials appeared on adjacent electrodes, we only used one unit. We then imported sorted waveforms and their timestamps to NeuroExplorer (Nex Technologies, Madison, AL) for further analysis.

4.4.4 Behavioral procedures

For the behavioral experiment, animals underwent similar procedures as described above for the recording experiments. On Day 1, rats were transported to the conditioning context (Context A) in squads of eight. Rats received either vehicle or CNO (3 mg/kg, i.p.) approximately 5-10 minutes before fear conditioning. After a 3 min stimulus free baseline period, all animals were conditioned with a single CS-US pairing (0.5 sec, 0.5 mA shock). Rats were removed from the conditioning chambers and underwent immediate extinction or no-extinction (i.e., context B exposure, no CS presentation) procedures in Context B.

Rats were transported to Context B in white transport boxes. The chamber was cleaned with 3% acetic acid and a metal pan containing a thin layer of the same solution had been placed under the grid floor. Ambient white lights illuminated the room. For animals undergoing immediate extinction, 45 CS-alone trials were presented (30 sec ITI) after a 3 min stimulus free baseline period. Rats undergoing no-extinction procedures remained in the chambers for the same amount of time, but no CS was presented. Animals were returned to their home cages at the end of the session. Animals were tested for extinction retrieval 48 hrs later. This extinction retrieval test was identical to the initial extinction session.

4.4.5 Drugs

D,L-propranolol hydrochloride was obtained from a commercial supplier (Sigma-Aldrich). The drug was dissolved in distilled water and injected systemically (10 mg/kg, i.p.). Clozapine *N*-oxide was obtained from the NIH and dissolved in 2.5% DMSO in distilled water and injected systemically (3 mg/kg i.p.).

4.4.6 Histology

After completion of the experiments, the rats were overdosed with pentobarbital. For rats implanted with a BLA array, electrolytic lesions were created by passing electrical current (80 μA, 10 sec; A365 stimulus isolator, World Precision Instruments, Sarasota, FL) through four of the recording wires (the 4 corners of the 4x4 array). Rats were then perfused transcardially with 0.9% saline followed by 10% formalin. Brains were extracted from the skull and post-fixed in a 10% formalin solution for 24 hours, followed by a 30% sucrose solution, where they remained for a minimum of 48 hours. Coronal brain sections

of the BLA (40 µm thickness) were cut on a cryostat (-20^{o} C, Leica Microsystems, Buffalo Grove, IL), mounted on subbed microscope slides, and stained with thionin (0.25%) to visualize electrode placements.

To visualize LC viral expression using immunohistochemistry, the following steps were carried out. First, brains were coronally sectioned (40 µm thickness) with a cryostat and stored in a 0.01% sodium azide solution until further processing. Sections were blocked in PBS with 0.1% Triton X-100 (TX) and 3% normal donkey serum (NDS, 2 ml/well) for one hour. All steps occurred in this PBS-TX-NDS solution at room temperature. Sections were then incubated in primary antibodies (mouse anti-tyrosine hydroxylase (TH) [1:2000] and rabbit anti-HA [1:1000]) for 24 hours. Sections were then rinsed three times (10 min each). Sections were then incubated in secondary antibodies (donkey anti-mouse Alexa Fluor 488 [1:500; for TH] and donkey anti-rabbit Alexa Fluor 594 [1:500; for HA]) for three hours. Afterward, sections were rinsed three times (10 min each). Next, the sections were mounted on microscope slides using PBS, and coverslipped using fluoromount (Diagnostic BioSystems, Pleasanton, CA). Images were obtained using a Zeiss AXIO Imager M2. The following suppliers were used for the above materials: NDS (EMD Millipore, Billerica, MA), Triton X (Sigma-Aldrich, St. Louis, MO), mouse anti-TH (ImmunoStar, Hudson, WI), rabbit anti-HA (Cell Signaling Technology, Danvers, MA), donkey anti-mouse IgG (H+L) Alexa Fluor 488 (Thermo Fisher Scientific/Invitrogen, Waltham, MA), donkey anti-rabbit IgG (H+L) Alexa Fluor 594 (Thermo Fisher Scientific/Invitrogen, Waltham, MA).

4.4.7 Statistics

The data were analyzed with conventional parametric statistics (StatView, SAS Institute). One-way and two-way analysis of variance (ANOVA) and repeated-measures ANOVA were used to assess general main effects and interactions ($\alpha = 0.05$). Results are shown as mean ± SEM. Chi-square analyses were used to examine differences in neuronal populations.

5. LOCUS COERULEUS TOGGLES RECIPROCAL PREFRONTAL FIRING TO REINSTATE FEAR*

5.1 Introduction

Learning to inhibit or "extinguish" fear when danger has passed is not only adaptive, but also central to behavioral therapies for many psychiatric disorders. However, the extinction of fear is short-lived and relapse occurs under a variety of conditions, including psychological stress. Considerable data indicate that the prelimbic (PL) and infralimbic (IL) subdivisions of the medial prefrontal cortex (mPFC) serve to regulate the expression and inhibition of learned fear, respectively (Giustino and Maren, 2015; Milad and Quirk, 2012). Projections from the locus coeruleus (LC) to the mPFC have a prominent role in stress-induced modulation of mPFC function (Giustino and Maren, 2018; Arnsten, 2009, 2015). Moreover, noradrenergic transmission mediates stress-induced decreases in IL spike firing and impairments in extinction learning (Fitzgerald et al., 2015; Giustino et al., 2017). This work suggests that noradrenergic neurons in the LC may trigger relapse by altering mPFC firing dynamics to drive fear expression while weakening fear inhibition. Here we explored this possibility using selective pharmacogenetic manipulation of LC noradrenergic neurons and mPFC single-unit recordings in rats undergoing relapse of extinguished fear.

* Reprinted with permission from PNAS Giustino TF, Fitzgerald PJ, Ressler RL, and Maren S. (2019). Locus coeruleus toggles reciprocal prefrontal firing to reinstate fear. ***Proc. Natl. Acad. Sci. USA.*** doi:10.1073/pnas.1814278116.

5.2 Results

5.2.1 Prefrontal correlates of low and high fear states

To characterize the neuronal correlates of extinction retrieval and fear relapse in the mPFC, we implanted animals with a single microelectrode array targeting both PL and IL and recorded single-unit activity using a novel within-subject behavioral design (Figure 5.1a, b). In this design, animals underwent standard auditory fear conditioning and extinction in distinct contexts; freezing behavior served as the index of fear (Figure 5.2). To facilitate the relapse of fear after extinction, animals received an unsignaled footshock unconditioned stimulus (US) in the conditioning context to reinstate the fear memory (Bouton, 2002). Single-unit recordings were then made in both the extinction context (where rats retrieved an extinction memory and expressed low levels of conditional freezing behavior) and a third distinct context (where rats retrieved a fear memory and expressed relatively higher levels of freezing behavior). Hence, this design combines two procedures that drive relapse of extinguished fear: reinstatement (re-exposure to the US) and renewal (a context-shift during retrieval testing); we refer this to as a "renewalment" procedure (Bouton, 2002; Goode and Maren, 2014). Importantly, the animals remained connected to the recording interface throughout these sessions so that the same mPFC neurons could be recorded during both retrieval tests (i.e., extinction retrieval and fear relapse).

During the test sessions, we recorded a total of 333 PL neurons and 288 IL neurons from 12 rats. CS-evoked activity was normalized by calculating *z*-scores for each post-

CS bin (200ms) relative to the firing rate in the 1-sec pre-CS period; these *z*-scores were averaged across the 5 CSs delivered during each test.

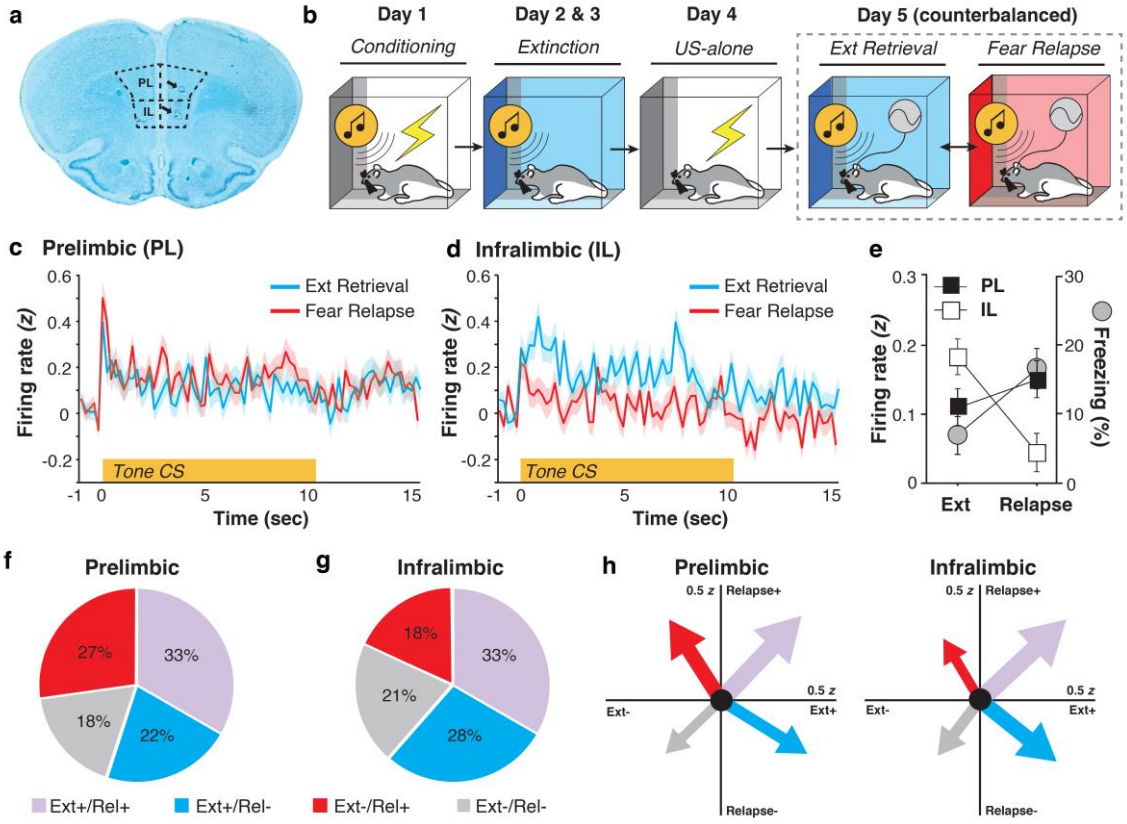

Figure 5.1 Extinction retrieval and fear relapse bidirectionally engage mPFC signaling. a) Representative histology of electrode placements in PL and IL. b) Schematized behavioral design. c, d) CS-evoked firing from PL and IL neurons in retrieval and renewal. e) Percentage of freezing (gray circles, mean ± SEM) across days; Freezing is overlaid with the 10-second summary of the CS-evoked firing responses. f, g) Pie charts displaying the proportion of PL and IL neurons in one of four categories based on how neurons responded to presentation of the CS in both retrieval and renewal. h) Vector plots depicting CS-evoked firing. The tips of the arrows point to the mean CS-evoked responding for a particular quadrant. The thickness of the arrow is proportional to the total number of neurons recorded in either PL or IL.

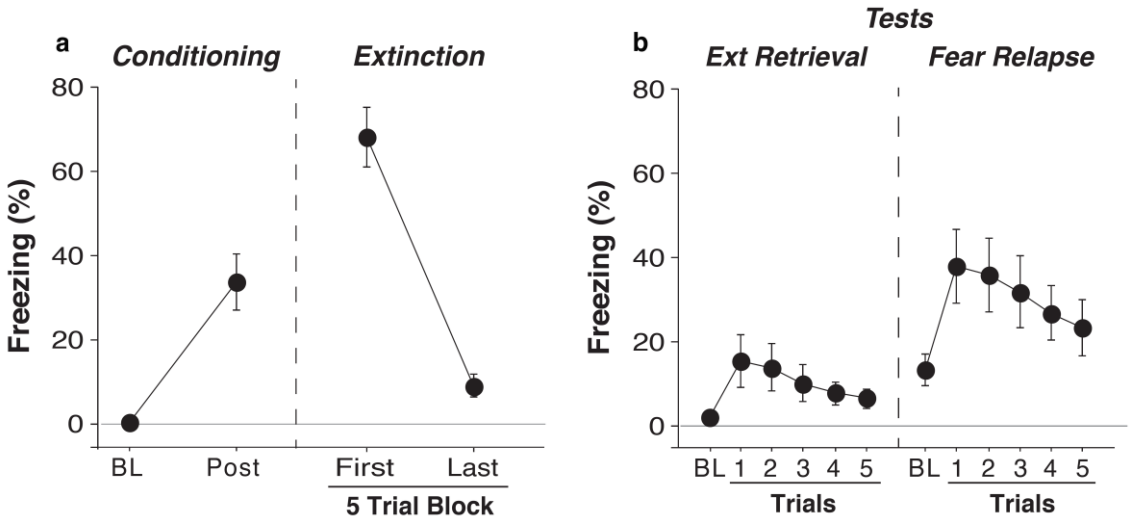

Figure 5.2 CS presentation outside the extinction context produces fear relapse. a) Percentage of freezing (mean ± SEM) from the conditioning and extinction sessions. Freezing is averaged across CSs and ITIs for all trial blocks in each session. All rats showed an increase in freezing behavior following conditioning [main effect of time, $F(1, 11) = 25.35$, $p < 0.001$]. The following two days rats showed a reduction in CS-evoked freezing behavior throughout extinction [main effect of time, $F(1, 11) = 44.05$, $p < 0.0001$]. b) Rats next underwent a dual retrieval-relapse test session. Rats showed low CS-evoked freezing in the retrieval context relative to the relapse context. The baseline freezing and the trial x trial data for the test session further illustrating that CS-evoked freezing was higher in relapse than in extinction retrieval [main effect of test, $F(1, 11) = 17.67$, $p < 0.01$].

As shown in Figure 5.1, single-unit activity recorded in PL (Figure 5.1c) and IL (Figure 5.1d) exhibited a reciprocal relationship in response to an identical auditory conditioned stimulus (CS) presented in the two distinct test contexts. Neurons in PL exhibited reliably higher CS-evoked firing in the relapse context relative to the extinction context, whereas the inverse was true among IL single-units. This observation was confirmed in an ANOVA which revealed a significant test context X brain region interaction on the average normalized firing rate in PL and IL to the test CSs [Figure 5.1e; $F(1, 629) = 11.73$, $p <$

0.001]. The reciprocal firing in PL and IL mirrored CS-elicited freezing behavior (normalized to the 3-min pre-CS baseline), which was low in the extinction context and high in the relapse context [Figure 5.1e, $F(1, 11) = 6.05$, $p < 0.05$].

Because we recorded the activity of the same prefrontal neurons during both retrieval tests, we were able to classify units according to four firing phenotypes defined by the direction of their CS-evoked response [excitatory (+, $z > 0$ for the 10-sec CS averaged across 5-trials) or inhibitory (-, $z < 0$ for the 10-sec CS averaged across 5-trials)] in each of the two contexts (extinction or relapse). As shown in Figure 1, these firing phenotypes were differently represented among the populations of neurons recorded in PL and IL (Figure 5.1f, g). A chi-square analysis revealed differences between PL and IL in terms of the proportion of neurons responding to the CS during extinction retrieval and fear relapse such that a larger proportion of PL neurons showed increased firing during relapse, whereas IL neurons were proportionately more active during extinction retrieval [$\chi^2(3) = 9.04$, $p < 0.05$]. Figure 5.1h depicts these data as population vectors that represent both the number of neurons in each phenotype (represented by arrow thickness) and the population mean of the average CS-evoked activity in each test context (indicated by the x,y coordinate of the arrow tip). These plots confirm that PL units fire preferentially in the relapse context, whereas IL units fire preferentially in the extinction context. Collectively, these data reveal that IL neurons showed more robust firing in response to the CS in the extinction context compared to the relapse context, whereas PL activity was higher relative to IL in the relapse context.

5.2.2 LC-DREADD validation

Given that noradrenergic transmission mediates stress-induced decreases in IL spike firing and impairments in extinction learning (Fitzgerald et al., 2015; Giustino et al., 2017), we hypothesized that noradrenergic neurons in the LC would drive fear relapse. To selectively target noradrenergic LC neurons (Figure 5.3a), we used custom excitatory and inhibitory DREADD vectors whose receptor expression is under control of the synthetic dopamine-ß-hydroxylase PRSx8 promoter (Vazey and Aston-Jones, 2014). To confirm the *in vivo* functional efficacy of these LC-specific DREADDs, animals expressing either AAV9-PRSx8-hM3Dq-HA (Figure 5.3c, an excitatory DREADD) or AAV9-PRSx8-hM4Di-HA (Figure 5.3d, an inhibitory DREADD) were anesthetized and implanted with a recording array for acute LC recordings. We used a within-subject design to record the activity of the same neurons (n = 54, hM3Dq; n = 100, hM4Di) in response to both vehicle (VEH) and clozapine *N*-oxide (CNO, 3 mg/kg, i.p.). After a 10 min baseline period rats were injected with VEH and recording continued for 30 min, upon which rats were then injected with CNO followed by recording for an additional 60 min to observe the changes in firing rate.

The spontaneous baseline LC firing rates prior to drug administration were as follows (mean ± SEM): hM3Dq (2.09 ± 0.12 Hz) and hM4Di (1.83 ± 0.09 Hz). To assess CNO-induced changes in spike firing, we normalized the post-injection firing rates (60-sec bins across the entire 100 min recording session) to the 10-min baseline period. As shown in Figure 5.3b-d, CNO induced statistically reliably changes in average LC firing

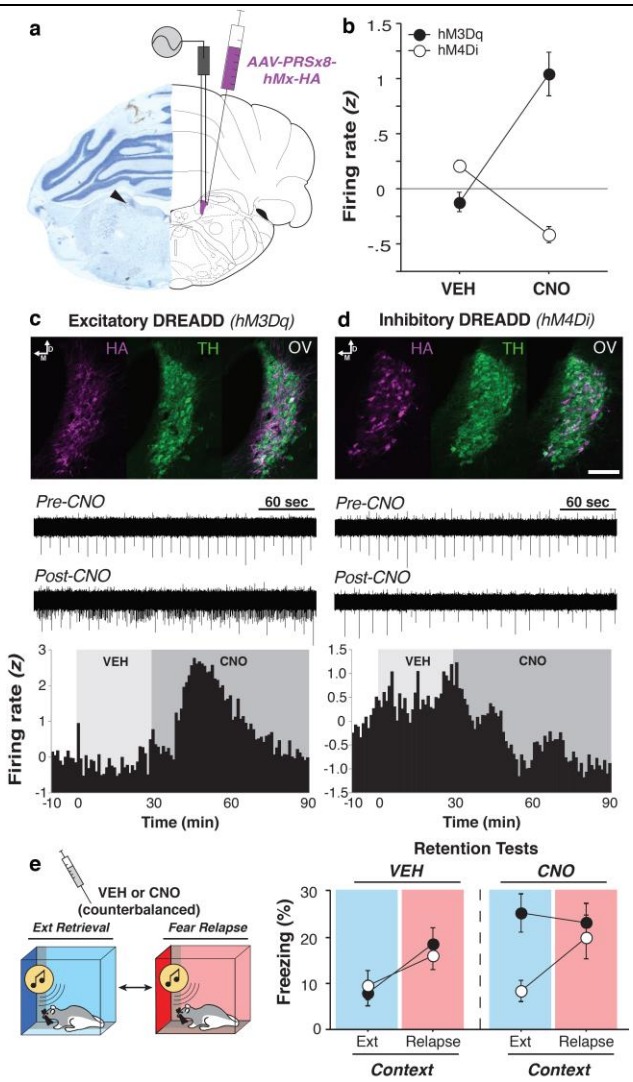

Figure 5.3 Locus coeruleus (LC)-specific DREADD functionality. a) Representative microelectrode placement in the LC (left) and a schematic (right) indicating the placement of electrodes and DREADDs in the LC. b) CNO administration bidirectionally regulates LC firing rates in animals expressing inhibitory (hM4Di) or excitatory (hM3Dq) DREADDs in the LC. c, d) Immunohistochemical localization (OV, overlay) of LC-DREADDs (HA, purple) in tyrosine hydroxylase-positive neurons (TH, green). CNO administration produced robust increases (c) and decreases (d) in LC spike firing illustrated in both the raw recording traces and the average firing rate of all neurons recorded. e) Percentage of freezing (mean ± SEM) for each group across days. CNO produced fear relapse in the hM3Dq group (black circles) whereas LC inhibition via hM4Di (white circles) had minimal effects on freezing in the extinction retrieval and fear relapse. Background colors within the freezing graphs correspond to each sessions context (i.e., blue = extinction/retrieval, red = relapse). Scale bar = 100 um

rate [Figure 5.3b, drug x virus interaction, $F(1, 122) = 117.7$, $p < 0.0001$]. It significantly increased LC firing in 76% (n = 41 of 54) of the neurons recorded in hM3Dq expressing rats [Figure 5.3c, main effect of time, $F(69, 3657) = 22.00$, $p < 0.0001$] and decreased LC firing in 65% (n = 65 of 100)of the neurons recorded in hM4Di expressing animals [Figure 5.3d, main effect of time, $F(69, 6831) = 15.11$, $p < 0.0001$].

5.2.3 LC-NE induces fear relapse

After confirming the functional efficacy of the LC DREADDs, we next determined whether manipulating LC activity would influence extinction retrieval and fear relapse in the within-subject "renewalment" design. As shown in Figure 5.3e, VEH-treated rats expressing inhibitory or excitatory LC DREADDs showed low levels of CS-elicited freezing (normalized to baseline) in the extinction context, but a marked increase in freezing in the relapse context. Interestingly, pharmacogenetic activation of noradrenergic neurons in the LC was sufficient to induce fear relapse; CNO administration in hM3Dq-expressing rats dramatically increased freezing in the extinction context. Inhibiting LC activity, however, did not prevent fear relapse [drug x context x virus interaction, $F(1, 40) = 5.17$, $p < 0.05$]. This is not surprising insofar as relapse associated with a context shift is independent of contextual fear (Maren et al., 2013). In addition to increasing freezing to the CS, CNO administration also produced significant increases in freezing prior to delivery of the CS (during the baseline period) in animals expressing hM3Dq in the LC (Figure 5.4). Note that this increase in baseline freezing was independent of the relapse effect, which was manifest as an increase in CS-evoked freezing normalized to the elevated baseline. The observation that LC activation increases freezing behavior is

consistent with recent work showing that LC activation induces anxiety-like behavior (McCall et al., 2017; Hirschberg et al., 2017). These results reveal that pharmacogenetic activation of noradrenergic LC neurons promotes the relapse of extinguished fear.

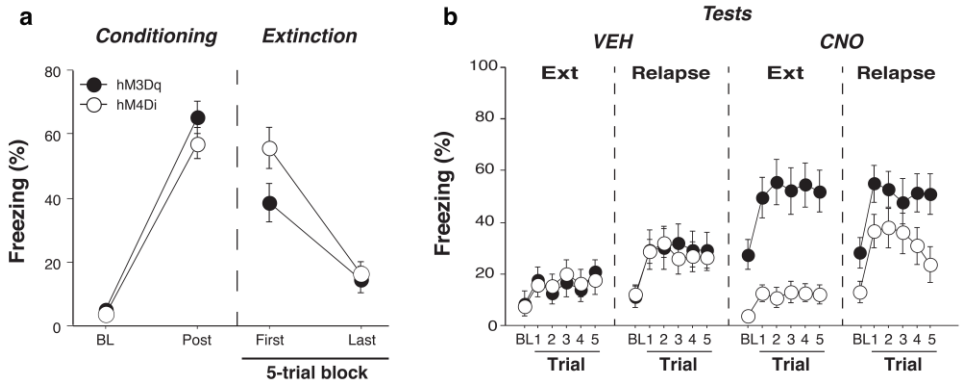

Figure 5.4 LC-NE activation produces fear relapse. a) Percentage of freezing (mean ± SEM); freezing is averaged across CSs and ITIs for all trial blocks in each session. Rats expressing either hM3Dq or hM4Di in the LC conditioned similarly as evidenced by an increase in freezing behavior from the baseline period to the post shock period [main effect of time, $F(1, 40) = 285.61$, $p < 0.0001$]. Likewise, both groups extinguished fear to the CS over the course of the extinction sessions [main effect of time, $F(1, 40) = 64.62$, $p < 0.0001$]. After extinction, the animals received within-subject retention tests to the CS in either the extinction context or a familiar, alternate relapse context after administration of either VEH or CNO. b) The 3 min baseline (BL) freezing is shown as well as the freezing for each trial. While CNO produced a nonspecific increase in baseline (BL) freezing prior to CS onset (in the hM3Dq group) this does not account for the differences in CS-evoked freezing [drug x test x virus interaction, $F(1, 40) = 12.68$, $p < 0.001$].

5.2.4 LC-NE drives changes in PL and IL firing to promote fear relapse

The relapse of extinguished fear is associated with the suppression of activity in IL-amygdala circuits involved in the inhibition of fear (Marek et al., 2018a; Knapska and Maren, 2009). Based on previous work showing that noradrenergic transmission mediates stress-induced reductions in IL firing (Fitzgerald et al., 2015), we hypothesized that LC-

driven fear relapse is mediated by a suppression of IL spike firing in the mPFC. To test this hypothesis, microelectrode-implanted rats (targeting PL and IL) expressing either AAV9-PRSx8-hM3Dq-HA or an AAV9-PRSx8-mCherry control virus (Figure 5.5) in the LC (Figure 6a) underwent fear conditioning, extinction, and retrieval tests in the extinction context after either VEH or CNO administration (Figure 5.6b, Figure 5.7). The number of neurons recorded in each group and brain area are as follows: VEH-mCherry [PL, n = 160; IL, n = 135]; CNO-mCherry [PL, n = 134; IL, n = 136]; VEH- hM3Dq [PL, n = 131; IL, n = 105]; CNO- hM3Dq [PL, n = 105; IL, n = 116]. As shown in Figure 5.6c-f, pharmacogenetic activation of noradrenergic LC neurons increased CS-evoked spike firing in PL and suppressed that in IL. In other words, LC activation shifted mPFC firing from a low-fear (IL>PL) to a high-fear profile (PL>IL) [Figure 5.6d, f; drug x virus x region interaction, $F(1,1010) = 6.80$, $p < 0.01$] *and* drove fear relapse [Figure 5.6d, f (gray circles), $F(1,11) = 7.93$, $p < 0.05$].

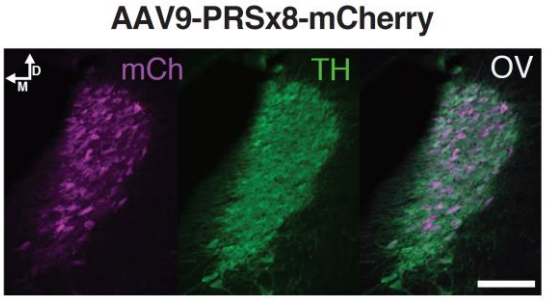

Figure 5.5 Control viral expression is restricted to the locus coeruleus. Immunohistochemical localization (OV, overlay) of the blank mCherry control virus (mCh, purple) in tyrosine hydroxylase-positive neurons (TH, green) in the LC. Scale bar = 100 um

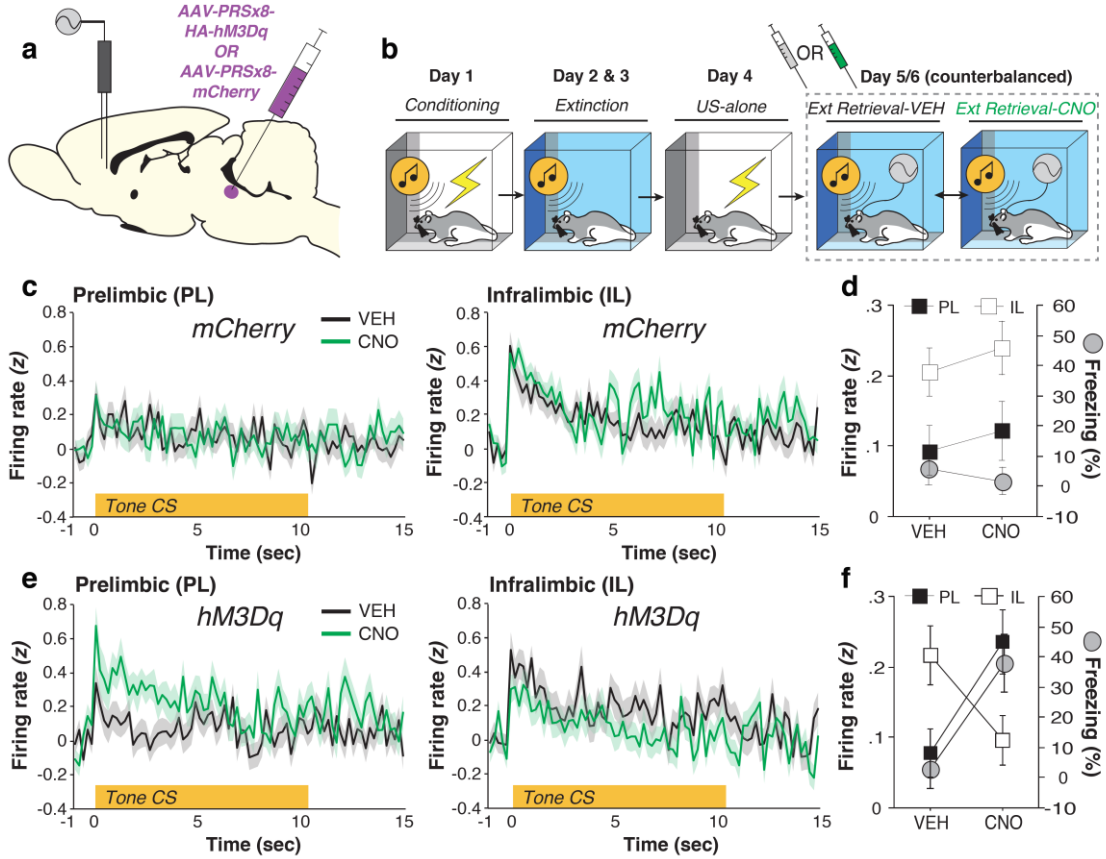

Figure 5.6 LC-NE drives PL CS-evoked activity and fear relapse. a) Schematic representation of experimental approach. b) Schematized behavioral design. c) CS-evoked responses in PL and IL during extinction retrieval following either VEH or CNO administration in animals expressing the blank mCherry vector. d) Percentage of freezing (gray circles, mean ± SEM) across test days; freezing is overlaid with the 10-second summary of the CS-evoked firing responses for each brain region. e) CS-evoked responses in PL and IL in animals expressing hM3Dq in the LC following either VEH or CNO administration. f) Percentage of freezing (gray circles, mean ± SEM) across test days; freezing is overlaid with the 10-second summary of the CS-evoked firing responses for each brain region.

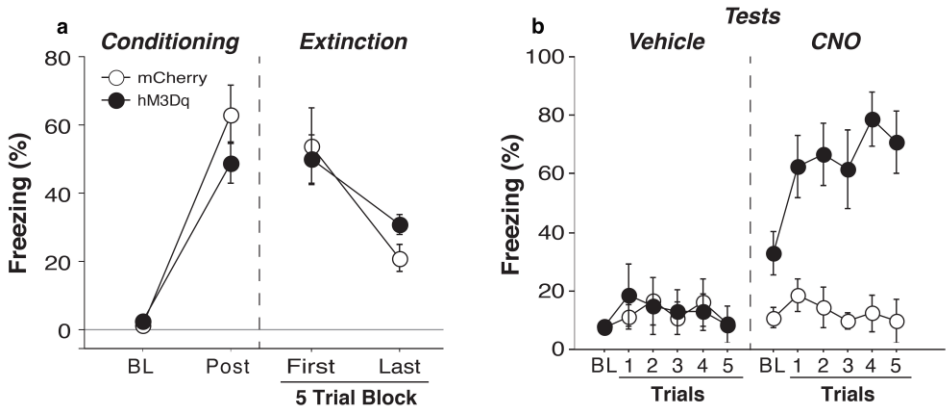

Figure 5.7 LC-NE induced fear relapse is independent of increases in baseline (pre-CS) freezing. a) Percentage of freezing (mean ± SEM) from the conditioning and extinction sessions. Animals expressing either hM3Dq or a blank mCherry virus in the LC showed similar increases in freezing from the pre-conditioning baseline period [main effect of time, $F(1,11) = 90.73$, $p < 0.0001$]. Both groups extinguished at a similar rate [main effect of time, $F(1,11) = 13.59$, $p < 0.01$]. b) The 3 min baseline (BL) freezing is shown as well as the freezing for each trial for the extinction retrieval sessions (each rat underwent one session after VEH or CNO administration). While CNO produced a nonspecific increase in baseline freezing in rats expressing hM3Dq, this does not account for the observed differences in CS-evoked freezing [time x drug x virus interaction, $F(5,55) = 3.53$, $p < 0.01$].

Collectively, these results reveal that LC activation toggles reciprocal firing in the mPFC by decreasing CS-evoked spike firing in IL on the one hand while increasing PL spike firing on the other. This inversion of extinction-related mPFC firing results in the relapse of extinguished fear.

5.2.5 LC-NE acts both directly in the PL and through the BLA to promote fear relapse

Although the previous data strongly implicate a role of LC-NE modulation of mPFC CS-evoked firing, they do not causally implicate LC projections to mPFC in the observed neural and behavioral changes. In order to address this question, we sought to

determine whether the propensity of CNO-induced LC activation to cause fear relapse could be antagonized by pharmacologically reducing NE release in the PL with intracranial infusions of clonidine, an alpha2 agonist). Because it has been suggested that LC actions in the BLA might also come to influence the mPFC (Giustino and Maren, 2018; Arnsten, 2009, 2015), we also included animals in which we reduced NE release in the amygdala. To this end, animals expressing AAV9-PRSx8-hM3Dq-HA were implanted with bilateral cannula targeting either the PL or BLA to examine if CNO-induced LC activation mediates its behavioral effect via one of these targets. Animals underwent fear conditioning, extinction, and retrieval tests in the extinction context (Figure 5.8). Using a within-subject design (Figure 5.9a), animals received intracranial infusions of either vehicle or clonidine (order counterbalanced) in either the PL or BLA (Figure 5.9b). After intracranial infusions, animals were injected with systemic VEH or CNO (order counterbalanced) and approximately 20 min later underwent extinction retrieval as in the previous experiments. In this design, each rat underwent four separate extinction test sessions. As shown in Figure 5.9c, intracranial clonidine infusions reduced CNO-induced increases in CS-evoked freezing relative to VEH controls [systemic drug x intracranial drug interaction, $F(1,18) = 8.04$, $p < 0.05$]. This effect was similar whether clonidine was infused in PL or BLA. These data reveal that NE release in both the PL and BLA mediate the behavioral effects of pharmacogenetic activation of the LC. This suggests that NE release in the BLA might drive, at least in part, the neuronal correlates of fear relapse observed in the PL.

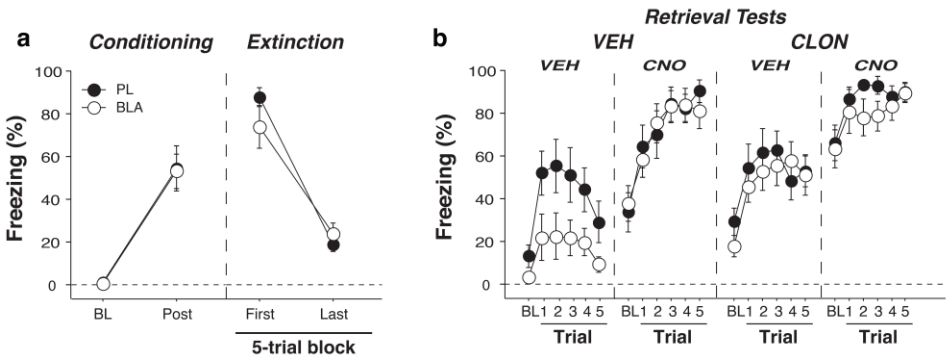

Figure 5.8 Local infusions of clonidine into either PL or the BLA block CNO-induced fear relapse independent of increases in baseline (pre-CS) freezing. a) Percentage of freezing (mean ± SEM) from the conditioning and extinction sessions split by brain region. Animals from both groups showed a similar increase in conditioned freezing from the baseline to the post conditioning period [main effect of time, $F(1,18) = 65.11$, $p < 0.0001$]. Both groups extinguished at a similar rate [main effect of time, $F(1,18) = 106.35$, $p < 0.0001$]. b) The 3-min baseline (BL) freezing is shown as well as the freezing for each trial for the extinction retrieval sessions (each rat underwent four total retrieval sessions). We replicate our finding where CNO-induced increases in LC-NE produce non-specific effects on baseline freezing, but this does not account for the differences in CS-evoked freezing. Local infusions of clonidine (alpha2-noradrenergic receptor agonist) into either the PL or BLA limit CS-evoked freezing in the presence of CNO [systemic drug x local infusion x time interaction, $F(5, 90) = 5.11$, $p < 0.001$].

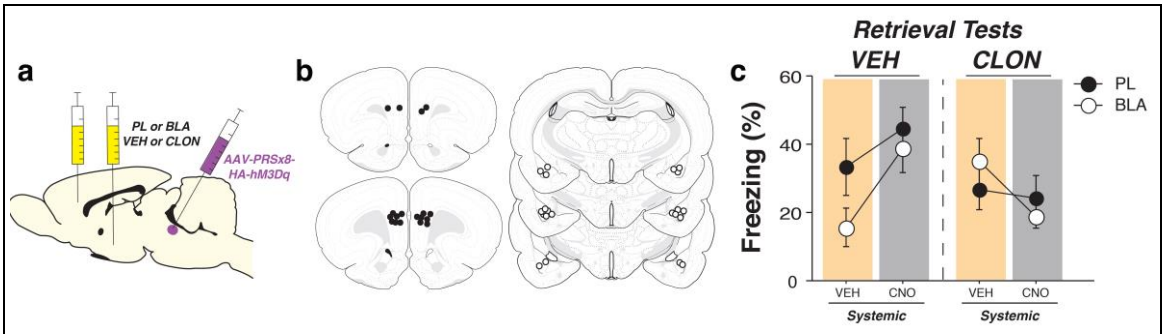

Figure 5.9 Local infusions of clonidine in either the PL or BLA block CNO-induced fear relapse to a previously extinguished CS. a) Schematic representation of the experimental approach. b) Schematic histology displaying location of cannula tips in either PL or BLA. c) Percentage of freezing (mean ± SEM) across test days split by brain region. Data are normalized by subtracting the 5-trial CS-evoked averages from the 3 min stimulus-free baseline period. Background colors depict systemic injections (orange = vehicle, gray = CNO).

5.3 Discussion

Collectively, these experiments uncover a novel role for LC modulation of mPFC spike firing in the relapse of extinguished fear. Specifically, we demonstrate that DREADD-induced increases in LC firing toggle reciprocal spike firing in the mPFC and drive relapse of extinguished fear. In particular, LC activation increased CS-evoked responding in PL while decreasing that in the IL, an inversion of the IL-dominated firing observed after extinction (Giustino and Maren, 2015; Quirk and Mueller, 2008; Maren et al., 2013). These data reveal that noradrenergic neurons in the LC modulate mPFC signaling to induce neuronal firing signatures associated with high fear states, which in turn drives relapse.

The current data confirm and extend previous work revealing dissociable roles of PL and IL in conditioned fear (Giustino et al., 2016a; Sotres-Bayon and Quirk, 2010; Quirk et al., 2000; Milad and Quirk, 2002; Milad et al., 2004; Vidal-Gonzalez et al., 2006; Ye et al., 2017). We demonstrate that CS-evoked firing in IL is most pronounced in the extinction context (where fear is low), whereas it is reliably lower in the relapse context (where fear is high). In contrast, this pattern is inverted in PL, where CS-evoked spike firing is relatively higher in the relapse compared to the extinction context. Although it is well established that PL and IL firing correlate with high and low fear states respectively, the neural circuitry and transmitter systems driving these differences is relatively unknown. We now demonstrate a critical role for the LC-NE system in driving differential responses in PL and IL: pharmacogenetic activation of the LC increased PL firing (relative to IL) and caused the relapse of extinguished fear. One possibility is that direct LC→PL

projections excite PL pyramidal cells which in turn inhibit the IL; others have shown that PL→IL connections can influence freezing behavior (Marek et al., 2018b). A second possibility is that these differences are driven by indirect pathways from the LC to the mPFC via the amygdala. Past work has shown that BLA projections to PL and IL mediate high and low fear states, respectively (Senn et al., 2014). The fact that reducing NE release in either the PL or BLA prevented LC-induced fear relapse suggests it may be a combination of these pathways that mediates the effects of LC activation on fear.

The LC-NE system has been widely studied in the context of stressor- and trauma-related disorders, such as PTSD (Bremner et al., 1996; Strawn and Geracioti, 2008; Koenigs and Grafman, 2009; Giustino and Maren, 2018). For example, prazosin, an alpha1-adrenoceptor antagonist, has had some success in reducing nightmares associated with PTSD (Taylor et al., 2008; Writer et al., 2014; Koola et al., 2014; Keeshin et al., 2017; Raskind et al., 2018). In addition, guanfacine and clonidine (alpha2-adrenoceptor agonists) as well as propranolol (beta1,2-adrenoceptor antagonist) have shown promise in alleviating PTSD symptomatology (Arnsten, 2015; Giustino and Maren, 2018; Giustino et al., 2016b). However, here we show no effect of pharmacogenetic inhibition of the LC on either extinction retrieval or fear relapse. This suggests that noradrenergic antagonists, such as propranolol, might not be effective in reducing the acute relapse of extinguished fear. Of course, it is possible that the degree of inhibition we obtained with inhibitory DREADDs was not sufficient to prevent NE release in LC terminals in the forebrain.

Overall, these data have important clinical implications insofar as elevated norepinephrine levels are observed in patients with PTSD and have been argued to

underlie, at least in part, the pathophysiology of this disorder (O'Donnell et al., 2004; Southwick et al., 1999c, 1999a; Giustino et al., 2016b). Consistent with this, noradrenergic transmission causes stress-induced decreases in IL firing and impairs extinction learning (Arnsten, 2009; Giustino and Maren, 2015; Milad and Quirk, 2012), which may underlie extinction learning deficits in individuals suffering from PTSD (Giustino et al., 2016b; Wessa and Flor, 2007; Milad et al., 2009; Arnsten, 2009, 2015). We now show that noradrenergic neurons in the LC influence mPFC spike firing to drive the return of fear once it has been extinguished. As such, noradrenergic tone along with mPFC activity may serve as a reliable biomarker to predict fear relapse. Moreover, pharmacotherapeutic interventions that moderate LC hyperactivity in PTSD might be particularly effective in promoting long-lasting extinction learning and preventing its relapse once learned (Giustino et al., 2016b; Fitzgerald et al., 2015; Giustino et al., 2017).

5.4 Materials and Methods

5.4.1 Subjects

Eighty-seven experimentally naïve adult male Long-Evans Blue Spruce rats (weighing 200-224 g; 50-57 days old) were obtained from a commercial supplier (Envigo, Indianapolis, IN). Upon arrival and throughout the experiments, rats were individually housed in cages within a humidity- and temperature-controlled vivarium, and kept on a 14:10 hr light/dark cycle (lights on at 7 am) with ad libitum access to food and water. All experiments were conducted in the daytime during the light phase. Rats were handled for ~30 seconds a day for 5 days to habituate them to the experimenter before any behavioral testing or surgical procedures were carried out. All procedures were conducted at Texas

A&M University and were performed in strict accordance with the guidelines and regulations set forth by the National Institutes of Health and Texas A&M University with full approval from its Animal Care and Use Committee.

5.4.2 Locus coeruleus-specific DREADDs and behavioral procedures

Rats were bilaterally infused with a locus coeruleus (LC)-specific DREADD (Designer Receptors Exclusively Activated by Designer Drugs). For viral infusion surgery, rats were anesthetized with isoflurane (5% induction, 2% maintenance) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). The scalp was incised and retracted, the head was tilted 15 degrees downward such that bregma skull surface was 2 mm below intersectional lambda skull surface in the horizontal plane. The skull overlying the left and right hemispheres of LC was removed. Both hemispheres of LC were then separately infused with either the LC-specific excitatory DREADD (AAV9-PRSx8-hM3Dq-HA), with an inhibitory one (AAV9-PRSx8-hM4Di-HA), or a blank control virus (AAV9-PRSx8-mCherry), using a hypodermic injector (Small Parts/Amazon, Seattle, WA) that was coupled to a Legato 101 infusion pump (KD Scientific, Holliston, MA) and 10 µl syringe (Hamilton Company, Reno, NV) using polyethylene tubing (Braintree Scientific, Braintree, MA). The coordinates for each infusion (relative to intersectional lambda skull surface) were as follows: AP: -3.8, ML: +/-1.4. Since these were LC-specific DREADDs(Vazey and Aston-Jones, 2014) and the depth of the LC is variable across rats, virus was infused at three separate depths from lambda skull surface (first infusion: -7.0, second: -6.5, third: -6.0), 0.5 µl was infused at each depth. The infusion rate was 0.25 µl/min, and after the third infusion the injector was left in the brain for an additional 5 min

to allow the virus to diffuse more effectively at the infusion site. At least two weeks were allowed for recovery, and for the virus to express in the LC, before experiments began.

A modified rodent behavioral chamber (30x24x21 cm, Med Associates, St. Albans, VT) enclosed in a sound-attenuating cabinet was used for most days (i.e., the two extinction sessions, two dual retrieval-renewal tests) of these behavioral experiments. This chamber was modified to allow for freely moving electrophysiological recordings as well (described later). The chamber comprised two aluminum side walls, a Plexiglas rear wall, a hinged Plexiglas door, and an open top. The grid floor consisted of 19 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center-to-center). A loudspeaker attached to the outside of a grating in one aluminum wall was used to play auditory tones. Locomotor activity of the rat was transduced by a load-cell under the floor of the chamber, and the output of the load-cell was recorded by an OmniPlex recording system (Plexon, Dallas, TX). Thus, all behavioral activity (and neural activity, for the freely moving recordings) was recorded automatically with this system. A separate but very similar (i.e., enclosed top) behavioral chamber (context A), located in an adjacent room, was used for fear conditioning and to deliver a reminder shock in a later session (see below); having a separate room for context A helped reduce fear generalization across contexts. No electrophysiological recordings took place in this chamber, and locomotor activity was recorded automatically using a computerized load-cell system. The rods comprising the grid floor were connected to a shock source and solid-state grid scrambler (Med Associates) for the delivery of footshocks.

On Day 1 of behavioral testing, each rat was individually fear conditioned in context A. In this procedure, the rat was transported to the room in a white plastic box and placed in the behavioral chamber. The chamber had been cleaned with 70% ethanol to provide a distinct olfactory cue, and a metal pan containing a thin layer of the same solution had been placed under the grid floor. The room was illuminated with white ambient lights, although the chamber house light was off and the sound attenuating chamber doors were closed, with a small window in one of the doors to allow some light in. A fan mounted within one wall of the sound-attenuating chamber was also turned on to provide constant, ambient background noise (context A). After a 3-min stimulus-free baseline period, the animal received three auditory tone-footshock pairings. The tones (conditioned stimuli; CS) were 10 sec, 80 dB, 2 kHz; the shocks (unconditioned stimuli; US) were 2 sec and 1 mA, where shock onset occurred at tone offset. There was a 1-min inter-trial interval (ITI) between shocks. The behavioral session continued for 1 min after the final shock, and then the rat was returned to its home cage.

On Day 2, the first of two fear extinction sessions took place, in the recording room adjacent to where fear conditioning was administered. The rat was transported to the room in a black plastic box and placed in the behavioral chamber. The chamber had been cleaned with 3% acetic acid to provide a distinct olfactory cue, a black plastic pan containing a thin layer of the same solution had been placed under the grid floor, the grid floor was covered with a transparent rubber mat, the back wall was covered with alternating black and white stripes, and the room was illuminated with ambient red lights (context B). After a 3-min stimulus-free baseline period, the animal was presented with

45 tone-alone trials (30-sec ITI); the rat remained in the chamber for 3 min after the final tone, and movement was recorded automatically throughout the session. Day 3 consisted of a second extinction session, identical to that of Day 2. On Day 4, the rat received an unsignaled (i.e., no tone was presented) reminder shock in the conditioning chamber (context A). After a 3-min baseline, this weaker shock (0.5 mA, 2 sec) was delivered, followed by a 3-min stimulus-free period. As in the Day 1 session, movement was recorded automatically.

On Day 5 (and Day 6 for experiments that had 2 test days), the rat received a dual retrieval-renewal test. For animals that received LC DREADDs and an mPFC array, the DREADD ligand, clozapine N-oxide (CNO; 3 mg/kg, i.p.) or a vehicle (VEH; 2.5% dimethyl sulfoxide [DMSO] in distilled water) injection was given 30 min before the start of testing. In this within-subjects design, each rat received CNO one day and VEH the other day of testing, with drug sequence counterbalanced across rats. For a given rat, whether retrieval or renewal came first within the test was held constant across Days 5 and 6, and this was also counterbalanced across rats. The following is a description of the behavioral procedure if retrieval took place first. The rat was transported to the recording room in a black plastic box and placed in the behavioral chamber (context B). After a 3-min stimulus-free baseline period, the animal was presented with 5 tone-alone trials (30-sec ITI) and remained in the chamber for 10 min after the final tone. The rat was then immediately placed in a large white plastic bucket with a layer of bedding in the bottom. The contextual cues were then rapidly (within approximately 5 min) altered as follows to prepare for the fear renewal session: 1% ammonium scent, white plastic pan beneath the

grid floor, no rubber mat, no striped walls, white ambient lighting (context C). The renewal protocol was the same as in retrieval: 3-min baseline, 5 tone-alone trials, 10-min stimulus-free period after the last tone. The rat was then returned to its home cage.

5.4.3 Electrophysiological characterization of LC DREAADs

Rats were anesthetized with isoflurane (5% induction, 2% maintenance) and placed in a stereotaxic apparatus (Kopf Instruments). The scalp was incised and retracted, the head was tilted downward 15 degrees as described above, and the skull was cleaned to allow for the acute insertion of the electrode array into LC. The microelectrode array (Innovative Neurophysiology, Durham, NC) comprised 16, 10.5 mm long wires. This 4 x 4 wire array had 200 µm center-to-center spacing of adjacent wires. Each wire was 50 µm in diameter and the conductor was tungsten. Using the same coordinates as described above for viral infusion in LC, the array was slowly lowered to a depth of 6-7 mm, while the experimenters listened to an audio output of the neural activity through our Plexon recording system. After lowering the electrode array into LC, we allowed the signal to stabilize for at least 30 min, and then the recording session began. After a 10 min baseline period, the rat was gently injected (i.p.) with VEH (the Plexon file was briefly paused for this), followed 30 min later by CNO (Plexon file again briefly paused); the recording session continued for 60 more minutes. Immediately afterward, the rat was deeply anesthetized with pentobarbital and perfused transcardially (see Histology section below).

Extracellular single-unit activity was recorded with a multichannel neurophysiological recording system (OmniPlex, Plexon, Dallas, TX). Wideband signals recorded on each channel were referenced to one of the recording wires (resulting in a

maximum of 15 channels of activity per rat), amplified (8,000x), digitized (40 kHz sampling rate), and saved on a PC for offline sorting and analysis. The recording reference wire we chose for each session was selected to optimize the quality of the recordings. After high-pass filtering the signal at 600 Hz, we sorted waveforms manually using 2-dimensional principal component analysis (Offline Sorter, Plexon). Only well-isolated units were used in our analysis. If two units with similar waveforms and identical time stamps for their action potentials appeared on adjacent electrodes, we only used one unit. We then imported sorted waveforms and their timestamps to NeuroExplorer (Nex Technologies, Madison, AL) for further analysis.

5.4.4 *In vivo* electrophysiology in freely moving rats

For surgeries in the animals that were implanted with a chronic microelectrode array targeting the mPFC, the rat was anesthetized with isoflurane and secured in a stereotaxic apparatus (Kopf Instruments). Some of these animals also received bilateral infusions of LC virus prior to mPFC array implantation: the scalp was incised and retracted, the head was tilted 15 degrees downward, and either the excitatory or blank control virus was bilaterally infused into LC as described above. The head was then returned to horizontally level for the rest of the surgery. The following description applies to all of the mPFC recording rats. Next, three-five burr holes were drilled for anchor screws. The region of the skull overlying mPFC was removed to allow for microelectrode implantation. The animal was then implanted with a 16-channel microelectrode array (Innovative Neurophysiology) targeting both PL (8 wires) and IL (8 wires) in the right hemisphere. This 2 x 8 wire microarray was constructed from two rows of 50 µm diameter tungsten

wires of two different lengths (PL, 6.9 mm; IL, 8.0 mm; see below for dorsal-ventral coordinates); the wires in each row and the rows themselves were spaced 200 µm apart (center-to-center). The array was positioned with its long axis parallel to the anteroposterior plane. Coordinates for the centermost wires of the array were (relative to bregma skull surface): +2.7 mm AP, +0.55 mm ML, -4.0 mm DV for PL; and +2.7 mm AP, +0.35 mm ML, -5.1 mm DV for IL. The array was secured to the skull with dental acrylic and at least two weeks were allowed for recovery and viral expression before *in vivo* recordings began.

As described above, a standard rodent behavioral chamber (Med Associates) enclosed in a sound-attenuating cabinet was modified to allow for electrophysiological recordings. Neural activity and locomotor output was recorded automatically by an OmniPlex recording system (Plexon). The behavioral procedure for these rats took place across five (mPFC only recording rats) or six (LC virus + mPFC recording rats) consecutive days, and was the same as described above except that neural recordings were obtained on the two test days (Days 5 and 6). For the recording days, the rat was connected to a headstage with a flexible cable (Plexon) before the session began, and then placed in the recording chamber. Immediately after the session, the rat was unplugged from the headstage and returned to its home cage. For the initial mPFC recording experiment (i.e., rats that did not receive LC DREADDs), the rat remained plugged in between the retrieval and renewal sessions while placed in the large white plastic bucket with a layer of bedding in the bottom, while the contexts were rapidly changed. For the LC virus + mPFC recording experiment, rats only underwent a retrieval session on both Day 5 and 6

following either VEH or CNO administration. Neural data were collected and analyzed as described above for the anesthetized recordings. The recording reference electrode we chose was typically one of the eight wires located in PL. The analysis of neural activity focused on CS-evoked activity during the Day 5 and 6 test sessions. For analysis of the CS-evoked activity, firing rate was binned in 200-msec increments around the time of the tones for individual neurons, and the evoked responses were z-score normalized to the 1-sec period prior to tone onset, averaged across the five tones.

5.4.5 Cannula implantation for intracranial infusions

For surgeries in animals that were implanted with bilateral cannula targeting PL or BLA, rats were anesthetized with isoflurane and secured in a stereotaxic apparatus (Kopf Instruments). They first received bilateral infusions of the AAV9-PRSx8-hM3Dq-HA virus in the LC as described above. After viral infusions, the head was placed in the horizontal plane for implantation of bilateral guide cannulae in the PL (8mm, 26 gauge; Plastics One; AP: + 2.7, ML: +/- 2.0 (insertion point), DV: -3.8 at a 20-degree angle) or the BLA (10mm, 26 gauge; Plastics One; AP: - 2.9, ML: +/- 4.8, DV: -8.55); all coordinates relative to bregma. Three-five burr holes were drilled in the skull for jeweler's screws and dental acrylic was applied to the skull to secure the cannula in place. Dummy cannula (33 gauge) were placed into the guide cannula upon completion of the surgery.

After recovery from surgery, intracranial infusions were performed as previously described (Giustino et al., 2017). Briefly, rats were transported to the infusion room in 5-gallon buckets. Dummies were removed and stainless steel injectors (33 gauge) were inserted into the guide cannula for local infusion of drug. All infusions were made using

Hamilton syringes mounted in an infusion pump and connected to the injectors with polyethylene tubing. The infusions were made approximately 25-30 minutes before the behavioral procedures. Clonidine hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in saline (5.83 ug/ul) and infused into either PL or BLA (0.3 ul/side at 0.25 ul/min); this dose (1.75 ug/side) has previously been shown to reduce conditioned freezing behavior (Schulz et al., 2002; Holmes et al., 2017). Injectors remained in place for 1 min after the infusion to allow for drug diffusion. After the infusions, clean dummies were inserted in the guide cannulas and the rats were injected with either systemic VEH or CNO in a counterbalanced fashion. Extinction retrieval tests commenced approximately 20 min following the systemic injections.

5.4.6 Histology

After completion of the experiment, the rats were overdosed with pentobarbital. For rats implanted with an mPFC array, electrolytic lesions were created by passing electrical current (80 µA, 10 sec; A365 stimulus isolator, World Precision Instruments, Sarasota, FL) through six of the recording wires (anterior, middle, posterior wires in both PL and IL). Rats were then perfused transcardially with 0.9% saline followed by 10% formalin. Brains were extracted from the skull and post-fixed in a 10% formalin solution for 24 hours, followed by a 30% sucrose solution, where they remained for a minimum of 48 hours. Coronal brain sections of the mPFC (40 µm thickness) were cut on a cryostat (-20^{o} C, Leica Microsystems, Buffalo Grove, IL), mounted on subbed microscope slides, and stained with thionin (0.25%) to visualize electrode or cannula placements.

To visualize LC viral expression using immunohistochemistry, the following steps were carried out. First, brains were coronally sectioned (40 µm thickness) with a cryostat and stored in a 0.01% sodium azide solution until further processing. Sections were blocked in PBS with 0.1% Triton X-100 (TX) and 3% normal donkey serum (NDS, 2 ml/well) for one hour. All steps occurred in this PBS-TX-NDS solution at room temperature. Sections were then incubated in primary antibodies (mouse anti-tyrosine hydroxylase (TH) [1:2000] and rabbit anti-HA [1:1000]) for 24 hours. Sections were then rinsed three times (10 min each). Sections were then incubated in secondary antibodies (donkey anti-mouse Alexa Fluor 488 [1:500; for TH] and donkey anti-rabbit Alexa Fluor 594 [1:500; for HA]) for three hours. Afterward, sections were rinsed three times (10 min each). Next, the sections were mounted on microscope slides using PBS, and coverslipped using fluoromount (Diagnostic BioSystems, Pleasanton, CA). Images were obtained using a Zeiss AXIO Imager M2. The following suppliers were used for the above materials: NDS (EMD Millipore, Billerica, MA), Triton X (Sigma-Aldrich, St. Louis, MO), mouse anti-TH (ImmunoStar, Hudson, WI), rabbit anti-HA (Cell Signaling Technology, Danvers, MA), donkey anti-mouse IgG (H+L) Alexa Fluor 488 (Thermo Fisher Scientific/Invitrogen, Waltham, MA), donkey anti-rabbit IgG (H+L) Alexa Fluor 594 (Thermo Fisher Scientific/Invitrogen, Waltham, MA).

5.4.7 Statistics

We analyzed the data with conventional parametric statistics (StatView, SAS Institute). Two-way analysis of variance (ANOVA) and repeated-measures ANOVA were used to assess general main effects and interactions ($\alpha = 0.05$). Results are shown as mean ± SEM.

6. CONCLUSIONS*

6.1 Norepinephrine as a target for PTSD and extinction deficits

Noradrenergic modulating drugs are used to treat an array of neuropsychiatric disorders, though the only two FDA approved drugs for PTSD are selective serotonin reuptake inhibitors (Tawa and Murphy, 2013; Arnsten et al., 2015; Steckler and Risbrough, 2012; Fitzgerald et al., 2014a). Drugs that either elevate or reduce NE transmission have been studied and used off-label for the treatment of PTSD and its symptoms with varying success (Holmes and Quirk, 2010; Giustino et al., 2016b; Bukalo et al., 2014; Southwick et al., 1999a, 1997, 1999c, 1999b). It has recently been shown that threat is associated with increased LC activity in healthy human volunteers and this acts to strengthen prioritized memory representations (Clewett et al., 2018). This suggests that heightened states of arousal may promote fear memory formation and maintenance. In addition, a number of studies have demonstrated that elevated NE plays a major role in the pathophysiology of PTSD (Geracioti et al., 2001; Strawn and Geracioti, 2008; Naegeli et al., 2017; Yehuda et al., 1992; Southwick et al., 1999a, 1997, 1999c; Bremner et al., 1996; Southwick et al., 1999b; Kosten et al., 1987). Despite this link, success with pharmacological erasure of fear memories and/or enhancement of extinction based cognitive behavioral therapies has been limited (Bos et al., 2014).

* Parts of this chapter have been reprinted with permission from Frontiers in Behavioral Neuroscience Giustino TF and Maren, S (2015). The role of the medial prefrontal cortex in the conditioning and extinction of fear. ***Front. Behav. Neurosc****i*, *9*, 298. http://doi.org/10.3389/fnbeh.2015.00298 Giustino TF, and Maren S. (2018). Noradrenergic modulation of fear conditioning and extinction. ***Front. Behav. Neurosc****i.* 12, 43. doi:10.3389/fnbeh.2018.00043.

It has been proposed that individuals with PTSD may "hypercondition" to fearful stimuli and this is coupled with impaired extinction, ultimately resulting in a heavy bias towards fearful responses in inappropriate situations (Blechert et al., 2007; Wessa and Flor, 2007; Guthrie and Bryant, 2006; Lissek et al., 2005; Orr et al., 2000; Pitman et al., 2012; VanElzakker et al., 2014; Milad et al., 2009, 2008; Norrholm et al., 2015). While the LC-NE system plays an important role in learning and memory, including extinction learning, (Sterpenich et al., 2006; Sara, 2015, 2009; Arnsten, 2009, 2015; Arnsten et al., 2015; Ramos and Arnsten, 2007), stress (and elevated NE beyond optimal levels) may only exacerbate these effects by impairing extinction learning and/or increasing generalization (Raio and Phelps, 2015; Raio et al., 2017, 2014; Hartley et al., 2014; Maren and Holmes, 2016; Dunsmoor et al., 2017). Indeed, yohimbine has been used in healthy human subjects to enhance fear learning and it also has been shown to hinder extinction learning (Soeter and Kindt, 2011b, 2012; van Stegeren et al., 2010; Visser et al., 2015). Despite this, there has been some interest in yohimbine as a pharmaceutical agent to augment the treatment of PTSD, which has yielded mixed results (Wangelin et al., 2013; Holmes and Quirk, 2010; Powers et al., 2009).

Clonidine and guanfacine are two α2-AR agonists that are used to treat a number of conditions. While the evidence for the efficacy of either drug is somewhat limited, these drugs may have some use in the treatment of PTSD and related disorders (Belkin and Schwartz, 2015; Arnsten et al., 2015). These NE-reducing agents have been shown to reduce symptoms of hyperarousal associated with PTSD as well as sleep disturbances (Porter and Bell, 1999; Boehnlein and Kinzie, 2007; Detweiler et al., 2016; Horrigan and

Barnhill, 1996; Kinzie and Leung, 1989). Importantly, both compounds have also shown safety and promise for treating PTSD in children (Harmon and Riggs, 1996; Connor et al., 2013). Unfortunately, some studies have found little evidence for the efficacy of α2-AR agonists in the treatment of PTSD (Davis et al., 2008; Neylan et al., 2006). It remains possible that these, or related drugs, may only be effective for individuals who have dysregulated/elevated NE signaling which may explain discrepant findings. Further research is warranted on the efficacy of these compounds.

Results with noradrenergic receptor antagonists, such as propranolol, have yielded mixed results in both healthy human volunteers and individuals with PTSD. Propranolol is already used safely in humans for other conditions and has been shown to reduce long-term memory for an emotionally arousing story (Cahill et al., 1994) and reduced the strength of context conditioning in healthy human subjects (Grillon et al., 2004). These data suggest that β-AR activation underlies memories for emotional events. However, some have suggested that propranolol treatment soon after trauma has no effects, although this was done in the absence of any extinction based therapy (McGhee et al., 2009; Nugent et al., 2010; Stein et al., 2007). In addition, one report found that propranolol has no effects on the acquisition or retention of extinction learning (Orr et al., 2006). Others have shown that propranolol impairs extinction learning in healthy subjects (Bos et al., 2012). At first glance, these conflicting reports would imply that researchers and clinicians alike should look elsewhere for the pharmaceutical adjunct for exposure therapy. However, we have argued that the timing of propranolol administration coupled with behavioral therapy is an often overlooked, and highly important, factor regulating the long-term outcome of

extinction learning (Giustino et al., 2016b). This may be due to differences in the prevailing level of noradrenergic arousal at the time of administration. There is some empirical evidence to support this idea in humans though more research coupling propranolol and extinction soon after trauma is needed (Pitman et al., 2002; Vaiva et al., 2003).

One area that has shown promise centers on blocking the reconsolidation of a fearful memory. Several studies in both healthy human volunteers and individuals with PTSD have suggested that propranolol can be used to disrupt reconsolidation (Poundja et al., 2012; Brunet et al., 2011, 2008; Lonergan et al., 2013; Brunet et al., 2014; Kindt et al., 2009; Soeter and Kindt, 2011a, 2012; Schwabe et al., 2012). As discussed previously, effects on reconsolidation may be subject to certain boundary conditions and memories do not necessarily even undergo reconsolidation unless new learning occurs (Sevenster et al., 2012). Moreover, many of these reconsolidation effects have not been replicated which further complicates approaches focusing on reconsolidation blockade as an effective treatment strategy for PTSD (Spring et al., 2015; Wood et al., 2015; Tollenaar et al., 2009; Bos et al., 2014). It seems unlikely that acute administration of propranolol, or any drug, would effectively eradicate a long-standing fear memory, such as those observed in individuals suffering from PTSD. However, this does not preclude the idea that propranolol may reduce fear under some circumstances and thus has utility moving forward (Giustino et al., 2016b; Kroes et al., 2016b, 2016a).

Another noradrenergic receptor antagonist that has been used primarily to combat disordered sleep in individuals with PTSD is the α1-AR antagonist prazosin. Prazosin has

shown promise in ameliorating nightmares and sleep disturbances associated with PTSD (Keeshin et al., 2017; Taylor et al., 2008; Raskind et al., 2003; Short et al., 2017; Writer et al., 2014; Miller et al., 2017; Koola et al., 2014; de Dassel et al., 2017). However, a recent clinical trial demonstrated that prazosin did not ameliorate sleep-related disturbances in military veterans with PTSD (Raskind et al., 2018). Less is known about how prazosin affects other aspects of PTSD symptomatology. A recent study in healthy human subjects suggests that prazosin delivered prior to fear conditioning enhanced future discrimination between fearful and safe stimuli during extinction (Homan et al., 2017). Further work is needed to examine the effects of prazosin as well as other NE-altering drugs in both healthy human volunteers and those with PTSD.

Overall, the LC-NE system critically regulates most aspects of emotional learning and memory in rodent models, healthy human subjects, and individuals suffering from trauma- and stressor-related disorders. Recent advances in technology for basic science research will be crucial to further our understanding of how stress and the LC-NE system regulate these effects in rodent models. Target-specific approaches have led to a new appreciation of LC function and while the precise effects of distinct LC subpopulations are not well characterized several recent papers have pinpointed unique contributions to learning and memory as well as anxiety in rodents (Uematsu et al., 2015, 2017; McCall et al., 2017; Li et al., 2016; Hirschberg et al., 2017; Schwarz and Luo, 2015; Schwarz et al., 2015).

An important area of research moving forward may center around individualized medicine based on differences in the LC-NE system and stress responsivity. Human

imaging protocols have improved to better isolate the LC (Betts et al., 2017; Song et al., 2017; Priovoulos et al., 2018; Tona et al., 2017; Krebs et al., 2017; Brooks et al., 2017; Murphy et al., 2014; Keren et al., 2009; Langley et al., 2017). Understanding if and how LC-NE is contributing to an individual's symptomatology will likely improve therapeutic outcomes. Patients often undergo several "rounds" of drug treatment as they (and their doctor) search for either a single or combination of agents that ameliorate their condition. An improved appreciation of how the LC-NE system contributes to aversive learning and memory and its subsequent extinction may help improve empirically driven treatment options.

Given technological and surgical limitations, the vast majority of therapeutic options for the treatment of human disease has been limited to systemic delivery of pharmacological agents. However, preclinical research has suggested that "therapeutic-like" effects observed on various learning and memory tasks can be localized to distinct brain regions or circuits, as we have observed throughout my doctoral work. Of particular interest when looking at manipulations of the stress system, is that both epinephrine and NE do not cross the blood-brain barrier (Weil-Malherbe et al., 1959). Because of this, a therapeutic target that serves as an interface or a gateway between the peripheral and central nervous system may serve as an interesting therapeutic approach. Indeed, the vagus nerve has been identified as a key player in relaying peripheral information to the central nervous system. In fact, vagal nerve stimulation is currently being examined as a therapeutic option for a number of disorders including epilepsy and depression (Sackeim et al., 2001; Handforth et al., 1998). Vagal nerve stimulation has also received some

attention for the treatment of stressor- and trauma- related disorders, such as PTSD (Noble et al., 2019).

The vagus nerve expresses beta-adrenoceptors (Lawrence et al., 1995; Miyashita and Williams, 2006; Schreurs et al., 1986). We have shown that beta adrenoceptors, particularly in the amygdala, play a critical role in extinction deficits. Interestingly, it has been shown that stimulation of the adrenal nerves or systemic administration of epinephrine results in increased evoked firing rates in the vagus nerve, an effect that is blocked by propranolol (Miyashita and Williams, 2006). Moreover, systemic epinephrine produces increased levels of NE in the amygdala suggesting the vagus nerve may act as an interface to relay information to and from the central nervous system (Williams et al., 1998; O'Carroll et al., 1999; Hurlemann et al., 2005; Chen and Williams, 2012).

Indeed, vagus nerve stimulation has shown promise as a therapeutic option for individuals suffering from PTSD. Rodent research has demonstrated that systemic injections of epinephrine have memory enhancing effects, thought to be mediated by the vagus nerve (Mccarty, 1981). Interestingly, these memory enhancing effects are sensitive to manipulations of the central norepinephrine system. That is, reducing or blocking NE transmission in the amygdala, with propranolol, mitigates the effects of systemic epinephrine (Liang et al., 1986, 1995; Williams et al., 1998, 2000; Hassert et al., 2004). The vagus nerve is well suited to serve as an interface between the periphery and the central nervous system in terms of anatomy. Past work has shown that vagal nerve projections synapse on the nucleus tractus solitarius, a brain stem nucleus that produces and releases NE (Sumal et al., 1983; Kalia and Mesulam, 1980). The nucleus tractus

solitarius in turn sends noradrenergic projections that innervate the BLA as well as the LC (Williams et al., 2000; Fallon et al., 1978; Riche et al., 1990; Van Bockstaele et al., 1999). Overall, the anatomy coupled with the interaction of peripheral epinephrine driving NE release in the amygdala make the vagus nerve an attractive and promising therapeutic target for the treatment of stress- and trauma- related disorders.

6.2 Next generation treatment approaches

Neuroscience research has seen an unprecedented wave of new technological advances for selectively isolating and manipulating both cell-specific and circuit-specific neuronal populations. While many obstacles remain before these technological advances may become clinically viable, these advanced approaches have begun a new wave of possibilities. Optogenetics and chemogenetics are virally-mediated techniques allowing for cell- and circuit-specific manipulations to selectively excite or suppress precise neuronal populations. Optogenetics requires the expression of exogenous light-sensitive ion channels to modulate neuronal activity with high temporal precision (Boyden et al., 2005; Fenno et al., 2011). A difficult obstacle to overcome for use in humans (beyond use in the retina) is the invasive manner in which certain wavelengths of light need to reach the tissue expressing these ion channels. One chemogenetic approach makes use of DREADDs (Designer Receptors Exclusively Activated by Designer Drugs), which are synthetic G-protein coupled receptors that respond selectively to the systemic injection of an inert ligand, clozapine-*N*-oxide (CNO) (Dong et al., 2010; Urban and Roth, 2015). These technologies provide an *in vivo* mechanism to control cellular physiology in intact

neural circuits and delineate the causal contribution of specific neuronal subtypes to behavior, and perhaps the treatment of human disorders.

An obvious problem to address before use in humans is the invasive nature of delivering these viral constructs (currently done via stereotaxic infusions in rodents). However, recent advances in our understanding of adeno-associated viruses may allow for noninvasive delivery methods, such as intravascular administration. For example, recent work has begun to delineate varying serotypes and viral constructs that, when delivered systemically, are capable of penetrating the blood-brain barrier (BBB) and expressing in central tissue (Merkel et al., 2017; Zhang et al., 2018; Hudry et al., 2018; Bourdenx et al., 2014). Perhaps most intriguing related to my doctoral work is a recent report demonstrating that the serotype AAV9 can reach and express in central tissue after intravascular administration (Merkel et al., 2017). This is particularly noteworthy given that the LC-DREADD constructs used in my work are also AAV9, suggesting the possibility that LC-NE activity can be up and downregulated in a noninvasive manner. Of course, an additional issue for implementing this technology to treat human disease lies in the specificity of the exogenous ligands. DREADDs are activated by a designer ligand, clozapine *N*-oxide (CNO), though recent work has brought into question the use of CNO. In particular, it has been shown that CNO does not even cross the BBB and is actually "back metabolized" into clozapine, an atypical antipsychotic that has been used to treat schizophrenia (Gomez et al., 2017). While this downfall can be worked around in basic research through the use of appropriate controls, this creates a major issue for translation to humans. New ligands are currently being developed such as compound 21 as well as

the FDA approved drug olanzapine (Weston et al., 2019). The combination of BBB penetrating AAVs as well as an FDA approved ligand may be a major breakthrough for restricting DREADD expression in a cell-specific manner. The next frontier will be discovering ways to implement these approaches in noninvasive, circuit-specific manners.

As mentioned above, tremendous progress has been made with imaging technology. We are now able to selectively isolate the LC with more precision in standard human imaging techniques which will allow for more informative treatment options based on LC activity. In basic research, a number of biosensors have been developed and are continuing to be optimized for the *in vivo* detection of fluctuations in calcium dyanmics (GCaMP), acetylcholine (GACh), dopamine ($GRAB_{DA}$, dLight), and norepinephrine ($GRAB_{NE}$) (Feng et al., 2019; Patriarchi et al., 2018; Akerboom et al., 2012; Jing et al., 2018; Sun et al., 2018). $GRAB_{NE}$ is a modified adrenoceptor that can be used to measure NE dynamics with 1,000-fold specificity from dopamine (Feng et al., 2019). While not yet developed, a future avenue for improving our understanding of NE in humans may be radioligands that can be used in positron emission tomography (PET) studies for the *in vivo* detection and visualization of the LC-NE system and its downstream targets, such as the mPFC and BLA, which have been heavily implicated in PTSD. We have delineated a circuit in which the LC-NE system dynamically regulates mPFC and BLA activity, in opposing manners, and these changes correspond with the long-term success of extinction learning. While these techniques are still in their scientific infancy, the rapid development and improvement of these advances are likely to be 0applied for the treatment of human disorders in the near future. It remains possible that future use of advanced imaging

techniques in humans may allow for real-time monitoring of aberrations and then corrections (whether via DREADDs or pharmacology or both) to these circuits in an attempt to improve therapeutic outcomes and improve the efficacy of behavioral therapies.

6.3 Parallels with Reward Circuitry

In appetitive paradigms, initial performance reflects goal-directed behavior which can shift to habitual behavior with extended training. Overlapping circuits and brain regions have been heavily examined in the context of reward and addiction. The mPFC and BLA are thought to play important roles in the acquisition, expression, extinction, and relapse of addictive behaviors. For example, a similar dichotomy of function has been proposed to regulate addiction in the mPFC (Peters et al., 2009), in which PL serves to drive drug seeking behavior (Capriles et al., 2003; McFarland and Kalivas, 2001) whereas IL acts to suppress this behavior following extinction (Moorman et al., 2015; Peters et al., 2009). Similar to fear, drug seeking also faces relapse phenomenon, which may in part, reflect mPFC dysfunction. Typically, it has been thought that PL activity is required for the execution of goal-direct behavior ("go"). In contrast, IL activity is thought to regulate behavioral inhibition ("stop"). It has been demonstrated that rats with PL lesions exhibit no sensitivity to goal value after initial or extensive training. IL lesions yield an opposing deficit in which rats show sensitivity to goal value independent of the level of training (Killcross and Coutureau, 2003). These data suggest that PL promotes flexibility whereas IL inhibits flexibility and promotes behavioral rigidity. Overall, there is an abundance of literature discussing the potential functional opposition of PL and IL in reward and drug seeking behavior (Moorman et al., 2015; Peters et al., 2008, 2009).

The opposing roles of the PL and IL in addiction and reward seeking-behavior suggest some symmetry between the circuits driving high and low fear states. For instance, my doctoral work suggests an important interaction between the BLA and the mPFC underlying extinction learning as well as fear relapse. Recent work has shown that the amygdala is a critical hub for reward learning (Tye et al., 2008; Murray, 2007; Janak and Tye, 2015; Tye and Janak, 2007; Luo et al., 2013; Burgos-Robles et al., 2017). Under most circumstances it appears that amygdala lesions or inactivation impair reward based behavior (Ishikawa et al., 2008; Maren, 1999; Cador et al., 1989; Hatfield et al., 1996; Hiroi and White, 1991; McDonald and White, 1993). Mirroring fear conditioning studies, it has been shown that LA synaptic plasticity is critical for cue reward learning (Tye et al., 2008; Tye and Janak, 2007; Schoenbaum et al., 1998). Some have suggested that the amygdala encodes valence insofar as it shows evoked responding to both pleasant and aversive stimuli (Young and Williams, 2010; Belova et al., 2007). It is not entirely clear if these neurons are distinct populations or overlapping. Past work suggests it may be a combination of the two and this may be task specific. For example, in a study examining BLA activity in response to stimuli that predict either positive or negative outcomes, it was found that distinct neuronal populations with the amygdala responded primarily to either positive or negative stimuli, but not both (Tye and Janak, 2007; Paton et al., 2006). In contrast, others have found some evidence for neurons that respond to aversive, safety, or reward cues which suggests some overlapping populations respond to multiple stimuli and may generally encode salience (Sangha et al., 2013; Shabel and Janak, 2009). Interestingly, these effects are also sensitive to manipulations of the NE system (Young

and Williams, 2010). Overall, there is compelling evidence to suggest that the circuitry we have examined during my doctoral work is not limited to aversive learning and memory procedures. In fact, our findings may have broad implications for the treatment of neuropsychiatric disorders associated with dysregulated NE, mPFC, and/or BLA activity such as addiction (Ebrahimi et al., 2019; Somerville et al., 2006; Johnsrude et al., 2000).

6.4 Summary

Overall, my doctoral work has demonstrated that the LC-NE system dynamically regulates the acquisition, extinction, and relapse of fear and these effects are dependent upon the prevailing level of stress (and NE) at the onset of learning. LC-NE likely influences learning and memory processes in a manner described by an inverted-U function, albeit acting in different brain regions/circuits to regulate learning and memory retrieval. We suggest that low levels of NE release prior to delayed extinction may enhance extinction learning by promoting mPFC function, which would, in turn, inhibit BLA output to enable extinction learning. In contrast, high levels of NE released under stress (such as that accompanying footshock) promotes fear expression while inhibiting new learning (as is observed with immediate extinction procedures) by strengthening BLA function and simultaneously impairing mPFC function via target-specific LC subpopulations. As such, understanding the involvement of the LC-NE system and its dynamic regulation of prefrontal and amygdala circuits may serve as a previously underappreciated therapeutic target for individuals suffering from stressor-and trauma-related disorders.

7. REFERENCES

Abercrombie, E. D., Keller, R. W., and Zigmond, M. J. (1988). Characterization of hippocampal norepinephrine release as measured by microdialysis perfusion: pharmacological and behavioral studies. *Neuroscience* 27, 897–904. doi:10.1016/0306-4522(88)90192-3.

Acca, G. M., Mathew, A. S., Jin, J., Maren, S., and Nagaya, N. (2017). Allopregnanolone induces state-dependent fear via the bed nucleus of the stria terminalis. *Horm. Behav.* 89, 137–144. doi:10.1016/j.yhbeh.2017.01.002.

Agster, K. L., Mejias-Aponte, C. A., Clark, B. D., and Waterhouse, B. D. (2013). Evidence for a regional specificity in the density and distribution of noradrenergic varicosities in rat cortex. *J. Comp. Neurol.* 521, 2195–2207. doi:10.1002/cne.23270.

Akerboom, J., Chen, T.-W., Wardill, T. J., Tian, L., Marvin, J. S., Mutlu, S., Calderón, N. C., Esposti, F., Borghuis, B. G., Sun, X. R., et al. (2012). Optimization of a GCaMP calcium indicator for neural activity imaging. *J. Neurosci.* 32, 13819–13840. doi:10.1523/JNEUROSCI.2601-12.2012.

Amano, T., Duvarci, S., Popa, D., and Paré, D. (2011). The fear circuit revisited: contributions of the basal amygdala nuclei to conditioned fear. *J. Neurosci.* 31, 15481–15489. doi:10.1523/JNEUROSCI.3410-11.2011.

Amaral, D. G., and Sinnamon, H. M. (1977). The locus coeruleus: neurobiology of a central noradrenergic nucleus. *Prog. Neurobiol.* 9, 147–196. doi:10.1016/0301-0082(77)90016-8.

Anagnostaras, S. G., Maren, S., and Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J. Neurosci.* 19, 1106–1114.

Anglada-Figueroa, D., and Quirk, G. J. (2005). Lesions of the basal amygdala block expression of conditioned fear but not extinction. *J. Neurosci.* 25, 9680–9685. doi:10.1523/JNEUROSCI.2600-05.2005.

Aoki, C., Go, C.-G., Venkatesan, C., and Kurose, H. (1994). Perikaryal and synaptic localization ofα2A-adrenergic receptor-like immunoreactivity. *Brain Res.* 650, 181–204. doi:10.1016/0006-8993(94)91782-5.

Arnsten, A. F., and Goldman-Rakic, P. S. (1984). Selective prefrontal cortical projections to the region of the locus coeruleus and raphe nuclei in the rhesus monkey. *Brain Res.* 306, 9–18. doi:10.1016/0006-8993(84)90351-2.

Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nat. Rev. Neurosci.* 10, 410–422. doi:10.1038/nrn2648.

Arnsten, A. F. T. (2015). Stress weakens prefrontal networks: molecular insults to higher cognition. *Nat. Neurosci.* 18, 1376–1385. doi:10.1038/nn.4087.

Arnsten, A. F. T., and Li, B.-M. (2005). Neurobiology of executive functions: catecholamine influences on prefrontal cortical functions. *Biol. Psychiatry.* 57, 1377–1384. doi:10.1016/j.biopsych.2004.08.019.

Arnsten, A. F. T., Raskind, M. A., Taylor, F. B., and Connor, D. F. (2015). The Effects of Stress Exposure on Prefrontal Cortex: Translating Basic Research into

Successful Treatments for Post-Traumatic Stress Disorder. *Neurobiol. Stress* 1, 89–99. doi:10.1016/j.ynstr.2014.10.002.

Aston-Jones, G., and Bloom, F. E. (1981a). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J. Neurosci.* 1, 876–886.

Aston-Jones, G., and Bloom, F. E. (1981b). Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *J. Neurosci.* 1, 887–900.

Aston-Jones, G., and Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu. Rev. Neurosci.* 28, 403–450. doi:10.1146/annurev.neuro.28.061604.135709.

Aston-Jones, G., Ennis, M., Pieribone, V. A., Nickell, W. T., and Shipley, M. T. (1986). The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. *Science (80-.).* 234, 734–737.

Aston-Jones, G., Rajkowski, J., and Cohen, J. (1999). Role of locus coeruleus in attention and behavioral flexibility. *Biol. Psychiatry.* 46, 1309–1320. doi:10.1016/S0006-3223(99)00140-7.

Aston-Jones, G., and Waterhouse, B. (2016). Locus coeruleus: From global projection system to adaptive regulation of behavior. *Brain Res.* 1645, 75–78. doi:10.1016/j.brainres.2016.03.001.

Aston-Jones, G., Zhu, Y., and Card, J. P. (2004). Numerous GABAergic afferents to locus ceruleus in the pericerulear dendritic zone: possible interneuronal pool. *J. Neurosci.* 24, 2313–2321. doi:10.1523/JNEUROSCI.5339-03.2004.

Bangasser, D. A., Wiersielis, K. R., and Khantsis, S. (2016). Sex differences in the locus coeruleus-norepinephrine system and its regulation by stress. *Brain Res.* 1641, 177–188. doi:10.1016/j.brainres.2015.11.021.

Belkin, M. R., and Schwartz, T. L. (2015). Alpha-2 receptor agonists for the treatment of posttraumatic stress disorder. *Drugs Context* 4, 212286. doi:10.7573/dic.212286.

Berridge, C. W., and Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev* 42, 33–84. doi:10.1016/S0165-0173(03)00143-7.

Betts, M. J., Cardenas-Blanco, A., Kanowski, M., Jessen, F., and Düzel, E. (2017). In vivo MRI assessment of the human locus coeruleus along its rostrocaudal extent in young and older adults. *Neuroimage* 163, 150–159. doi:10.1016/j.neuroimage.2017.09.042.

Birnbaum, S. G., Yuan, P. X., Wang, M., Vijayraghavan, S., Bloom, A. K., Davis, D. J., Gobeske, K. T., Sweatt, J. D., Manji, H. K., and Arnsten, A. F. T. (2004). Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science (80-.).* 306, 882–884. doi:10.1126/science.1100021.

Blechert, J., Michael, T., Vriends, N., Margraf, J., and Wilhelm, F. H. (2007). Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of

autonomic, experiential, and behavioural responses. *Behav. Res. Ther.* 45, 2019–2033. doi:10.1016/j.brat.2007.02.012.

Bliss, T. V., Goddard, G. V., and Riives, M. (1983). Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. *J. Physiol. (Lond)* 334, 475–491. doi:10.1113/jphysiol.1983.sp014507.

Van Bockstaele, E. J., Colago, E. E., and Valentino, R. J. (1998). Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites: substrate for the co-ordination of emotional and cognitive limbs of the stress response. *J. Neuroendocrinol.* 10, 743–757. doi:10.1046/j.1365-2826.1998.00254.x.

Boehnlein, J. K., and Kinzie, J. D. (2007). Pharmacologic reduction of CNS noradrenergic activity in PTSD: the case for clonidine and prazosin. *J. Psychiatr. Pract.* 13, 72–78. doi:10.1097/01.pra.0000265763.79753.c1.

Booze, R. M., Crisostomo, E. A., and Davis, J. N. (1993). Beta-adrenergic receptors in the hippocampal and retrohippocampal regions of rats and guinea pigs: autoradiographic and immunohistochemical studies. *Synapse* 13, 206–214. doi:10.1002/syn.890130303.

Bos, M. G. N., Beckers, T., and Kindt, M. (2014). Noradrenergic blockade of memory reconsolidation: a failure to reduce conditioned fear responding. *Front. Behav. Neurosci.* 8, 412. doi:10.3389/fnbeh.2014.00412.

Bos, M. G. N., Beckers, T., and Kindt, M. (2012). The effects of noradrenergic blockade on extinction in humans. *Biol. Psychol.* 89, 598–605. doi:10.1016/j.biopsycho.2012.01.007.

Bourdenx, M., Dutheil, N., Bezard, E., and Dehay, B. (2014). Systemic gene delivery to the central nervous system using Adeno-associated virus. *Front. Mol. Neurosci.* 7, 50. doi:10.3389/fnmol.2014.00050.

Bouret, S., and Sara, S. J. (2004). Reward expectation, orientation of attention and locus coeruleus-medial frontal cortex interplay during learning. *Eur. J. Neurosci.* 20, 791–802. doi:10.1111/j.1460-9568.2004.03526.x.

Bouton, M. E. (2000). A learning theory perspective on lapse, relapse, and the maintenance of behavior change. *Health Psychol.* 19, 57–63.

Bouton, M. E. (2002). Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biol. Psychiatry.* 52, 976–986. doi:10.1016/S0006-3223(02)01546-9.

Bouton, M. E., Westbrook, R. F., Corcoran, K. A., and Maren, S. (2006). Contextual and temporal modulation of extinction: behavioral and biological mechanisms. *Biol. Psychiatry.* 60, 352–360. doi:10.1016/j.biopsych.2005.12.015.

Boyajian, C. L., Loughlin, S. E., and Leslie, F. M. (1987). Anatomical evidence for alpha-2 adrenoceptor heterogeneity: differential autoradiographic distributions of [3H]rauwolscine and [3H]idazoxan in rat brain. *J. Pharmacol. Exp. Ther.* 241, 1079–1091.

Bremner, J. D., Krystal, J. H., Southwick, S. M., and Charney, D. S. (1996). Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. *Synapse* 23, 28–38. doi:10.1002/(SICI)1098-2396(199605)23:1<28::AID-SYN4>3.0.CO;2-J.

Brooks, J. C. W., Davies, W.-E., and Pickering, A. E. (2017). Resolving the brainstem contributions to attentional analgesia. *J. Neurosci.* 37, 2279–2291. doi:10.1523/JNEUROSCI.2193-16.2016.

Brunet, A., Orr, S. P., Tremblay, J., Robertson, K., Nader, K., and Pitman, R. K. (2008). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *J. Psychiatr. Res.* 42, 503–506. doi:10.1016/j.jpsychires.2007.05.006.

Brunet, A., Poundja, J., Tremblay, J., Bui, E., Thomas, E., Orr, S. P., Azzoug, A., Birmes, P., and Pitman, R. K. (2011). Trauma reactivation under the influence of propranolol decreases posttraumatic stress symptoms and disorder: 3 open-label trials. *J Clin Psychopharmacol* 31, 547–550. doi:10.1097/JCP.0b013e318222f360.

Brunet, A., Thomas, É., Saumier, D., Ashbaugh, A. R., Azzoug, A., Pitman, R. K., Orr, S. P., and Tremblay, J. (2014). Trauma reactivation plus propranolol is associated with durably low physiological responding during subsequent script-driven traumatic imagery. *Can J Psychiatry* 59, 228–232. doi:10.1177/070674371405900408.

Bryant, R. A. (2002). Early interventions following psychological trauma. *CNS Spectr* 7, 650–654. doi:10.1017/S1092852900022136.

Buffalari, D. M., and Grace, A. A. (2007). Noradrenergic modulation of basolateral amygdala neuronal activity: opposing influences of alpha-2 and beta receptor

activation. *J. Neurosci.* 27, 12358–12366. doi:10.1523/JNEUROSCI.2007-07.2007.

Bukalo, O., Pinard, C. R., and Holmes, A. (2014). Mechanisms to medicines: elucidating neural and molecular substrates of fear extinction to identify novel treatments for anxiety disorders. *Br. J. Pharmacol.* 171, 4690–4718. doi:10.1111/bph.12779.

Bukalo, O., Pinard, C. R., Silverstein, S., Brehm, C., Hartley, N. D., Whittle, N., Colacicco, G., Busch, E., Patel, S., Singewald, N., et al. (2015). Prefrontal inputs to the amygdala instruct fear extinction memory formation. *Sci. Adv.* 1. doi:10.1126/sciadv.1500251.

Burgos-Robles, A., Kimchi, E. Y., Izadmehr, E. M., Porzenheim, M. J., Ramos-Guasp, W. A., Nieh, E. H., Felix-Ortiz, A. C., Namburi, P., Leppla, C. A., Presbrey, K. N., et al. (2017). Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. *Nat. Neurosci.* 20, 824–835. doi:10.1038/nn.4553.

Burgos-Robles, A., Vidal-Gonzalez, I., and Quirk, G. J. (2009). Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *J. Neurosci.* 29, 8474–8482. doi:10.1523/JNEUROSCI.0378-09.2009.

Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E., and Quirk, G. J. (2007). Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. *Neuron* 53, 871–880. doi:10.1016/j.neuron.2007.02.021.

Bush, D. E. A., Caparosa, E. M., Gekker, A., and Ledoux, J. (2010). Beta-adrenergic receptors in the lateral nucleus of the amygdala contribute to the acquisition but not the consolidation of auditory fear conditioning. *Front. Behav. Neurosci.* 4, 154. doi:10.3389/fnbeh.2010.00154.

Cahill, L., Prins, B., Weber, M., and McGaugh, J. L. (1994). Beta-adrenergic activation and memory for emotional events. *Nature* 371, 702–704. doi:10.1038/371702a0.

Cedarbaum, J. M., and Aghajanian, G. K. (1978). Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. *J. Comp. Neurol.* 178, 1–16. doi:10.1002/cne.901780102.

Chandler, D. J., Gao, W.-J., and Waterhouse, B. D. (2014). Heterogeneous organization of the locus coeruleus projections to prefrontal and motor cortices. *Proc. Natl. Acad. Sci. USA* 111, 6816–6821. doi:10.1073/pnas.1320827111.

Chandler, D. J., Lamperski, C. S., and Waterhouse, B. D. (2013). Identification and distribution of projections from monoaminergic and cholinergic nuclei to functionally differentiated subregions of prefrontal cortex. *Brain Res.* 1522, 38–58. doi:10.1016/j.brainres.2013.04.057.

Chandler, D., and Waterhouse, B. D. (2012). Evidence for broad versus segregated projections from cholinergic and noradrenergic nuclei to functionally and anatomically discrete subregions of prefrontal cortex. *Front. Behav. Neurosci.* 6, 20. doi:10.3389/fnbeh.2012.00020.

Chang, C., Berke, J. D., and Maren, S. (2010). Single-unit activity in the medial prefrontal cortex during immediate and delayed extinction of fear in rats. *PLoS One* 5, e11971. doi:10.1371/journal.pone.0011971.

Chang, C., and Maren, S. (2009). Early extinction after fear conditioning yields a context-independent and short-term suppression of conditional freezing in rats. *Learn. Mem.* 16, 62–68. doi:10.1101/lm.1085009.

Chang, C., and Maren, S. (2010). Strain difference in the effect of infralimbic cortex lesions on fear extinction in rats. *Behav. Neurosci.* 124, 391–397. doi:10.1037/a0019479.

Chaulk, P. C., and Harley, C. W. (1998). Intracerebroventricular norepinephrine potentiation of the perforant path-evoked potential in dentate gyrus of anesthetized and awake rats: A role for both α- and β-adrenoceptor activation. *Brain Res.* 787, 59–70. doi:10.1016/S0006-8993(97)01460-1.

Chen, F. J., and Sara, S. J. (2007). Locus coeruleus activation by foot shock or electrical stimulation inhibits amygdala neurons. *Neuroscience* 144, 472–481. doi:10.1016/j.neuroscience.2006.09.037.

Chen, W., Wang, Y., Wang, X., and Li, H. (2017). Neural circuits involved in the renewal of extinguished fear. *IUBMB Life* 69, 470–478. doi:10.1002/iub.1636.

Cho, J.-H., Deisseroth, K., and Bolshakov, V. Y. (2013). Synaptic encoding of fear extinction in mPFC-amygdala circuits. *Neuron* 80, 1491–1507. doi:10.1016/j.neuron.2013.09.025.

Ciocchi, S., Herry, C., Grenier, F., Wolff, S. B. E., Letzkus, J. J., Vlachos, I., Ehrlich, I., Sprengel, R., Deisseroth, K., Stadler, M. B., et al. (2010). Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature* 468, 277–282. doi:10.1038/nature09559.

Clewett, D. V., Huang, R., Velasco, R., Lee, T.-H., and Mather, M. (2018). Locus coeruleus activity strengthens prioritized memories under arousal. *J. Neurosci.* 38, 1558–1574. doi:10.1523/JNEUROSCI.2097-17.2017.

Connor, D. F., Grasso, D. J., Slivinsky, M. D., Pearson, G. S., and Banga, A. (2013). An open-label study of guanfacine extended release for traumatic stress related symptoms in children and adolescents. *J Child Adolesc Psychopharmacol* 23, 244–251. doi:10.1089/cap.2012.0119.

Corcoran, K. A., and Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J. Neurosci.* 21, 1720–1726.

Corcoran, K. A., and Quirk, G. J. (2007). Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. *J. Neurosci.* 27, 840–844. doi:10.1523/JNEUROSCI.5327-06.2007.

Dahl, D., and Sarvey, J. M. (1989). Norepinephrine induces pathway-specific long-lasting potentiation and depression in the hippocampal dentate gyrus. *Proc. Natl. Acad. Sci. USA* 86, 4776–4780.

Dahlström, A., and Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Experientia* 20, 398–399.

De Dassel, T., Wittmann, L., Protic, S., Höllmer, H., and Gorzka, R. J. (2017). Association of posttraumatic nightmares and psychopathology in a military sample. *Psychol. Trauma*. doi:10.1037/tra0000319.

Davis, L. L., Ward, C., Rasmusson, A., Newell, J. M., Frazier, E., and Southwick, S. M. (2008). A placebo-controlled trial of guanfacine for the treatment of posttraumatic stress disorder in veterans. *Psychopharmacol Bull* 41, 8–18.

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annu. Rev. Neurosci.* 15, 353–375. doi:10.1146/annurev.ne.15.030192.002033.

Day, H. E., Campeau, S., Watson, S. J., and Akil, H. (1997). Distribution of alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brain and spinal cord. *J Chem Neuroanat* 13, 115–139.

Dazzi, L., Seu, E., Cherchi, G., and Biggio, G. (2005). Chronic administration of the SSRI fluvoxamine markedly and selectively reduces the sensitivity of cortical serotonergic neurons to footshock stress. *Eur. Neuropsychopharmacol.* 15, 283–290. doi:10.1016/j.euroneuro.2004.11.003.

Deahl, M. (2000). Psychological debriefing: controversy and challenge. *Aust. N. Z. J. Psychiatry* 34, 929–939. doi:10.1080/000486700267.

Dębiec, J., Bush, D. E. A., and LeDoux, J. E. (2011). Noradrenergic enhancement of reconsolidation in the amygdala impairs extinction of conditioned fear in rats--a possible mechanism for the persistence of traumatic memories in PTSD. *Depress. Anxiety* 28, 186–193. doi:10.1002/da.20803.

Debiec, J., and Ledoux, J. E. (2004). Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience* 129, 267–272. doi:10.1016/j.neuroscience.2004.08.018.

Debiec, J., and LeDoux, J. E. (2006). Noradrenergic signaling in the amygdala contributes to the reconsolidation of fear memory: treatment implications for PTSD. *Ann. N. Y. Acad. Sci.* 1071, 521–524. doi:10.1196/annals.1364.056.

Dejean, C., Courtin, J., Rozeske, R. R., Bonnet, M. C., Dousset, V., Michelet, T., and Herry, C. (2015). Neuronal circuits for fear expression and recovery: recent advances and potential therapeutic strategies. *Biol. Psychiatry.* 78, 298–306. doi:10.1016/j.biopsych.2015.03.017.

Descarries, L., and Lapierre, Y. (1973). Noradrenergic axon terminals in the cerebral cortex of rat. I. Radioautographic visualization after topical application of dl-[3H]norepinephrine. *Brain Res.* 51, 141–160. doi:10.1016/0006-8993(73)90369-7.

Descarries, L., Watkins, K. C., and Lapierre, Y. (1977). Noradrenergic axon terminals in the cerebral cortex of rat. III. Topometric ultrastructural analysis. *Brain Res.* 133, 197–222. doi:10.1016/0006-8993(77)90759-4.

Deschaux, O., Motanis, H., Spennato, G., Moreau, J.-L., and Garcia, R. (2011). Re-emergence of extinguished auditory-cued conditioned fear following a sub-conditioning procedure: effects of hippocampal and prefrontal tetanic stimulations. *Neurobiol. Learn. Mem.* 95, 510–518. doi:10.1016/j.nlm.2011.03.002.

Detweiler, M. B., Pagadala, B., Candelario, J., Boyle, J. S., Detweiler, J. G., and Lutgens, B. W. (2016). Treatment of Post-Traumatic Stress Disorder Nightmares at a Veterans Affairs Medical Center. *J Clin Med* 5. doi:10.3390/jcm5120117.

Do-Monte, F. H., Manzano-Nieves, G., Quiñones-Laracuente, K., Ramos-Medina, L., and Quirk, G. J. (2015). Revisiting the role of infralimbic cortex in fear extinction with optogenetics. *J. Neurosci.* 35, 3607–3615. doi:10.1523/JNEUROSCI.3137-14.2015.

Domyancic, A. V., and Morilak, D. A. (1997). Distribution of α1A adrenergic receptor m RNA in the rat brain visualized by in situ hybridization. *Journal of Comparative Neurology*.

Dunsmoor, J. E., Otto, A. R., and Phelps, E. A. (2017). Stress promotes generalization of older but not recent threat memories. *Proc. Natl. Acad. Sci. USA* 114, 9218–9223. doi:10.1073/pnas.1704428114.

Dunwiddie, T. V., Taylor, M., Heginbotham, L. R., and Proctor, W. R. (1992). Long-term increases in excitability in the CA1 region of rat hippocampus induced by beta-adrenergic stimulation: possible mediation by cAMP. *J. Neurosci.* 12, 506–517.

Duvarci, S., and Pare, D. (2014). Amygdala microcircuits controlling learned fear. *Neuron* 82, 966–980. doi:10.1016/j.neuron.2014.04.042.

Fadok, J. P., Krabbe, S., Markovic, M., Courtin, J., Xu, C., Massi, L., Botta, P., Bylund, K., Müller, C., Kovacevic, A., et al. (2017). A competitive inhibitory circuit for

selection of active and passive fear responses. *Nature* 542, 96–100. doi:10.1038/nature21047.

Fallon, J. H., Koziell, D. A., and Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. *J. Comp. Neurol.* 180, 509–532. doi:10.1002/cne.901800308.

Falls, W. A., Miserendino, M. J., and Davis, M. (1992). Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. *J. Neurosci.* 12, 854–863.

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

Fanselow, M. S., and LeDoux, J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* 23, 229–232. doi:10.1016/S0896-6273(00)80775-8.

Farb, C. R., Chang, W., and Ledoux, J. E. (2010). Ultrastructural characterization of noradrenergic axons and Beta-adrenergic receptors in the lateral nucleus of the amygdala. *Front. Behav. Neurosci.* 4, 162. doi:10.3389/fnbeh.2010.00162.

Farrell, M. R., Sayed, J. A., Underwood, A. R., and Wellman, C. L. (2010). Lesion of infralimbic cortex occludes stress effects on retrieval of extinction but not fear conditioning. *Neurobiol. Learn. Mem.* 94, 240–246. doi:10.1016/j.nlm.2010.06.001.

Farrell, M. R., Sengelaub, D. R., and Wellman, C. L. (2013). Sex differences and chronic stress effects on the neural circuitry underlying fear conditioning and extinction. *Physiol. Behav.* 122, 208–215. doi:10.1016/j.physbeh.2013.04.002.

Feng, J., Zhang, C., Lischinsky, J. E., Jing, M., Zhou, J., Wang, H., Zhang, Y., Dong, A., Wu, Z., Wu, H., et al. (2019). A genetically encoded fluorescent sensor for rapid and specific in vivo detection of norepinephrine. *Neuron*. doi:10.1016/j.neuron.2019.02.037.

Ferry, B., Roozendaal, B., and McGaugh, J. L. (1999a). Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between beta- and alpha1-adrenoceptors. *J. Neurosci.* 19, 5119–5123.

Ferry, B., Roozendaal, B., and McGaugh, J. L. (1999b). Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: a critical involvement of the amygdala. *Biol. Psychiatry*. 46, 1140–1152. doi:10.1016/S0006-3223(99)00157-2.

Finlay, J. M., Zigmond, M. J., and Abercrombie, E. D. (1995). Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. *Neuroscience* 64, 619–628. doi:10.1016/0306-4522(94)00331-X.

Fitzgerald, P. J., Giustino, T. F., Seemann, J. R., and Maren, S. (2015). Noradrenergic blockade stabilizes prefrontal activity and enables fear extinction under stress. *Proc. Natl. Acad. Sci. USA* 112, E3729–37. doi:10.1073/pnas.1500682112.

Fitzgerald, P. J., Seemann, J. R., and Maren, S. (2014a). Can fear extinction be enhanced? A review of pharmacological and behavioral findings. *Brain Res. Bull.* 105, 46–60. doi:10.1016/j.brainresbull.2013.12.007.

Fitzgerald, P. J., Whittle, N., Flynn, S. M., Graybeal, C., Pinard, C. R., Gunduz-Cinar, O., Kravitz, A. V., Singewald, N., and Holmes, A. (2014b). Prefrontal single-unit firing associated with deficient extinction in mice. *Neurobiol. Learn. Mem.* 113, 69–81. doi:10.1016/j.nlm.2013.11.002.

Foote, S. L., Aston-Jones, G., and Bloom, F. E. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proc. Natl. Acad. Sci. USA* 77, 3033–3037.

Foote, S. L., Bloom, F. E., and Aston-Jones, G. (1983). Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. *Physiol. Rev.* 63, 844–914. doi:10.1152/physrev.1983.63.3.844.

Forneris, C. A., Gartlehner, G., Brownley, K. A., Gaynes, B. N., Sonis, J., Coker-Schwimmer, E., Jonas, D. E., Greenblatt, A., Wilkins, T. M., Woodell, C. L., et al. (2013). Interventions to prevent post-traumatic stress disorder: a systematic review. *Am. J. Prev. Med.* 44, 635–650. doi:10.1016/j.amepre.2013.02.013.

Foy, M. R., Stanton, M. E., Levine, S., and Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behav Neural Biol* 48, 138–149. doi:10.1016/S0163-1047(87)90664-9.

Frankland, P. W., Cestari, V., Filipkowski, R. K., McDonald, R. J., and Silva, A. J. (1998). The dorsal hippocampus is essential for context discrimination but not

for contextual conditioning. *Behav. Neurosci.* 112, 863–874. doi:10.1037/0735-7044.112.4.863.

Fucich, E. A., Paredes, D., and Morilak, D. A. (2016). Therapeutic effects of extinction learning as a model of exposure therapy in rats. *Neuropsychopharmacology* 41, 3092–3102. doi:10.1038/npp.2016.127.

Galvez, R., Mesches, M. H., and McGaugh, J. L. (1996). Norepinephrine release in the amygdala in response to footshock stimulation. *Neurobiol. Learn. Mem.* 66, 253–257. doi:10.1006/nlme.1996.0067.

Garfinkel, S. N., Abelson, J. L., King, A. P., Sripada, R. K., Wang, X., Gaines, L. M., and Liberzon, I. (2014). Impaired contextual modulation of memories in PTSD: an fMRI and psychophysiological study of extinction retention and fear renewal. *J. Neurosci.* 34, 13435–13443. doi:10.1523/JNEUROSCI.4287-13.2014.

George, S. A., Knox, D., Curtis, A. L., Aldridge, J. W., Valentino, R. J., and Liberzon, I. (2013). Altered locus coeruleus-norepinephrine function following single prolonged stress. *Eur. J. Neurosci.* 37, 901–909. doi:10.1111/ejn.12095.

Geracioti, T. D., Baker, D. G., Ekhator, N. N., West, S. A., Hill, K. K., Bruce, A. B., Schmidt, D., Rounds-Kugler, B., Yehuda, R., Keck, P. E., et al. (2001). CSF norepinephrine concentrations in posttraumatic stress disorder. *Am. J. Psychiatry* 158, 1227–1230. doi:10.1176/appi.ajp.158.8.1227.

Gerfen, C. R., and Clavier, R. M. (1979). Neural inputs to the prefrontal agranular insular cortex in the rat: Horseradish peroxidase study. *Brain Res. Bull.* 4, 347–353. doi:10.1016/S0361-9230(79)80012-X.

Girotti, M., Adler, S. M., Bulin, S. E., Fucich, E. A., Paredes, D., and Morilak, D. A. (2017). Prefrontal cortex executive processes affected by stress in health and disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* doi:10.1016/j.pnpbp.2017.07.004.

Giustino, T. F., Fitzgerald, P. J., and Maren, S. (2016a). Fear expression suppresses medial prefrontal cortical firing in rats. *PLoS One* 11, e0165256. doi:10.1371/journal.pone.0165256.

Giustino, T. F., Fitzgerald, P. J., and Maren, S. (2016b). Revisiting propranolol and PTSD: Memory erasure or extinction enhancement? *Neurobiol. Learn. Mem.* 130, 26–33. doi:10.1016/j.nlm.2016.01.009.

Giustino, T. F., Fitzgerald, P. J., Ressler, R. L., and Maren, S. (2019). Locus coeruleus toggles reciprocal prefrontal firing to reinstate fear. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.1814278116.

Giustino, T. F., and Maren, S. (2018). Noradrenergic modulation of fear conditioning and extinction. *Front. Behav. Neurosci.* 12, 43. doi:10.3389/fnbeh.2018.00043.

Giustino, T. F., and Maren, S. (2015). The role of the medial prefrontal cortex in the conditioning and extinction of fear. *Front. Behav. Neurosci.* 9, 298. doi:10.3389/fnbeh.2015.00298.

Giustino, T. F., Seemann, J. R., Acca, G. M., Goode, T. D., Fitzgerald, P. J., and Maren, S. (2017). β-Adrenoceptor Blockade in the Basolateral Amygdala, But Not the Medial Prefrontal Cortex, Rescues the Immediate Extinction Deficit. *Neuropsychopharmacology* 42, 2537–2544. doi:10.1038/npp.2017.89.

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., Ellis, R. J., Richie, C. T., Harvey, B. K., Dannals, R. F., et al. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science (80-.).* 357, 503–507. doi:10.1126/science.aan2475.

Goode, T. D., and Maren, S. (2014). Animal models of fear relapse. *ILAR J.* 55, 246–258. doi:10.1093/ilar/ilu008.

Goosens, K. A., Hobin, J. A., and Maren, S. (2003). Auditory-Evoked Spike Firing in the Lateral Amygdala and Pavlovian Fear Conditioning. *Neuron* 40, 1013–1022. doi:10.1016/S0896-6273(03)00728-1.

Goosens, K. A., and Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn. Mem.* 8, 148–155. doi:10.1101/lm.37601.

Goosens, K. A., and 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, 738–750. doi:10.1037/0735-7044.117.4.738.

Gresch, P. J., Sved, A. F., Zigmond, M. J., and Finlay, J. M. (1994). Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. *J. Neurochem.* 63, 575–583. doi:10.1046/j.1471-4159.1994.63020575.x.

Grillon, C., Cordova, J., Morgan, C. A., Charney, D. S., and Davis, M. (2004). Effects of the beta-blocker propranolol on cued and contextual fear conditioning in humans. *Psychopharmacology (Berl).* 175, 342–352. doi:10.1007/s00213-004-1819-5.

Grzanna, R., and Molliver, M. E. (1980). The locus coeruleus in the rat: an immunohistochemical delineation. *Neuroscience* 5, 21–40. doi:10.1016/0306-4522(80)90068-8.

Guthrie, R. M., and Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosom. Med.* 68, 307–311. doi:10.1097/01.psy.0000208629.67653.cc.

Hajós-Korcsok, E., Robinson, D. D., Yu, J. H., Fitch, C. S., Walker, E., and Merchant, K. M. (2003). Rapid habituation of hippocampal serotonin and norepinephrine release and anxiety-related behaviors, but not plasma corticosterone levels, to repeated footshock stress in rats. *Pharmacol. Biochem. Behav.* 74, 609–616.

Hansen, N. (2017). The Longevity of Hippocampus-Dependent Memory Is Orchestrated by the Locus Coeruleus-Noradrenergic System. *Neural Plast.* 2017, 2727602. doi:10.1155/2017/2727602.

Harley, C. W. (2007). Norepinephrine and the dentate gyrus. *Prog. Brain Res.* 163, 299–318. doi:10.1016/S0079-6123(07)63018-0.

Harmon, R. J., and Riggs, P. D. (1996). Clonidine for posttraumatic stress disorder in preschool children. *J. Am. Acad. Child Adolesc. Psychiatry* 35, 1247–1249. doi:10.1097/00004583-199609000-00022.

Hartley, C. A., Gorun, A., Reddan, M. C., Ramirez, F., and Phelps, E. A. (2014). Stressor controllability modulates fear extinction in humans. *Neurobiol. Learn. Mem.* 113, 149–156. doi:10.1016/j.nlm.2013.12.003.

Hatfield, T., Spanis, C., and McGaugh, J. L. (1999). Response of amygdalar norepinephrine to footshock and GABAergic drugs using in vivo microdialysis and HPLC. *Brain Res.* 835, 340–345. doi:10.1016/S0006-8993(99)01566-8.

Haubensak, W., Kunwar, P. S., Cai, H., Ciocchi, S., Wall, N. R., Ponnusamy, R., Biag, J., Dong, H.-W., Deisseroth, K., Callaway, E. M., et al. (2010). Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* 468, 270–276. doi:10.1038/nature09553.

Helmstetter, F. J., and Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behav. Neurosci.* 108, 1005–1009. doi:10.1037/0735-7044.108.5.1005.

Hermans, D., Craske, M. G., Mineka, S., and Lovibond, P. F. (2006). Extinction in human fear conditioning. *Biol. Psychiatry.* 60, 361–368. doi:10.1016/j.biopsych.2005.10.006.

Herry, C., Ciocchi, S., Senn, V., Demmou, L., Müller, C., and Lüthi, A. (2008). Switching on and off fear by distinct neuronal circuits. *Nature* 454, 600–606. doi:10.1038/nature07166.

Herry, C., Ferraguti, F., Singewald, N., Letzkus, J. J., Ehrlich, I., and Lüthi, A. (2010). Neuronal circuits of fear extinction. *Eur. J. Neurosci.* 31, 599–612. doi:10.1111/j.1460-9568.2010.07101.x.

Herry, C., and Johansen, J. P. (2014). Encoding of fear learning and memory in distributed neuronal circuits. *Nat. Neurosci.* 17, 1644–1654. doi:10.1038/nn.3869.

Herry, C., Trifilieff, P., Micheau, J., Lüthi, A., and Mons, N. (2006). Extinction of auditory fear conditioning requires MAPK/ERK activation in the basolateral amygdala. *Eur. J. Neurosci.* 24, 261–269. doi:10.1111/j.1460-9568.2006.04893.x.

Hirschberg, S., Li, Y., Randall, A., Kremer, E. J., and Pickering, A. E. (2017). Functional dichotomy in spinal- vs prefrontal-projecting locus coeruleus modules splits descending noradrenergic analgesia from ascending aversion and anxiety in rats. *Elife* 6. doi:10.7554/eLife.29808.

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

Hobin, J. A., Ji, J., and Maren, S. (2006). Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. *Hippocampus* 16, 174–182. doi:10.1002/hipo.20144.

Hollis, F., Sevelinges, Y., Grosse, J., Zanoletti, O., and Sandi, C. (2016). Involvement of CRFR1 in the basolateral amygdala in the immediate fear extinction deficit. *Eneuro* 3. doi:10.1523/ENEURO.0084-16.2016.

Holmes, A., and Quirk, G. J. (2010). Pharmacological facilitation of fear extinction and the search for adjunct treatments for anxiety disorders--the case of yohimbine. *Trends Pharmacol. Sci.* 31, 2–7. doi:10.1016/j.tips.2009.10.003.

Holmes, A., and Wellman, C. L. (2009). Stress-induced prefrontal reorganization and executive dysfunction in rodents. *Neurosci. Biobehav. Rev.* 33, 773–783. doi:10.1016/j.neubiorev.2008.11.005.

Holmes, N. M., Crane, J. W., Tang, M., Fam, J., Westbrook, R. F., and Delaney, A. J. (2017). α2-adrenoceptor-mediated inhibition in the central amygdala blocks fear-conditioning. *Sci. Rep.* 7, 11712. doi:10.1038/s41598-017-12115-x.

Holt, W., and Maren, S. (1999). Muscimol inactivation of the dorsal hippocampus impairs contextual retrieval of fear memory. *J. Neurosci.* 19, 9054–9062.

Homan, P., Lin, Q., Murrough, J. W., Soleimani, L., Bach, D. R., Clem, R. L., and Schiller, D. (2017). Prazosin during threat discrimination boosts memory of the safe stimulus. *Learn. Mem.* 24, 597–601. doi:10.1101/lm.045898.117.

Hopkins, W. F., and Johnston, D. (1988). Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. *J. Neurophysiol.* 59, 667–687.

Horrigan, J. P., and Barnhill, L. J. (1996). The suppression of nightmares with guanfacine. *J. Clin. Psychiatry.* 57, 371.

Hudry, E., Andres-Mateos, E., Lerner, E. P., Volak, A., Cohen, O., Hyman, B. T., Maguire, C. A., and Vandenberghe, L. H. (2018). Efficient Gene Transfer to the Central Nervous System by Single-Stranded Anc80L65. *Mol. Ther. Methods Clin. Dev.* 10, 197–209. doi:10.1016/j.omtm.2018.07.006.

Hugues, S., Garcia, R., and Léna, I. (2007). Time course of extracellular catecholamine and glutamate levels in the rat medial prefrontal cortex during and after extinction of conditioned fear. *Synapse* 61, 933–937. doi:10.1002/syn.20448.

Ishimatsu, M., and Williams, J. T. (1996). Synchronous activity in locus coeruleus results from dendritic interactions in pericoerulear regions. *J. Neurosci.* 16, 5196–5204.

Ishizuka, Y., Ishida, Y., Jin, Q., Kato, K., Kunitake, T., Mitsuyama, Y., and Kannan, H. (2000). Differential profiles of nitric oxide and norepinephrine releases in the paraventricular nucleus region in response to mild footshock in rats. *Brain Res.* 862, 17–25.

Izquierdo, A., Wellman, C. L., and Holmes, A. (2006). Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. *J. Neurosci.* 26, 5733–5738. doi:10.1523/JNEUROSCI.0474-06.2006.

Izumi, Y., and Zorumski, C. F. (1999). Norepinephrine promotes long-term potentiation in the adult rat hippocampus in vitro. *Synapse*.

Jackson, M. E., and Moghaddam, B. (2006). Distinct patterns of plasticity in prefrontal cortex neurons that encode slow and fast responses to stress. *Eur. J. Neurosci.* 24, 1702–1710. doi:10.1111/j.1460-9568.2006.05054.x.

Ji, G., Sun, H., Fu, Y., Li, Z., Pais-Vieira, M., Galhardo, V., and Neugebauer, V. (2010). Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation. *J. Neurosci.* 30, 5451–5464. doi:10.1523/JNEUROSCI.0225-10.2010.

Jin, J., and Maren, S. (2015a). Fear renewal preferentially activates ventral hippocampal neurons projecting to both amygdala and prefrontal cortex in rats. *Sci. Rep.* 5, 8388. doi:10.1038/srep08388.

Jin, J., and Maren, S. (2015b). Prefrontal-Hippocampal Interactions in Memory and Emotion. *Front. Syst. Neurosci.* 9, 170. doi:10.3389/fnsys.2015.00170.

Jing, M., Zhang, P., Wang, G., Feng, J., Mesik, L., Zeng, J., Jiang, H., Wang, S., Looby, J. C., Guagliardo, N. A., et al. (2018). A genetically encoded fluorescent acetylcholine indicator for in vitro and in vivo studies. *Nat. Biotechnol.* 36, 726–737. doi:10.1038/nbt.4184.

Jodo, E., Chiang, C., and Aston-Jones, G. (1998). Potent excitatory influence of prefrontal cortex activity on noradrenergic locus coeruleus neurons. *Neuroscience* 83, 63–79. doi:10.1016/S0306-4522(97)00372-2.

Johansen, J. P., Cain, C. K., Ostroff, L. E., and LeDoux, J. E. (2011). Molecular mechanisms of fear learning and memory. *Cell* 147, 509–524. doi:10.1016/j.cell.2011.10.009.

Johnson, R. D., and Minneman, K. P. (1985). α1-Adrenergic receptors and stimulation of [3H]inositol metabolism in rat brain: Regional distribution and parallel inactivation. *Brain Res.* 341, 7–15. doi:10.1016/0006-8993(85)91466-0.

Jones, B. E., and Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. *Brain Res.* 127, 25–53. doi:10.1016/0006-8993(77)90378-X.

Jones, B. E., and Yang, T. Z. (1985). The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. *J. Comp. Neurol.* 242, 56–92. doi:10.1002/cne.902420105.

Jones, L. S., Gauger, L. L., and Davis, J. N. (1985). Anatomy of brain alpha 1-adrenergic receptors: in vitro autoradiography with [125I]-heat. *J. Comp. Neurol.* 231, 190–208. doi:10.1002/cne.902310207.

Jovanovic, T., Norrholm, S. D., Blanding, N. Q., Davis, M., Duncan, E., Bradley, B., and Ressler, K. J. (2010). Impaired fear inhibition is a biomarker of PTSD but not depression. *Depress. Anxiety* 27, 244–251. doi:10.1002/da.20663.

Katsuki, H., Izumi, Y., and Zorumski, C. F. (1997). Noradrenergic regulation of synaptic plasticity in the hippocampal CA1 region. *J. Neurophysiol.* 77, 3013–3020. doi:10.1152/jn.1997.77.6.3013.

Keeshin, B. R., Ding, Q., Presson, A. P., Berkowitz, S. J., and Strawn, J. R. (2017). Use of Prazosin for Pediatric PTSD-Associated Nightmares and Sleep Disturbances: A Retrospective Chart Review. *Neurol. Ther.* doi:10.1007/s40120-017-0078-4.

Keren, N. I., Lozar, C. T., Harris, K. C., Morgan, P. S., and Eckert, M. A. (2009). In vivo mapping of the human locus coeruleus. *Neuroimage* 47, 1261–1267. doi:10.1016/j.neuroimage.2009.06.012.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., and Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* 62, 593–602. doi:10.1001/archpsyc.62.6.593.

Kessler, R. C., Sonnega, A., Bromet, E., Hughes, M., and Nelson, C. B. (1995). Posttraumatic stress disorder in the National Comorbidity Survey. *Arch. Gen. Psychiatry* 52, 1048–1060. doi:10.1001/archpsyc.1995.03950240066012.

Kim, J. J., and Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science (80-.).* 256, 675–677. doi:10.1126/science.1585183.

Kim, J., Lee, S., Park, H., Song, B., Hong, I., Geum, D., Shin, K., and Choi, S. (2007). Blockade of amygdala metabotropic glutamate receptor subtype 1 impairs fear extinction. *Biochem. Biophys. Res. Commun.* 355, 188–193. doi:10.1016/j.bbrc.2007.01.125.

Kim, S. C., Jo, Y. S., Kim, I. H., Kim, H., and Choi, J.-S. (2010). Lack of medial prefrontal cortex activation underlies the immediate extinction deficit. *J. Neurosci.* 30, 832–837. doi:10.1523/JNEUROSCI.4145-09.2010.

Kindt, M., Soeter, M., and Vervliet, B. (2009). Beyond extinction: erasing human fear responses and preventing the return of fear. *Nat. Neurosci.* 12, 256–258. doi:10.1038/nn.2271.

Kinzie, J. D., and Leung, P. (1989). Clonidine in Cambodian patients with posttraumatic stress disorder. *J. Nerv. Ment. Dis.* 177, 546–550.

Klavir, O., Prigge, M., Sarel, A., Paz, R., and Yizhar, O. (2017). Manipulating fear associations via optogenetic modulation of amygdala inputs to prefrontal cortex. *Nat. Neurosci.* 20, 836–844. doi:10.1038/nn.4523.

Knapska, E., Macias, M., Mikosz, M., Nowak, A., Owczarek, D., Wawrzyniak, M., Pieprzyk, M., Cymerman, I. A., Werka, T., Sheng, M., et al. (2012). Functional anatomy of neural circuits regulating fear and extinction. *Proc. Natl. Acad. Sci. USA* 109, 17093–17098. doi:10.1073/pnas.1202087109.

Knapska, E., and Maren, S. (2009). Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. *Learn. Mem.* 16, 486–493. doi:10.1101/lm.1463909.

Koenen, K. C., Stellman, S. D., Sommer, J. F., and Stellman, J. M. (2008). Persisting posttraumatic stress disorder symptoms and their relationship to functioning in Vietnam veterans: a 14-year follow-up. *J. Trauma. Stress* 21, 49–57. doi:10.1002/jts.20304.

Koenigs, M., and Grafman, J. (2009). Posttraumatic stress disorder: the role of medial prefrontal cortex and amygdala. *Neuroscientist* 15, 540–548. doi:10.1177/1073858409333072.

Koola, M. M., Varghese, S. P., and Fawcett, J. A. (2014). High-dose prazosin for the treatment of post-traumatic stress disorder. *Ther Adv Psychopharmacol* 4, 43–47. doi:10.1177/2045125313500982.

Korf, J., Aghajanian, G. K., and Roth, R. H. (1973). Increased turnover of norepinephrine in the rat cerebral cortex during stress: role of the locus

coeruleus. *Neuropharmacology* 12, 933–938. doi:10.1016/0028-3908(73)90024-5.

Kosten, T. R., Mason, J. W., Giller, E. L., Ostroff, R. B., and Harkness, L. (1987). Sustained urinary norepinephrine and epinephrine elevation in post-traumatic stress disorder. *Psychoneuroendocrinology* 12, 13–20. doi:10.1016/0306-4530(87)90017-5.

Krebs, R. M., Park, H. R. P., Bombeke, K., and Boehler, C. N. (2017). Modulation of locus coeruleus activity by novel oddball stimuli. *Brain Imaging Behav.* 12, 1–8. doi:10.1007/s11682-017-9700-4.

Kroes, M. C. W., Schiller, D., LeDoux, J. E., and Phelps, E. A. (2016a). Translational approaches targeting reconsolidation. *Current topics in behavioral neurosciences* 28, 197–230. doi:10.1007/7854_2015_5008.

Kroes, M. C. W., Tona, K.-D., den Ouden, H. E. M., Vogel, S., van Wingen, G. A., and Fernández, G. (2016b). How Administration of the Beta-Blocker Propranolol Before Extinction can Prevent the Return of Fear. *Neuropsychopharmacology* 41, 1569–1578. doi:10.1038/npp.2015.315.

Krystal, J. H., Abdallah, C. G., Pietrzak, R. H., Averill, L. A., Harpaz-Rotem, I., Levy, I., Kelmendi, B., and Southwick, S. M. (2018). Locus coeruleus hyperactivity in posttraumatic stress disorder: answers and questions. *Biol. Psychiatry.* 83, 197–199. doi:10.1016/j.biopsych.2017.09.027.

Lacaille, J. C., and Harley, C. W. (1985). The action of norepinephrine in the dentate gyrus: beta-mediated facilitation of evoked potentials in vitro. *Brain Res.* 358, 210–220. doi:10.1016/0006-8993(85)90965-5.

Langley, J., Huddleston, D. E., Liu, C. J., and Hu, X. (2017). Reproducibility of locus coeruleus and substantia nigra imaging with neuromelanin sensitive MRI. *MAGMA* 30, 121–125. doi:10.1007/s10334-016-0590-z.

Lapierre, Y., Beaudet, A., Demianczuk, N., and Descarries, L. (1973). Noradrenergic axon terminals in the cerebral cortex of rat. II. Quantitative data revealed by light and electron microscope radioautography of the frontal cortex. *Brain Res.* 63, 175–182. doi:10.1016/0006-8993(73)90086-3.

Lattal, K. M., and Abel, T. (2001). An immediate-shock freezing deficit with discrete cues: a possible role for unconditioned stimulus processing mechanisms. *J Exp Psychol Anim Behav Process* 27, 394–406.

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cell Mol. Neurobiol.* 23, 727–738.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184. doi:10.1146/annurev.neuro.23.1.155.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., and Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J. Neurosci.* 10, 1062–1069.

Lee, S., Kim, S.-J., Kwon, O.-B., Lee, J. H., and Kim, J.-H. (2013). Inhibitory networks of the amygdala for emotional memory. *Front. Neural Circuits* 7, 129. doi:10.3389/fncir.2013.00129.

Li, Y., Hickey, L., Perrins, R., Werlen, E., Patel, A. A., Hirschberg, S., Jones, M. W., Salinas, S., Kremer, E. J., and Pickering, A. E. (2016). Retrograde optogenetic characterization of the pontospinal module of the locus coeruleus with a canine adenoviral vector. *Brain Res.* 1641, 274–290. doi:10.1016/j.brainres.2016.02.023.

Liberzon, I., and Sripada, C. S. (2008). The functional neuroanatomy of PTSD: a critical review. *Prog. Brain Res.* 167, 151–169. doi:10.1016/S0079-6123(07)67011-3.

Lim, E. P., Dawe, G. S., and Jay, T. M. (2017). Activation of beta- and alpha-2-adrenoceptors in the basolateral amygdala has opposing effects on hippocampal-prefrontal long-term potentiation. *Neurobiol. Learn. Mem.* 137, 163–170. doi:10.1016/j.nlm.2016.11.020.

Lin, C.-C., Tung, C.-S., Lin, P.-H., Huang, C.-L., and Liu, Y.-P. (2016). Traumatic stress causes distinctive effects on fear circuit catecholamines and the fear extinction profile in a rodent model of posttraumatic stress disorder. *Eur. Neuropsychopharmacol.* 26, 1484–1495. doi:10.1016/j.euroneuro.2016.06.004.

Linnman, C., Zeidan, M. A., Furtak, S. C., Pitman, R. K., Quirk, G. J., and Milad, M. R. (2012). Resting amygdala and medial prefrontal metabolism predicts functional activation of the fear extinction circuit. *Am. J. Psychiatry* 169, 415–423. doi:10.1176/appi.ajp.2011.10121780.

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., and Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behav. Res. Ther.* 43, 1391–1424. doi:10.1016/j.brat.2004.10.007.

Liu, K. Y., Marijatta, F., Hämmerer, D., Acosta-Cabronero, J., Düzel, E., and Howard, R. J. (2017). Magnetic resonance imaging of the human locus coeruleus: A systematic review. *Neurosci. Biobehav. Rev.* 83, 325–355. doi:10.1016/j.neubiorev.2017.10.023.

Lonergan, M. H., Olivera-Figueroa, L. A., Pitman, R. K., and Brunet, A. (2013). Propranolol's effects on the consolidation and reconsolidation of long-term emotional memory in healthy participants: a meta-analysis. *J. Psychiatry Neurosci.* 38, 222–231. doi:10.1503/jpn.120111.

Loughlin, S. E., Foote, S. L., and Bloom, F. E. (1986). Efferent projections of nucleus locus coeruleus: topographic organization of cells of origin demonstrated by three-dimensional reconstruction. *Neuroscience* 18, 291–306. doi:10.1016/0306-4522(86)90155-7.

Loughlin, S. E., Foote, S. L., and Fallon, J. H. (1982). Locus coeruleus projections to cortex: Topography, morphology and collateralization. *Brain Res. Bull.* 9, 287–294. doi:10.1016/0361-9230(82)90142-3.

Lu, K. T., Walker, D. L., and Davis, M. (2001). Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. *J. Neurosci.* 21, RC162.

MacDonald, E., Kobilka, B. K., and Scheinin, M. (1997). Gene targeting — homing in on α2-adrenoceptor-subtype function. *Trends Pharmacol. Sci.* 18, 211–219. doi:10.1016/S0165-6147(97)90625-8.

MacPherson, K., Whittle, N., Camp, M., Gunduz-Cinar, O., Singewald, N., and Holmes, A. (2013). Temporal factors in the extinction of fear in inbred mouse strains differing in extinction efficacy. *Biol. Mood Anxiety Disord.* 3, 13. doi:10.1186/2045-5380-3-13.

Mansdorf, I. J. (2008). Psychological interventions following terrorist attacks. *Br Med Bull* 88, 7–22. doi:10.1093/bmb/ldn041.

Marek, R., Jin, J., Goode, T. D., Giustino, T. F., Wang, Q., Acca, G. M., Holehonnur, R., Ploski, J. E., Fitzgerald, P. J., Lynagh, T., et al. (2018a). Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear. *Nat. Neurosci.* 21, 384–392. doi:10.1038/s41593-018-0073-9.

Marek, R., Xu, L., Sullivan, R. K. P., and Sah, P. (2018b). Excitatory connections between the prelimbic and infralimbic medial prefrontal cortex show a role for the prelimbic cortex in fear extinction. *Nat. Neurosci.* 21, 654–658. doi:10.1038/s41593-018-0137-x.

Maren, S. (2000). Auditory fear conditioning increases CS-elicited spike firing in lateral amygdala neurons even after extensive overtraining. *Eur. J. Neurosci.* 12, 4047–4054. doi:10.1046/j.1460-9568.2000.00281.x.

Maren, S. (2014). Nature and causes of the immediate extinction deficit: a brief review. *Neurobiol. Learn. Mem.* 113, 19–24. doi:10.1016/j.nlm.2013.10.012.

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annu. Rev. Neurosci.* 24, 897–931. doi:10.1146/annurev.neuro.24.1.897.

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

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *J. Neurosci.* 18, 3088–3097.

Maren, S. (2011). Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. *Neuron* 70, 830–845. doi:10.1016/j.neuron.2011.04.023.

Maren, S., Aharonov, G., and Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behav. Brain Res.* 88, 261–274. doi:10.1016/S0166-4328(97)00088-0.

Maren, S., and Chang, C. (2006). Recent fear is resistant to extinction. *Proc. Natl. Acad. Sci. USA* 103, 18020–18025. doi:10.1073/pnas.0608398103.

Maren, S., and Hobin, J. A. (2007). Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala. *Learn. Mem.* 14, 318–324. doi:10.1101/lm.477007.

Maren, S., and Holmes, A. (2016). Stress and fear extinction. *Neuropsychopharmacology* 41, 58–79. doi:10.1038/npp.2015.180.

Maren, S., Phan, K. L., and Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat. Rev. Neurosci.* 14, 417–428. doi:10.1038/nrn3492.

Maren, S., and Quirk, G. J. (2004). Neuronal signalling of fear memory. *Nat. Rev. Neurosci.* 5, 844–852. doi:10.1038/nrn1535.

Maroun, M., and Richter-Levin, G. (2003). Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. *J. Neurosci.* 23, 4406–4409.

Marshall, I., Burt, R. P., and Chapple, C. R. (1999). Signal transduction pathways associated with alpha1-adrenoceptor subtypes in cells and tissues including human prostate. *Eur. Urol.* 36 Suppl 1, 42–7; discussion 65. doi:52317.

McCall, J. G., Al-Hasani, R., Siuda, E. R., Hong, D. Y., Norris, A. J., Ford, C. P., and Bruchas, M. R. (2015). CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. *Neuron* 87, 605–620. doi:10.1016/j.neuron.2015.07.002.

McCall, J. G., Siuda, E. R., Bhatti, D. L., Lawson, L. A., McElligott, Z. A., Stuber, G. D., and Bruchas, M. R. (2017). Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior. *Elife* 6. doi:10.7554/eLife.18247.

McCune, S. K., Voigt, M. M., and Hill, J. M. (1993). Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. *Neuroscience* 57, 143–151. doi:10.1016/0306-4522(93)90116-W.

McDonald, A. J., and Augustine, J. R. (1993). Localization of GABA-like immunoreactivity in the monkey amygdala. *Neuroscience* 52, 281–294.

McGarry, L. M., and Carter, A. G. (2016). Inhibitory gating of basolateral amygdala inputs to the prefrontal cortex. *J. Neurosci.* 36, 9391–9406. doi:10.1523/JNEUROSCI.0874-16.2016.

McGaugh, J. L. (2000). Memory--a century of consolidation. *Science (80-.).* 287, 248–251. doi:10.1126/science.287.5451.248.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu. Rev. Neurosci.* 27, 1–28. doi:10.1146/annurev.neuro.27.070203.144157.

McGaugh, J. L., and Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Curr. Opin. Neurobiol.* 12, 205–210. doi:10.1016/S0959-4388(02)00306-9.

McGhee, L. L., Maani, C. V., Garza, T. H., Desocio, P. A., Gaylord, K. M., and Black, I. H. (2009). The effect of propranolol on posttraumatic stress disorder in burned service members. *J Burn Care Res* 30, 92–97. doi:10.1097/BCR.0b013e3181921f51.

McIntyre, C. K., McGaugh, J. L., and Williams, C. L. (2012). Interacting brain systems modulate memory consolidation. *Neurosci. Biobehav. Rev.* 36, 1750–1762. doi:10.1016/j.neubiorev.2011.11.001.

Merkel, S. F., Andrews, A. M., Lutton, E. M., Mu, D., Hudry, E., Hyman, B. T., Maguire, C. A., and Ramirez, S. H. (2017). Trafficking of adeno-associated virus vectors across a model of the blood-brain barrier; a comparative study of

transcytosis and transduction using primary human brain endothelial cells. *J. Neurochem.* 140, 216–230. doi:10.1111/jnc.13861.

Merz, C. J., Hamacher-Dang, T. C., and Wolf, O. T. (2014). Exposure to stress attenuates fear retrieval in healthy men. *Psychoneuroendocrinology* 41, 89–96. doi:10.1016/j.psyneuen.2013.12.009.

Merz, C. J., Hamacher-Dang, T. C., and Wolf, O. T. (2016). Immediate extinction promotes the return of fear. *Neurobiol. Learn. Mem.* 131, 109–116. doi:10.1016/j.nlm.2016.03.013.

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., and Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. *J. Psychiatr. Res.* 42, 515–520. doi:10.1016/j.jpsychires.2008.01.017.

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., Zeidan, M. A., Handwerger, K., Orr, S. P., and Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol. Psychiatry.* 66, 1075–1082. doi:10.1016/j.biopsych.2009.06.026.

Milad, M. R., and Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: ten years of progress. *Annu Rev Psychol* 63, 129–151. doi:10.1146/annurev.psych.121208.131631.

Milad, M. R., and Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420, 70–74. doi:10.1038/nature01138.

Milad, M. R., Vidal-Gonzalez, I., and Quirk, G. J. (2004). Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behav. Neurosci.* 118, 389–394. doi:10.1037/0735-7044.118.2.389.

Miller, K. E., Brownlow, J. A., Woodward, S., and Gehrman, P. R. (2017). Sleep and dreaming in posttraumatic stress disorder. *Curr Psychiatry Rep* 19, 71. doi:10.1007/s11920-017-0827-1.

Milner, T. A., Shah, P., and Pierce, J. P. (2000). β-Adrenergic receptors primarily are located on the dendrites of granule cells and interneurons but also are found on astrocytes and a few presynaptic profiles in the rat dentate gyrus. *Synapse*.

Miracle, A. D., Brace, M. F., Huyck, K. D., Singler, S. A., and Wellman, C. L. (2006). Chronic stress impairs recall of extinction of conditioned fear. *Neurobiol. Learn. Mem.* 85, 213–218. doi:10.1016/j.nlm.2005.10.005.

Moore, R. Y., and Bloom, F. E. (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annu. Rev. Neurosci.* 2, 113–168. doi:10.1146/annurev.ne.02.030179.000553.

Morilak, D. A., Barrera, G., Echevarria, D. J., Garcia, A. S., Hernandez, A., Ma, S., and Petre, C. O. (2005). Role of brain norepinephrine in the behavioral response to stress. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 29, 1214–1224. doi:10.1016/j.pnpbp.2005.08.007.

Morrison, J. H., Molliver, M. E., and Grzanna, R. (1979). Noradrenergic innervation of cerebral cortex: widespread effects of local cortical lesions. *Science (80-.).* 205, 313–316.

Mueller, D., Porter, J. T., and Quirk, G. J. (2008). Noradrenergic signaling in infralimbic cortex increases cell excitability and strengthens memory for fear extinction. *J. Neurosci.* 28, 369–375. doi:10.1523/JNEUROSCI.3248-07.2008.

Muller, J., Corodimas, K. P., Fridel, Z., and 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. *Behav. Neurosci.* 111, 683–691. doi:10.1037/0735-7044.111.4.683.

Murphy, P. R., O'Connell, R. G., O'Sullivan, M., Robertson, I. H., and Balsters, J. H. (2014). Pupil diameter covaries with BOLD activity in human locus coeruleus. *Hum. Brain Mapp.* 35, 4140–4154. doi:10.1002/hbm.22466.

Myers, K. M., and Davis, M. (2007). Mechanisms of fear extinction. *Mol. Psychiatry* 12, 120–150. doi:10.1038/sj.mp.4001939.

Myers, K. M., Ressler, K. J., and Davis, M. (2006). Different mechanisms of fear extinction dependent on length of time since fear acquisition. *Learn. Mem.* 13, 216–223. doi:10.1101/lm.119806.

Naegeli, C., Zeffiro, T., Piccirelli, M., Jaillard, A., Weilenmann, A., Hassanpour, K., Schick, M., Rufer, M., Orr, S. P., and Mueller-Pfeiffer, C. (2017). Locus Coeruleus Activity Mediates Hyper-Responsiveness in Posttraumatic Stress Disorder. *Biol. Psychiatry.* 83, 254–262. doi:10.1016/j.biopsych.2017.08.021.

Nagai, T., Satoh, K., Imamoto, K., and Maeda, T. (1981). Divergent projections of catecholamine neurons of the locus coeruleus as revealed by fluorescent

retrograde double labeling technique. *Neurosci. Lett.* 23, 117–123. doi:10.1016/0304-3940(81)90027-6.

Neuman, R. S., and Harley, C. W. (1983). Long-lasting potentiation of the dentate gyrus population spike by norepinephrine. *Brain Res.* 273, 162–165. doi:10.1016/0006-8993(83)91106-X.

Neylan, T. C., Lenoci, M., Samuelson, K. W., Metzler, T. J., Henn-Haase, C., Hierholzer, R. W., Lindley, S. E., Otte, C., Schoenfeld, F. B., Yesavage, J. A., et al. (2006). No improvement of posttraumatic stress disorder symptoms with guanfacine treatment. *Am. J. Psychiatry* 163, 2186–2188. doi:10.1176/appi.ajp.163.12.2186.

Nicholas, A. P., Pieribone, V. A., and Hökfelt, T. (1993a). Cellular localization of messenger RNA for beta-1 and beta-2 adrenergic receptors in rat brain: an in situ hybridization study. *Neuroscience* 56, 1023–1039. doi:10.1016/0306-4522(93)90148-9.

Nicholas, A. P., Pieribone, V., and Hökfelt, T. (1993b). Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study. *J. Comp. Neurol.* 328, 575–594. doi:10.1002/cne.903280409.

Nitecka, L., and Ben-Ari, Y. (1987). Distribution of GABA-like immunoreactivity in the rat amygdaloid complex. *J. Comp. Neurol.* 266, 45–55. doi:10.1002/cne.902660105.

Norrholm, S. D., Glover, E. M., Stevens, J. S., Fani, N., Galatzer-Levy, I. R., Bradley, B., Ressler, K. J., and Jovanovic, T. (2015). Fear load: The psychophysiological

over-expression of fear as an intermediate phenotype associated with trauma reactions. *Int. J. Psychophysiol.* 98, 270–275. doi:10.1016/j.ijpsycho.2014.11.005.

Norrholm, S. D., Vervliet, B., Jovanovic, T., Boshoven, W., Myers, K. M., Davis, M., Rothbaum, B., and Duncan, E. J. (2008). Timing of extinction relative to acquisition: a parametric analysis of fear extinction in humans. *Behav. Neurosci.* 122, 1016–1030. doi:10.1037/a0012604.

Nugent, N. R., Christopher, N. C., Crow, J. P., Browne, L., Ostrowski, S., and Delahanty, D. L. (2010). The efficacy of early propranolol administration at reducing PTSD symptoms in pediatric injury patients: a pilot study. *J. Trauma. Stress* 23, 282–287. doi:10.1002/jts.20517.

O'Donnell, T., Hegadoren, K. M., and Coupland, N. C. (2004). Noradrenergic mechanisms in the pathophysiology of post-traumatic stress disorder. *Neuropsychobiology* 50, 273–283. doi:10.1159/000080952.

Olschowka, J. A., Molliver, M. E., Grzanna, R., Rice, F. L., and Coyle, J. T. (1981). Ultrastructural demonstration of noradrenergic synapses in the rat central nervous system by dopamine-beta-hydroxylase immunocytochemistry. *J. Histochem. Cytochem.* 29, 271–280. doi:10.1177/29.2.7019303.

Ordway, G. A., O'Donnell, J. M., and Frazer, A. (1987). Effects of clenbuterol on central beta-1 and beta-2 adrenergic receptors of the rat. *J. Pharmacol. Exp. Ther.* 241, 187–195.

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., and Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *J. Abnorm. Psychol.* 109, 290–298. doi:10.1037//0021-843X.109.2.290.

Orr, S. P., Milad, M. R., Metzger, L. J., Lasko, N. B., Gilbertson, M. W., and Pitman, R. K. (2006). Effects of beta blockade, PTSD diagnosis, and explicit threat on the extinction and retention of an aversively conditioned response. *Biol. Psychol.* 73, 262–271. doi:10.1016/j.biopsycho.2006.05.001.

Orsini, C. A., Kim, J. H., Knapska, E., and Maren, S. (2011). Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *J. Neurosci.* 31, 17269–17277. doi:10.1523/JNEUROSCI.4095-11.2011.

Oznur, T., Akarsu, S., Celik, C., Bolu, A., Ozdemir, B., Akcay, B. D., Ozselek, S., Bozkurt, A., and Ozmenler, K. N. (2014). Is transcranial magnetic stimulation effective in treatment-resistant combat related posttraumatic stress disorder? *Neurosciences (Riyadh).* 19, 29–32.

Palacios, J. M., Hoyer, D., and Cortés, R. (1987). α1-adrenoceptors in the mammalian brain: similar pharmacology but different distribution in rodents and primates. *Brain Res.* 419, 65–75. doi:10.1016/0006-8993(87)90569-5.

Papadopoulos, G. C., and Parnavelas, J. G. (1990). Distribution and synaptic organization of serotoninergic and noradrenergic axons in the lateral geniculate nucleus of the rat. *J. Comp. Neurol.* 294, 345–355. doi:10.1002/cne.902940304.

Papadopoulos, G. C., Parnavelas, J. G., and Buijs, R. (1987). Monoaminergic fibers form conventional synapses in the cerebral cortex. *Neurosci. Lett.* 76, 275–279. doi:10.1016/0304-3940(87)90414-9.

Papadopoulos, G. C., Parnavelas, J. G., and Buijs, R. M. (1989). Light and electron microscopic immunocytochemical analysis of the noradrenaline innervation of the rat visual cortex. *J Neurocytol* 18, 1–10. doi:10.1007/BF01188418.

Pape, H.-C., and Pare, D. (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol. Rev.* 90, 419–463. doi:10.1152/physrev.00037.2009.

Paré, D., Quirk, G. J., and Ledoux, J. E. (2004). New vistas on amygdala networks in conditioned fear. *J. Neurophysiol.* 92, 1–9. doi:10.1152/jn.00153.2004.

Paré, D., and Smith, Y. (1993). Distribution of GABA immunoreactivity in the amygdaloid complex of the cat. *Neuroscience* 57, 1061–1076.

Parsons, R. G., and Ressler, K. J. (2013). Implications of memory modulation for post-traumatic stress and fear disorders. *Nat. Neurosci.* 16, 146–153. doi:10.1038/nn.3296.

Passerin, A. M., Cano, G., Rabin, B. S., Delano, B. A., Napier, J. L., and Sved, A. F. (2000). Role of locus coeruleus in foot shock-evoked Fos expression in rat brain. *Neuroscience* 101, 1071–1082. doi:10.1016/S0306-4522(00)00372-9.

Patriarchi, T., Cho, J. R., Merten, K., Howe, M. W., Marley, A., Xiong, W.-H., Folk, R. W., Broussard, G. J., Liang, R., Jang, M. J., et al. (2018). Ultrafast neuronal

imaging of dopamine dynamics with designed genetically encoded sensors. *Science (80-.).* 360. doi:10.1126/science.aat4422.

Pervanidou, P., and Chrousos, G. P. (2010). Neuroendocrinology of post-traumatic stress disorder. *Prog. Brain Res.* 182, 149–160. doi:10.1016/S0079-6123(10)82005-9.

Peters, J., Dieppa-Perea, L. M., Melendez, L. M., and Quirk, G. J. (2010). Induction of fear extinction with hippocampal-infralimbic BDNF. *Science (80-.).* 328, 1288–1290. doi:10.1126/science.1186909.

Pezzone, M. A., Lee, W. S., Hoffman, G. E., Pezzone, K. M., and Rabin, B. S. (1993). Activation of brainstem catecholaminergic neurons by conditioned and unconditioned aversive stimuli as revealed by c-Fos immunoreactivity. *Brain Res.* 608, 310–318. doi:10.1016/0006-8993(93)91472-5.

Phelps, E. A., Delgado, M. R., Nearing, K. I., and LeDoux, J. E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron* 43, 897–905. doi:10.1016/j.neuron.2004.08.042.

Phillips, R. G., and LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* 106, 274–285. doi:10.1037/0735-7044.106.2.274.

Pickel, V. M., Segal, M., and Bloom, F. E. (1974). A radioautographic study of the efferent pathways of the nucleus locus coeruleus. *J. Comp. Neurol.* 155, 15–42. doi:10.1002/cne.901550103.

Pieribone, V. A., Nicholas, A. P., Dagerlind, A., and Hökfelt, T. (1994). Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes. *J. Neurosci.* 14, 4252–4268.

Pitman, R. K. (1988). Post-traumatic stress disorder, conditioning, and network theory. *Psychiatric Annals* 18, 182–189.

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., Milad, M. R., and Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nat. Rev. Neurosci.* 13, 769–787. doi:10.1038/nrn3339.

Pitman, R. K., Sanders, K. M., Zusman, R. M., Healy, A. R., Cheema, F., Lasko, N. B., Cahill, L., and Orr, S. P. (2002). Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biol. Psychiatry.* 51, 189–192.

Porter, D. M., and Bell, C. C. (1999). The use of clonidine in post-traumatic stress disorder. *J Natl Med Assoc* 91, 475–477.

Poundja, J., Sanche, S., Tremblay, J., and Brunet, A. (2012). Trauma reactivation under the influence of propranolol: an examination of clinical predictors. *Eur. J. Psychotraumatol.* 3. doi:10.3402/ejpt.v3i0.15470.

Powers, M. B., Smits, J. A. J., Otto, M. W., Sanders, C., and Emmelkamp, P. M. G. (2009). Facilitation of fear extinction in phobic participants with a novel cognitive enhancer: a randomized placebo controlled trial of yohimbine augmentation. *J. Anxiety Disord.* 23, 350–356. doi:10.1016/j.janxdis.2009.01.001.

Priovoulos, N., Jacobs, H. I. L., Ivanov, D., Uludağ, K., Verhey, F. R. J., and Poser, B. A. (2018). High-resolution in vivo imaging of human locus coeruleus by magnetization transfer MRI at 3T and 7T. *Neuroimage* 168, 427–436. doi:10.1016/j.neuroimage.2017.07.045.

Prouty, E. W., Waterhouse, B. D., and Chandler, D. J. (2017). Corticotropin releasing factor dose-dependently modulates excitatory synaptic transmission in the noradrenergic nucleus locus coeruleus. *Eur. J. Neurosci.* 45, 712–722. doi:10.1111/ejn.13501.

Quirarte, G. L., Galvez, R., Roozendaal, B., and McGaugh, J. L. (1998). Norepinephrine release in the amygdala in response to footshock and opioid peptidergic drugs. *Brain Res.* 808, 134–140. doi:10.1016/S0006-8993(98)00795-1.

Quirk, G. J., and Beer, J. S. (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Curr. Opin. Neurobiol.* 16, 723–727. doi:10.1016/j.conb.2006.07.004.

Quirk, G. J., Likhtik, E., Pelletier, J. G., and Paré, D. (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *J. Neurosci.* 23, 8800–8807.

Quirk, G. J., and Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology* 33, 56–72. doi:10.1038/sj.npp.1301555.

Quirk, G. J., Repa, C., and LeDoux, J. E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron* 15, 1029–1039. doi:10.1016/0896-6273(95)90092-6.

Quirk, G. J., Russo, G. K., Barron, J. L., and Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J. Neurosci.* 20, 6225–6231.

Rainbow, T. C., and Biegon, A. (1983). Quantitative autoradiography of [3H]prazosin binding sites in rat forebrain. *Neurosci. Lett.* 40, 221–226. doi:10.1016/0304-3940(83)90042-3.

Rainbow, T. C., Parsons, B., and Wolfe, B. B. (1984). Quantitative autoradiography of beta 1- and beta 2-adrenergic receptors in rat brain. *Proc. Natl. Acad. Sci. USA* 81, 1585–1589.

Raio, C. M., Brignoni-Perez, E., Goldman, R., and Phelps, E. A. (2014). Acute stress impairs the retrieval of extinction memory in humans. *Neurobiol. Learn. Mem.* 112, 212–221. doi:10.1016/j.nlm.2014.01.015.

Raio, C. M., Hartley, C. A., Orederu, T. A., Li, J., and Phelps, E. A. (2017). Stress attenuates the flexible updating of aversive value. *Proc. Natl. Acad. Sci. USA* 114, 11241–11246. doi:10.1073/pnas.1702565114.

Raio, C. M., and Phelps, E. A. (2015). The influence of acute stress on the regulation of conditioned fear. *Neurobiol. Stress* 1, 134–146. doi:10.1016/j.ynstr.2014.11.004.

Ramos, B. P., and Arnsten, A. F. T. (2007). Adrenergic pharmacology and cognition: focus on the prefrontal cortex. *Pharmacol. Ther.* 113, 523–536. doi:10.1016/j.pharmthera.2006.11.006.

Ramos, B. P., Stark, D., Verduzco, L., van Dyck, C. H., and Arnsten, A. F. T. (2006). Alpha2A-adrenoceptor stimulation improves prefrontal cortical regulation of

behavior through inhibition of cAMP signaling in aging animals. *Learn. Mem.* 13, 770–776. doi:10.1101/lm.298006.

Raskind, M. A., Peskind, E. R., Chow, B., Harris, C., Davis-Karim, A., Holmes, H. A., Hart, K. L., McFall, M., Mellman, T. A., Reist, C., et al. (2018). Trial of Prazosin for Post-Traumatic Stress Disorder in Military Veterans. *N. Engl. J. Med.* 378, 507–517. doi:10.1056/NEJMoa1507598.

Raskind, M. A., Peskind, E. R., Kanter, E. D., Petrie, E. C., Radant, A., Thompson, C. E., Dobie, D. J., Hoff, D., Rein, R. J., Straits-Tröster, K., et al. (2003). Reduction of nightmares and other PTSD symptoms in combat veterans by prazosin: a placebo-controlled study. *Am. J. Psychiatry* 160, 371–373. doi:10.1176/appi.ajp.160.2.371.

Rauch, S. L., Shin, L. M., and Phelps, E. A. (2006). Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research--past, present, and future. *Biol. Psychiatry.* 60, 376–382. doi:10.1016/j.biopsych.2006.06.004.

Repa, J. C., Muller, J., Apergis, J., Desrochers, T. M., Zhou, Y., and LeDoux, J. E. (2001). Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nat. Neurosci.* 4, 724–731. doi:10.1038/89512.

Riga, D., Matos, M. R., Glas, A., Smit, A. B., Spijker, S., and Van den Oever, M. C. (2014). Optogenetic dissection of medial prefrontal cortex circuitry. *Front. Syst. Neurosci.* 8, 230. doi:10.3389/fnsys.2014.00230.

Rive, M. M., van Rooijen, G., Veltman, D. J., Phillips, M. L., Schene, A. H., and Ruhé, H. G. (2013). Neural correlates of dysfunctional emotion regulation in major depressive disorder. A systematic review of neuroimaging studies. *Neurosci. Biobehav. Rev.* 37, 2529–2553. doi:10.1016/j.neubiorev.2013.07.018.

Robbins, T. W., and Arnsten, A. F. T. (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annu. Rev. Neurosci.* 32, 267–287. doi:10.1146/annurev.neuro.051508.135535.

Rodrigues, S. M., LeDoux, J. E., and Sapolsky, R. M. (2009). The influence of stress hormones on fear circuitry. *Annu. Rev. Neurosci.* 32, 289–313. doi:10.1146/annurev.neuro.051508.135620.

Rodriguez-Romaguera, J., Sotres-Bayon, F., Mueller, D., and Quirk, G. J. (2009). Systemic propranolol acts centrally to reduce conditioned fear in rats without impairing extinction. *Biol. Psychiatry.* 65, 887–892. doi:10.1016/j.biopsych.2009.01.009.

Room, P., Postema, F., and Korf, J. (1981). Divergent axon collaterals of rat locu Demonstration by a fluorescent double labeling technique. *Brain Res.* 221, 219–230. doi:10.1016/0006-8993(81)90773-3.

Roozendaal, B., Hui, G. K., Hui, I. R., Berlau, D. J., McGaugh, J. L., and Weinberger, N. M. (2006a). Basolateral amygdala noradrenergic activity mediates corticosterone-induced enhancement of auditory fear conditioning. *Neurobiol. Learn. Mem.* 86, 249–255. doi:10.1016/j.nlm.2006.03.003.

Roozendaal, B., Okuda, S., Van der Zee, E. A., and McGaugh, J. L. (2006b). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proc. Natl. Acad. Sci. USA* 103, 6741–6746. doi:10.1073/pnas.0601874103.

Rosin, D. L., Talley, E. M., Lee, A., Stornetta, R. L., Gaylinn, B. D., Guyenet, P. G., and Lynch, K. R. (1996a). Distribution of α2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *Journal of Comparative Neurology*.

Rosin, D. L., Talley, E. M., Lee, A., Stornetta, R. L., Gaylinn, B. D., Guyenet, P. G., and Lynch, K. R. (1996b). Distribution of α2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *Journal of Comparative Neurology*.

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

Sabban, E. L., Serova, L. I., Newman, E., Aisenberg, N., and Akirav, I. (2018). Changes in Gene Expression in the Locus Coeruleus-Amygdala Circuitry in Inhibitory Avoidance PTSD Model. *Cell Mol. Neurobiol.* 38, 273–280. doi:10.1007/s10571-017-0548-3.

Sara, S. J. (2015). Locus Coeruleus in time with the making of memories. *Curr. Opin. Neurobiol.* 35, 87–94. doi:10.1016/j.conb.2015.07.004.

Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nat. Rev. Neurosci.* 10, 211–223. doi:10.1038/nrn2573.

Sara, S. J., and Hervé-Minvielle, A. (1995). Inhibitory influence of frontal cortex on locus coeruleus neurons. *Proc. Natl. Acad. Sci. USA* 92, 6032–6036.

Sara, S. J., and Segal, M. (1991). "Plasticity of sensory responses of locus coeruleus neurons in the behaving rat: implications for cognition," in *Neurobiology of the locus coeruleus* Progress in brain research. (Elsevier), 571–585. doi:10.1016/S0079-6123(08)63835-2.

Scheinin, M., Lomasney, J. W., Hayden-Hixson, D. M., Schambra, U. B., Caron, M. G., Lefkowitz, R. J., and Fremeau, R. T. (1994). Distribution of α2-adrenergic receptor subtype gene expression in rat brain. *Molecular Brain Research* 21, 133–149. doi:10.1016/0169-328X(94)90386-7.

Schiff, H. C., Johansen, J. P., Hou, M., Bush, D. E. A., Smith, E. K., Klein, J. E., LeDoux, J. E., and Sears, R. M. (2017). β-Adrenergic Receptors Regulate the Acquisition and Consolidation Phases of Aversive Memory Formation Through Distinct, Temporally Regulated Signaling Pathways. *Neuropsychopharmacology* 42, 895–903. doi:10.1038/npp.2016.238.

Schiller, D., Levy, I., Niv, Y., LeDoux, J. E., and Phelps, E. A. (2008). From fear to safety and back: reversal of fear in the human brain. *J. Neurosci.* 28, 11517–11525. doi:10.1523/JNEUROSCI.2265-08.2008.

Schulz, B., Fendt, M., and Schnitzler, H.-U. (2002). Clonidine injections into the lateral nucleus of the amygdala block acquisition and expression of fear-potentiated startle. *Eur. J. Neurosci.* 15, 151–157. doi:10.1046/j.0953-816x.2001.01831.x.

Schwabe, L., Nader, K., Wolf, O. T., Beaudry, T., and Pruessner, J. C. (2012). Neural signature of reconsolidation impairments by propranolol in humans. *Biol. Psychiatry.* 71, 380–386. doi:10.1016/j.biopsych.2011.10.028.

Schwarz, L. A., and Luo, L. (2015). Organization of the locus coeruleus-norepinephrine system. *Curr. Biol.* 25, R1051–R1056. doi:10.1016/j.cub.2015.09.039.

Schwarz, L. A., Miyamichi, K., Gao, X. J., Beier, K. T., Weissbourd, B., DeLoach, K. E., Ren, J., Ibanes, S., Malenka, R. C., Kremer, E. J., et al. (2015). Viral-genetic tracing of the input-output organization of a central noradrenaline circuit. *Nature* 524, 88–92. doi:10.1038/nature14600.

Segal, M., and Landis, S. (1974). Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. *Brain Res.* 78, 1–15. doi:10.1016/0006-8993(74)90349-7.

Segal, M., Markram, H., and Richter-Levin, G. (1991). "Actions of norepinephrine in the rat hippocampus," in *Neurobiology of the locus coeruleus* Progress in brain research. (Elsevier), 323–330. doi:10.1016/S0079-6123(08)63819-4.

Sehlmeyer, C., Schöning, S., Zwitserlood, P., Pfleiderer, B., Kircher, T., Arolt, V., and Konrad, C. (2009). Human fear conditioning and extinction in neuroimaging: a systematic review. *PLoS One* 4, e5865. doi:10.1371/journal.pone.0005865.

Selden, N. R., Everitt, B. J., Jarrard, L. E., and Robbins, T. W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience* 42, 335–350. doi:10.1016/0306-4522(91)90379-3.

Senn, V., Wolff, S. B. E., Herry, C., Grenier, F., Ehrlich, I., Gründemann, J., Fadok, J. P., Müller, C., Letzkus, J. J., and Lüthi, A. (2014). Long-range connectivity defines behavioral specificity of amygdala neurons. *Neuron* 81, 428–437. doi:10.1016/j.neuron.2013.11.006.

Sevenster, D., Beckers, T., and Kindt, M. (2012). Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. *Neurobiol. Learn. Mem.* 97, 338–345. doi:10.1016/j.nlm.2012.01.009.

Shin, L. M., and Handwerger, K. (2009). Is posttraumatic stress disorder a stress-induced fear circuitry disorder? *J. Trauma. Stress* 22, 409–415. doi:10.1002/jts.20442.

Shipley, M. T., Fu, L., Ennis, M., Liu, W. L., and Aston-Jones, G. (1996). Dendrites of locus coeruleus neurons extend preferentially into two pericoerulear zones. *J. Comp. Neurol.* 365, 56–68. doi:10.1002/(SICI)1096-9861(19960129)365:1<56::AID-CNE5>3.0.CO;2-I.

Short, N. A., Allan, N. P., Stentz, L., Portero, A. K., and Schmidt, N. B. (2017). Predictors of insomnia symptoms and nightmares among individuals with post-traumatic stress disorder: an ecological momentary assessment study. *J Sleep Res* 27, 64–72. doi:10.1111/jsr.12589.

Singewald, N., Salchner, P., and Sharp, T. (2003). Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. *Biol. Psychiatry.* 53, 275–283. doi:10.1016/S0006-3223(02)01574-3.

Skelly, M. J., Ariwodola, O. J., and Weiner, J. L. (2017). Fear conditioning selectively disrupts noradrenergic facilitation of GABAergic inhibition in the basolateral amygdala. *Neuropharmacology* 113, 231–240. doi:10.1016/j.neuropharm.2016.10.003.

Smith, M. A., Banerjee, S., Gold, P. W., and Glowa, J. (1992). Induction of c-fos mRNA in rat brain by conditioned and unconditioned stressors. *Brain Res.* 578, 135–141. doi:10.1016/0006-8993(92)90240-A.

Soeter, M., and Kindt, M. (2011a). Disrupting reconsolidation: pharmacological and behavioral manipulations. *Learn. Mem.* 18, 357–366. doi:10.1101/lm.2148511.

Soeter, M., and Kindt, M. (2011b). Noradrenergic enhancement of associative fear memory in humans. *Neurobiol. Learn. Mem.* 96, 263–271. doi:10.1016/j.nlm.2011.05.003.

Soeter, M., and Kindt, M. (2012). Stimulation of the noradrenergic system during memory formation impairs extinction learning but not the disruption of reconsolidation. *Neuropsychopharmacology* 37, 1204–1215. doi:10.1038/npp.2011.307.

Song, A. H., Kucyi, A., Napadow, V., Brown, E. N., Loggia, M. L., and Akeju, O. (2017). Pharmacological modulation of noradrenergic arousal circuitry disrupts

functional connectivity of the locus ceruleus in humans. *J. Neurosci.* 37, 6938–6945. doi:10.1523/JNEUROSCI.0446-17.2017.

Sotres-Bayon, F., Bush, D. E. A., and LeDoux, J. E. (2007). Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. *Neuropsychopharmacology* 32, 1929–1940. doi:10.1038/sj.npp.1301316.

Sotres-Bayon, F., and Quirk, G. J. (2010). Prefrontal control of fear: more than just extinction. *Curr. Opin. Neurobiol.* 20, 231–235. doi:10.1016/j.conb.2010.02.005.

Southwick, S. M., Bremner, J. D., Rasmusson, A., Morgan, C. A., Arnsten, A., and Charney, D. S. (1999a). Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol. Psychiatry.* 46, 1192–1204. doi:10.1016/S0006-3223(99)00219-X.

Southwick, S. M., Krystal, J. H., Bremner, J. D., Morgan, C. A., Nicolaou, A. L., Nagy, L. M., Johnson, D. R., Heninger, G. R., and Charney, D. S. (1997). Noradrenergic and serotonergic function in posttraumatic stress disorder. *Arch. Gen. Psychiatry* 54, 749–758.

Southwick, S. M., Morgan, C. A., Charney, D. S., and High, J. R. (1999b). Yohimbine use in a natural setting: effects on posttraumatic stress disorder. *Biol. Psychiatry.* 46, 442–444. doi:10.1016/S0006-3223(99)00107-9.

Southwick, S. M., Paige, S., Morgan, C. A., Bremner, J. D., Krystal, J. H., and Charney, D. S. (1999c). Neurotransmitter alterations in PTSD: catecholamines and serotonin. *Semin. Clin. Neuropsychiatry* 4, 242–248. doi:10.153/SCNP00400242.

Spampanato, J., Polepalli, J., and Sah, P. (2011). Interneurons in the basolateral amygdala. *Neuropharmacology* 60, 765–773. doi:10.1016/j.neuropharm.2010.11.006.

Spring, J. D., Wood, N. E., Mueller-Pfeiffer, C., Milad, M. R., Pitman, R. K., and Orr, S. P. (2015). Prereactivation propranolol fails to reduce skin conductance reactivity to prepared fear-conditioned stimuli. *Psychophysiology* 52, 407–415. doi:10.1111/psyp.12326.

Stafford, J. M., Maughan, D. K., Ilioi, E. C., and Lattal, K. M. (2013). Exposure to a fearful context during periods of memory plasticity impairs extinction via hyperactivation of frontal-amygdalar circuits. *Learn. Mem.* 20, 156–163. doi:10.1101/lm.029801.112.

Stafford, J. M., Raybuck, J. D., Ryabinin, A. E., and Lattal, K. M. (2012). Increasing histone acetylation in the hippocampus-infralimbic network enhances fear extinction. *Biol. Psychiatry.* 72, 25–33. doi:10.1016/j.biopsych.2011.12.012.

Stanton, P. K., and Sarvey, J. M. (1985a). Blockade of norepinephrine-induced long-lasting potentiation in the hippocampal dentate gyrus by an inhibitor of protein synthesis. *Brain Res.* 361, 276–283. doi:10.1016/0006-8993(85)91299-5.

Stanton, P. K., and Sarvey, J. M. (1985b). Depletion of norepinephrine, but not serotonin, reduces long-term potentiation in the dentate gyrus of rat hippocampal slices. *J. Neurosci.* 5, 2169–2176.

Stanton, P. K., and Sarvey, J. M. (1987). Norepinephrine regulates long-term potentiation of both the population spike and dendritic EPSP in hippocampal dentate gyrus. *Brain Res. Bull.* 18, 115–119. doi:10.1016/0361-9230(87)90039-6.

Steckler, T., and Risbrough, V. (2012). Pharmacological treatment of PTSD - established and new approaches. *Neuropharmacology* 62, 617–627. doi:10.1016/j.neuropharm.2011.06.012.

Van Stegeren, A. H., Roozendaal, B., Kindt, M., Wolf, O. T., and Joëls, M. (2010). Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. *Neurobiol. Learn. Mem.* 93, 56–65. doi:10.1016/j.nlm.2009.08.004.

Stein, M. B., Kerridge, C., Dimsdale, J. E., and Hoyt, D. B. (2007). Pharmacotherapy to prevent PTSD: Results from a randomized controlled proof-of-concept trial in physically injured patients. *J. Trauma. Stress* 20, 923–932. doi:10.1002/jts.20270.

Steindler, D. A. (1981). Locus coerules neurons have axons that branch to the forebrain and cerebellum. *Brain Res.* 223, 367–373. doi:10.1016/0006-8993(81)91149-5.

Sterpenich, V., D'Argembeau, A., Desseilles, M., Balteau, E., Albouy, G., Vandewalle, G., Degueldre, C., Luxen, A., Collette, F., and Maquet, P. (2006). The locus ceruleus is involved in the successful retrieval of emotional memories in humans. *J. Neurosci.* 26, 7416–7423. doi:10.1523/JNEUROSCI.1001-06.2006.

Strawn, J. R., and Geracioti, T. D. (2008). Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. *Depress. Anxiety* 25, 260–271. doi:10.1002/da.20292.

Summers, R. J., Papaioannou, M., Harris, S., and Evans, B. A. (1995). Expression of beta 3-adrenoceptor mRNA in rat brain. *Br. J. Pharmacol.* 116, 2547–2548.

Sun, F., Zeng, J., Jing, M., Zhou, J., Feng, J., Owen, S. F., Luo, Y., Li, F., Wang, H., Yamaguchi, T., et al. (2018). A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. *Cell* 174, 481–496.e19. doi:10.1016/j.cell.2018.06.042.

Sved, A. F., Cano, G., Passerin, A. M., and Rabin, B. S. (2002). The locus coeruleus, Barrington's nucleus, and neural circuits of stress. *Physiol. Behav.* 77, 737–742.

Swanson, L. W. (1976). The locus coeruleus: a cytoarchitectonic, Golgi and immunohistochemical study in the albino rat. *Brain Res.* 110, 39–56. doi:10.1016/0006-8993(76)90207-9.

Swanson, L. W., and Hartman, B. K. (1975). The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. *J. Comp. Neurol.* 163, 467–505. doi:10.1002/cne.901630406.

Tawa, J., and Murphy, S. (2013). Psychopharmacological treatment for military posttraumatic stress disorder: an integrative review. *J Am Assoc Nurse Pract* 25, 419–423. doi:10.1111/1745-7599.12016.

Taylor, H. R., Freeman, M. K., and Cates, M. E. (2008). Prazosin for treatment of nightmares related to posttraumatic stress disorder. *Am. J. Health Syst. Pharm.* 65, 716–722. doi:10.2146/ajhp070124.

Thierry, A. M., Javoy, F., Glowinski, J., and Kety, S. S. (1968). Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat. I. Modifications of norepinephrine turnover. *J. Pharmacol. Exp. Ther.* 163, 163–171.

Tollenaar, M. S., Elzinga, B. M., Spinhoven, P., and Everaerd, W. (2009). Psychophysiological responding to emotional memories in healthy young men after cortisol and propranolol administration. *Psychopharmacology (Berl).* 203, 793–803. doi:10.1007/s00213-008-1427-x.

Tona, K.-D., Keuken, M. C., de Rover, M., Lakke, E., Forstmann, B. U., Nieuwenhuis, S., and van Osch, M. J. P. (2017). In vivo visualization of the locus coeruleus in humans: quantifying the test-retest reliability. *Brain Struct. Funct.*, 1–15. doi:10.1007/s00429-017-1464-5.

Uematsu, A., Tan, B. Z., and Johansen, J. P. (2015). Projection specificity in heterogeneous locus coeruleus cell populations: implications for learning and memory. *Learn. Mem.* 22, 444–451. doi:10.1101/lm.037283.114.

Uematsu, A., Tan, B. Z., Ycu, E. A., Cuevas, J. S., Koivumaa, J., Junyent, F., Kremer, E. J., Witten, I. B., Deisseroth, K., and Johansen, J. P. (2017). Modular organization of the brainstem noradrenaline system coordinates opposing learning states. *Nat. Neurosci.* 20, 1602–1611. doi:10.1038/nn.4642.

Unnerstall, J. R., Kopajtic, T. A., and Kuhar, M. J. (1984). Distribution of α2 agonist binding sites in the rat and human central nervous system: Analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. *Brain Res. Rev.* 7, 69–101. doi:10.1016/0165-0173(84)90030-4.

Vaiva, G., Ducrocq, F., Jezequel, K., Averland, B., Lestavel, P., Brunet, A., and Marmar, C. R. (2003). Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. *Biol. Psychiatry.* 54, 947–949. doi:10.1016/S0006-3223(03)00412-8.

Valentino, R. J., and Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *Eur. J. Pharmacol.* 583, 194–203. doi:10.1016/j.ejphar.2007.11.062.

VanElzakker, M. B., Dahlgren, M. K., Davis, F. C., Dubois, S., and Shin, L. M. (2014). From Pavlov to PTSD: the extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiol. Learn. Mem.* 113, 3–18. doi:10.1016/j.nlm.2013.11.014.

Vazey, E. M., and Aston-Jones, G. (2014). Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. *Proc. Natl. Acad. Sci. USA* 111, 3859–3864. doi:10.1073/pnas.1310025111.

Ventura, R., Latagliata, E. C., Morrone, C., La Mela, I., and Puglisi-Allegra, S. (2008). Prefrontal norepinephrine determines attribution of "high" motivational salience. *PLoS One* 3, e3044. doi:10.1371/journal.pone.0003044.

Vervliet, B., Craske, M. G., and Hermans, D. (2013). Fear extinction and relapse: state of the art. *Annu. Rev. Clin. Psychol.* 9, 215–248. doi:10.1146/annurev-clinpsy-050212-185542.

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S. L., and Quirk, G. J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learn. Mem.* 13, 728–733. doi:10.1101/lm.306106.

Visser, R. M., Kunze, A. E., Westhoff, B., Scholte, H. S., and Kindt, M. (2015). Representational similarity analysis offers a preview of the noradrenergic modulation of long-term fear memory at the time of encoding. *Psychoneuroendocrinology* 55, 8–20. doi:10.1016/j.psyneuen.2015.01.021.

Wagatsuma, A., Okuyama, T., Sun, C., Smith, L. M., Abe, K., and Tonegawa, S. (2018). Locus coeruleus input to hippocampal CA3 drives single-trial learning of a novel context. *Proc. Natl. Acad. Sci. USA* 115, E310–E316. doi:10.1073/pnas.1714082115.

Wang, R., Macmillan, L. B., Fremeau, R. T., Magnuson, M. A., Lindner, J., and Limbird, L. E. (1996). Expression of alpha 2-adrenergic receptor subtypes in the mouse brain: evaluation of spatial and temporal information imparted by 3 kb of 5’ regulatory sequence for the alpha 2A AR-receptor gene in transgenic animals. *Neuroscience* 74, 199–218. doi:10.1016/0306-4522(96)00116-9.

Wangelin, B. C., Powers, M. B., Smits, J. A. J., and Tuerk, P. W. (2013). Enhancing exposure therapy for PTSD with yohimbine HCL: protocol for a double-blind,

randomized controlled study implementing subjective and objective measures of treatment outcome. *Contemp. Clin. Trials* 36, 319–326. doi:10.1016/j.cct.2013.08.003.

Waterhouse, B. D., and Chandler, D. J. (2016). Heterogeneous organization and function of the central noradrenergic system. *Brain Res.* 1641, v–x. doi:10.1016/j.brainres.2015.12.050.

Waterhouse, B. D., Lin, C. S., Burne, R. A., and Woodward, D. J. (1983). The distribution of neocortical projection neurons in the locus coeruleus. *J. Comp. Neurol.* 217, 418–431. doi:10.1002/cne.902170406.

Wessa, M., and Flor, H. (2007). Failure of extinction of fear responses in posttraumatic stress disorder: evidence from second-order conditioning. *Am. J. Psychiatry* 164, 1684–1692. doi:10.1176/appi.ajp.2007.07030525.

Weston, C. S. E. (2014). Posttraumatic stress disorder: a theoretical model of the hyperarousal subtype. *Front. Psychiatry* 5, 37. doi:10.3389/fpsyt.2014.00037.

Weston, M., Kaserer, T., Wu, A., Mouravlev, A., Carpenter, J. C., Snowball, A., Knauss, S., von Schimmelmann, M., During, M. J., Lignani, G., et al. (2019). Olanzapine: A potent agonist at the hM4D(Gi) DREADD amenable to clinical translation of chemogenetics. *Sci. Adv.* 5, eaaw1567. doi:10.1126/sciadv.aaw1567.

Wilber, A. A., Walker, A. G., Southwood, C. J., Farrell, M. R., Lin, G. L., Rebec, G. V., and Wellman, C. L. (2011). Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction. *Neuroscience* 174, 115–131. doi:10.1016/j.neuroscience.2010.10.070.

Wilensky, A. E., Schafe, G. E., Kristensen, M. P., and 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, 12387–12396. doi:10.1523/JNEUROSCI.4316-06.2006.

Wiltgen, B. J., Sanders, M. J., Anagnostaras, S. G., Sage, J. R., and Fanselow, M. S. (2006). Context fear learning in the absence of the hippocampus. *J. Neurosci.* 26, 5484–5491. doi:10.1523/JNEUROSCI.2685-05.2006.

Wolff, S. B. E., Gründemann, J., Tovote, P., Krabbe, S., Jacobson, G. A., Müller, C., Herry, C., Ehrlich, I., Friedrich, R. W., Letzkus, J. J., et al. (2014). Amygdala interneuron subtypes control fear learning through disinhibition. *Nature* 509, 453–458. doi:10.1038/nature13258.

Wood, N. E., Rosasco, M. L., Suris, A. M., Spring, J. D., Marin, M.-F., Lasko, N. B., Goetz, J. M., Fischer, A. M., Orr, S. P., and Pitman, R. K. (2015). Pharmacological blockade of memory reconsolidation in posttraumatic stress disorder: three negative psychophysiological studies. *Psychiatry Res.* 225, 31–39. doi:10.1016/j.psychres.2014.09.005.

Writer, B. W., Meyer, E. G., and Schillerstrom, J. E. (2014). Prazosin for military combat-related PTSD nightmares: a critical review. *J. Neuropsychiatry Clin. Neurosci.* 26, 24–33. doi:10.1176/appi.neuropsych.13010006.

Yang, H.-W., Lin, Y.-W., Yen, C.-D., and Min, M.-Y. (2002). Change in bi-directional plasticity at CA1 synapses in hippocampal slices taken from 6-

hydroxydopamine-treated rats: the role of endogenous norepinephrine. *Eur. J. Neurosci.* 16, 1117–1128. doi:10.1046/j.1460-9568.2002.02165.x.

Yavich, L., Jäkälä, P., and Tanila, H. (2005). Noradrenaline overflow in mouse dentate gyrus following locus coeruleus and natural stimulation: real-time monitoring by in vivo voltammetry. *J. Neurochem.* 95, 641–650. doi:10.1111/j.1471-4159.2005.03390.x.

Ye, X., Kapeller-Libermann, D., Travaglia, A., Inda, M. C., and Alberini, C. M. (2017). Direct dorsal hippocampal-prelimbic cortex connections strengthen fear memories. *Nat. Neurosci.* 20, 52–61. doi:10.1038/nn.4443.

Yehuda, R., Southwick, S., Giller, E. L., Ma, X., and Mason, J. W. (1992). Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. *J. Nerv. Ment. Dis.* 180, 321–325.

Young, S. L., Bohenek, D. L., and Fanselow, M. S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure. *Behav. Neurosci.* 108, 19–29. doi:10.1037/0735-7044.108.1.19.

Young, W. S., and Kuhar, M. J. (1980). Noradrenergic alpha 1 and alpha 2 receptors: light microscopic autoradiographic localization. *Proc. Natl. Acad. Sci. USA* 77, 1696–1700.

Yu, K., Ahrens, S., Zhang, X., Schiff, H., Ramakrishnan, C., Fenno, L., Deisseroth, K., Zhao, F., Luo, M.-H., Gong, L., et al. (2017). The central amygdala controls

learning in the lateral amygdala. *Nat. Neurosci.* 20, 1680–1685. doi:10.1038/s41593-017-0009-9.

Zelikowsky, M., Pham, D. L., and Fanselow, M. S. (2012). Temporal factors control hippocampal contributions to fear renewal after extinction. *Hippocampus* 22, 1096–1106. doi:10.1002/hipo.20954.

Zeng, D., and Lynch, K. R. (1991). Distribution of α2-adrenergic receptor mRNAs in the rat CNS. *Molecular Brain Research* 10, 219–225. doi:10.1016/0169-328X(91)90064-5.

Zhang, H.-T., Huang, Y., Mishler, K., Roerig, S. C., and O'Donnell, J. M. (2005). Interaction between the antidepressant-like behavioral effects of beta adrenergic agonists and the cyclic AMP PDE inhibitor rolipram in rats. *Psychopharmacology (Berl).* 182, 104–115. doi:10.1007/s00213-005-0055-y.

Zhang, X., He, T., Chai, Z., Samulski, R. J., and Li, C. (2018). Blood-brain barrier shuttle peptides enhance AAV transduction in the brain after systemic administration. *Biomaterials* 176, 71–83. doi:10.1016/j.biomaterials.2018.05.041.