1-1-2016

Analgesia Followed By Long-Term Hyperalgesia Generated By Disinhibition Of The Basolateral Amygdala

Derek Atchley
Wayne State University,

ANALGESIA FOLLOWED BY LONG-TERM HYPERALGESIA GENERATED BY DISINHIBITION OF THE BASOLATERAL AMYGDALA

by

DEREK E. ATCHLEY

THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF ARTS

2016

MAJOR: PSYCHOLOGY

Approved by:

_____

Advisor Date

DEDICATION

I dedicate this thesis to my mom and grandma for always being my biggest fan and for encouraging me to be my absolute best.

ACKNOWLEDGMENTS

First and foremost I would like to acknowledge my advisor Dr. George S. Borszcz because without his help and guidance absolutely none of this would be possible. I would also like to express my sincerest gratitude to the other members of my committee. Dr. Michelle Tomaszycki and Dr. Susanne Brummelte have been instrumental in making this project the best it could be. Finally I would like to thank my colleagues Sean Callan, Zeb Keehn, Jennifer Kott, Sean Mooney-Leber, and Ben Biermeier-Hanson for statistical help and offering me helpful insights.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1 INTRODUCTION

Pain is an unpleasant sensory and emotional experience in response to actual, perceived, or potential tissue damage (IASP, 1984). Pain is a warning signal that something is wrong and can have protective effects necessary for survival. The aversive quality of pain motivates an individual to withdraw from a dangerous situation and to seek help or medical attention in order to prevent further damage (Institute of Medicine, 2011). Perception of pain is unique to the individual and can be based on biological (sensory), psychological (emotional and cognitive), and social factors (Institute of Medicine, 2011). When pain becomes chronic, however, it is no longer protective and can negatively impact the well being of the suffering individual. It is estimated that 116 million people suffer from common chronic pain conditions including headaches and lower back pain. The economic cost of chronic pain is estimated to be between $560 and 635 billion dollars annually (Institute of Medicine, 2011).

1.1 Stress & Pain

Stress produces bimodal effects on pain perception. During exposure to a stressor responses to painful stimuli are inhibited, i.e. stress-induced analgesia (Butler & Finn, 2009); however, following exposure to a long-term stressor, increases in responsiveness to painful stimuli may develop; stress-induced hyperalgesia (Jennings, Okine, Roche, & Finn, 2014). Stressors activate a well-characterized subcortical circuit that detects threatening stimuli and produces innate defensive behaviors (Chapman, Tuckett, & Song, 2008; LeDoux, 2000). Here, I evaluated how a key component of the subcortical defense circuit, the basolateral amygdala, contributes to production of both stress-induced analgesia and hyperalgesia.

1.2 Defense Circuit

A well-characterized subcortical defense circuit generates defensive behaviors, such as vocalizations, in response to a threat. This network developed as evolutionary pressures selected for a mechanism to encourage the avoidance of threats (Mobbs et al., 2010). For example, rodents that have been exposed to a natural predator will display freezing or flight behavior (Mobbs et al., 2010). This circuit is composed primarily of hypothalamic nuclei, the periaqueductal gray (PAG), and medullary nuclei (Keay & Bandler, 2001).

Stress exposure results in a bimodal response of either active or passive coping strategies (Keay & Bandler, 2001). Active coping strategies can include motor activity, vigilance, hyperreactivity, and tachycardia, while passive strategies are associated with immobility, hypotension, and decreased vigilance (Keay & Bandler, 2001). An active or passive coping strategy is mediated by the PAG. The columnar organization within the PAG gives rise to these opposing responses (see Figure 1). Active coping strategies are activated by the lateral (lPAG) or dorsolateral (dlPAG) columns, while the ventrolateral (vlPAG) column is associated with passive strategies. A calm animal will respond as if there is a threat following activation of the lPAG or dlPAG (Keay & Bandler, 2001). Activation in the rostral region of these columns results in confrontational behavior and is associated with extracranial blood flow (Keay & Bandler, 2001). Activation in the caudal area leads to a flight or escape response and increased blood flow to skeletal muscles (Keay & Bandler, 2001). In contrast, activation of the vlPAG in calm prey animals, such as rats, results in immobility and bradycardia (Keay & Bandler, 2001). Activation of these different columns also results in differences in pain sensitivity. Non-opioid, short-term analgesia can be evoked by stimulation of the lPAG and dlPAG. Longer lasting, opioid mediated analgesia can be evoked following vlPAG stimulation (Keay & Bandler, 2001).

In addition to different coping strategies, the different PAG columns have different afferent and efferent projections. Projections from lPAG and vlPAG terminate in the rostral ventral medulla (RVM) (Keay & Bandler, 2001). The dlPAG projects to the cuneiform nucleus, which then projects to the ventrolateral medulla (VLM) (Keay & Bandler, 2001). Therefore the PAG activates spinopetal inhibitory projections via the RVM and VLM (Basbaum & Fields, 1984; Keay & Bandler, 2001). Spinal projections to the PAG terminate in the lateral and ventrolateral columns, with sensory information from the face and forelimbs projecting to the rostral regions and the lower half of the body projecting to the caudal region (Keay & Bandler, 2001). The dlPAG does not receive spinal input directly, and therefore is likely activated by non-physical stressors (Keay & Bandler, 2001).

The dlPAG and interconnected dorsomedial ventromedial hypothalamus (dmVMH) both show increased c-Fos activation following exposure to threatening stimuli (Canteras & Goto, 1999; Comoli, Ribeiro-Barbosa, & Canteras, 2003). Stimulation of these structures generates defensive behaviors and vocalizations even when not in the presence of a threat (Fernandez De Molina & Hunsperger, 1962). For example, electrical or chemical stimulation of the dmVMH elicits vocalization after discharge (VAD)-like vocalizations in rats, and subthreshold chemical stimulation of dmVMH selectively lowered the threshold of tailshock elicited VADs and facilitated fear conditioning supported by tailshock (Borszcz, 2006). In humans, stimulation of these sites leads to reports of fear and anxiety (Ervin, Mark, & Stevens, 1969; Iacono & Nashold, 1982). The dmVMH sends glutamatergic projections to the dlPAG, which then coordinates activity within the brainstem to generate behavioral defensive and vocal responses (Jurgens, 2002; Schubert, Shaikh, & Siegel, 1996). In response, the dlPAG sends excitatory projections back to the dmVMH to create a positive feedback loop that sustains defensive responding during exposure

to an environmental threat (Bhatt, Gregg, & Siegel, 2003). Figure 2 shows a complete diagram of the brain structures and connections within the subcortical defense circuit.

The amygdala, which responds to threatening stimuli and attaches emotional significance to them (LeDoux, 2007), provides excitatory input to the subcortical defense circuit to generate defensive behaviors to a perceived threat (Shaikh, Schubert, & Siegel, 1994). As the prototypical threat is exposure to noxious stimuli, the amygdala receives nociceptive input to the central nucleus (CeA) through the spinoamygdaloid and the spinopontoamygdaloid pathways (Bernard, 1990; Jasmin, Burkey, Card, & Basbaum, 1997; Newman, Stevens, & Apkarian, 1996). The BLA also receives pain input through from the thalamus and cortex (Millan, 1999).

Together these structures generate the defensive behaviors and modulate pain responding. Fanselow and Lester (1988) suggest that the differences in behaviors, generated by the defense circuit, and subsequent effects on pain can be explained by the predator imminence theory. Defensive responding is determined by the prey's perception of the chance of consumption by a predator that generates the appropriate response. Imminence is determined by spatial and temporal distance of the threat as well as threat characteristics (Fanselow, 2016). Each animal has a repertoire of defensive behaviors that can be divided into pre-encounter, post-encounter, and circa-strike behaviors. Pre-encounter behaviors include cautiousness to leave the nest, meal reorganization and hypervigilance (Fanselow and Lester, 1988). Post-encounter defensive behaviors include freezing in rats to avoid detection by the threat. Once contact with the threat or predator is imminent, circa-strike behaviors are used, including biting, jumping, and vocalizations in rats (Fanselow, 2016). Helmstetter and Fanselow (1993) have shown that as shock density increases, defensive behaviors increase within each stage until moving along this continuum to the next type of behavior. Therefore, predatory imminence determines the type of defensive behavior

that is generated which includes the subject's response to noxious stimulation (see below). This is consistent with the active and passive coping strategies and columnar organization of the PAG presented by Keay and Bandler (2001). As threat level increases coping strategy will shift from a passive strategy, such as freezing, to a more active strategy of flight or fight if escape is not possible. These shifts in behavior can be explained by the defense circuit and PAG subcolumns (Keay and Bandler, 2001).

1.3 Stress-Induced Analgesia

Fear and pain have been described as competing systems and, during a threatening situation fear is given priority while pain perception is suppressed (Bolles & Fanselow, 1982). This idea is also in line with the predator imminence theory. If contact with a predator is imminent, injury is likely to occur causing the animal to direct behavior toward the injured site detract from production of circa-strike behaviors. Situations activating circa-strike behaviors also activate endogenous analgesia systems (Fanselow and Lester, 1988). Endogenous analgesia is important for survival and prevents pain behaviors from disrupting the species-specific defense responding (Fanselow, 1984). Thus aversive events generate defensive responding as well as analgesia, a phenomenon known as conditional analgesia or fear-conditioned analgesia. Exposure to a cat increased the shock threshold necessary to induce a tail-flick response and increased tail-flick latency to radiant heat (Lichtman and Fanselow, 1990). Further support for fear mediation of conditional analgesia is that midazolam and diazepam (GABA agonists) decrease freezing to a conditional stimulus previously paired with a footshock and increase recuperative behavior following shock (Fanselow & Helmstetter, 1988). Although, the analgesic effect during conditioning can be reversed with the opioid antagonists naloxone and naltrexone, it suggests that benzodiazepines ($GABA_A$ agonists) act at a site before the fear and analgesic systems diverge (Fanselow & Helmstetter, 1988).

The amygdala is critical for Pavlovian fear conditioning and is the proposed site of integration of sensory and motor information underlying the expression of conditioned fear (Borszcz & Leaton, 2003). Diazepam microinjected into the basolateral amygdala (BLA) reduced the amount of burying (a measure of anxiety) as well as burying latency in the burying behavior test in addition to reducing pain symptoms associated with uric acid injection into the knee. A similar effect was seen when diazepam was microinjected into the dlPAG (Jimenez-Velazquez, Lopez-Munoz, & Fernandez-Guasti, 2010). The simultaneous anxiolytic and analgesic effect of diazepam suggest that these processes are related and can influence one another (Jimenez-Velazquez et al., 2010). Lesions to the CeA elevated shock thresholds to elicit VADs in addition to preventing conditional vocalizations (Borszcz & Leaton, 2003). Further support for the role of the amygdala in conditional analgesia is the finding that muscimol, a $GABA_A$ agonist, prevented the expression of conditional fear as well as fear conditional analgesia (Rea, Roche, & Finn, 2011). Additionally, GABA levels were reduced in the BLA and dlPAG in animals undergoing fear conditioning (Rea, Lang, & Finn, 2009).

Amygdalar stimulation reduces tonic pain, as evidenced by decreased pain scores in the formalin test, based on time spent elevating, licking, or shaking the paw (Mena, Mathur, & Nayar, 1995). Additionally, amygdalar activation decreases phasic pain as measured by increased tail flick latency to noxious heat (Mena et al., 1995). Electrical or chemical stimulation of the amygdala also alleviated affective pain, suggested by an increase in vocalization during discharge (VDS) and VAD thresholds to tail shock that was mediated via activation of the vlPAG (Mena et al., 1995; Spuz & Borszcz, 2012, 2014).

Furthermore, morphine administered directly into the BLA increased VAD thresholds. These elevated responses were attenuated with a morphine antagonist (Nandigama & Borszcz,

2003). Additionally, the mu opioid agonist, DAMGO, when injected bilaterally into the BLA, increased tail flick latencies evoked by radiant heat (Helmstetter, Tershner, Poore, & Bellgowan, 1998). This antinociceptive effect was mediated by both the RVM and vlPAG; lidocaine injected into these areas following morphine in the BLA attenuated the antinociceptive effect (Helmstetter et al., 1998). These findings suggest that spinal nociceptive reflexes can be modulated through a descending circuit that includes the amygdala as well as the PAG and RVM (Helmstetter et al., 1998).

1.4 Stress-Induced Hyperalgesia

While the analgesic effect of stress is well documented, the hyperalgesic effect associated with prolonged stress is less understood (Olango and Finn, 2014). Rhudy and Meagher (1999) suggest that this could be due to a failure of animal models and human studies to distinguish between fear and anxiety. Fear is a response to a present threat and mobilizes the organism to take action, whereas anxiety is a fear response to a perceived or potential threat that leads to increased environmental scanning and sensory receptivity (Rhudy & Meagher, 2000). This idea is also consistent with the predatory imminence theory. When a threat is not present, animals will maintain their preferred activities such as foraging and searching for a mate (Fanselow and Lester, 1988). However, in anticipation of a threat, even when not present, these behaviors are readjusted to activate pre-encounter behaviors, which may reduce foraging, increase hypervigilance, and increase cautiousness to leave the nest (Fanselow and Lester, 1988). These behaviors increase reactivity to environmental stimuli, including noxious stimulation (Fanselow and Lester, 1988).

In order to observe the divergent effects on pain by fear and anxiety, Rhudy and Meager (2000) exposed human participants to a shock, a threat of shock, or a neutral condition and measured finger withdrawal latencies in a radiant heat test. Participants exposed to the shocks had

longer withdrawal latencies compared to the neutral condition (i.e., analgesia or antinociception), while participants who were told there may be shocks given had shorter withdrawal latencies compared to the neutral condition (i.e., hyperalagesia; Rhudy & Meagher, 2000). These results suggest the presence of two different phenomena, fear-induced analgesia and anxiety-induced hyperalgesia (Rhudy & Meagher, 2000).

Animal models have supported the phenomenon of anxiety-induced hyperalgesia as rats exposed to repeated social defeat stress exhibit anxiety-like behavior in the elevated plus-maze (EPM) as well as sensory hypersensitivity in the Von-Frey filament test, as well as increased pain scores in the formalin test (Rivat et al., 2010). Elevated pain scores following social defeat were reduced to control levels by chlordiazepoxide ($GABA_A$ agonist) as well as systemic administration of a CCK (cholecystokinin) antagonist (Rivat et al., 2010). Exposure to the EPM has been shown to be a stressor itself, and following exposure rats exhibited decreased paw withdrawal latencies in the hotplate test, which was reversed by a muscimol injection into the RVM (Cornelio, Nunes-de-Souza, & Morgan, 2012).

Chronic stress has been shown to increase dendritic branching and spine density in the basolateral amygdala (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005) and to increase spontaneous firing of BLA neurons (Rosenkranz, Venheim, & Padival, 2010). The BLA projects directly to the CeA through glutamatergic projections and indirectly through GABAergic interneurons located in the intercalated cell masses (ITC) (Ren & Neugebauer, 2010). In an arthritic model of pain, excitatory input from the BLA to the CeA increased, whereas inhibitory input decreased (Ren & Neugebauer, 2010). Additionally, reciprocal connections were strengthened between the CeA and the parabrachial nucleus (Neugebauer, Galhardo, Maione, &

Mackey, 2009). This pain related input from the BLA with nociceptive specific input from the spinal cord (Neugebauer et al., 2009).

Chronic stress also affects the inhibitory regulation of the medial prefrontal cortex (mPFC) to the BLA. The mPFC and BLA have reciprocal projections that are important for emotional regulation as well as the generation of defensive responding (Quirk and Beer, 2006). In contrast to the amygdala, dendritic branches in the mPFC are reduced following chronic stress (Cook and Wellman, 2004). This reduction in the mPFC allows the amygdala to be more responsive to stressors. Indeed, lesion of the mPFC increased neuronal firing rate in the amygdala in response to acute cold stress (Correll, Rosenkranz, and Grace, 2005). Additionally, electrolytic lesion of the mPFC increased recovery of an extinguished fear (Quirk and Russo, 2000). Together, Decreased regulation by the mPFC and increased amygdala responsiveness to stress suggest an important contribution of the BLA to increased defensive responding leading to environmental hypervigilance that may contribute to the development of hyperalgesia.

1.5 Hypotheses

In the current study bicuculline methiodide (a $GABA_A$ antagonist) was used to mimic the effects on the brain during exposure to a stressful stimulus. As stress hormones act within the BLA to reduce GABAergic signaling (Anderjaska, 2007), disinhibition of the BLA will allow for a similar effect within the brain without the need for a behavioral stressor. Bicuculline acts as a competitive antagonist of $GABA_A$ receptors by reducing chloride ion channel open times and opening frequency reducing GABAs normal induction of Inhibitory Post-Synaptic Potentials (IPSPs) (Johnston, 2013). Behaviorally, microinjection of bicuculline into the BLA of rodents resulted in an increase in heart rate and blood pressure (Sanders, 1991) similar to that observed follow stressor exposure. Peak behavioral effects were reached within 11.5 minutes and lasted for

23 minutes (Sanders, 1991). Microinjection of 20pmol of bicuculline into the BLA resulted in decreased social interaction, indicative of anxiety-like behavior (Shekhar, 1995). Additionally, .1µg of bicuculline in the BLA decreased time spent in the open arms of the elevated plus-maze as well as the number of open arm entries with no effect on total number of arm entries suggesting an anxiogenic effect (Zarrindast, 2008). Together these findings suggest that microinjection of bicuculline methiodide into the BLA is a good model to examine the effects of stress on pain sensitivity. However it is important to note that this only mimics one aspect of the stress response in one brain region. Chronic stress can have a multitude of effects in multiple brain areas as well as the body. Figure 13 illustrates other ways in which stress may affect the brain and can influence amygdalar activity through indirect routes as well.

The hypotheses for this study were as follows: First, immediately following bicuculline administration to the BLA, I expected that pain responsiveness would decrease as measured by amplitudes of VADs and VDSs. This acute effect of bicuculline is hypothesized to mimic exposure to stressor (i.e., high predatory imminence - fear) and engage endogenous antinociceptive mechanisms. Likewise, as exposure to a predator can inhibit withdrawal reflexes, I expected a decrease in Spinal Motor Reflex (SMR) immediately after intracerebral injection of bicuculline. I expected to see an increase in VADs and VDS, with no change in SMRs, in the days and weeks following bicuculline administration. This delayed hyperalgesia is hypothesized to reflect the aftereffects of stressor exposure (i.e., lower predatory imminence – anxiety) that produces environmental hypervigilance and reactivity to potential threats including noxious stimuli (i.e., hyperalgesia). I also expected that pain responsiveness in animals treated with saline injection would remain stable over time with no significant differences. Similarly, administration of bicuculline into brain sites surrounding the BLA should have also resulted in stable pain

responsiveness over time. Finally, I hypothesized that bicuculline would have no effect on the animals' ability to vocalize and that changes in vocalizations were due to changes in pain sensitivity rather than an increase or decrease in ability to vocalize.

CHAPTER 2 METHOD

2.1 Subjects

Subjects were adult Male Long-Evans rats (between 226-250g upon arrival) (Charles River, Wilmington, MA). All subjects were housed in pairs in polycarbonate cages in a climate-controlled vivarium and were given access to food and water *ad libitum*. The vivarium was on a 12:12 light-dark cycle with lights on at 0700 hours. All procedures were conducted between 0800 and 1800 hours. Upon arrival, rats were given one week to acclimate and then handled once per day for 1 week to minimize the possibility of stress due to human contact.

2.2 Cannulation Surgeries

The Institutional Animal Care and Use Committee of Wayne State University approved all procedures and surgeries were performed under aseptic conditions. Rats were anesthetized with isoflurane (induced at 5% and maintained between 2 and 3% throughout the procedure. Prior to incision, a local analgesic injection of lidocaine (.05 mg/kg**)** and bupivacaine (1.5 mg/kg) was applied locally. Additionally, an IP injection of meloxicam (1mg/kg) was given prior to the start of surgery. Rats were implanted bilaterally with 26 gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) above the BLA (2.8mm posterior to Bregma, 4.9 mm lateral from midline, and 6.4mm ventral from the top of the skull) using a stereotaxic apparatus. Coordinates were determined according to the rat brain atlas of Paxinos and Watson (Paxinos and Watson 1998). Guide cannulae were attached to the skull with 3 stainless steel bone screws (3/16") and dental cement. Rats were allowed 7 days to recover before initiation of behavioral testing.

2.3 Testing Apparatus

Rats were placed in custom made Velcro suits and restrained to a Plexiglas pedestal to restrict movement during injections and behavioral testing. Testing was conducted in a lit, sound-attenuating chamber with a ventilation fan. A window located on the door of the chamber allows for the monitoring of animals during testing. Tailshock was delivered through electrodes (0-guage stainless steel insect pins) in 20ms pulses at 25Hz for 1,000ms. Electrodes were placed intracutaneously on opposite sides of the tail. Spinal Motor Reflexes (SMR) were measured with a semi-isotonic displacement transducer (Lafayette Instruments Model 76614, Lafayette, IN) attached to the rat's tail with cotton thread. SMR was defined as movement of the transducer arm by at least 1.0mm following shock onset. Latency (ms), amplitude (mm), and magnitude (cm x ms) of tail movement were measured for each trial. Vocalizations were recorded by an omnidirectional boundary microphone (Optimus, Barcelona, Spain). The microphone was placed on the wall of the testing chamber approximately 15cm from the rat's head. Peak intensity was recorded in decibels as well as latency (ms) and duration (ms) of vocalizations during the shock presentations (VDS) and for 2,000ms following shock termination referred to as vocalizations after discharge (VADs).

2.4 Pain Testing

Prior to the onset of testing rats were exposed to the Velcro suit and testing chamber for 20 minutes per day for 2 days in order to reduce the effects of restraint stress. Testing began immediately following intracerebral injections. Baseline shock level was first determined based on vocalization and response rate. All animals began testing with a shock intensity of .4 milliamps. Vocalizations around 70 decibels or a 50% response rate maintained baseline at .4 milliamps. If vocalizations and response rate were higher, the shock intensity was reduced to .3ma. Decreased

vocalizations and response rates resulted in an increase in shock intensity to .5ma. Once established, this shock intensity was used throughout the remainder of the experiment. Trials consisted of 6 shocks and one catch-trial, in random order, to assess false alarm rates. There was a minimum inter-trial interval of 30 seconds, however the following shock was not given until vocalization and movement from the previous shock had ceased. The chamber was cleaned with 5% ammonium hydroxide following each testing session in order to eliminate potential stress odors. Pain behaviors were assessed immediately following bicuculline administration (day 1) as well as 4,7,14, and 21 days following intracerebral injections (see figure 3).

2.5 Drug Injections

Intracerebral injections were given through each cannula with a 33-gauge injector that extends 2.2mm beyond the end of the guide cannula. Bicuculline or saline was injected at a constant volume of .25µl delivered over one minute via an infusion pump (Harvard). Injectors remained in place for 2 minutes to allow the drug to diffuse into the tissue. A dose of 100 pmol of bicuculline was delivered in each hemisphere.

2.6 Groups

Subjects were divided into 4 groups. All groups received an injection of saline prior to baseline pain testing. The following day began test day 1. An experimental group (n = 10) received an injection of bicuculline into the BLA on day 1 as described above. A drug control group (n = 12) received another injection of saline on day 1. The anatomical control group (n = 6) had cannula surgically implanted as described above but in sites surrounding the BLA. All anatomical control animals received bicuculline on day 1. A vocal ability control group received cannula implantation to the BLA and followed all procedures as the experimental group, however during pain testing

they were exposed to 20 shocks of varying intensity ranging from .01 milliamps to 2.0 milliamps in order to elicit vocalizations despite bicuculline treatment .

2.7 Histology

Following the last testing session rats were sacrificed by carbon dioxide asphyxiation and were then injected intracerebrally with .25µl of safrin-O dye in order to mark injection sites. Brains were then removed and placed in sugar buffered formalin for at least 24 hours. Brains were sectioned at 50µm on a freezing microtome. The site of injection was confirmed with the aid Paxino and Watson's rat brain atlas (Paxino and Watson 1998).

2.8 Data Analysis

Data were analyzed in two separate mixed factorial Analysis of Variance (ANOVA) in order to separately compare the analgesic and hyperalgesic components of the experiment. The first ANOVA compared baseline testing to test day 1 for SMR, VDS, and VAD measures. The second mixed factorial ANOVA compared baseline testing to days 4, 7, 14, and 21 for SMR, VDS, and VAD. Follow up analyses included separate repeated measures ANOVAs comparing saline or bicuculline over time. Additionally *t*-tests were run comparing saline to bicuculline at different test days of interest, as needed based on findings from the previous analyses. Sidak corrections were used to correct for multiple comparisons in the repeated measures ANOVAs and *t*-tests. One-way repeated measures ANOVAs with Dunnett's post-hoc tests were used to analyze anatomical control subjects for SMR, VDS, and VAD measures comparing each test day to baseline responding. The same analysis was used for the vocal ability control group.

CHAPTER 3 RESULTS

3.1 Vocalizations

Amplitudes for VADs and VDSs were analyzed using two mixed factorial ANOVAs. The first compared baseline to test day 1 for bicuculline and saline treated rats (see figures 4 and 5). There was a significant main effect of time for both VADs and VDSs ($F(1, 130) = 12.85$, $p < .001$ and $F(1, 130) = 49.17$, $p < .001$ respectively). There was also a significant main effect of drug for VADs ($F(1, 130) = 147.33$, $p < .001$) and VDSs ($F(1, 130) = 271.67$, $p < .001$). The Time x Drug interaction was also significant ($F(1, 130) = 27.08$, $p < .001$ and $F(1, 130) = 27.08$, $p < .001$). To further understand the effects, separate paired samples t-tests were conducted for saline and bicuculline for VADs and VDSs. A paired samples t-test comparing bicuculline animals showed significant decrease in VAD and VDS amplitude on test day 1 compared to baseline ($t(59) = 6.83$, $p < .001$ and $t(59) = 7.39$, $p < .001$ respectively). There was no difference in saline animals on day 1 compared to baseline for VADs ($t(71) = 1.11$, $p = 0.27$) or VDSs ($t(71) = 1.58$, $p = 0.12$). VAD amplitudes for saline and bicuculline treated rats did not differ at baseline ($t(130) = .761$, $p = .45$) and there was a significant difference between saline and bicuculline on day 1 ($t(130) = -7.25$, $p < .001$). A similar effect was observed for VDS amplitudes. There was no difference between saline and bicuculline at baseline ($t(130) = -.569$, $p = .570$). There was, however, a significant difference on test day 1 ($t(130) = -6.619$, $p < .001$. These findings demonstrate that immediately following bicuculline treatment the capacity of noxious tailshock to generate VADs and VDSs was reduced indicating an antinociceptive action of bicuculline injected into BLA. Indeed, of the 10 rats tested only 1 produced VADs and 3 produced VDSs immediately following the injection of bicuculline into BLA.

The hyperalgesic component was measured using a mixed factorial ANOVA comparing baseline and test days 4, 7, 14, and 21 for both VADs and VDSs. There was a significant main effect of time ($F(3.68, 520) = 8.65$, $p < .001$ and $F(3.63, 520) = 5.69$, $p < .001$ respectively). A

significant main effect of drug was also observed for both VADs ($F(1, 130) = 270.62$, $p < .001$) and VDSs ($F(1, 130) = 618.79$, $p < .001$). The Time x Drug interaction for VADs was not significant ($F(3.68, 520) = 1.68$, $p = .16$) but was significant for VDSs ($F(1, 130) = 27.08$, $p < .001$). In order to further understand these effects separate one-way repeated measures ANOVAs were conducted. There was a significant main effect of bicuculline for both VADs and VDSs ($F(3.423, 202.0) = 6.119$, $p < .001$) and Dunnett's post-hoc tests revealed that VAD amplitudes were elevated on days 4 and 14 while VDS amplitudes were elevated on days 7 and 14 compared to baseline. There was a significant main effect of saline on VADs ($F(3.573, 253.7) = 6.352$) but not for VDSs ($F(3.659, 259.8) = 2.87$). Dunnett's post-hoc tests revealed that VAD amplitudes were significantly increased on day 14 and 21. VAD amplitude in bicuculline treated rats was significantly greater than saline rats on day 4 ($t(130) = 2.52$, $p < .013$) while no difference was observed on days 14 ($t(130) = .926$, $p = .356$) or 21($t(130) = .052$, $p = .958$). VDSs were significantly greater in bicuculline rats compared to saline on day 7 ($t(130) = 3.31$, $p = .001$) but not on day 14 ($t(130) = .597$, $p = .55$). Locations of bicuculline and saline injections are shown in figure 6 as well as a photomicrograph of an injection site (figure 7). These findings suggest that VAD amplitudes are enhanced 4 days and VDS amplitudes are enhanced 7 days following bicuculline administration into the BLA, indicating a hyperalgesic effect.

3.2 Spinal Motor Reflex

A mixed factorial ANOVA compared baseline testing to day 1 for bicuculline and saline rats (see figure 8). In contrast to findings on vocalization amplitudes, there was no significant main effect of time ($F(1, 130) = .075$, $p = .78$) but there was a significant effect of drug ($F(1, 130) = 1099.17$, $p < .001$). No significant Time x Drug interaction ($F(1, 130) = 3.28$, $p = .07$) was observed. The

reduction in vocalization amplitudes observed immediately following bicuculline treatment was not observed for SMR amplitude.

Baseline SMR amplitude was compared to testing days 4, 7, 14 and 21 for bicuculline and saline treated animals (see figure 8). There was no significant main effect of time ($F(3.506, 520) = 1.26$, $p = .29$). A main effect of drug ($F(1, 130) = 2211.11$, $p < .001$). There was a significant Day x Drug interaction ($F(3.506, 520) = 3.94$, $p < .01$). Within subjects contrasts were used to compare days 4, 7, 14, and 21 to baseline separately for saline and bicuculline rats. Bicuculline had no effect on SMR amplitude over time, ($F(3.21, 236) = 1.70$, $p = .15$). There was a significant effect of saline over time, ($F(3.65, 284) = 4.09$, $p < .01$). Dunnett's post hoc tests showed that SMR amplitude decreased in saline rats on days 4, 7, and 14 compared to baseline responding.

3.3 Anatomical Controls

One-way repeated measures ANOVA of VAD (see figure 9a) and VDS (see figure 9b) amplitudes revealed significant changes across days of testing (VAD, $F(5, 175) = 10.79$, $p < .001$; VDS, $F(5,175) = 4.72$, $p < .01$). Dunnett's post-hoc analysis comparing baseline amplitudes with amplitudes on each test day showed that VAD amplitudes were elevated on days 7, 14, and 21 and VDS amplitudes were elevated on day 14. A one-way repeated measures ANOVA revealed no significant effect of bicuculline on SMR (see figure 9c) responding over time ($F(5, 175) = 2.42$, $p = .07$). These findings suggest that the analgesic effect observed following bicuculline administration is specific to the BLA and not due to spread of the drug to surrounding brain areas. Injection sites and a photomicrograph of an injection site can be seen in figure 10.

3.4 Vocal Ability Controls

In order to confirm that decreases in vocalizations were not due to bicuculline causing an inability to vocalize, a separate group of rats were exposed to varying shock levels from .01mA to

1.5mA. Vocalizations within the .3mA to .5mA range were analyzed in order to maintain comparisons with other experimental groups. A paired samples t-test revealed no significant difference in VAD, VDS, or SMR on day 1 compared to baseline responding. Similarly, a one-way repeated measures ANOVA revealed no significant differences in responding on day 4, 7, or 14 compared to baseline for VAD ($F(4, 56) = 1.35$, $p = .26$) VDS ($F(4, 56) = .93$, $p = .45$), or SMR ($F(4, 56) = .811$) see figures 11a, b, and c respectively. Additionally, response thresholds, or the minimum shock intensity necessary to elicit a response, were measured. A one-way repeated measures ANOVA revealed no significant main effect of bicuculline administration on VAD threshold over time, $F(4,16) = 3.06$, $p = .094$. Similarly a one-way repeated measures ANOVA showed no significant effect of bicuculline administration on VDS threshold over time ($F(4,16) = 4.18$, $p = .08$). There was no main effect of bicuculline administration on SMR threshold over time ($F(4,16) = 2.11$, $p = .18$). These findings suggest that analgesic effects observed on vocalization measures was not due to an inability to vocalize following bicuculline injection into the BLA. Injection sites for vocal control rats can be seen in figure 12.

CHAPTER 4 DISCUSSION

The primary purpose of the present study was to evaluate how a key component of the defense circuit, the basolateral amygdala, contributes to the bimodal influence of stressors on pain responsiveness. In this study, intracerebral injections of bicuculline in the BLA were used to simulate the effects of stress on this region and pain responsiveness was assessed at various time points. I demonstrated a bimodal response to bicuculline as pain responding decreased immediately following injection yet increased in the days and weeks following injection. To my knowledge, this is the first study to exhibit both stress-induced analgesia and stress-induced hyperalgesia in the same animals over time. Additionally this is the first study to show that

bicuculline injection into the BLA results in analgesia. The findings of this study could have profound effects on establishing a baseline from which to study the transition of the facilitating effects of stress (analgesia) to the debilitating effects of stress (hyperalgesia) on pain responsiveness.

4.1 Stress-induced analgesia

Under normal circumstances the BLA is responsible for attaching emotional salience to environmental stimuli (Ledoux, 2000). Chronic stressors were shown to facilitate sensory transmission within the BLA through disinhibition of the GABAergic tone. Indeed chronic exposure to urocortin, a CRF 1 and 2 agonist, resulted in a decrease in spontaneous and stimulated IPSPs within the BLA, and an increase in the excitability of BLA projection neurons to afferent stimulation. Similarly this effect was seen following bicuculline administration as well (Rannie et al., 2004). Behavioral changes were also observed as urocortin injected in BLA resulted in increased heart rate and respiration rate and also resulted in decreased open arm exploration in the EPM – a behavioral measure of anxiety in rats (Rannie et al., 2004). Again, similar changes were observed following bicuculline injection into BLA, suggesting it is a good model to mimic the effects of stress.

The analgesic effects of bicuculline injection into the BLA observed in the current study were strong and had anatomical specificity. Immediately following injection of bicuculline into the BLA pain responding decreased as measured by a decrease in the amplitude of both VDS and VAD. On the other hand, the amplitude of SMR was not influence by the intra-BLA administration of bicuculline. As VAD and VDS are respectively organized at forebrain and medullary levels of the neuraxis, and SMR within the spinal cord these findings indicate that bicuculline injected into BLA preferentially suppresses pain transmission at supraspinal levels of the neuraxis. The failure

of bicuculline to suppress SMR amplitude does not reflect the insensitivity of this response to antinociceptive treatments. In previous studies, administration of morphine into the rostral ventromedial medulla or vPAG produced significant suppression of VAD, VDS, and SMR (Blanchard, Rodgers, Hendrie, & Hori, 1988; Borszcz, 2002), and the intrathecal administration of morphine, 5-HT, or norepinephrine was equally effective in suppressing VAD, VDS, and SMR (Borszcz, Johnson, Anderson, & Young, 1992). Similar to the present results, administration of 5-HT, a 5-HT agonist or morphine into either the amygdala or thalamus produced selective suppression of VAD and VDS (Floyd, Price, Ferry, Keay, & Bandler, 2000; Gracely, McGrath, & Dubner, 1978; Maren, 2001; Mark, Ervin, & Yakovlev, 1963). Therefore, the capacity of suppress SMR appears to be dependent on the site at which antinociceptive treatments are administered.

Several studies reported suppression of the spinally organized tail flick response to radiant heat in rats following administration of morphine into BLA. Helmstetter and colleagues reported that administration of morphine or μ-opiate agonists into BLA of pentobarbital-anesthetized rats produced suppression in tail-flick, and that this effect was expressed through the vPAG and RVM (Helmstetter, Bellgowan, & Poore, 1995; Helmstetter, Bellgowan, & Tershner, 1993; Helmstter, Tershner, Poore, & Bellgowan, 1998). McGaraughty and Heinricher (2002) also reported suppression of tail flick in barbiturate-anesthetized rats following the injection of morphine into the BLA. However, barbiturate anesthesia reportedly modifies the antinociceptive action of intracerebrally administered opiates in the tail-flick test (Ossipov & Gebhart, 1984; Smith, Robertson, & Monroe, 1992). Whether barbiturate anesthesia modifies the antinociceptive action of opiates administered into the amygdala has not been directly tested.

However, Nandiagama and Borszcz (2003) reported that injection of morphine into the BLA in conscious rats suppressed VAD and VDS but not SMR – findings similar to those found

in the present study. This finding is consistent with other reports that microinjection of morphine, μ-opiates, enkephalinase inhibitors, or acetylcholine agonists into various forebrain sites including the amygdala and medial thalamus failed to alter withdrawal reflexes but suppressed supraspinally organized pain behaviors in conscious rats (Al-Rodhan, Chipkin, & Yaksh, 1990; Carr & Bak, 1988; Dupouy & Zajac, 1997; Harte, Lagman, & Borszcz, 2000; Rodgers, 1978; Yeung, Yaksh, & Rudy, 1978). Furthermore, administration of methysergide (serotonin receptor antagonist) into the amygdala failed to alter increases in SMR threshold produced by microinjection of morphine into vPAG, but reduced increases in VAD and VDS thresholds (Borszcz, 1999; Borszcz & Streltsov, 2000). Similarly, inactivation of the amygdala, via microinjection of lidocaine, did not alter the suppression of tail-flick generated by electrical stimulation of vPAG (Oliviera & Prado, 2001). On balance, the available data indicates that these forebrain sites do not to contribute directly to suppression of withdrawal reflexes, or are only marginally involved in this suppression.

The suppression of VDS and VAD amplitude immediately following bicuculline injection into BLA may reflect engagement of endogenous antinociceptive systems that occur during execution of circa-strike behaviors (responses that occur immediately before, during and immediately after confrontation with a predator) when predatory imminence is high. For the rat these behaviors include biting, jumping, vocalizations, and attempts to escape. Engagement of endogenous antinociceptive systems during these times enables the individual to continue executing defensive behaviors in the face of painful injury (Bolles, 1980; Fanselow & Lester, 1988; Perusini and Fanselow, 2016). For example, analgesia has been reported when rats are exposed to a cat (Lictman and Fanselow, 1990; Lester & Fanselow, 1985) and following conditioned fear (Fanselow, 1986; Fanselow & Helmstetter 1988; Rea, Lang, & Finn, 2009; Rea, Roche, & Finn, 2011). Odors from stressed rats also resulted in opiate analgesia in unstressed rats

(Fanselow, 1985; Fanselow & Sigmundi, 1986). I propose that the injection of bicuculline into BLA and subsequent placement in the testing chamber could pose an imminent threat and therefore activate circa-strike behaviors including activation of the endogenous analgesic system. Consistent with this suggestion are reports that administration of bicuculline into BLA produces behavioral and autonomic responses that mimic threat exposure (Sanders, 1991; Shekhar 1995). The findings presented here also highlight a possible mechanism for the analgesic effect produced by fear. As the amygdala provides excitatory inputs into the defense circuit, a reduction in GABAergic signaling is a likely candidate for the effects of fear and stress on pain responsiveness (Shaikh, Schubert, & Siegel, 1994). Consistent with this notion is the recent finding that muscimol, a $GABA_A$ agonist administered into the BLA, had the opposite effect and blocked the expression of fear-induced analgesia (Rea, Roche, & Finn, 2011). Together, these findings suggest an important role of the GABAergic system in regulating amygdala output to the defense circuit during times of fear and subsequently expression of stress-induced analgesia.

Although audible vocalizations are part of the circa-strike behavioral repertoire that are designed to disorient the predator and facilitate escape, pain-induced vocalizations during these times may be suppressed. As pain-induced vocalizations (especially VAD) are a validated measure of rats' emotional response to pain it would be beneficial for successful escape that emotional responses to pain are suppressed to the circa-strike epoch. That is, brain processing of pain (as reflected in pain-induce vocalizations) would be suppressed during this time so as not to interfere with the brain's organization of innate behavioral patterns designed to enable the rat to cope with a highly imminent predator. This interpretation suggests that are multiple inputs to the defensive aggression circuit and that during imminent threat by a predator those sensory inputs that represent the predator activate the defense circuit and generate behaviors (including

vocalizations) that enable the rat respond to that threat. At the same time, pain inputs to the defense circuit are suppressed; thereby, preventing pain inflicted by the predator to redirect behavior toward the injury. This selective suppression of pain inputs to the defense circuit represents an antinociceptive action rather than a general suppression of defensive responding. This interpretation can account for my observation that whereas injection of bicuculline into BLA nearly eliminated pain-induced vocalizations, rats vocalized when being placed into or being removed from the testing chamber. Presumably the experimenter (who is temporally associated with BLA injections) comes to represent a predator and evokes vocalizations while handling the rat, but pain-elicited vocalizations are suppressed.

While emotional responding to pain during the execution of circa-strike behaviors are suppressed it may still be beneficial for pain-elicited spinally organized withdrawal reflexes to remain intact. During confrontation with the predator the rat's ability to reflexively withdrawal a limb that in under attack may minimize damage to that limb so preserve the capacity to engage in escape behaviors. This interpretation could account for our finding that whereas emotional responding to pain (vocalizations) is suppressed following bicuculline treatment spinal withdrawal reflexes (SMR) remain intact.

A potential mechanism to explain the analgesia produced by bicuculline administration into BLA could lie in projections from the BLA to the vlPAG. The vlPAG is a nodal structure of the endogenous analgesia system (Borszcz, 1999; Basbaum & Fields, 1984). Previous work from this laboratory demonstrated that injection of morphine into vlPAG produces preferential suppression of VAD and VDS, which is mediated by its reciprocal connections with intralaminar thalamic nuclei and the amygdala (Borszcz, 1999; Borszcz & Strelstov, 2000; Munn et al., 2009). Although the BLA sends neuronal projections to the vlPAG, it appears that the primary input from

the BLA to vlPAG includes a synapse within the central amygdaloid nucleus (CeA). Earlier studies reported that the antinociceptive action of opiates injected into BLA were blocked by inactivation of the vlPAG, but the impact of CeA inactivation to this antinociceptive action has not been evaluated. I hypothesize that following bicuculline administration to the BLA, reduced GABA signaling increases excitatory glutamatergic projections to the CeA, which then activates antinociceptive projection neurons in the vlPAG. This activation results in μ-opioid mediated analgesia (da Costa Gomez, 1995). Indeed, bilateral injection of NMDA into the CeA resulted in antinociception as measured by a selective increase in VAD and VDS thresholds (Spuz and Borszcz, 2014). Likewise, NMDA injection to the CeA increased c-Fos in the vlPAG, and microinjection of a μ-opioid receptor antagonist in vlPAG blocked the increases in VAD and VDS thresholds that followed NMDA injection into CeA (Spuz and Borszcz, 2014).

Exposure to environmental threats also elicits a stress response that includes activation of the HPA-axis. The hypothalamus releases CRF into the portal system of the anterior pituitary causing the release of ACTH into the bloodstream. ACTH acts on the adrenal cortex and adrenal medulla to release glucocorticoids and catecholamines, respectively. These stress hormones provide feedback (directly and indirectly) to the brain circuits that control sensitivity and reactivity to threatening stimuli. It is well established that the BLA is the principal site within the defense circuit where stress hormones act to enhance responding to threatening stimuli (Fig. 5). Epinephrine, which does not cross the blood–brain barrier, induces the release of norepinephrine (NE) in the BLA by activating vagal afferents to the nucleus of the solitary tract (NTS). Noradrenergic neurons in the NTS project directly to the BLA, and indirectly via the locus coeruleus (LC). Glucocorticoids (GCs) freely enter the brain and bind to glucocorticoid receptors in brainstem noradrenergic neurons to potentiate norepinephrine release in the BLA, as well as

postsynaptically on BLA neurons. NE and GCs bind to GABAergic interneurons in BLA to decrease $GABA_A$ receptor-mediated inhibition of BLA projection neurons (Duvarci & Pare 2007, Rodriguez Manzanares et al. 2005, Tully et al. 2007), thereby allowing for increased excitability of BLA projection neurons to sensory inputs. With high levels of predatory imminence, I hypothesize that BLA outputs to the dmVMH and dlPAG are enhanced so to promote execution of circa-strike behaviors and at the same time outputs to CeA are engaged that activate endogenous antinociceptive systems that reduce emotional responding to injury during confrontation with the threat.

4.2 Stress-induced hyperalgesia

The hyperalgesic effect observed in the current study were not as strong as the analgesic effect and had limited anatomical specificity. In the days following bicuculline administration the analgesic effects wore off and an increase in pain responsiveness was observed. Vocalizations During Shock were elevated 7 and 14 days following injection. No effects of saline injection on VDS over time was observed. Similarly VADs were elevated in bicuculline treated animals were elevated at 4 and 14 days following injection.

Stress-induced hyperalgesia is not as well studied as analgesia, despite its role in human chronic pain conditions. As mentioned previously, this could be due to an inability to distinguish clearly between fear and anxiety in animal models (Rhudy and Meager, 1999). As fear suppresses pain leading to an analgesic effect, anxiety increases environmental scanning and sensory receptivity, thus leading to a hyperalgesic effect (Rhudy and Meager, 2000). The differences in pain responding during fear and anxiety could be the driving force behind the bimodal responses observed in the current study.

Upon initial injection of bicuculline and subsequent pain testing there is an analgesic effect. This is likely because the time of injection aided by exposure to the experimenter and placement into the testing chamber could be considered a present stressor and thus results in a fear response. In the days following, however, anticipation of further injections and pain testing could result in anxiety. A symptom of PTSD and other anxiety disorders is hypervigilance, which is reflected in the preoccupation with possible unknown threats, constantly watching and scanning surroundings coupled with a persistent sense of insecurity. Somatic hypervigilance produces over-interpretation of the threat posed to the individual by a noxious stimulus or injury; thereby, augmenting their emotional response to pain (Asmundson & Katz 2008; Defrin et al., 2008; Sharp & Harvey, 2001). The increases in the amplitude of VAD and VDS that developed in the present study may reflect development of somatic hyperalgesia. Again, this idea is consistent with Fanselow's predator imminence theory. Bicuculline administration may result in the development of an anxiety state and therefore result in pre- and post- encounter behaviors such as hypervigilance.

Interestingly, VADs were elevated 14 and 21 days following saline injection to the BLA. This increase in vocalizations could be due anxiety produced following repeated testing. It is important to note, however, that there were no measures of anxiety (i.e., elevated plus maze) taken over the course of this study.

The findings here are in line with other studies examining stress and hyperalgesia. Repeated social defeat stress in rodents resulted in increases in anxiety-like behavior as well as increased pain sensitivity (Rivat et al., 2010). Stress hormones act within the amygdala to decrease GABAergic signaling (Anderjaska, 2007), therefore it is not surprising that in the days following bicuculline injection there is an increase in pain responding. This also helps to explain the increase in VADs noted following saline injection. This finding is supported by the finding that stress

induced increases in pain scores were reduced with systemic treatment by the benzodiazepine, chlordiazepoxide which is a $GABA_A$ agonist (Rivat et al., 2010).

The reduction in GABAergic tone in the BLA following repeated stressors, or in this case through injection of bicuculline, allows increased activity within BLA projection neurons. As the amygdala provides emotional meaning to sensory inputs (Ledoux, 2000), increased BLA activity through decreased IPSPs (Rannie, 2004) as well as reduced after-hyperpolarizations (Atchley, 2012) could provide an opportunity for increased responding to emotionally significant stimuli as well as responding to stimuli that were not previously significant (i.e. an anxiety disorder develops). For example, LTP develops within the amygdala following chronic immobilization stress (Suvrathan, 2015). After 10 days of immobilization stress there was an increase in EPSP slope, an increase in the NMDAR to AMPAR ratio, and a general increase in NMDAR (Suvrathan, 2015). Furthermore, there was also an increased number of silent synapses, those that contain NMDARs but no AMPARs (Suvrathan, 2015). These synapses will increase the magnitude of LTP and may provide a mechanism for the difference in normal fear compared to pathological fear. Based on this evidence, chronic stress can lead to plasticity within the amygdala allowing it to become hypersensitive to environmental stimuli thus generating affective disorders (Suvrathan, 2015). Because of the increase in excitation and a reduction in GABAergic inhibition, BLA projection neurons send excitatory projections to the dmVMH and mPFC (Porrino, Crane, & Goldman-Rakic, 1981) which then send excitatory projections to the dlPAG (Keay and Bandler, 2001) which in turn increases defensive responding such as an increase in vocalizations (see figure 13).

Within the context of the present model, I propose that sustained activation of the HPA-axis that follows exposure to a fear-inducing stimulus (i.e., predator) leads to long-lasting down-

regulation of GABAergic inhibition of BLA project neurons (see figure 13). This down-regulation leads to enhanced excitability of BLA projection neurons to glutamatergic sensory afferents. Electrophysiological studies indicated that previous stress and repeated administration of corticotrophin-releasing factor receptor agonist into the BLA reduces GABAergic neurotransmission in this structure, increases the response of BLA projection neurons to sensory stimulation (Rodrıguez Manzanares et al, 2005; Rainnie et al, 2004). This enhanced excitability promotes NMDA-mediated synaptic plasticity within the BLA resulting in long-term heightened sensitivity of BLA projection neurons to sensory inputs. The augmented output of glutamatergic BLA projection neurons to the defense circuit (i.e., dlPAG and dmVMH) also promotes NMDA-mediated synaptic plasticity within these sites. As a result, augmented defense responses are elicited to threatening stimuli and non-threatening stimuli are capable of eliciting defense responses (i.e., an anxiety disorder develops). As the dlPAG and dmVMH receive nociceptive afferents the BLA/stress-mediated neuroplasticity within these sites likely underlies long-term increases in pain sensitivity that accompanies anxiety disorders.

I hypothesize that a lower level of arousal is associated with anxiety compared to that associated with fear produced by direct confrontation with a threat. For example, Rainnie and colleagues (2004) found that repeated administration of a low dose of urocortin, a CRF 1 and 2 agonist, increased BLA excitability at doses that had no acute effects on anxiety-like behavior. The reduced arousal with anxiety may reflect a muted but steady state activation BLA projection neurons which may be sufficient to activate the defense circuit but not sufficient to activate the putative projections from CeA to the vlPAG. As a result, anxiety produces a steady state somatic hypervigilance through activation of the defense circuit that facilitates pain processing within the defense circuit; thereby, producing persistent augmented responding to painful stimuli. Because

the BLA outputs to CeA are not engaged during anxiety the hypersensitivity of the defense circuit to pain input is not opposed by activation of the endogenous analgesia circuit and hyperalgesia results (Fig 13). Alternately, the CeA neurons in CeA that receive inputs from BLA projection neurons may have a higher threshold of activation than those in the defense circuit. The figure shows that as stress hormones act within the BLA to reduce the tonic GABAergic inhibition, the BLA becomes more susceptible to the glutamatergic sensory input resulting in BLA projections to the dlPAG and dmVMH that results in the expression of defensive responses and hyperalgesia in response to the anxiety-inducing stimuli (i.e. anxiety-induced hyperalgesia).

Reciprocal projections between the BLA and mPFC can also explain the generation of hyperalgesia following stress exposure. Bidirectional connections between the BLA and mPFC are important for fear conditioning and extinction (Marek, 2013, see Fig 2). In humans, fMRI studies show that fear-inducing stimuli activate the amygdala (Morris, 1996) and the PFC is activated during fear extinction (LaBar, 1998). Additionally, Mobbs *et al.*, 2010 showed shifting activation between the PFC and amygdala based on threat distance. When a tarantula was placed close to a participant's foot via real-time camera feed in an fMRI scanner, there was greater activation in the amygdala compared to greater activation in the PFC from a more distant tarantula. Similarly, stimulation of BLA projections to the mPFC using optogenetic approaches produced anxiety-like behavior in the EPM and reduced social behavior while inhibition produced anxiolytic effects (Tye, 2016).

Inhibitory control of the mPFC over the BLA is decreased following stress as evidenced by decreased dendritic branching (Cook and Wellman, 2004) and an increase in amygdalar firing rate following mPFC lesion (Correll, Rosenkranz, and Grace, 2005). This decrease in inhibition allows for the activation of BLA projections to the mPFC that then facilitate anxiety responses

(Tye, 2016). Thus, the mPFC appears to have a dual role capable of producing anxiogenic effects as well as anxiolytic effects (Lisboa, 2010). While further work is needed to elucidate these differential effects there is evidence to suggest that these opposing responses are generated by the prelimbic cortex (PL) and the infralimbic cortex (IL). Inhibition of the PL resulted in an increase in heart rate following acute restraint stress, while inhibition of the IL resulted in a decrease in heart rate (Tavares, 2009). These findings suggest that the IL facilitates restraint-evoked responses while the PL reduces these responses (Tavares, 2009). These structures also differ in their cortical and subcortical projections (Vertes 2004). Of particular interest here is that there are dense projections from the IL to the dlPAG (Chiba, Kayahara, and Nakano, 2001).

In context of the current experiment, I hypothesize that in the days following bicuculline injection, increased activity within the BLA amplifies activation of projections to the infralimbic cortex which then sends excitatory signals to the dlPAG generating defensive responding and thus contributing to the development of the hyperalgesic effect observed here.

4.3 Anatomical Specificity

Administration of bicuculline into brain sites surrounding the BLA yielded similar results to that of saline injections within the BLA. There was an increase in VAD responding compared to baseline on day 7, 14, and 21. VDS responding was elevated on day 14 compared to baseline. There was no effect on SMR responding following injection to sites surrounding the BLA. These findings suggest that the hyperalgesic effects following injection of bicuculline into BLA is generated by its spread to sites outside the BLA. Given the limited number of rats tested at each extra-BLA site it is not possible to conclude which site(s) outside the BLA may account for the hyperalgesia observed in the present study. On the other hand, the spread of intracerebral bicuculline injections made in .25µl is estimated to be in the range of .145mm - .28mm (radius)

which is outside the range of the extra-BLA sites sampled in the present study. Smith & Berridge (2005) mapped the effects of bicuculline microinjected into the ventral pallidum on eating and taste 'hedonic' reactions in rats. They assessed the spread of their injections by measuring plumes of Fos-like protein immunoreactivity around the microinjection sites. As stated by the authors the Fos plume reflects a local zone of functional modulation induced by the drug and provides quantitative information on intensity and size of the local activation zone. The boundaries of the plume reveal where the drug has the most intense functional impact, even if drug molecules spread further beyond the plume in lower concentrations insufficient to trigger gene transcription. Thus, a Fos plume provides objective information on the extent of tissue activation most likely to mediate functional effects of drug microinjections. Their Fos plume analysis revealed that .2 μg bicuculline injected in .5 μl produced an intense inner zone of activation with a radius of .29 mm and an outer zone of low activation with an additional .27 mm radius = total zone of activation of .56 mm (radius). In the current study, the dose of bicuculline was 1/4 (100 pmol = .05 μg) and the injection volume was 1/2 (.25 μl) that used by Smith & Berridge. A conservative estimate of the functional spread of bicuculline in the current study is .145 mm (inner zone) - .28 mm (total zone) (see figure 14).

These findings strongly suggest that the analgesic effect observed during bicuculline administration to the BLA is specific to this brain area as there is no analgesic effect found when bicuculline is injected to sites surrounding the BLA. The current results suggest that amygdala activation is required for the analgesic effect observed here.

The findings from the anatomical specificity study also suggest that the hyperalgesic effect observed following saline injection into the BLA could be due to anxiety developed from the stress of repeated pain testing. It is possible that administration of bicuculline sped up or increased this

development of anxiety, as VADs were first elevated 4 days following injection compared to 7 days in the anatomical specificity animals and 14 days for saline injected animals. A similar effect was seen in VDS responding with vocalizations first elevated in bicuculline-injected animals after 7 days. The first elevated VDS responding wasn't seen in anatomical specificity animals until 14 days after injection, and no elevation was observed in animals injected with saline into the BLA.

4.4 Vocalization Controls

In order to verify that the analgesic effect observed wasn't an inability of the animals to vocalize due to bicuculline administration, shocks of varying intensity were given before, during, and in the days following injection. Injection of bicuculline into the BLA had no effect on VAD, VDS, or SMR threshold, or the minimum intensity stimulus necessary to elicit a response. Animals were able to vocalize at a variety of shock intensities, suggesting that bicuculline did not interrupt the vocal ability of the rats. Contrary to the earlier findings in this study, the vocalization control group did not exhibit an analgesic or hyperalgesic effect when vocalizations between .3 and .5mA only were measured. This failure to replicate the previously observed effects could be due to inherent differences within the rats, as these experiments were completed in months following completion of the other experimental and control groups. Furthermore, the stronger shock intensities may have decreased the analgesic effect of bicuculline administration as it may have been perceived as more stressful and therefore facilitated a hyperalgesic response. It is also important to note that the rats in the vocalization control group had a significantly higher baseline compared to the other experimental groups, suggesting they may have been more sensitive to the experimental procedures to begin with. This increase in baseline measures would also explain the lack of an observed increase in vocalization behaviors around day 14 that was seen in the experimental group, saline controls, and anatomical controls.

4.5 Future Directions

The findings presented in the current study create numerous routes for future research. First, it has been noted that while I am hypothesizing the hyperalgesic effect observed in the days following bicuculline administration is due to the development of anxiety, it is not directly measured in these experiments. I plan to add a measure of anxiety using the elevated plus-maze to confirm that these changes in pain responsiveness are consistent with the development of anxiety. In the current experiments, I have shown that disinhibition of the BLA leads to biphasic effects on pain responsiveness. Future research should focus effects of bicuculline administration on other brain areas, such as the CeA, dmVMH, and dlPAG as a function of time. It is important to establish the diverging mechanisms in which stress, and more specifically disinhibition of the BLA, contributes to the opposing pain responses observed here.

4.6 Conclusions

The findings presented here support my hypotheses. I first hypothesized that bicuculline administration would immediately decrease pain responsiveness as measured by VADs and VDS. The results of this experiment confirmed this hypothesis. Increases in vocalizations observed in VADs and VDS on days 4 and 14 indicate long-term changes in pain sensitivity. As expected, no decrease was found immediately following saline injection in the BLA. In contradiction to my hypothesis, there was an increase in VADs on days 14 and 21 following saline treatment and no changes in VDS observed. Similarly to the saline treated animals, bicuculline administration to sites outside of the BLA did not result in a decrease in pain responsiveness immediately following injection. There was however, a significant increase in VADs and VDS on 7,14, and 21, and day 14 respectively. Consistent with my hypothesis, bicuculline administration does not appear to

interrupt the animals' ability to vocalize, as bicuculline administration did not prevent responding at varying shock levels.

In conclusion, I have shown here that a single bicuculline injection into the BLA can have biphasic effects on pain responsiveness as a function of time. The findings here support stress-induced analgesia as well as stress induced hyperalgesia. These findings seem to be specific to the BLA and are not due to changes in vocalization ability.

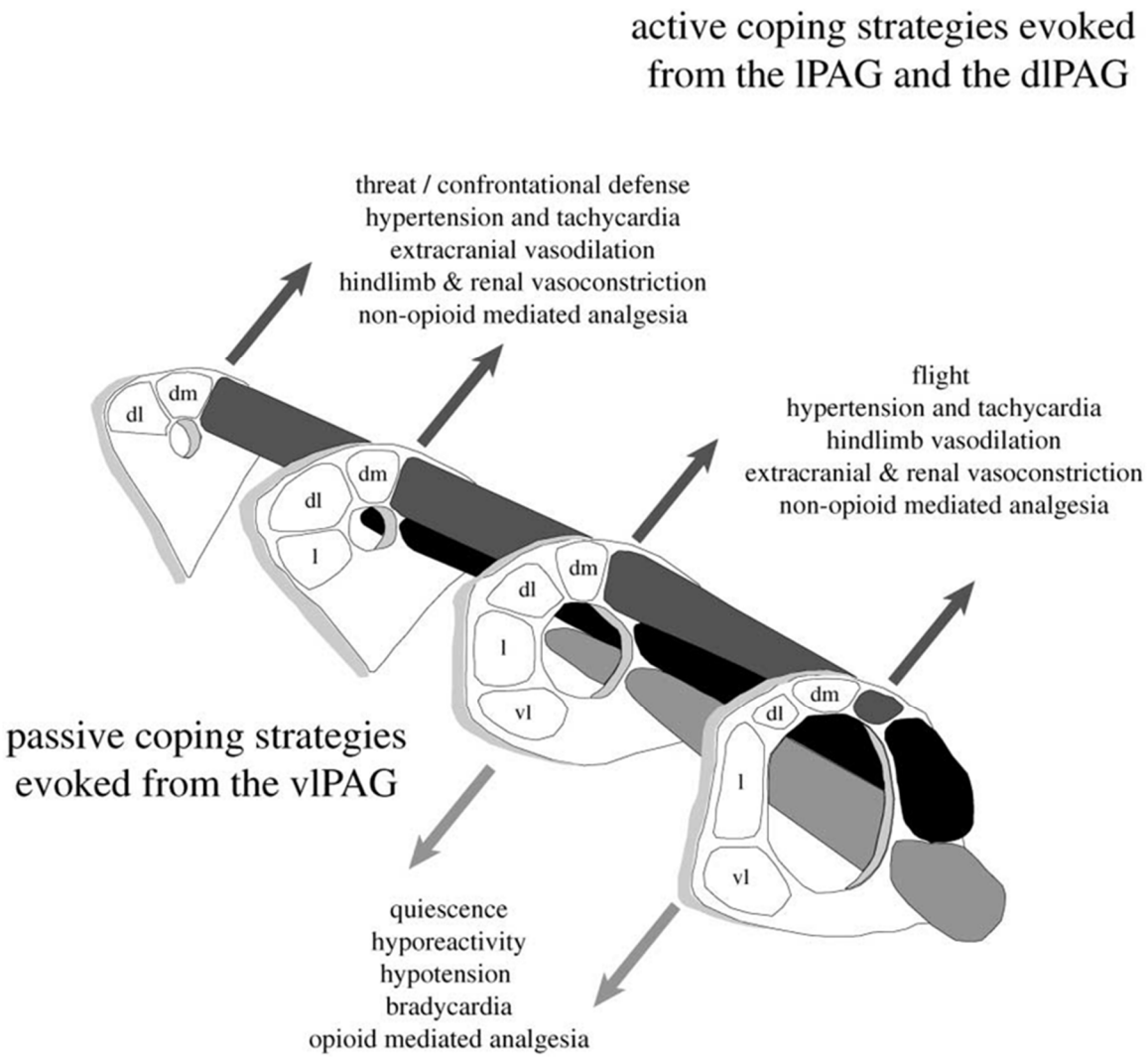


*Figure 1***.** Columnar Organization of the PAG. This figure from Keay and Bandler (2001) describes the 3 columns of the PAG. The different columns play roles in different types of coping behaviors and lead to differences in defensive responding.

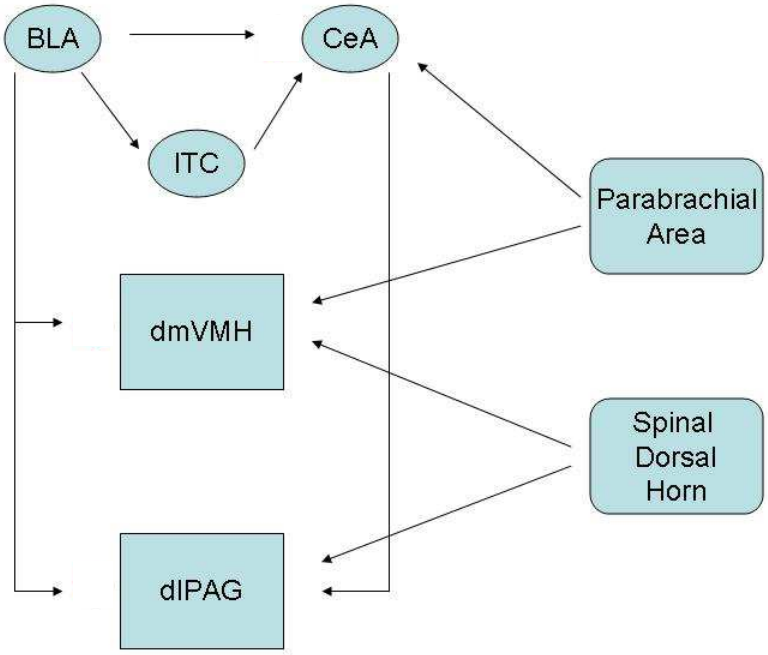


Figure 2. The subcortical defense circuit. This figure shows the connections between structures of the subcortical defense circuit and sites of nociceptive input to this circuit.

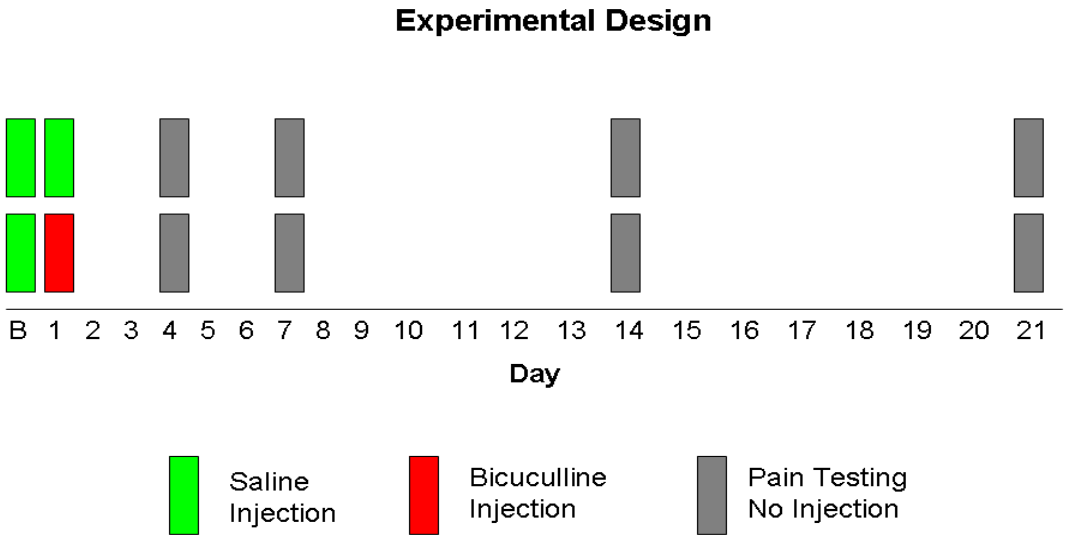


Figure 3. Experimental design timeline. All animals were given intracerebral injections of saline for baseline pain testing. The following day either saline or bicuculline was injected to the BLA or surrounding sites. Each rectangle represents a session of pain testing.

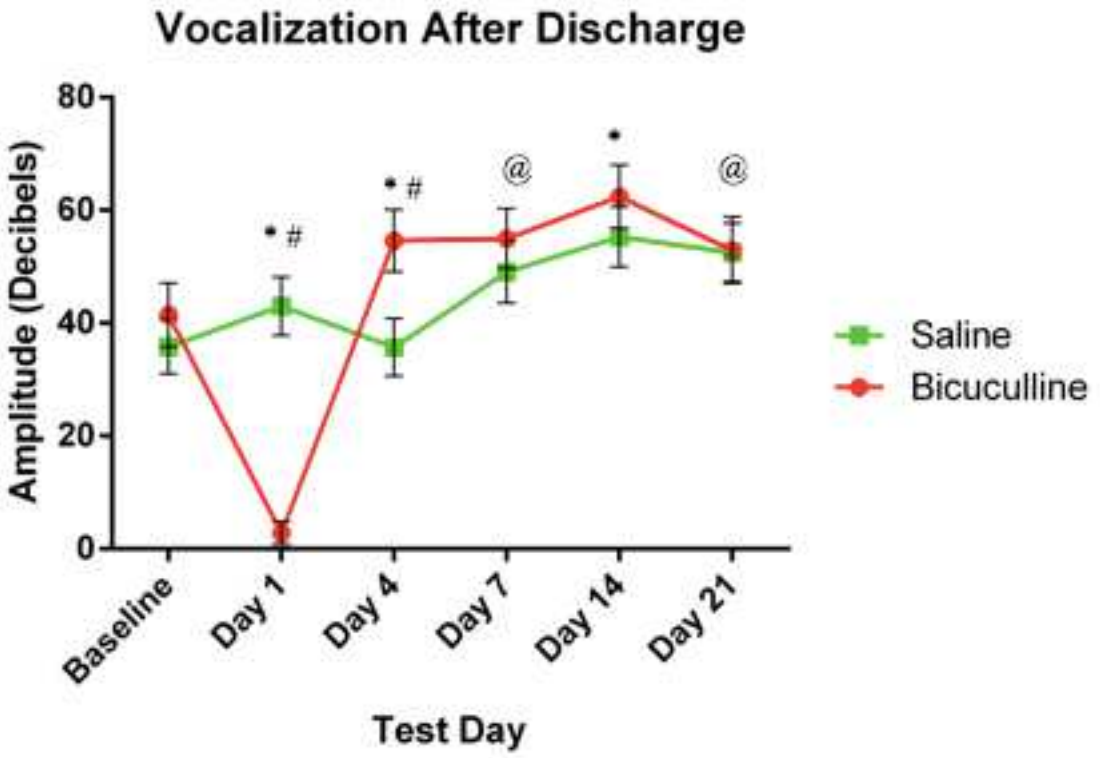


Figure 4. Vocalization after discharge. VAD amplitudes (mean ± SEM) were measured following tail shock over time. Bicuculline injection resulted in an immediate decrease in vocalization amplitude followed by an increase on subsequent test days. * = Bicuculline significantly different from baseline. @ = Saline significantly different from baseline. # = Bicuculline significantly different compared to saline.

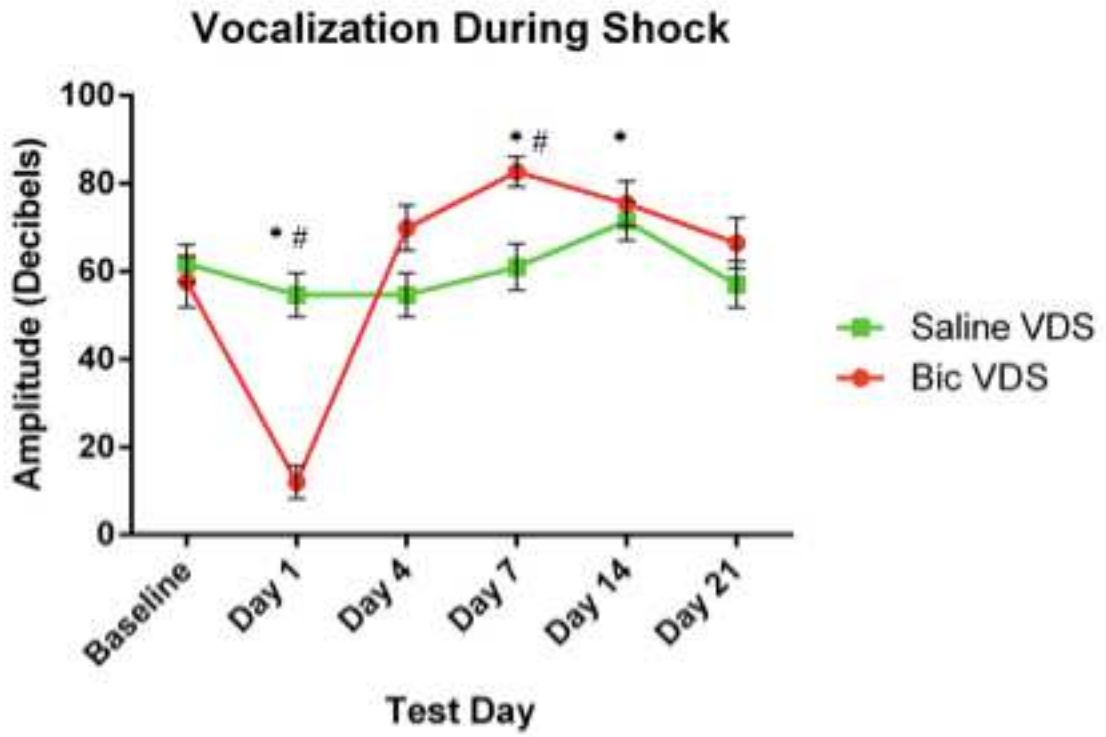


Figure 5. Vocalization during shock. Amplitudes of VDSs (mean ± SEM<) were measured at different time points. Bicuculline injection resulted in an immediate decrease in amplitude followed by an increase on subsequent test days. * = Bicuculline significantly different from baseline. # = Bicuculline significantly different compared to saline.

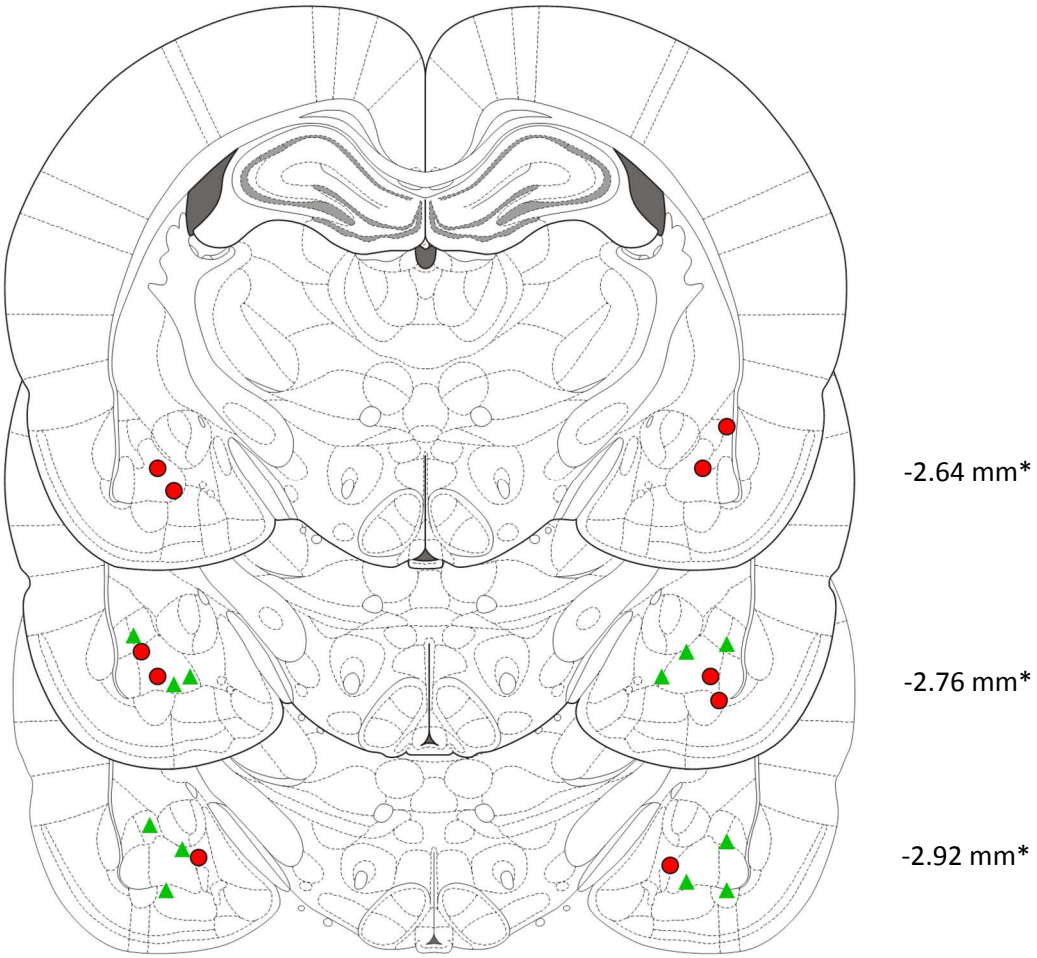
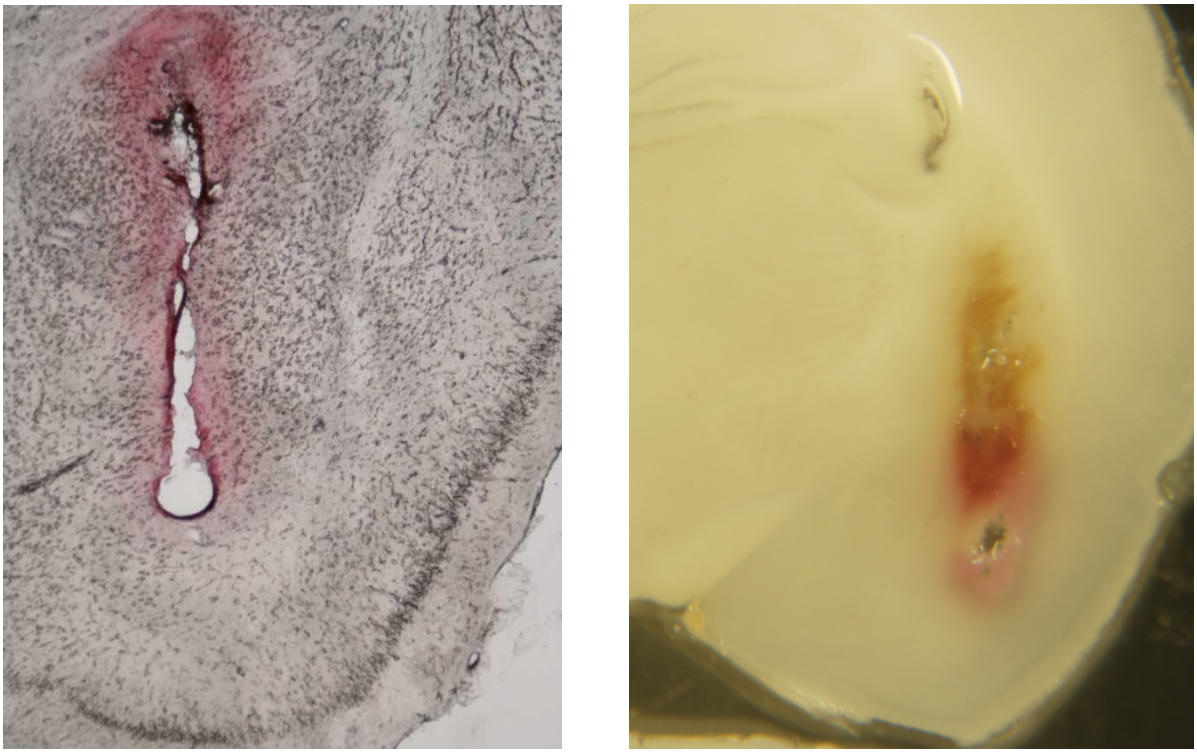


Figure 6. Location of injection sites. The location of injection was determined with an injection of safranin-O dye following conclusion of the experiment. Red circles represent bilateral bicuculline injections. Green triangles represent bilateral saline injections. * mm from bregma (Paxinos and Watson, 1998).



A. B.

Figure 7. Injection site photomicrographs. An example of the confirmation of injection site following injection of safranin-O dye. Note the location of the tissue damage surrounded by pink tissue located within the teardrop shape of the BLA. (A) Saline injection. (B) Bicuculline injection.

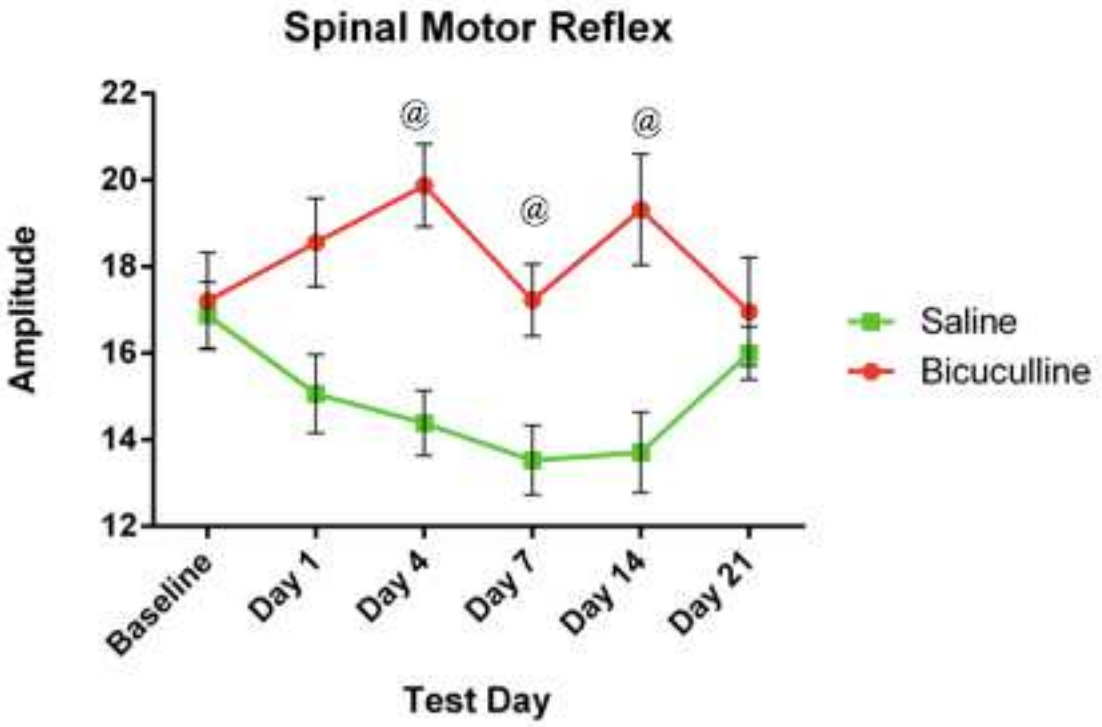
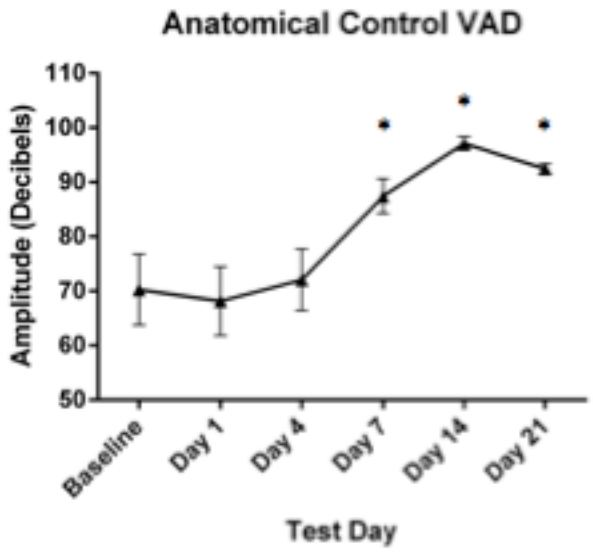
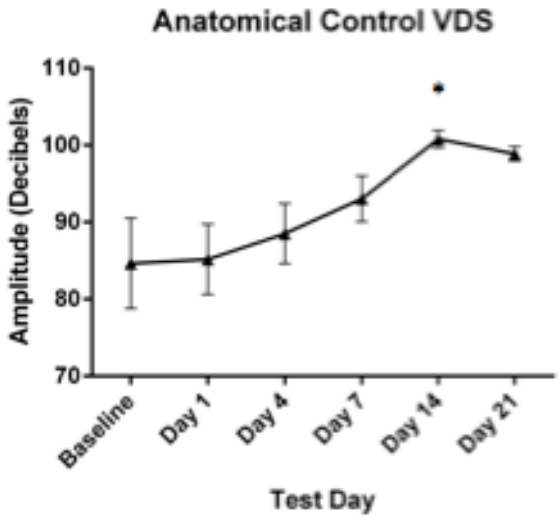


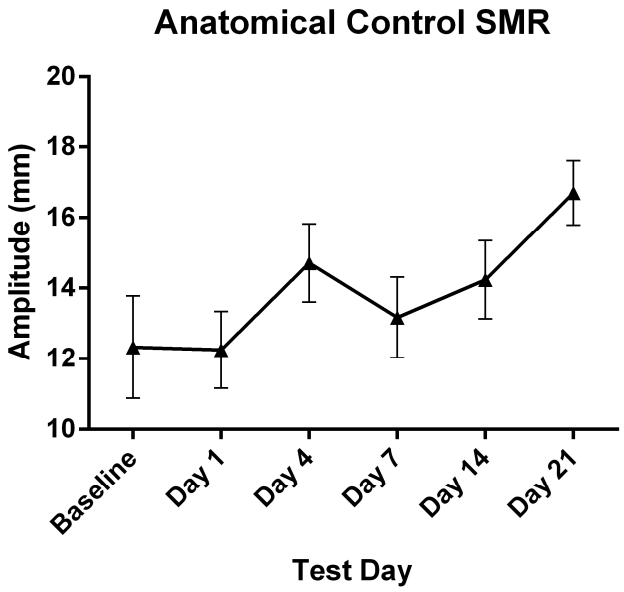
Figure 8. Spinal Motor Reflex. The SMR amplitude (mean ± SEM) was measured by a displacement transducer attached to the rats tail by a cotton thread. Bicuculline injection had no effect on SMR. There was a decrease in SMRs in saline treated animals. @ = Saline responding significantly different compared to baseline.



A.



B.



C.

Figure 9. Anatomical Specificity of analgesic and hyperalgesic effects. In order to verify that the effects of bicuculline injection on VADs, VDSs, and SMRs, bicuculline was injected to sites surrounding the BLA. There were no immediate effects of bicuculline injection on any measure (day 1), however elevated vocalizations were observed in days following injection. * = significantly different compared to baseline responding

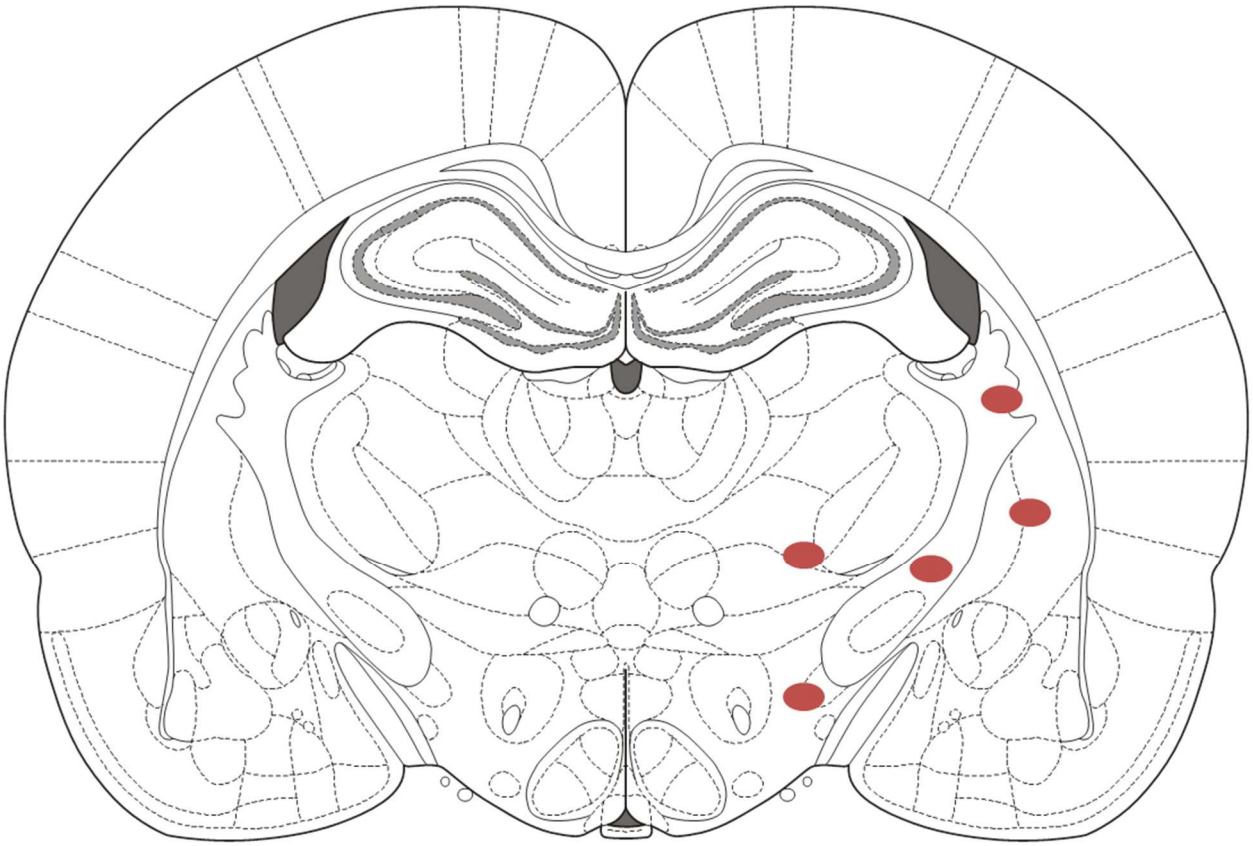
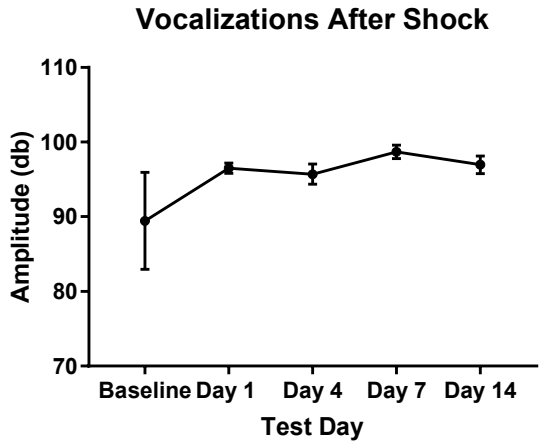
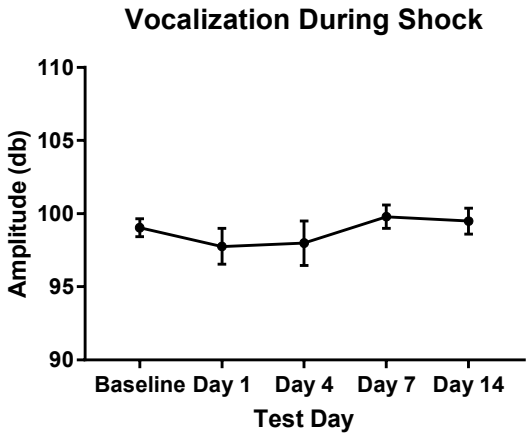


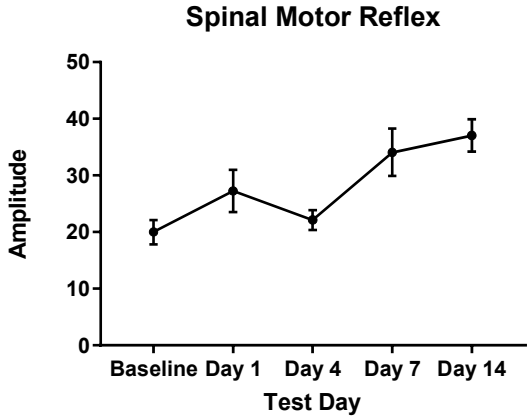
Figure 10. Anatomical control injection locations. Injection sites are indicated by red ovals. Overlaid to the left of the plate is a photomicrograph of an actual injection location confirmed with safranin-O dye.



A.



B.



C.

Figure 11. Vocal ability controls. In order to verify that the analgesic effect observed during vocalization measures, varying shock intensities were delivered in order to elicit vocalizations from bicuculline treated animals for VADs (A) and VDSs (B). SMR response (C) was also analyzed. Data are represented as mean ± SEM.

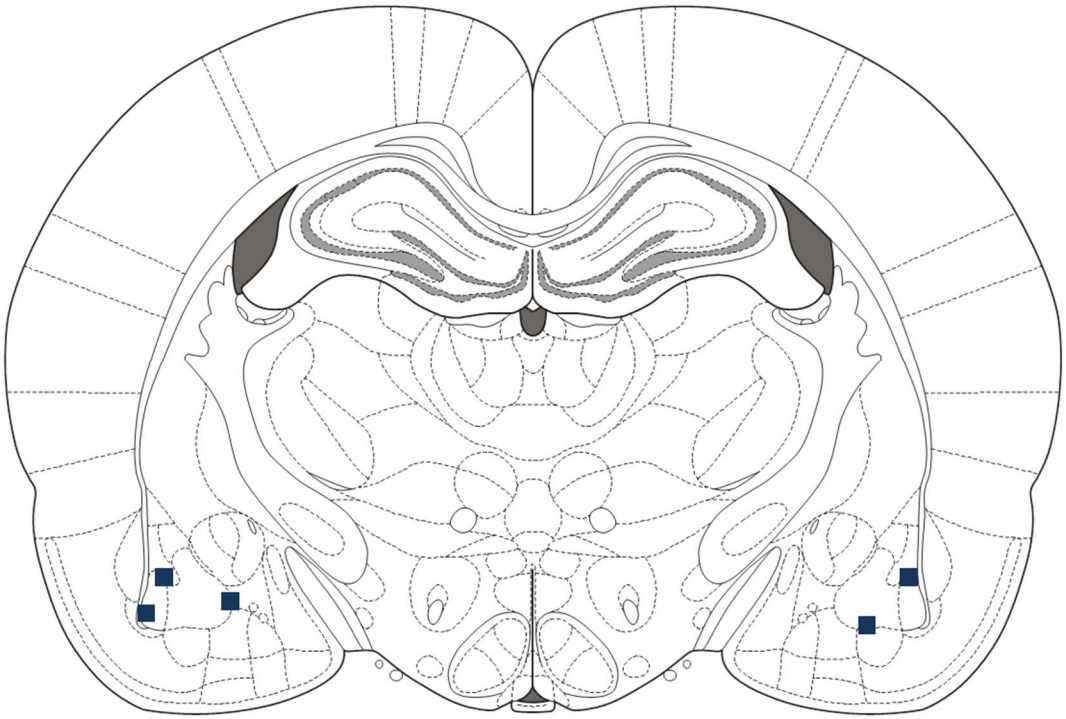


Figure 12. Vocal ability control injection sites. Blue squares represent the location within the BLA in which a bilateral injection of bicuculline was delivered.

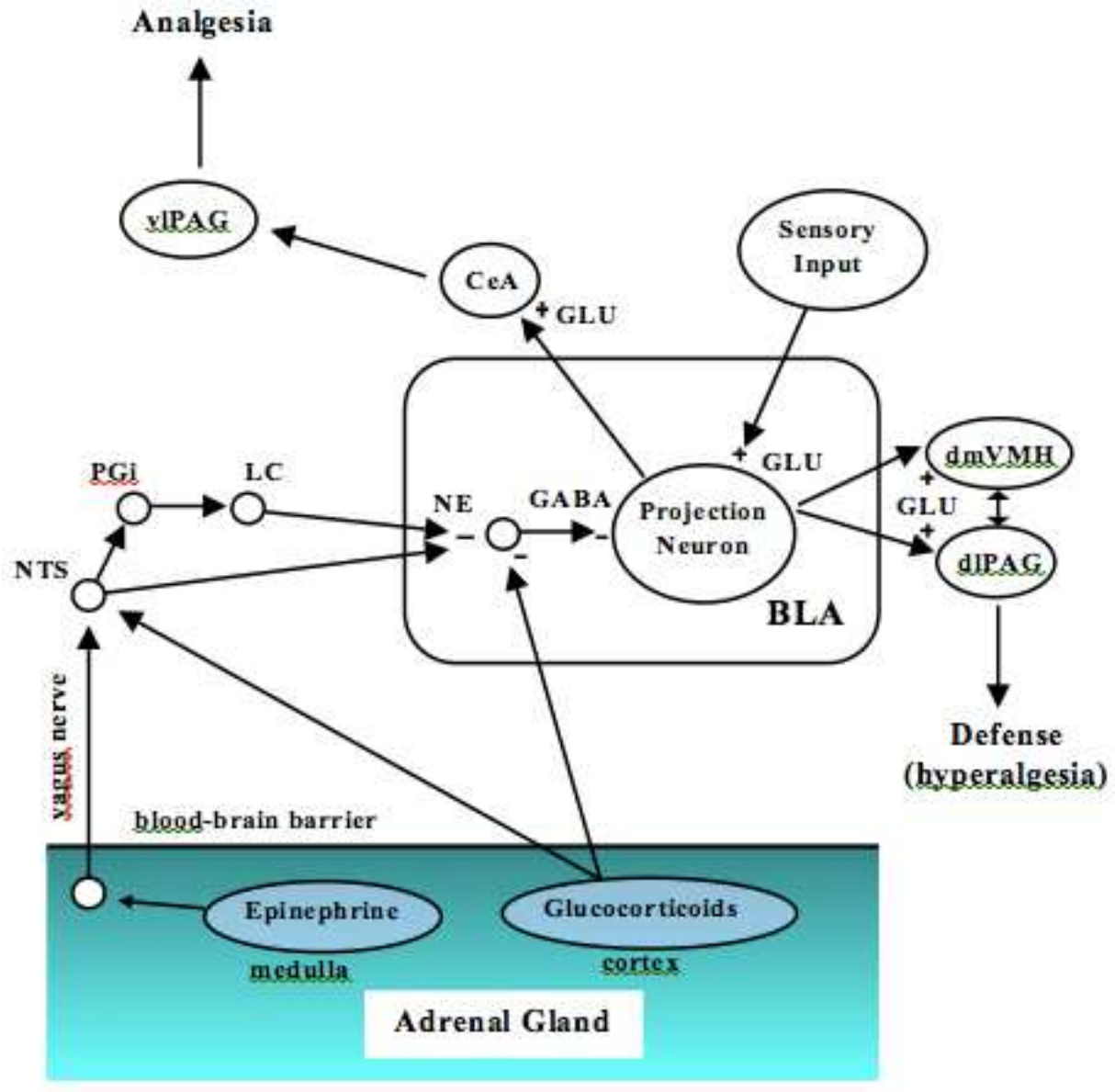


Figure 13. Proposed mechanism of analgesia and hyperalgesia. Diagram whereby stress hormones augment emotional responding to pain (see text for details). Stress hormones induce synaptic plasticity in the basolateral amygdala (BLA) thereby augmenting its response to sensory input. Augmented output of BLA to the dorsomedial ventromedial hypothalamus (dmVMH) and dorsolateral periaqueductal gray (dlPAG) produced enhanced emotional reactivity to threatening stimuli, including noxious stimuli. NTS = nucleus of the solitary tract, PGi = nucleus paragigantocellularis, LC = locus coeruleus, CRF = corticotropin-releasing factor, NE = norepinephrine, GABA ((gamma-aminobutyric acid), GLU = glutamate. CeA = central amygdaloid nucleus. Adapted from de Quervain et al., 2009.

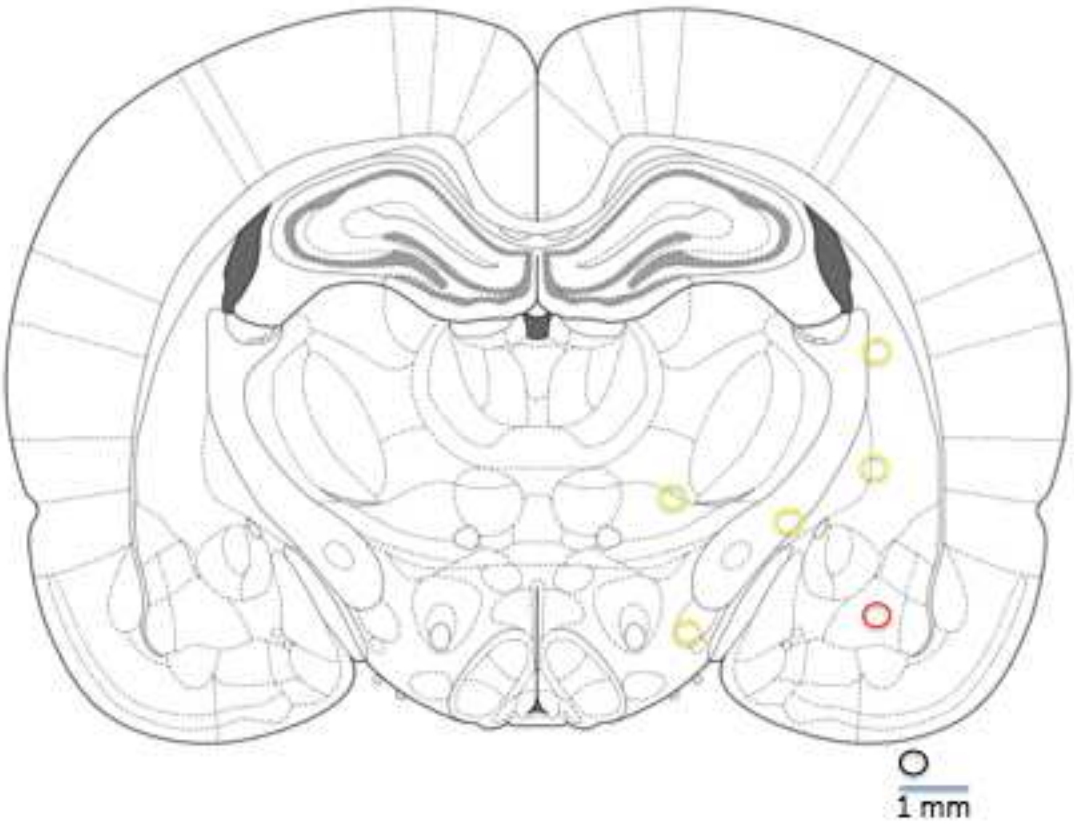


Figure 14. An estimation of functional spread of Bicuculline. Based on dose and total injection volume compared to calculations from Smith and Berridge (2005), a total functional radius of .28mm is estimated and shown here. Red circle indicates bicuculline injection within the BLA and yellow circles indicate bicuculline injections in this study outside of the BLA.

REFERENCES

al-Rodhan, N., Chipkin, R., & Yaksh, T. L. (1990). The antinociceptive effects of SCH-32615, a neutral endopeptidase (enkephalinase) inhibitor, microinjected into the periaqueductal, ventral medulla and amygdala. *Brain Res, 520*(1-2), 123-130.

Asmundson, G. J., & Katz, J. (2008). Understanding pain and posttraumatic stress disorder comorbidity: do pathological responses to trauma alter the perception of pain? *Pain, 138*(2), 247-249. doi:10.1016/j.pain.2008.06.020

Atchley, D., Hankosky, E. R., Gasparotto, K., & Rosenkranz, J. A. (2012). Pharmacological enhancement of calcium-activated potassium channel function reduces the effects of repeated stress on fear memory. *Behav Brain Res, 232*(1), 37-43. doi:10.1016/j.bbr.2012.03.037

Basbaum, A. I., & Fields, H. L. (1984). Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci, 7*, 309-338. doi:10.1146/annurev.ne.07.030184.001521

Bernard JF, B. J. (1990). The spino(trigemino)pontoamygdaloid pathway: Electrophysiological evidence for an involvement in pain process. *Journal of Neurophysology, 63*, 473-490.

Bhatt, S., Gregg, T. R., & Siegel, A. (2003). NK1 receptors in the medial hypothalamus potentiate defensive rage behavior elicited from the midbrain periaqueductal gray of the cat. *Brain Res, 966*(1), 54-64.

Blanchard, D. C., Rodgers, R. J., Hendrie, C. A., & Hori, K. (1988). 'Taming' of wild rats (Rattus rattus) by 5HT1A agonists buspirone and gepirone. *Pharmacol Biochem Behav, 31*(2), 269-278.

Bolles, R. C. (1980). Wundt and after. *Science, 208*(4445), 715-716.

doi:10.1126/science.208.4445.715

Bolles, R. C., & Fanselow, M. S. (1982). Endorphins and behavior. *Annu Rev Psychol, 33*, 87-101. doi:10.1146/annurev.ps.33.020182.000511

Borszcz, G. S. (1999). Differential contributions of medullary, thalamic, and amygdaloid serotonin to the antinociceptive action of morphine administered into the periaqueductal gray: a model of morphine analgesia. *Behav Neurosci, 113*(3), 612-631.

Borszcz, G. S. (2006). Contribution of the ventromedial hypothalamus to generation of the affective dimension of pain. *Pain, 123*(1-2), 155-168. doi:10.1016/j.pain.2006.02.026

Borszcz, G. S., Johnson, C. P., Anderson, M. E., & Young, B. J. (1992). Characterization of tailshock elicited withdrawal reflexes in intact and spinal rats. *Physiol Behav, 52*(6), 1055-1062.

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

Borszcz, G. S., & Streltsov, N. G. (2000). Amygdaloid-thalamic interactions mediate the antinociceptive action of morphine microinjected into the periaqueductal gray. *Behav Neurosci, 114*(3), 574-584.

Butler, R. K., & Finn, D. P. (2009). Stress-induced analgesia. *Prog Neurobiol, 88*(3), 184-202. doi:10.1016/j.pneurobio.2009.04.003

Canteras, N. S., & Goto, M. (1999). Fos-like immunoreactivity in the periaqueductal gray of rats exposed to a natural predator. *Neuroreport, 10*(2), 413-418.

Chapman, C. R., Tuckett, R. P., & Song, C. W. (2008). Pain and stress in a systems perspective: reciprocal neural, endocrine, and immune interactions. *J Pain, 9*(2), 122-145. doi:10.1016/j.jpain.2007.09.006

Comoli, E., Ribeiro-Barbosa, E. R., & Canteras, N. S. (2003). Predatory hunting and exposure to a live predator induce opposite patterns of Fos immunoreactivity in the PAG. *Behav Brain Res, 138*(1), 17-28.

Cook, S. C., & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol, 60*(2), 236-248. doi:10.1002/neu.20025

Cornelio, A. M., Nunes-de-Souza, R. L., & Morgan, M. M. (2012). Contribution of the rostral ventromedial medulla to post-anxiety induced hyperalgesia. *Brain Res, 1450*, 80-86. doi:10.1016/j.brainres.2012.02.037

Correll, C. M., Rosenkranz, J. A., & Grace, A. A. (2005). Chronic cold stress alters prefrontal cortical modulation of amygdala neuronal activity in rats. *Biol Psychiatry, 58*(5), 382-391. doi:10.1016/j.biopsych.2005.04.009

da Costa Gomez, T. M., & Behbehani, M. M. (1995). An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors. *Brain Res, 689*(1), 21-31.

Defrin, R., Ginzburg, K., Solomon, Z., Polad, E., Bloch, M., Govezensky, M., & Schreiber, S. (2008). Quantitative testing of pain perception in subjects with PTSD--implications for the mechanism of the coexistence between PTSD and chronic pain. *Pain, 138*(2), 450-459. doi:10.1016/j.pain.2008.05.006

Dupouy, V., & Zajac, J. M. (1997). Neuropeptide FF receptors control morphine-induced analgesia in the parafascicular nucleus and the dorsal raphe nucleus. *Eur J Pharmacol, 330*(2-3), 129-137.

Duvarci, S., & Pare, D. (2007). Glucocorticoids enhance the excitability of principal basolateral amygdala neurons. *J Neurosci, 27*(16), 4482-4491. doi:10.1523/JNEUROSCI.0680-

07.2007

Ervin, F. R., Mark, V. H., & Stevens, J. (1969). Behavioral and affective responses to brain stimulation in man. *Proc Annu Meet Am Psychopathol Assoc, 58*, 54-65.

Fanselow, M. S. (1984). Shock-induced analgesia on the formalin test: effects of shock severity, naloxone, hypophysectomy, and associative variables. *Behav Neurosci, 98*(1), 79-95.

Fanselow, M. S. (1985). Odors released by stressed rats produce opioid analgesia in unstressed rats. *Behav Neurosci, 99*(3), 589-592.

Fanselow, M. S. (1986). Conditioned fear-induced opiate analgesia: a competing motivational state theory of stress analgesia. *Ann N Y Acad Sci, 467*, 40-54.

Fanselow, M. S., & Helmstetter, F. J. (1988). Conditional analgesia, defensive freezing, and benzodiazepines. *Behav Neurosci, 102*(2), 233-243.

Fanselow, M. S., & Sigmundi, R. A. (1986). Species-specific danger signals, endogenous opioid analgesia, and defensive behavior. *J Exp Psychol Anim Behav Process, 12*(3), 301-309.

Felix-Ortiz, A. C., Burgos-Robles, A., Bhagat, N. D., Leppla, C. A., & Tye, K. M. (2016). Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. *Neuroscience, 321*, 197-209. doi:10.1016/j.neuroscience.2015.07.041

Fernandez De Molina, A., & Hunsperger, R. W. (1962). Organization of the subcortical system governing defense and flight reactions in the cat. *J Physiol, 160*, 200-213.

Floyd, N. S., Price, J. L., Ferry, A. T., Keay, K. A., & Bandler, R. (2000). Orbitomedial prefrontal cortical projections to distinct longitudinal columns of the periaqueductal gray in the rat. *J Comp Neurol, 422*(4), 556-578.

Gracely, R. H., McGrath, P., & Dubner, R. (1978). Validity and sensitivity of ratio scales of

sensory and affective verbal pain descriptors: manipulation of affect by diazepam. *Pain, 5*(1), 19-29.

Harte, S. E., Lagman, A. L., & Borszcz, G. S. (2000). Antinociceptive effects of morphine injected into the nucleus parafascicularis thalami of the rat. *Brain Res, 874*(1), 78-86.

Helmstetter, F. J., Bellgowan, P. S., & Poore, L. H. (1995). Microinfusion of mu but not delta or kappa opioid agonists into the basolateral amygdala results in inhibition of the tail flick reflex in pentobarbital-anesthetized rats. *J Pharmacol Exp Ther, 275*(1), 381-388.

Helmstetter, F. J., Bellgowan, P. S., & Tershner, S. A. (1993). Inhibition of the tail flick reflex following microinjection of morphine into the amygdala. *Neuroreport, 4*(5), 471-474.

Helmstetter, F. J., Tershner, S. A., Poore, L. H., & Bellgowan, P. S. (1998). Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. *Brain Res, 779*(1-2), 104-118.

Iacono, R. P., & Nashold, B. S., Jr. (1982). Mental and behavioral effects of brain stem and hypothalamic stimulation in man. *Hum Neurobiol, 1*(4), 273-279.

Jasmin, L., Burkey, A. R., Card, J. P., & Basbaum, A. I. (1997). Transneuronal labeling of a nociceptive pathway, the spino-(trigemino-)parabrachio-amygdaloid, in the rat. *J Neurosci, 17*(10), 3751-3765.

Jennings, E. M., Okine, B. N., Roche, M., & Finn, D. P. (2014). Stress-induced hyperalgesia. *Prog Neurobiol, 121*, 1-18. doi:10.1016/j.pneurobio.2014.06.003

Jimenez-Velazquez, G., Lopez-Munoz, F. J., & Fernandez-Guasti, A. (2010). Parallel anxiolytic-like and antinociceptive actions of diazepam in the anterior basolateral amygdala and dorsal periaqueductal gray. *Brain Res, 1349*, 11-20. doi:10.1016/j.brainres.2010.06.014

Johnston, G. A. (2013). Advantages of an antagonist: bicuculline and other GABA antagonists.

Br J Pharmacol, 169(2), 328-336. doi:10.1111/bph.12127

Jurgens, U. (2002). Neural pathways underlying vocal control. *Neurosci Biobehav Rev, 26*(2), 235-258.

Keay, K. A., & Bandler, R. (2001). Parallel circuits mediating distinct emotional coping reactions to different types of stress. *Neurosci Biobehav Rev, 25*(7-8), 669-678.

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

LeDoux, J. (2007). The amygdala. *Curr Biol, 17*(20), R868-874. doi:10.1016/j.cub.2007.08.005

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

Lichtman, A. H., & Fanselow, M. S. (1990). Cats produce analgesia in rats on the tail-flick test: naltrexone sensitivity is determined by the nociceptive test stimulus. *Brain Res, 533*(1), 91-94.

Lisboa, S. F., Stecchini, M. F., Correa, F. M., Guimaraes, F. S., & Resstel, L. B. (2010). Different role of the ventral medial prefrontal cortex on modulation of innate and associative learned fear. *Neuroscience, 171*(3), 760-768. doi:10.1016/j.neuroscience.2010.09.048

Marek, R., Strobel, C., Bredy, T. W., & Sah, P. (2013). The amygdala and medial prefrontal cortex: partners in the fear circuit. *J Physiol, 591*(10), 2381-2391. doi:10.1113/jphysiol.2012.248575

Maren, S. (2000). Auditory fear conditioning increases CS-elicited spike firing in lateral amygdala neurons even after extensive overtraining. *Eur J Neurosci, 12*(11), 4047-4054.

Mark, V. H., Ervin, F. R., & Yakovlev, P. I. (1963). The verification of anatomical lesion sites in the human thalamus. *Arch Neurol, 8*, 528-538.

McGaraughty, S., & Heinricher, M. M. (2002). Microinjection of morphine into various amygdaloid nuclei differentially affects nociceptive responsiveness and RVM neuronal activity. *Pain, 96*(1-2), 153-162.

Mena, N. B., Mathur, R., & Nayar, U. (1995). Amygdalar involvement in pain. *Indian J Physiol Pharmacol, 39*(4), 339-346.

Millan, M. J. (1999). The induction of pain: an integrative review. *Prog Neurobiol, 57*(1), 1-164.

Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proc Natl Acad Sci U S A, 102*(26), 9371-9376. doi:10.1073/pnas.0504011102

Mobbs, D., Yu, R., Rowe, J. B., Eich, H., FeldmanHall, O., & Dalgleish, T. (2010). Neural activity associated with monitoring the oscillating threat value of a tarantula. *Proc Natl Acad Sci U S A, 107*(47), 20582-20586. doi:10.1073/pnas.1009076107

Morris, J. S., Frith, C. D., Perrett, D. I., Rowland, D., Young, A. W., Calder, A. J., & Dolan, R. J. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature, 383*(6603), 812-815. doi:10.1038/383812a0

Munn, E. M., & Borszcz, G. S. (2002). Increases in the release and metabolism of serotonin in nucleus parafascicularis thalami following systemically administered morphine in the rat. *Neurosci Lett, 332*(3), 151-154.

Munn, E. M., Harte, S. E., Lagman, A., & Borszcz, G. S. (2009). Contribution of the periaqueductal gray to the suppression of pain affect produced by administration of morphine into the intralaminar thalamus of rat. *J Pain, 10*(4), 426-435.

doi:10.1016/j.jpain.2008.10.011

Nandigama, P., & Borszcz, G. S. (2003). Affective analgesia following the administration of morphine into the amygdala of rats. *Brain Res, 959*(2), 343-354.

Neugebauer, V., Galhardo, V., Maione, S., & Mackey, S. C. (2009). Forebrain pain mechanisms. *Brain Res Rev, 60*(1), 226-242. doi:10.1016/j.brainresrev.2008.12.014

Neugebauer, V., Li, W., Bird, G. C., & Han, J. S. (2004). The amygdala and persistent pain. *Neuroscientist, 10*(3), 221-234. doi:10.1177/1073858403261077

Newman, H. M., Stevens, R. T., & Apkarian, A. V. (1996). Direct spinal projections to limbic and striatal areas: anterograde transport studies from the upper cervical spinal cord and the cervical enlargement in squirrel monkey and rat. *J Comp Neurol, 365*(4), 640-658. doi:10.1002/(SICI)1096-9861(19960219)365:4<640::AID-CNE10>3.0.CO;2-L

Olango, W. M., & Finn, D. P. (2014). Neurobiology of stress-induced hyperalgesia. *Curr Top Behav Neurosci, 20*, 251-280. doi:10.1007/7854_2014_302

Oliveira, M. A., & Prado, W. A. (2001). Role of PAG in the antinociception evoked from the medial or central amygdala in rats. *Brain Res Bull, 54*(1), 55-63.

Ossipov, M. H., & Gebhart, G. F. (1984). Light pentobarbital anesthesia diminishes the antinociceptive potency of morphine administered intracranially but not intrathecally in the rat. *Eur J Pharmacol, 97*(1-2), 137-140.

Perusini, J. N., Meyer, E. M., Long, V. A., Rau, V., Nocera, N., Avershal, J., . . . Fanselow, M. S. (2016). Induction and Expression of Fear Sensitization Caused by Acute Traumatic Stress. *Neuropsychopharmacology, 41*(1), 45-57. doi:10.1038/npp.2015.224

Porrino, L. J., Crane, A. M., & Goldman-Rakic, P. S. (1981). Direct and indirect pathways from the amygdala to the frontal lobe in rhesus monkeys. *J Comp Neurol, 198*(1), 121-136.

doi:10.1002/cne.901980111

Quirk, G. J., & Beer, J. S. (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Curr Opin Neurobiol, 16*(6), 723-727. doi:10.1016/j.conb.2006.07.004

Quirk, G. J., Russo, G. K., Barron, J. L., & Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J Neurosci, 20*(16), 6225-6231.

Rainnie, D. G., Bergeron, R., Sajdyk, T. J., Patil, M., Gehlert, D. R., & Shekhar, A. (2004). Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. *J Neurosci, 24*(14), 3471-3479. doi:10.1523/JNEUROSCI.5740-03.2004

Rea, K., Lang, Y., & Finn, D. P. (2009). Alterations in extracellular levels of gamma-aminobutyric acid in the rat basolateral amygdala and periaqueductal gray during conditioned fear, persistent pain and fear-conditioned analgesia. *J Pain, 10*(10), 1088-1098. doi:10.1016/j.jpain.2009.04.019

Rea, K., Roche, M., & Finn, D. P. (2011). Modulation of conditioned fear, fear-conditioned analgesia, and brain regional c-Fos expression following administration of muscimol into the rat basolateral amygdala. *J Pain, 12*(6), 712-721. doi:10.1016/j.jpain.2010.12.010

Rhudy, J. L., & Meagher, M. W. (2000). Fear and anxiety: divergent effects on human pain thresholds. *Pain, 84*(1), 65-75.

Rivat, C., Becker, C., Blugeot, A., Zeau, B., Mauborgne, A., Pohl, M., & Benoliel, J. J. (2010). Chronic stress induces transient spinal neuroinflammation, triggering sensory hypersensitivity and long-lasting anxiety-induced hyperalgesia. *Pain, 150*(2), 358-368. doi:10.1016/j.pain.2010.05.031

Rodgers, R. J. (1978). Influence of intra-amygdaloid opiate injections on shock thresholds, tail-flick latencies and open field behaviour in rats. *Brain Res, 153*(1), 211-216.

Rosenkranz, J. A., Venheim, E. R., & Padival, M. (2010). Chronic stress causes amygdala hyperexcitability in rodents. *Biol Psychiatry, 67*(12), 1128-1136. doi:10.1016/j.biopsych.2010.02.008

Sanders, S. K., & Shekhar, A. (1991). Blockade of GABAA receptors in the region of the anterior basolateral amygdala of rats elicits increases in heart rate and blood pressure. *Brain Res, 567*(1), 101-110.

Sanders, S. K., & Shekhar, A. (1995). Regulation of anxiety by GABAA receptors in the rat amygdala. *Pharmacol Biochem Behav, 52*(4), 701-706.

Schubert, K., Shaikh, M. B., & Siegel, A. (1996). NMDA receptors in the midbrain periaqueductal gray mediate hypothalamically evoked hissing behavior in the cat. *Brain Res, 726*(1-2), 80-90.

Shaikh, M. B., Schubert, K., & Siegel, A. (1994). Basal amygdaloid facilitation of midbrain periaqueductal gray elicited defensive rage behavior in the cat is mediated through NMDA receptors. *Brain Res, 635*(1-2), 187-195.

Sharp, T. J., & Harvey, A. G. (2001). Chronic pain and posttraumatic stress disorder: mutual maintenance? *Clin Psychol Rev, 21*(6), 857-877.

Smith, D. J., Robertson, B., & Monroe, P. J. (1992). Antinociception from the administration of beta-endorphin into the periaqueductal gray of rat is enhanced while that of morphine is inhibited by barbiturate anesthesia. *Neurosci Lett, 146*(2), 143-146.

Spuz, C. A., & Borszcz, G. S. (2012). NMDA or non-NMDA receptor antagonism within the amygdaloid central nucleus suppresses the affective dimension of pain in rats: evidence

for hemispheric synergy. *J Pain, 13*(4), 328-337. doi:10.1016/j.jpain.2011.12.007

Tavares, R. F., Correa, F. M., & Resstel, L. B. (2009). Opposite role of infralimbic and prelimbic cortex in the tachycardiac response evoked by acute restraint stress in rats. *J Neurosci Res, 87*(11), 2601-2607. doi:10.1002/jnr.22070

Tully, K., Li, Y., Tsvetkov, E., & Bolshakov, V. Y. (2007). Norepinephrine enables the induction of associative long-term potentiation at thalamo-amygdala synapses. *Proc Natl Acad Sci U S A, 104*(35), 14146-14150. doi:10.1073/pnas.0704621104

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse, 51*(1), 32-58. doi:10.1002/syn.10279

Yeung, J. C., Yaksh, T. L., & Rudy, T. A. (1978). Effect on the nociceptive threshold and EEG activity in the rat of morphine injected into the medial thalamus and the periaqueductal gray. *Neuropharmacology, 17*(7), 525-532.

Zarrindast, M. R., Solati, J., Oryan, S., & Parivar, K. (2008). Effect of intra-amygdala injection of nicotine and GABA receptor agents on anxiety-like behaviour in rats. *Pharmacology, 82*(4), 276-284. doi:10.1159/000161129

ABSTRACT

ANALGESIA FOLLOWED BY LONG-TERM HYPERALGESIA GENERATED BY DISINHIBITION OF THE BASOLATERAL AMYGDALA

by

DEREK ATCHLEY

August 2016

Advisor: Dr. George S. Borszcz

Major: Psychology (Behavioral and Cognitive Neuroscience)

Degree: Master of Arts

Stress produces bimodal effects on pain peception. During exposure to a stressor pain responses are inibited (i.e. stress-induced analgesia). However, following long-term exposure to a stressor increases in responsiveness to painful stimuli may develop (i.e. stress-induced hyperalgesia). Here I evaluated how a key component of the subcortical defense circuit and target of stress hormones contributes to the development of both stress-induced analgesia and hyperalgesia. Bicuculline methiodide, a $GABA_A$ antagonist, injected into the basolateral amygdala was used to mimic the neural effects of a stressor or threat exposure. Immediately following injection pain responsiveness was decreased as measured by vocalizations after discharge and vocolizations during shock following a tailshock. In the days and weeks following bicuculline injection pain responsiveness became elevated compared to control rats. These findings suggest that pain responsiveness can be mediated by a reduction in GABAergic signalling within the basolateral amygdala following stress exposure.

AUTOBIOGRAPHICAL STATEMENT

I am currently a doctoral student at Wayne State University in the Department of Psychology majoring in Behavioral and Cognitive Neuroscience. I graduated from Lake Forest College in 2010 with a Bachelor of Arts in Psychology with a neuroscience minor. Currently I am a member of the Affective Neuroscience lab at Wayne State.