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# Brain Derived Neurotrophic Factor Modulates Behavioral and Brain Responses to Social Stress

Elizabeth Jeffress

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BRAIN DERIVED NEUROTROPHIC FACTOR MODULATES BEHAVIORAL AND BRAIN  
RESPONSES TO SOCIAL STRESS

by

ELIZABETH CARNEY JEFFRESS THORSTEINSON

Under the Direction of Kim L. Huhman, PhD

ABSTRACT

Social stress is a prevalent factor in society that can cause or exacerbate neuropsychiatric disorders including depression and posttraumatic stress disorder. According to the National Institutes of Health, 6.9% of adults in this country currently suffer from depression, and 4.1% suffer from an anxiety disorder. Unfortunately, current treatments are ineffective in reducing or alleviating symptoms in a majority of these patients. Thus, it is critical to understand how social stress changes in brain and behavior so that we might develop alternative treatments. Brain derived neurotrophic factor (BDNF), which binds to tyrosine kinase B (TrkB) receptors, plays a role in fear learning and in behavioral responses to stress, although we do not currently know whether BDNF promotes or prevents these responses. The purpose of this project was to understand how BDNF alters brain and behavior in response to social stress using a model of social stress in Syrian hamsters, termed conditioned defeat (CD). CD refers to the marked increase in submissive and defensive behavior following social defeat. Specific Aim (SA) 1 tested

the hypothesis that BDNF, via TrkB receptors, promotes CD learning. Instead, we found that BDNF and a selective TrkB receptor agonist *reduced* CD and that a TrkB receptor antagonist *enhanced* CD. SA 2 tested the hypothesis that the behavioral response observed following systemic administration of TrkB-active drugs is mediated via their action in specific nodes of the neural circuit underlying CD. Unfortunately, the vehicle in which these drugs are dissolved independently activates immediate early gene expression making interpretation of these data impossible. Finally, SA 3 tested the hypothesis that BDNF alters defeat-induced neural activation at least in part by acting in the medial prefrontal cortex (mPFC). We demonstrated that BDNF microinjected into the mPFC site-specifically altered defeat-induced neural activation in the CD neural circuit supporting this hypothesis. Overall, these data suggest that BDNF acts to prevent social stress-induced changes in behavior, at least in part via the basolateral amygdala and the mPFC, and that BDNF-active drugs might be a useful avenue to pursue to discover new treatments for patients that suffer from stress-related neuropsychiatric disorders.

INDEX WORDS: Tyrosine Kinase B Receptors, Basolateral Amygdala, Prelimbic Cortex, Infralimbic Cortex, Conditioned Defeat, Synaptic Plasticity, Resilience

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ELIZABETH CARNEY JEFFRESS THORSTEINSON

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2015

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2015

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ELIZABETH CARNEY JEFFRESS THORSTEINSON

Committee Chair: Kim Huhman

Committee: Laura Carruth

Marise Parent

Kerry Ressler

Electronic Version Approved:

Office of Graduate Studies

College of Arts and Sciences

Georgia State University

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## DEDICATION

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## LIST OF ABBREVIATIONS

- 5HT Serotonin
- 7,8-DHF 7,8-Dihydroxyflavone
- ANA-12 ([N2-2-2-Oxoazepan-3-yl amino] carbonyl phenyl benzo (b) thiophene-2-carboxamide)
- ANOVA Analysis of Variance
- ANS Autonomic Nervous System
- BA Brodmann's Area
- BBB Blood Brain Barrier
- BDNF Brain Derived Neurotrophic Factor
- BLA Basolateral Amygdala
- cAMP cyclic adenosine monophosphate
- CD Conditioned Defeat
- CeA Central Amygdala
- CNS Central Nervous System
- CPu Caudate Putamen
- CREB cAMP response element-binding protein
- CRH corticotropin-releasing hormone
- DA Dopamine
- DAB nickel-3, 39-diaminobenzidene
- DRN Dorsal Raphe Nucleus
- DMSO Dimethylsulfoxide
- ELISA Enzyme-Linked Immunoabsorbent Assay
- FOS-IR FOS-immunoreactive
- GABA Gamma-amino butyric acid
- HPA Hypothalamic-pituitary Adrenal

IL Infralimbic Cortex

IP Intraperitoneal

KPBS Potassium Buffered Saline

LTM Long-term Memory

LTP Long-term Potentiation

MeA Medial Amygdala

mRNA Messenger ribonucleic acid

mPFC medial Prefrontal Cortex

NaC Nucleus Accumbens

NAI Nonaggressive Intruder

NGF Nerve Growth Factor

NGS Normal Goat Serum

PL Prelimbic Cortex

PTSD Posttraumatic Stress Disorder

RA Resident Aggressor

SA Specific Aims

TrK Tyrosine Kinase

## 1 GENERAL INTRODUCTION

Social stress is the most common source of stress experienced by humans in modern society (Bjorkqvist, 2001). Given the frequency with which humans interact with one another at work, in school or at home (Agid et al., 2000; Kelleher et al., 2008), it is not surprising that social stress is so prevalent. Stress, in general, is usually defined as a challenge or a perturbation that disturbs homeostasis. Stressors, including social stress, activate the hypothalamic pituitary adrenal (HPA) axis and the autonomic nervous system (ANS), the 2 main stress response systems of the body. Activation of these 2 systems, in turn, increases the release of cortisol and catecholamines (Dickerson and Kemeny, 2004; Kirschbaum et al., 2004). Increases in these neurochemicals, particularly if sustained, can have numerous detrimental effects on the health of an individual. Stress suppresses the immune system thereby increasing an individual's susceptibility to illness (Segerstrom and Miller, 2004). Stressed individuals are also more likely to experience changes in weight (Block et al., 2009), increases in blood pressure (Ghiadoni et al., 2000), and an increased risk of arteriosclerosis, hypertension, and other metabolic disorders (Chrousos, 2009). Furthermore, exposure to stress reduces telomere length (Epel et al., 2004), which is related to early onset of age-related diseases and which may explain in part why stressed individuals are at a higher risk of disrupted homeostasis, health problems, and decreased quality of life (Russ et al., 2012).

Stress unfortunately is also thought to be a critical factor that places individuals at an increased risk for developing neuropsychiatric disorders (Sapolsky, 2000; Karatsoreos & McEwen, 2011) including depression, posttraumatic stress disorder (PTSD), and other anxiety disorders (Gilbert & Allan 1998; Heim & Nemeroff 2001; McEwen & Stellar 1993; Pêgo et al., 2010; Shin & Liberzon, 2010). According to 12-month prevalence data from the National Institutes of Mental Health, 6.9 percent of adults in this country suffer from depression, and 4.1% of the adult population suffers from an anxiety disorder such as PTSD (NIH, NIH). Furthermore, the World Health Organization reports that depression is the third most



important cause of disease burden worldwide (WHO). Even more troubling is the fact that many individuals with disorders such as depression do not experience relief from their symptoms after taking many of the currently recommended treatments (Trivedi et al., 2006a; Trivedi et al., 2006b). To date, there is no treatment that is completely effective, and the emotional and economic burden is mounting (Kalia, 2002; Hoffman et al., 2008). Thus, it is critical that we improve our understanding of the mechanisms whereby stressors, such as social stress, lead to changes in brain and behavior so that we might discover alternative treatment options for individuals suffering from stress-related mental illness.

Recent research has suggested that brain derived neurotrophic factor (BDNF) systems may present a novel treatment option for depressed individuals. BDNF is a neurotrophic protein found in the brain and periphery. BDNF is secreted by neurons and binds primarily to tyrosine kinase B receptors (TrkB) (Chao et al., 2006; Huang and Reichardt, 2003; Reichardt, 2006), which are found on neurons and glia cells (Zhou et al., 1998; Zhou et al., 1993). BDNF promotes neuronal growth, survival, plasticity, learning and memory, and neuronal development (reviewed in Yoshii and Constantine-Paton, 2010; Chao et al., 2006; Minichiello, 2009). Importantly, BDNF is often suppressed following exposure to stress in humans, and reduced BDNF protein in serum is observed in patients with numerous psychiatric illnesses, including major depressive disorder (Karege et al., 2002; reviewed by Duman and Monteggia, 2006; Castren & Rantamaki, 2010), PTSD (Dell'osso et al., 2009; review by Rakofsky et al., 2012; Suliman, Hemmings, & Seedat, 2013) as well as in blood platelets of suicidal patients (reviewed in Dwivedi, 2010).

Supporting the potential therapeutic effect of BDNF in depression, numerous labs using animal models have reported that BDNF is increased following antidepressant treatment (Karege et al., 2002; reviewed by Duman and Monteggia, 2006; Castren & Rantamaki, 2010; Suliman, Hemmings, Seedat, 2013). Furthermore, blocking BDNF receptor activation in the prefrontal cortex prevents antidepressant effects in mice as measured in the forced swim test

(Saarelinen et al., 2003). Although these data are intriguing and encouraging, additional research is needed to explore how BDNF changes following social stress and whether manipulations that alter BDNF neurotransmission are effective in changing behavioral responses to social stress.

Animal models provide a non-human alternative to discover the mechanisms whereby exposure to social conflict leads to adverse physical and psychological outcomes (Bartolomucci 2007). One of the most prevalent animal models used to study social conflict is the resident-intruder model. This model involves social conflict between conspecifics that compete for a desired resource such as a territory or a mate (reviewed by Tamashiro et al., 2005; Blanchard, McKittrick, and Blanchard, 2001; Koolhass et al., 2013). Resident-intruder models have been developed in numerous species including mice, rats, hamsters, and non-human primates and have been shown to produce depression-like and anxiety-like changes in behavior much like those observed in humans following exposure to social stress (reviewed by Tamashiro et al., 2005). The overarching purpose of the current project is to use an animal model of social conflict to gain a better understanding of the role BDNF plays in brain and behavioral responses to social stress.

Our lab studies a unique model of social stress in Syrian hamsters, which are naturally solitary and are highly territorial. Thus, unlike some other species (e.g., rats) used in resident-intruder models, complex social housing arrangements are not required to induce aggression in hamsters. In addition, both male and female Syrian hamsters spontaneously exhibit aggressive behavior towards conspecific intruders (Huhman et al., 2003) making them ideal subjects for the study of social conflict. Additionally, their agonistic behavior is highly ritualized and few injuries occur as a result. In spite of these natural aggressive tendencies, after a brief social defeat hamsters no longer defend their home cage or territory, even against a smaller, non-aggressive intruder (NAI). Instead of attacking the NAI, a previously defeated hamster subsequently exhibits no aggression and instead displays submissive and defensive behavior

toward the intruder. This dramatic behavioral change following social defeat has been termed conditioned defeat (CD) (Potegal et al., 1993). In hamsters, the submissive behavior and the absence of aggression that characterizes CD can last at least a month in the majority of subjects after brief social defeat (Huhman et al., 2003). Defeated animals also exhibit increased plasma adrenocorticotropin, beta-endorphin, and cortisol, suggesting that social defeat is a salient stressor for these animals (Huhman et al., 1990; Huhman et al., 1991a; Huhman et al., 1991b; Huhman et al., 1992).

Beyond the hormonal and behavioral impacts of social defeat, our lab has examined the involvement of numerous neurochemical signals and brain nuclei in CD. Neurochemicals including serotonin (5HT), cAMP response element-binding protein (CREB), gamma-aminobutyric acid (GABA), dopamine (DA), corticotropin-releasing hormone (CRH), glutamate, and BDNF have all been examined to gain a better understanding of the mechanisms underlying social stress-induced behavioral change in hamsters (Cooper & Huhman 2005; Cooper & Huhman 2007; Day et al., 2011; Jasnow & Huhman, 2001; McDonald et al., 2012). Over the past several years our lab has also worked to identify a putative neural circuit that underlies CD (Markham & Huhman, 2008; Markham et al., 2009; Markham et al., 2010; McDonald et al., 2012). As depicted in the circuit diagram (Figure 1), several nuclei are involved in the acquisition and/or expression of CD. The basolateral amygdala (BLA), in particular, seems to play a central role in this circuit as the BLA is necessary for both the acquisition and expression of CD (Jasnow, 2005; Markham et al., 2010; Day et al., 2011), and synaptic plasticity in the BLA is required for CD learning to occur (Jasnow, 2005; Markham et al., 2010). Other brain areas such as the medial prefrontal cortex (mPFC) appear to be necessary only for the acquisition of CD (Markham et al., 2012). [Note: the mPFC is composed of the infralimbic cortex (IL) and prelimbic cortex (PL) in rodents and is analogous to area Brodmann's Areas (BA) 25 and 32 respectively in humans (Milad et al., 2007; Myers-Schulz & Koenigs, 2012; Quirk & Beer, 2006; Slattery, Neumann, & Cryan, 2011).]. Interestingly, the projections between the amygdala and

the mPFC are thought to play a role in neuropsychiatric illnesses such as PTSD and depression. For example, decreased activity of the PFC in disease states such as PTSD (Rabinak et al., 2014; Jovanovic et al., 2013) and depression (Rive et al., 2013; Erk et al., 2010) may fail to inhibit or even cause an over-active amygdala (Hamilton et al., 2008) in humans.

Current findings regarding changes in BDNF following social stress are somewhat inconsistent. For example, BDNF protein expression is increased in the mPFC by repeated social stress in mice (Nikulina et al., 2012) and in the amygdala following chronic stress (reviewed by Boyle, 2013). Others, however, report that social stress decreases BDNF protein in the amygdala, the hypothalamus, and the hippocampus (Fanous et al., 2010; Pizarro et al., 2004). Still other experiments indicate that BDNF mRNA is reduced 24 h after social defeat stress in the hippocampus, BLA, and parts of the cortex in mice (Pizarro et al., 2004), but we have shown that a brief social defeat increases BDNF mRNA in the BLA as compared to controls (Taylor et al., 2011). Thus, the current data are inconclusive as to the response of BDNF mRNA and protein to various stressors and to the role that BDNF plays in behavioral responses to stress. Some data indicate that BDNF may promote behavioral changes following stress, while other findings indicate that BDNF may protect against deleterious effects of stress and may thus act as a sort of antidepressant. It is possible that BDNF is altered by social stress in a site-specific manner (reviewed in Sakata, 2013) and that peripheral assays of BDNF, as reported in humans, are representative of a net effect of complex regional changes.

Our finding that social defeat stress increases BDNF mRNA in the amygdala suggests that BDNF might act in the amygdala to promote subordinate behavioral responses to social defeat in hamsters. To determine if inhibiting BDNF signaling would reduce the acquisition of CD and restore territorial aggression, Taylor and colleagues injected a non-specific Trk receptor antagonist, k252a, directly into the BLA (Taylor et al., 2011). K252a administered site-specifically in the BLA before defeat reduced the display of submission towards an NAI 24 h after defeat, indirectly supporting the hypothesis that BDNF promotes CD learning (Taylor et

al., 2011). However, site-specific injections cannot be used in humans, thus it is also important to determine if systemic injections that target the BDNF system are also effective in altering social stress-induced behavioral changes observed in socially defeated hamsters. Since the completion of our earlier study using k252a, novel compounds have been developed that specifically target TrkB receptors (Cazorla et al., 2011; Jang et al., 2010). These drugs offer a much improved way to test specifically the role of BDNF and TrkB receptors than does k252a, which is a non-specific Trk receptor antagonist that binds to both TrkA and TrkB receptors, as well as to a wide variety of other kinases (Arthur et al., 2005; Berg et al., 1992; Koizumi et al., 1988; Sofroniew et al., 2001). These novel TrkB compounds also allow us to examine the effect of peripherally administered BDNF-active compounds on social stress-induced behavioral changes and to determine if these compounds promote or prevent these changes in hamsters. Although these peripheral injections do not promote our understanding of the neural circuitry underlying the drugs' effects, the peripheral route of administration has potential translational implications for the utility of these drugs to alter responses to social stress. Based on our earlier findings, we expected that the peripheral administration of a specific TrkB receptor agonist would promote CD and that a specific TrkB receptor antagonist would decrease CD. This prediction will be tested in Specific Aim 1 of the current project.

Beyond the potential behavioral effect that these novel compounds may have when given peripherally in hamsters, it is also important to determine where in the brain these drugs are acting. Understanding where peripheral compounds are acting in the brain will 1) direct future studies for investigating the role of BDNF within specific nuclei, and 2) identify how changes in BDNF may alter neuronal activity in a nucleus dependent manner within the putative CD circuit. The purpose of Aim 2 is to determine in which brain nuclei a peripherally administered TrkB receptor agonist might act to alter neuronal activation following a defeat experience. FOS is the protein product of *c-fos*, an immediate early gene that is transiently activated in response to a cellular stimuli and has been used as a proxy to examine neuronal activation. In Aim 2, we

measured neuronal activation using FOS immunohistochemistry (FOS-IR). Specifically, we administered a TrkB agonist immediately following social defeat and examined FOS-IR 60 min later in the PL, IL, and the BLA. These 3 nuclei were selected because 1) they are part of the putative CD circuit (Figure 1), 2) TrkB receptors are found in these nuclei (Fryer et al., 1996), 3) BDNF is reported to play a role in stress responding in these nuclei (reviewed in Duman & Monteggia, 2006), and 4) defeat-induced neural activity as measured via FOS is robust in these areas (Bourne et al., 2013; Kollack-Walker et al., 1997; Kollack-Walker et al., 1999).

As mentioned above, the mPFC is necessary for the acquisition of CD (Markham et al., 2012). The mPFC, specifically area BA 32 in humans, is analogous to the rodent prelimbic cortex, whereas area BA 25 is analogous to the infralimbic cortex in rodents (Milad et al., 2007; Myers-Schulz & Koenigs, 2012; Quirk & Beer, 2006; Slattery, Neumann, & Cryan, 2011). Interestingly, both of these nuclei are active following stress, as measured by immediate early genes, such as *c-fos*. Repeated social defeat, for example, increases delta FOS B in the IL and PL (Nikulina et al., 2012), suggesting that both the PL and IL are active following this stressor. Studies examining the IL and PL individually have found region-specific differences in neural activation. Morrison and colleagues, for example, found that FOS-IR is increased in the IL of dominant as compared to subordinate Syrian hamsters (Morrison et al., 2012). When muscimol is site-specifically administered in the IL of dominant hamsters, then CD is increased (Morrison et al., 2013), suggesting that the IL is important for resistance to defeat stress. Similarly, lesions of the IL eliminate the stress-reducing effects of enriched housing environments on social defeat in mice (Lehmann and Herkenham, 2011). Labs that have studied both the IL and PL have found that stimulation of the IL *reduces* conditioned fear (Milad, Vidal-Gonzalez, & Quirk, 2004), whereas stimulation of the PL *increases* the expression of conditioned fear (Vidal-Gonzalez et al., 2006). Furthermore, recent work suggests that the PL underlies susceptibility to social defeat. Specifically, over expression of delta FOSB in the PL and infusion of a CCK agonist into the PL promote depression-like and anxiety-like behaviors after social defeat (Vialou et al.,

2014). Recent work using BDNF knockout mice has found that BDNF in the PL is necessary for the consolidation of fear learning (Choi et al., 2010; Choi et al., 2012), whereas BDNF in the IL is necessary for extinction of fear (Peters et al., 2010), thus providing additional evidence that these 2 nuclei may act in opposition and that BDNF is involved. Even with the differences in neuronal activation in the PL and IL, we still expected to see activation due to stress, and therefore we predicted that FOS-IR would increase in the IL and the PL following social defeat in Syrian hamsters. It was also possible, however, that activation was higher in the PL given that it appears to be involved in stress susceptibility. Given the role of BDNF in the IL and PL, we further predicted that defeat-induced FOS-IR will be enhanced in the PL following the peripheral administration of a TrkB receptor agonist. This prediction was tested in Specific Aim 2 of the current project.

Given the key role the BLA plays in CD, we also expected to see an increase in FOS-IR expression in this nucleus following social defeat, as observed previously (Markham et al., 2010). We, therefore, predicted that FOS-IR would increase in the BLA following social defeat in Syrian hamsters. Furthermore, we tested whether a TrkB receptor agonist enhances this neural activation given that BDNF in the BLA appears to play a key role in CD learning following social stress. These predictions were tested in Specific Aim 2 of the current project.

Much attention has been paid to the projections between the amygdala and the mPFC and their potential dysregulation in neuropsychiatric illnesses in humans. Interestingly, work in animal models has found that reciprocal projections between the amygdala and the mPFC are involved in fear learning and in threat identification (Ishikawa & Nakamura, 2003; Marek et al., 2013; Ongur & Price, 2000). Specifically tract tracing studies have found that the IL and PL project to different sub-nuclei within the amygdala (Vertes, 2004; Gabbott, 2005) and that stimulation of the PL enhances freezing (Vidal-Gonzalez et al., 2006) whereas stimulation of the IL reduces freezing (Milad & Quirk, 2002; Milad et al., 2004). Other data indicate that these 2 sub-nuclei may act in opposition as the PL is necessary for fear expression, whereas the IL is

necessary in fear extinction (Sierra-Mercado et al., 2011). It has previously been suggested that the projections from the PL to the basal nucleus of the amygdala are excitatory and, in turn, increase stress-induced freezing via projections to the central amygdala (Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011). In contrast, projections of the IL synapse on the intercalated cells, which then inhibit the central amygdala to reduce freezing behavior (Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011).

Recent work suggests that BDNF acts in a nucleus dependent manner in the PL and the IL to regulate fear learning (Choi et al., 2010; Choi et al., 2012; Peters et al., 2010). Our third specific aim was therefore designed to examine the hypothesis that BDNF in the mPFC modifies, in a site-specific manner, neural activation in the BLA following social defeat. Based on the aforementioned connections as laid out in Figure 2 (adapted from Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011), we expected that BDNF administered into the PL would enhance defeat-induced FOS-IR in the BLA and CeA, whereas BDNF administered into the IL would suppress defeat-induced FOS-IR in the BLA and CeA.

In conclusion, it was the goal of this dissertation to test the hypothesis that BDNF, via its action at TrkB receptors, enhances the effects of social stress and that it does so, at least in part, by binding in the PL, which in turn enhances the response of the BLA to social defeat. This hypothesis was addressed by the following specific aims (SAs):

*SA 1: Does peripheral administration of drugs that activate or inhibit TrkB receptors have stress-promoting or stress-preventing effects, respectively?*

*SA 2: Does peripheral administration of a TrkB receptor agonist in combination with social defeat decrease neural activity as measured by FOS in the IL and increase FOS in the PL and BLA?*

*SA 3: Do microinjections of BDNF into the IL inhibit neural activity in the BLA?*



## 1.1 References

- Adachi N, Numakawa T, Richards M, Nakajima S, Kunugi H. 2014. New insight in expression, transport, and secretion of brain-derived neurotrophic factor: Implications in brain-related diseases. *World journal of biological chemistry* 5: 409-28
- Agid O, Kohn Y, Lerer B. 2000. Environmental stress and psychiatric illness. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 54: 135-41
- Arthur DB, Akassoglou K, Insel PA. 2005. P2Y2 receptor activates nerve growth factor/TrkA signaling to enhance neuronal differentiation. *Proceedings of the National Academy of Sciences of the United States of America* 102: 19138-43
- Bartolomucci A. 2007. Social stress, immune functions and disease in rodents. *Frontiers in neuroendocrinology* 28: 28-49
- Berg MM, Sternberg DW, Parada LF, Chao MV. 1992. K-252a inhibits nerve growth factor-induced trk proto-oncogene tyrosine phosphorylation and kinase activity. *The Journal of biological chemistry* 267: 13-6
- Bjorkqvist K. 2001. Social defeat as a stressor in humans. *Physiology & behavior* 73: 435-42
- Blanchard RJ, McKittrick CR, Blanchard DC. 2001. Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiology & behavior* 73: 261-71
- Block JP, He Y, Zaslavsky AM, Ding L, Ayanian JZ. 2009. Psychosocial stress and change in weight among US adults. *American journal of epidemiology* 170: 181-92
- Bourne AR, Mohan G, Stone MF, Pham MQ, Schultz CR, et al. 2013. Olfactory cues increase avoidance behavior and induce Fos expression in the amygdala, hippocampus and prefrontal cortex of socially defeated mice. *Behavioural brain research* 256: 188-96
- Boyle LM. 2013. A neuroplasticity hypothesis of chronic stress in the basolateral amygdala. *The Yale journal of biology and medicine* 86: 117-25
- Castren E, Rantamaki T. 2010a. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Developmental neurobiology* 70: 289-97
- Castren E, Rantamaki T. 2010b. Role of brain-derived neurotrophic factor in the aetiology of depression: implications for pharmacological treatment. *CNS drugs* 24: 1-7
- Cazorla M, Premont J, Mann A, Girard N, Kellendonk C, Rognan D. 2011. Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. *The Journal of clinical investigation* 121: 1846-57
- Chao MV, Rajagopal R, Lee FS. 2006. Neurotrophin signalling in health and disease. *Clinical science* 110: 167-73
- Choi DC, Gourley SL, Ressler KJ. 2012. Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning. *Translational psychiatry* 2: e205
- Choi DC, Maguschak KA, Ye K, Jang SW, Myers KM, Ressler KJ. 2010. Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2675-80

- Chrousos GP. 2009. Stress and disorders of the stress system. *Nature reviews. Endocrinology* 5: 374-81
- Cooper MA, Huhman KL. 2005. Corticotropin-releasing factor type II (CRF-sub-2) receptors in the bed nucleus of the stria terminalis modulate conditioned defeat in Syrian hamsters (*Mesocricetus auratus*). *Behavioral neuroscience* 119: 1042-51
- Cooper MA, Huhman KL. 2007. Corticotropin-releasing factor receptors in the dorsal raphe nucleus modulate social behavior in Syrian hamsters. *Psychopharmacology* 194: 297-307
- Day DE, Cooper MA, Markham CM, Huhman KL. 2011. NR2B subunit of the NMDA receptor in the basolateral amygdala is necessary for the acquisition of conditioned defeat in Syrian hamsters. *Behavioural brain research* 217: 55-9
- Dell'Osso L, Carmassi C, Del Debbio A, Catena Dell'Osso M, Bianchi C, et al. 2009. Brain-derived neurotrophic factor plasma levels in patients suffering from post-traumatic stress disorder. *Progress in neuro-psychopharmacology & biological psychiatry* 33: 899-902
- Dickerson SS, Kemeny ME. 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological bulletin* 130: 355-91
- Duman RS, Monteggia LM. 2006. A neurotrophic model for stress-related mood disorders. *Biological psychiatry* 59: 1116-27
- Dwivedi Y. 2010. Brain-derived neurotrophic factor and suicide pathogenesis. *Annals of medicine* 42: 87-96
- Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, et al. 2004. Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences of the United States of America* 101: 17312-5
- Erk S, Mikschl A, Stier S, Ciaramidaro A, Gapp V, et al. 2010. Acute and sustained effects of cognitive emotion regulation in major depression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30: 15726-34
- Fanous S, Hammer RP, Jr., Nikulina EM. 2010. Short- and long-term effects of intermittent social defeat stress on brain-derived neurotrophic factor expression in mesocorticolimbic brain regions. *Neuroscience* 167: 598-607
- Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ. 2005. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. *The Journal of comparative neurology* 492: 145-77
- Ghiadoni L, Donald AE, Cropley M, Mullen MJ, Oakley G, et al. 2000. Mental stress induces transient endothelial dysfunction in humans. *Circulation* 102: 2473-8
- Gilbert P, Allan S. 1998. The role of defeat and entrapment (arrested flight) in depression: an exploration of an evolutionary view. *Psychological medicine* 28: 585-98
- Hamilton JP, Gotlib IH. 2008. Neural substrates of increased memory sensitivity for negative stimuli in major depression. *Biological psychiatry* 63: 1155-62
- Heim C, Nemeroff CB. 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological psychiatry* 49: 1023-39
- Hoffman DL, Dukes EM, Wittchen HU. 2008. Human and economic burden of generalized anxiety disorder. *Depression and anxiety* 25: 72-90
- Huang EJ, Reichardt LF. 2003. Trk receptors: roles in neuronal signal transduction. *Annual review of biochemistry* 72: 609-42

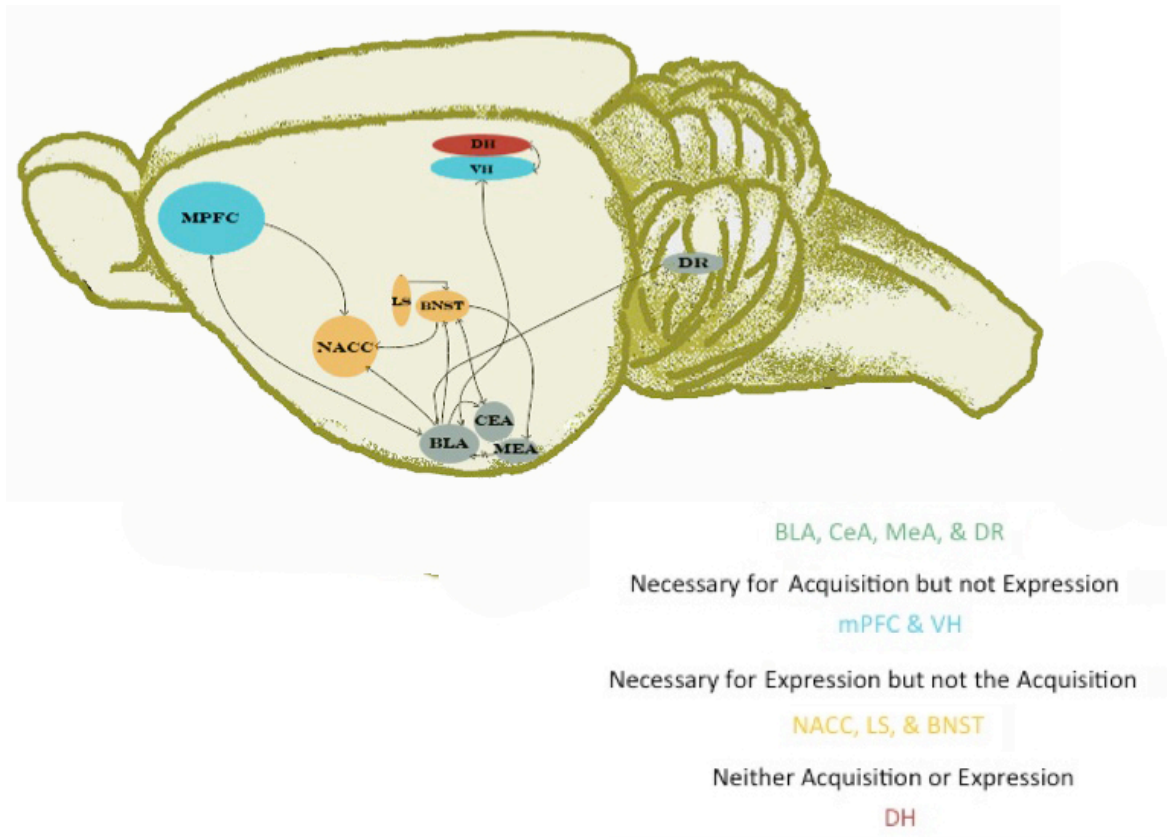
- Huhman KL, Bunnell BN, Mougey EH, Meyerhoff JL. 1990. Effects of social conflict on POMC-derived peptides and glucocorticoids in male golden hamsters. *Physiology & behavior* 47: 949-56
- Huhman KL, Hebert MA, Meyerhoff JL, Bunnell BN. 1991a. Plasma cyclic AMP increases in hamsters following exposure to a graded footshock stressor. *Psychoneuroendocrinology* 16: 559-63
- Huhman KL, Moore TO, Ferris CF, Mougey EH, Meyerhoff JL. 1991b. Acute and repeated exposure to social conflict in male golden hamsters: increases in plasma POMC-peptides and cortisol and decreases in plasma testosterone. *Hormones and behavior* 25: 206-16
- Huhman KL, Moore TO, Mougey EH, Meyerhoff JL. 1992. Hormonal responses to fighting in hamsters: separation of physical and psychological causes. *Physiology & behavior* 51: 1083-6
- Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, et al. 2003. Conditioned defeat in male and female Syrian hamsters. *Hormones and behavior* 44: 293-9
- Ishikawa A, Nakamura S. 2003. Convergence and interaction of hippocampal and amygdalar projections within the prefrontal cortex in the rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23: 9987-95
- Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, et al. 2010. A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2687-92
- Jasnow AM, Huhman KL. 2001. Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain research* 920: 142-50
- Jasnow AM, Shi C, Israel JE, Davis M, Huhman KL. 2005. Memory of social defeat is facilitated by cAMP response element-binding protein overexpression in the amygdala. *Behavioral neuroscience* 119: 1125-30
- Kalia M. 2002. Assessing the economic impact of stress--the modern day hidden epidemic. *Metabolism: clinical and experimental* 51: 49-53
- Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. 2002. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry research* 109: 143-8
- Kelleher I, Harley M, Lynch F, Arseneault L, Fitzpatrick C, Cannon M. 2008. Associations between childhood trauma, bullying and psychotic symptoms among a school-based adolescent sample. *The British journal of psychiatry : the journal of mental science* 193: 378-82
- Koizumi S, Contreras ML, Matsuda Y, Hama T, Lazarovici P, Guroff G. 1988. K-252a: a specific inhibitor of the action of nerve growth factor on PC 12 cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 8: 715-21
- Kollack-Walker S, Don C, Watson SJ, Akil H. 1999. Differential expression of c-fos mRNA within neurocircuits of male hamsters exposed to acute or chronic defeat. *Journal of neuroendocrinology* 11: 547-59
- Kollack-Walker S, Watson SJ, Akil H. 1997. Social stress in hamsters: defeat activates specific neurocircuits within the brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17: 8842-55

- Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P, Timmermans PJ. 2013. The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *Journal of visualized experiments : JoVE*: e4367
- Lehmann ML, Herkenham M. 2011. Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31: 6159-73
- Marek R, Strobel C, Bredy TW, Sah P. 2013. The amygdala and medial prefrontal cortex: partners in the fear circuit. *The Journal of physiology* 591: 2381-91
- Markham CM, Huhman KL. 2008. Is the medial amygdala part of the neural circuit modulating conditioned defeat in Syrian hamsters? *Learning & memory* 15: 6-12
- Markham CM, Luckett CA, Huhman KL. 2012. The medial prefrontal cortex is both necessary and sufficient for the acquisition of conditioned defeat. *Neuropharmacology* 62: 933-9
- Markham CM, Norvelle A, Huhman KL. 2009. Role of the bed nucleus of the stria terminalis in the acquisition and expression of conditioned defeat in Syrian hamsters. *Behavioural brain research* 198: 69-73
- Markham CM, Taylor SL, Huhman KL. 2010. Role of amygdala and hippocampus in the neural circuit subserving conditioned defeat in Syrian hamsters. *Learning & memory* 17: 109-16
- McDonald MM, Markham CM, Norvelle A, Albers HE, Huhman KL. 2012. GABAA receptor activation in the lateral septum reduces the expression of conditioned defeat and increases aggression in Syrian hamsters. *Brain research* 1439: 27-33
- McEwen BS, Stellar E. 1993. Stress and the individual. Mechanisms leading to disease. *Archives of internal medicine* 153: 2093-101
- Milad MR, Quirk GJ. 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420: 70-4
- Milad MR, Vidal-Gonzalez I, Quirk GJ. 2004. Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behavioral neuroscience* 118: 389-94
- Minichiello L. 2009. TrkB signalling pathways in LTP and learning. *Nature reviews. Neuroscience* 10: 850-60
- Morrison KE, Bader LR, McLaughlin CN, Cooper MA. 2013. Defeat-induced activation of the ventral medial prefrontal cortex is necessary for resistance to conditioned defeat. *Behavioural brain research* 243: 158-64
- Morrison KE, Curry DW, Cooper MA. 2012. Social status alters defeat-induced neural activation in Syrian hamsters. *Neuroscience* 210: 168-78
- NIH. Major Depression Among Adults.
- NIH. Post-Traumatic Stress Disorder Among Adults.
- Nikulina EM, Lacagnina MJ, Fanous S, Wang J, Hammer RP, Jr. 2012. Intermittent social defeat stress enhances mesocorticolimbic DeltaFosB/BDNF co-expression and persistently activates corticotegmental neurons: implication for vulnerability to psychostimulants. *Neuroscience* 212: 38-48
- Ongur D, Price JL. 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral cortex* 10: 206-19

- Pego JM, Sousa JC, Almeida OF, Sousa N. 2010. Stress and the neuroendocrinology of anxiety disorders. *Current topics in behavioral neurosciences* 2: 97-117
- Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. 2010. Induction of fear extinction with hippocampal-infralimbic BDNF. *Science* 328: 1288-90
- Pizarro JM, Lumley LA, Medina W, Robison CL, Chang WE, et al. 2004. Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain research* 1025: 10-20
- Potegal M, Huhman K, Moore T, Meyerhoff J. 1993. Conditioned defeat in the Syrian golden hamster (*Mesocricetus auratus*). *Behavioral and neural biology* 60: 93-102
- Rakofsky JJ, Ressler KJ, Dunlop BW. 2012. BDNF function as a potential mediator of bipolar disorder and post-traumatic stress disorder comorbidity. *Molecular psychiatry* 17: 22-35
- Reichardt LF. 2006. Neurotrophin-regulated signalling pathways. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 361: 1545-64
- Rive MM, van Rooijen G, Veltman DJ, Phillips ML, Schene AH, Ruhe HG. 2013. Neural correlates of dysfunctional emotion regulation in major depressive disorder. A systematic review of neuroimaging studies. *Neuroscience and biobehavioral reviews* 37: 2529-53
- Russ TC, Stamatakis E, Hamer M, Starr JM, Kivimaki M, Batty GD. 2012. Association between psychological distress and mortality: individual participant pooled analysis of 10 prospective cohort studies. *Bmj* 345: e4933
- Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, et al. 2003. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23: 349-57
- Sakata K, Martinowich K, Woo NH, Schloesser RJ, Jimenez DV, et al. 2013. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. *Proceedings of the National Academy of Sciences of the United States of America* 110: 15103-8
- Seegerstrom SC, Miller GE. 2004. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychological bulletin* 130: 601-30
- Shin LM, Liberzon I. 2010. The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 35: 169-91
- Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. 2011. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 36: 529-38
- Sofroniew MV, Howe CL, Mobley WC. 2001. Nerve growth factor signaling, neuroprotection, and neural repair. *Annual review of neuroscience* 24: 1217-81
- Suliman S, Hemmings SM, Seedat S. 2013. Brain-Derived Neurotrophic Factor (BDNF) protein levels in anxiety disorders: systematic review and meta-regression analysis. *Frontiers in integrative neuroscience* 7: 55
- Tamashiro KL, Nguyen MM, Sakai RR. 2005. Social stress: from rodents to primates. *Frontiers in neuroendocrinology* 26: 27-40

- Taylor SL, Stanek LM, Ressler KJ, Huhman KL. 2011. Differential brain-derived neurotrophic factor expression in limbic brain regions following social defeat or territorial aggression. *Behavioral neuroscience* 125: 911-20
- Trivedi MH, Fava M, Wisniewski SR, Thase ME, Quitkin F, et al. 2006a. Medication augmentation after the failure of SSRIs for depression. *The New England journal of medicine* 354: 1243-52
- Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, et al. 2006b. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR\*D: implications for clinical practice. *The American journal of psychiatry* 163: 28-40
- Vertes RP. 2004. Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse* 51: 32-58
- Vialou V, Bagot RC, Cahill ME, Ferguson D, Robison AJ, et al. 2014. Prefrontal cortical circuit for depression- and anxiety-related behaviors mediated by cholecystokinin: role of DeltaFosB. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34: 3878-87
- Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ. 2006. Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learning & memory* 13: 728-33
- WHO. 2008. The Global Burden of Disease: 2004 Update. Geneva, Switzerland: WHO Press
- Yoshii A, Constantine-Paton M. 2010. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Developmental neurobiology* 70: 304-22
- Zhou XF, Chie ET, Rush RA. 1998. Distribution of brain-derived neurotrophic factor in cranial and spinal ganglia. *Exp Neurol* 149: 237-42
- Zhou XF, Parada LF, Soppet D, Rush RA. 1993. Distribution of trkB tyrosine kinase immunoreactivity in the rat central nervous system. *Brain research* 622: 63-70

## 1.2 Figures



**Figure 1.1** Putative Circuit Mediating the Acquisition and Expression of Conditioned Defeat

## 2 BDNF REDUCES BEHAVIORAL RESPONSES TO SOCIAL STRESS IN SYRIAN HAMSTERS

### 2.1 Abstract

Brain derived neurotrophic factor (BDNF) via its action at its cognate receptor, TrkB, promotes learning and synaptic plasticity. Peripheral administration of 7,8-Dihydroxyflavone (7,8-DHF), a novel TrkB receptor agonist, increases conditioned fear learning following cued fear conditioning. Furthermore, our lab has demonstrated that a non-specific Trk receptor antagonist administered into the BLA prior to defeat reduces later submission, suggesting that neurotrophins are also necessary for fear learning in social situations and that defeat-induced behavioral changes are mediated at least in part in the BLA. The purpose of this study was to test whether BDNF acts via TrkB receptors to promote conditioned defeat, and if so, to test whether BDNF acts directly in the BLA. First, we administered rhBDNF (0.2ng/200nl; 0.4ng/200nl) directly into the BLA immediately prior to defeat and found that BDNF *reduced* submission in defeated hamsters when they were tested 24 h after defeat training. Similarly, the specific TrkB receptor agonist 7,8-DHF given immediately prior to (0.0, 2.5; 5.0; 10mg/kg IP) or immediately after (0.0; 10mg/kg IP) defeat training also reduced submission in defeated hamsters during testing. Finally, we demonstrated that the TrkB receptor antagonist ANA-12 (0.0; 0.5; 1mg/kg IP) enhanced submission during testing. Counter to our prediction that BDNF acting at TrkB receptors would promote CD learning, these results suggest that BDNF is a molecule that can reduce behavioral responses to stress and may thus promote a state of resistance to stress.



## 2.2 Introduction

Brain derived neurotrophic factor (BDNF) has gained increasing attention for its role in stress responding, learning, and memory (Chao et al., 2006; Minichiello, 2009; Adachi et al., 2014). This protein binds to tyrosine kinase B (TrkB) receptors in both brain (e.g., cerebral cortex, basal forebrain, striatum and hippocampus) and periphery, and BDNF is known to be involved in neuron growth, plasticity, and synapse development (Conner et al., 1997; Chao et al., 2006; Minichiello, 2009). Of particular interest is the role of BDNF in synaptogenesis and long-term potentiation (see Cunha et al., 2010 for review), as it appears that BDNF enhances long-term memory (LTM) (e.g., fear learning). Furthermore, BDNF and LTM are both altered by stress (Yang et al., 2013; see Pittenger & Duman, 2008 for review), thus leading to complex and context-dependent changes in brain and behavior. It is therefore not surprising that the intersection of BDNF, learning, and stress has gained increasing attention, especially given the potential clinical applications (e.g., potential treatment of mood and anxiety disorders). As the data regarding BDNF converges between different scientific subfields, it appears that there is support for 2 seemingly opposing ways that BDNF can alter the response to stressful experiences: one promoting fear learning and the other suggesting that BDNF is a molecule that reduces the effects of stress and instead promotes resiliency.

A large body of research indicates that BDNF/TrkB signaling is necessary for fear learning in several nuclei including the mPFC and the amygdala (reviewed in Mahan & Ressler, 2012). Pre-training administration of a non-specific Trk receptor antagonist directly into the BLA decreases subsequent fear-potentiated startle, suggesting that BDNF/TrkB signaling within the BLA is necessary for the acquisition of conditioned fear (Rattiner et al., 2004; reviewed in Mahan & Ressler, 2012). Similarly, we have demonstrated that k252a, a non-specific Trk receptor antagonist, administered directly into the BLA prior to defeat reduces submissive behavior 24 h later during testing (Taylor et al., 2011). The finding that BDNF is also necessary for the consolidation of fear learning is confirmed in several site-specific BDNF knockout

models that demonstrate that BDNF in the mPFC is necessary for fear learning (Choi et al., 2010; Choi et al., 2012). These studies clearly support the idea that BDNF promotes learning and the acquisition or consolidation of conditioned fear.

Furthermore, BDNF is altered in circuits that are important for fear learning and in nuclei that are stress responsive. For example, chronic stress in rats and repeated social stress in mice increases BDNF protein in the amygdala (Lakshminarasimhan & Chattarji, 2012; see also review by Boyle, 2013). Ten days of social defeat stress in mice increases BDNF protein levels in the nucleus accumbens (NAc) 24 h and 4 weeks later (Berton et al., 2006). Our lab has also reported that BDNF mRNA is increased in the BLA 24 h after social defeat. However, these results conflict with other reports that stress instead decreases BDNF protein or mRNA. Fanous and colleagues, for example, report that social stress decreases BDNF protein in the amygdala (Fanous et al., 2010). BDNF mRNA is also reported to decrease in the BLA 24 h after social defeat stress in mice (Pizarro et al., 2004). It is clear the BDNF changes in response to stressful experiences, but the directionality of these changes is not at all certain.

In opposition to its role in promoting fear learning, there is also evidence to suggest BDNF is protective against stress-induced behavioral changes. One of the most exciting frontiers in the field of depression research is the finding that BDNF may play a key role in this debilitating disorder. BDNF is suppressed following exposure to stress in humans, and reduced BDNF protein in serum is observed in patients with numerous psychiatric illnesses, including major depressive disorder (Karege et al., 2002; reviewed by Duman & Monteggia, 2006; Castren & Rantamaki, 2010), posttraumatic stress disorder (PTSD) (Dell'osso et al., 2009; review by Rakofsky et al., 2012; Suliman, Hemmings, & Seedat, 2013), as well as in blood cells of suicidal patients (Dwivedi, 2010). Numerous labs using animal models have reported that BDNF is increased following antidepressant treatment (Karege et al., 2002; reviewed by Duman and Monteggia, 2006; Castren & Rantamaki, 2010; Suliman, Hemmings, & Seedat, 2013). Furthermore, blocking BDNF receptor activation in the prefrontal cortex prevents

antidepressant effects in mice as measured in the forced swim test (Saarelainen et al., 2003). These data suggest that BDNF is a molecule that promotes “stress resilience”, as defined by a reduced behavioral response to stress, and that alterations of this neurotrophin system is one of the mechanisms that underlies the effectiveness of antidepressants.

Given the apparent contradictions regarding the role of BDNF in stress and LTM, it is important that additional work be completed. One model that may help to clarify the role of BDNF is conditioned defeat (CD), which refers to the increase in submissive behavior that is observed following a social defeat experience. CD can be viewed as an ethologically relevant model of fear conditioning, and many of the manipulations that alter fear conditioning also alter CD similarly. Given the literature supporting a role for BDNF in fear conditioning, we expected BDNF to promote CD learning as evidenced by increasing submission following social defeat. This finding would be consistent with our previous findings that BDNF mRNA increases in the BLA following defeat and that a non-specific TrkB antagonist in the BLA decreases the acquisition of CD. The purpose of this project was therefore to clarify the role of BDNF in behavioral responses to social stress following the site-specific injection of BDNF into the BLA and following peripheral administration of a selective TrkB receptor agonist and antagonist.

## **2.3 Materials & Methods**

### ***2.3.1 Animals & Housing Conditions***

Subjects in all experiments were male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories, New York, NY) that weighed 120-140 g and were between 9-10 weeks old at the time of testing. All hamsters were handled for five days prior to behavioral procedures to acclimate them to experimenter handling and were singly housed in polycarbonate cages (20x 40 x 20cm) with corncob bedding and wire mesh tops in a temperature ( $20 \pm 2^\circ$  C) and humidity controlled room. The colony room was maintained on a 14:10 light: dark cycle with lights off at 10:00 am. Food and water were available *ad libitum*. Older hamsters (>6 months),

which were individually housed (>1 month) and weighed at least 180g served as resident aggressors (RA) in defeat training, whereas smaller (110g-120g), younger (~ 7 weeks) hamsters were group housed and served as non-aggressive intruders (NAI) during CD testing. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### ***2.3.2 Surgical Procedures***

Subjects in Experiment 1 were deeply anesthetized via exposure to 5% isoflurane mixed with 100% oxygen, placed in a stereotaxic frame, and maintained under anesthesia for the duration of the cannula implantation surgery via a nose cone that delivered 2-3% isoflurane. Breathing and body temperature were monitored while animals were under anesthesia. 4mm, 26-gauge guide cannulae (Plastics One, Roanoke, VA) were bilaterally implanted and aimed at the BLA using the following stereotaxic coordinates: 0.2mm posterior and  $\pm 3.8$ mm lateral to bregma and 1.9 mm below dura. During the injection procedure, a 33-gauge needle with a 4.2 mm projection from the base of the cannula guide was lowered into the BLA to give a final dorsal-ventral depth of 6.3mm. This was done in order to minimize tissue damage in and around the amygdala. Lambda and bregma were leveled prior to placement of the guide cannula. Following surgery, dummy stylets were placed in the guide cannula to help maintain patency, and 1 cc of physiological saline and 0.1 cc of ketoprofen were injected subcutaneously in order to aid in recovery. Hamsters were allowed 5-7 days to recover prior to the start of behavioral testing during which time they were monitored daily to ensure that there were no adverse outcomes from the surgery.

### ***2.3.3 Social Defeat & Behavioral Testing***

The CD model has been described in detail elsewhere (Huhman et al., 2003). Prior to training, hamsters were matched by weight and then randomly assigned to groups. All animals

were transported in their home cage to the behavioral testing suite in the animal vivarium 30 min prior to all behavioral manipulations in order to acclimate. All behavioral procedures were performed in the first 3 h of the dark phase of the daily light: dark cycle to minimize any circadian effects. Social defeat training occurred in the home cage of the RA for 15 min (Experiment 1) or 5 min (Experiments 2a, 2b, & 3). In all experiments, no-defeat controls were placed into an empty RA cage to expose animals to odors but no physical interaction. Behavioral testing occurred 24 h after training when an NAI was placed in the home cage of the experimental animal for a 5 min test.

#### ***2.3.4 Behavioral Analysis***

All social defeat training and behavioral testing was recorded via video camera, transferred to an external hard drive, and later scored by a observer blind to experimental condition using Observer for Windows (Noldus Information Technology B.V., Wageningen, The Netherlands; version 7.0). Total durations for 4 classes of behavior were scored during testing including: (1) Social (stretch, approach, sniff, nose touching, and flank marking); (2) Non-social (locomotion, exploration, grooming, nesting, feeding and sleeping); (3) Submissive (flight, avoidance, tail up, upright and side defense, full submissive posture, stretch attend, attempted escape from cage); and (4) Aggressive (upright and side offense, chase and attack, including bite). In Experiment 3 wherein the drug treatment was given before the initial social defeat, the behavior of the resident aggressors and subjects was also scored during training to ensure that (1) all animals received similar defeats, and (2) all subjects were able to produce normal submissive behavior in response to attack.

#### ***2.3.5 Drug Administration***

In Experiment 1, rhBDNF (Sigma; St. Louis, MO) dissolved in physiological saline was administered via microinjection bilaterally into the BLA to freely moving hamsters immediately prior to defeat. All microinjections were administered over 2 min using a 33-gauge injection needle connected via polyethylene tubing to a Hamilton syringe. Hamilton syringes were

mounted on a syringe pump (Harvard Apparatus). The 2 dosages (0.2ng/200 nl; 0.4ng/200 nl) administered were based on previous work using site-specific microinjections of rhBDNF in rats in the periaqueductal grey (Casarotto et al., 2010).

In Experiments 2a & 2b, animals received either a TrkB receptor agonist, 7,8-Dihydroxyflavone (7,8-DHF) (TCI America; Montgomeryville, PA) dissolved in 40% Dimethylsulfoxide (DMSO)/60% saline (Experiments 2a & 2b) or vehicle (40% DMSO /60% saline). Animals in Experiment 2a received 7,8-DHF (2.5mg/kg; 5mg/kg; 10mg/kg) or vehicle immediately prior to a 5min defeat. A dose response curve was based on dosages used previously (Andero et al., 2010; Andero et al., 2011). Animals in Experiment 2b received the effective dose (determined in Experiment 2a) of 7,8-DHF (10mg/kg) or vehicle immediately after a 5min defeat. Post-training administration of 7,8-DHF has been used previously (Andero et al., 2010).

In Experiment 3, animals received either a TrkB receptor antagonist; ANA-12 (0.5mg/kg; 1.0mg/kg;) ([N2-2-2-Oxoazepan-3-yl amino] carbonyl phenyl benzo (b) thiophene-2-carboxamide) (Sigma; St. Louis, MO) dissolved in 100% DMSO or vehicle (100% DMSO) immediately after a 5min defeat. Dosages of ANA-12 were obtained from the literature (Cazorla et al., 2011). Post-training drug administration was used in Experiments 2b and 3 because the TrkB drugs were only soluble in DMSO solutions, and these vehicle solutions caused the subjects to have an odor that was discernable to the investigators. Administration of one of the DMSO solutions after defeat does not interfere with conspecific behavioral encounters.

### **2.3.6 Site Verification**

At the conclusion of Experiment 1, animals were administered an overdose of sodium pentobarbital and were bilaterally infused with 200nl of India ink to verify needle placements. Brains were then removed and placed in 10% buffered formalin before being sectioned on a cryostat (Leica CM 3050 S). Sections were stained with Neutral red, cover slipped with DPX mountant, and examined using a light microscope for ink in the BLA. Only animals with

bilateral ink injections within 0.3mm of the BLA, as determined by 2 observers blind to experimental group, were included in the final analysis.

### ***2.3.7 Experiment 1: Does a microinjection of BDNF into the BLA enhance the acquisition of CD?***

The purpose of Experiment 1 was to determine whether infusion of BDNF into the BLA enhances the acquisition of CD. Animals (n=19) were randomly assigned to one of 3 defeat groups that received one of the 2 dosages (0.2ng/200 nl; 0.4ng/200 nl) of rhBDNF in physiological saline or vehicle (physiological saline). All injections were administered immediately prior to a 15 min defeat. Animals were tested drug free 24 h later in their home cage with an NAI for 5 min, as described above in Experiment 1.

### ***2.3.8 Experiment 2a: Does systemic injection of a TrkB receptor agonist enhance the acquisition of CD?***

The goal of Experiment 2a was to determine whether an IP injection of a specific TrkB receptor agonist, 7, 8-DHF enhances the acquisition of CD. We predicted that 7,8-DHF would increase submissive behavior despite the site-specific effects of BDNF in the BLA because 1) the strong evidence that BDNF is necessary for fear learning and 2) the possibility that BDNF might act in opposing ways within nodes of the conditioned defeat neural circuit and peripheral injections would result in an overall net effect of the drug (i.e., enhanced submission). Animals (n=42) were randomly assigned to one of 4 defeat groups that received one of 3 dosages of 7,8-DHF (2.5mg/kg; 5mg/kg; 10mg/kg) dissolved in 40% Dimethylsulfoxide (DMSO) and 60% saline or vehicle (40% DMSO /60% saline) immediately prior to a 5 min defeat. A suboptimal 5 min defeat was used in this experiment to avoid a potential ceiling effect because we had initially expected this treatment to enhance CD. Animals were tested drug free 24 h later in their home cage with an NAI for 5 min, as described above in Experiment 1.

### ***2.3.9 Experiment 2b: Does systemic injection of a TrkB receptor agonist enhance the consolidation of CD?***

Given the unexpected findings that BDNF and a TrkB agonist administered prior to defeat reduce submission (Experiments 1 & 2a), the goal of Experiment 2b was to determine whether an IP injection of a specific TrkB receptor agonist, 7, 8-DHF enhances the consolidation of CD. Animals (n=40) were randomly assigned to groups (defeat or no defeat) and within these 2 conditions, animals received either 10mg/kg of 7,8-DHF dissolved in 40% Dimethylsulfoxide (DMSO) and 60% saline or vehicle (40% DMSO /60% saline) immediately after a 5 min defeat. A suboptimal 5 min defeat was used in this experiment to avoid a potential ceiling effect because we expected this treatment to enhance CD. Animals were tested drug free 24 h later in their home cage with an NAI for 5 min, as described above in Experiment 1.

### ***2.3.10 Experiment 3: Does a systemic injection of a TrkB receptor antagonist reduce the consolidation of CD?***

Given the unexpected findings that BDNF and a TrkB agonist reduce submission (Experiments 1-2b), the goal of Experiment 3 was to determine whether an IP injection of a specific TrkB receptor antagonist, ANA-12, enhances the consolidation of CD. Animals (n=38) received either ANA-12 (1.0mg/kg; 0.5mg/kg) dissolved in 100% DMSO or vehicle (100% DMSO) immediately after a suboptimal, 5 min defeat. Within the aforementioned drug groups defeat experience (defeat vs. no-defeat) was also examined. Animals were tested drug free 24 h later in their home cage with an NAI for 5 min, as described above in Experiment 2b.

### ***2.3.11 Statistical Analysis***

The total duration (seconds) of submission, social and non-social behaviors were determined. All durations were converted and represented as percent of vehicle control for statistical analysis because the baseline submission following defeat was variable due to the different durations of defeat training and natural variation in the behavior among experiments.



An analysis of variance (ANOVA) was used to test differences in durations of behavior between groups. When the variances in our behavioral data violated homogeneity (Levene's Test,  $P > 0.05$ ) or normality assumptions (normality of the behavioral data was examined using the Kolmogorov Smirnov test for Experiments 1, 2b, & 3), then non-parametric tests (Kruskal-Wallis using Mann Whitney U for post-hoc analysis) were completed, when appropriate. Analyses were performed with IBM SPSS software (20.0.0). Criterion for significance was set to  $p < 0.05$ .

## **2.4 Results**

### ***2.4.1 Experiment 1: BDNF infused into the BLA reduces the acquisition of CD***

Figure 1 shows the injections sites for all animals in Experiment 1, and Figure 2 is a photomicrograph of a representative coronal section of the BLA showing the location of the injection needle as indicated by ink injection.

As shown in Figure 3, infusion of BDNF into the BLA prior to defeat training significantly reduced the display of submissive behaviors (Kruskal Wallis,  $p < 0.05$ ) in defeated hamsters that received BDNF as compared to those receiving vehicle when tested 24 h later. No significant differences were observed for aggressive, social, and non-social behaviors in defeated hamsters that received BDNF as compared to vehicle (Kruskal Wallis,  $p > 0.05$ ). There were also no significant differences between drug and vehicle groups in the aggressive behavior produced by the RAs during defeat training or in the behavioral response exhibited by the experimental animals in response to attack (data not shown), indicating that rhBDNF did not alter the behavior of the RAs or the experimental animals during the initial defeat training.

### **2.4.2 Experiment 2a: 7, 8-DHF administered systemically reduces the acquisition of CD**

As shown in Figure 4, there was a main effect of drug on submission ( $F(3,39)=2.946$ ,  $p<0.05$ ) and on social behavior ( $F(3,39)=3.703$ ,  $p<0.05$ ). Least significant difference post-hoc tests revealed that hamsters receiving a 10mg/kg IP injection of 7,8-DHF prior to defeat displayed a significant decrease in submissive and defensive behavior when tested 24 h later as compared to animals receiving vehicle ( $p=0.007$ ). Post-hoc tests also revealed that hamsters receiving a 10mg/kg IP injection of 7,8-DHF prior to defeat displayed a significant increase in social behaviors as compared to animals receiving vehicle ( $p=0.049$ ). Animals receiving 2.5mg/kg of 7,8-DHF prior to defeat also displayed a significant increase in social behaviors as compared to animals receiving vehicle ( $p=0.002$ ). No significant differences were observed among groups for non-social behavior.

### **2.4.3 Experiment 2b: 7, 8-DHF administered systemically reduces the consolidation of CD**

As shown in Figure 5, hamsters receiving a 10mg/kg IP injection of 7,8-DHF after defeat displayed a significant decrease in submissive and defensive behavior when tested 24 h later (Kruskal Wallis,  $p<0.05$ ). No significant differences were observed for non-social, or social behavior. Aggression was not observed in any defeated animals.

7, 8-DHF did not have an effect on behavior in no-defeat controls (Table 1; Kruskal Wallis,  $p>0.05$ ). Furthermore, all defeated hamsters given vehicle showed a significant increase in submissive behavior as compared to the no-defeat, vehicle group (found in Table 1; Kruskal Wallis,  $p<0.05$ ) indicating that exposure to social defeat resulted in CD as evidenced by a significant increase in the duration of submissive/defensive behavior following defeat.

#### **2.4.4 Experiment 3: ANA-12 administered systemically increases the consolidation of CD**

As shown in Figure 6, hamsters receiving a 1mg/kg IP injection of ANA-12 after defeat displayed a significant increase in submissive and defensive behavior (Kruskal Wallis,  $p < 0.05$ ) when tested 24 h later. Administration of ANA-12 did not significantly alter social or non-social behavior in defeated hamsters. Aggression was not observed in any defeated animals.

ANA-12 did not have an effect on behavior in no-defeat controls (Table 1; Kruskal Wallis,  $p > 0.05$ ). Furthermore, all defeated hamsters given vehicle showed a significant increase in submissive behavior as compared to the appropriate no-defeat, vehicle hamsters, (Table 1; Kruskal Wallis,  $p < 0.05$ ), indicating that exposure to social defeat resulted in conditioned defeat as evidenced by a significant increase in the duration of submissive/defensive behavior following defeat.

## **2.5 Discussion**

Our current results were initially surprising to us, given that previous work from our lab and others had suggested that BDNF is necessary for fear learning and that BDNF mRNA increases in numerous brain regions following social defeat (Rattiner et al., 2004; Taylor et al., 2011; reviewed in Mahan & Ressler, 2012). Our current findings that a TrkB receptor agonist *reduces* submissive behavior and that a TrkB receptor antagonist *enhances* submissive behavior suggest instead that BDNF may act to prevent stress-induced changes in behavior and that this action occurs, at least in part, within the BLA.

Despite the results being initially surprising, they are consistent with an alternative hypothesis that BDNF promotes resiliency to stress (reviewed by Russo et al., 2012; Karatsoreos & McEwen, 2011) and that BDNF may underlie the therapeutic response to antidepressant treatment in depressed individuals (reviewed by Rothman and Mattson, 2013). Interestingly, BDNF is suppressed following exposure to stress in humans, and reduced BDNF protein in

serum is observed in patients with numerous psychiatric illnesses, as discussed above (Karege et al., 2002; reviewed by Duman and Monteggia, 2006; Castren & Rantamaki, 2010; Dell'osso et al., 2009; review by Rakofsky et al., 2012; Suliman, Hemmings, & Seedat, 2013; Dwivedi, 2010).

Given the potential translational applications of BDNF as an antidepressant or a molecule that reduces the effects of stress and promotes resiliency, it is of particular interest to note that our peripheral injection of a TrkB receptor agonist produced a pro-resilient response (a decrease in the behavioral response to social stress), supporting the possibility that BDNF-active drugs could be an effective treatment for those suffering from several psychopathologies, including PTSD and depression (Altar 1999, Duman 2004, Duman & Monteggia 2006). Theoretically, this work suggests that TrkB receptor agonists could be administered to humans immediately after a trauma occurs to prevent consolidation of information that promotes stress-induced behavioral change. Our model of CD, therefore, provides us the opportunity to 1) determine potential translational effects of BDNF-active drugs via systemic injections following exposure to social stress and 2) to begin to identify where in the neural circuit these changes may be mediated specifically.

Furthermore all findings in the current project, both site-specific and peripheral are consistent in that BDNF protects against the deleterious effects of stress as measured by a decrease in submissive behaviors. These findings were very surprising, given that k252a, a nonspecific Trk receptor antagonist, administered site-specifically into the BLA prior to defeat also decreased submissive behavior (Taylor et al., 2011). Although it was originally widely used and promoted as an acceptable TrkB receptor antagonist, k252a is now recognized to be a non-specific Trk receptor antagonist that binds to both TrkA and TrkB receptors, as well as to a wide variety of other protein kinases (Arthur et al., 2005; Berg et al., 1992; Koizumi et al., 1988; Sofroniew et al., 2001). k252a is reported to act on numerous protein kinases, thus it is possible that this non-specific Trk antagonist is inhibiting down stream kinases involved in synaptic plasticity and LTM. This idea is of particular interest given that BDNF is reported to promote

and be critical for LTP, synaptogenesis, neural growth, and in addition have neuroprotective effects (Chao et al., 2006; Minichiello 2009; Autry & Monteggia, 2012; Gray et al., 2013). Interestingly, these neuroprotective effects are blocked by both chemical inhibitors of the ERK and PI3K pathways, and the protein synthesis inhibitor anisomycin (Almeida et al., 2005). As we have previously shown that anisomycin in the BLA reduces submission (Markham & Huhman, 2008), the aforementioned findings suggest that the k252a-induced reduction in CD may be mediated by an inhibition of synaptic plasticity in the BLA. In conclusion, additional studies will be necessary to determine the mechanism of action whereby k252a reduced submission as observed by Taylor and colleagues (2011).

It is possible that BDNF interacted with gamma-amino butyric acid (GABA) to mediate the decrease in submission observed in the present study. Reductions in BDNF protein and mRNA are related to decreases in genes coding for GABA interneuron related peptides (e.g., somatostatin) in the amygdala of women suffering from major depressive disorder (Guilloux et al., 2012). In addition, GABA induces BDNF transcription in cortical cells (Fukuchi et al., 2014), and BDNF regulates maintenance and maturation of GABAergic synapses (Seil & Drake-Baumann, 2000; Kuczewski et al., 2010) as well as the cellular localization of GABA receptors (Brunig et al., 2001; Elmariah et al., 2004; Wuchter et al., 2012). A dynamic interaction between BDNF and GABAergic systems is extremely interesting given that our lab has previously shown that administration of a GABA agonist, muscimol, blocks the acquisition and expression of CD (Jasnow et al., 2001) as measured by a decrease in submissive behaviors. The observed decrease in submission in both studies thus could be the result of the dynamic interaction between GABA and BDNF. Finally, the interactions between BDNF and GABA appear to be nucleus-dependent (Brunig et al., 2001; Mou et al., 2011), something that should be considered in future studies given the variable effects of stress on GABA, BDNF, and neuronal plasticity in different nuclei.

The current findings combined with recent literature on BDNF lead us to speculate that BDNF may be a dynamic molecule that has differing effects depending on where

it is administered (reviewed in Sakata, 2013). Specifically, BDNF may promote stress responses when administered in some brain nuclei but reduce stress responses when administered into other nuclei. Given that we have established a putative neural circuit underlying CD, we maintain that this is an ideal model with which to explore the role of BDNF as a stress-reducing and stress-promoting molecule. Future studies should examine the effects of BDNF microinjections site-specifically into different nuclei within the putative CD circuit. While we predict that BDNF may have opposing effects in different component of a neural circuit, we must also be cognizant that BDNF in one nucleus likely interacts with and alters BDNF expression in other nuclei within the same circuit (Taliaz et al., 2013). This likelihood also underscores the importance of including peripheral manipulations, such as those used in this project, to determine what the overall sum of these different actions will be.

## 2.6 References

- Adachi N, Numakawa T, Richards M, Nakajima S, Kunugi H. 2014. New insight in expression, transport, and secretion of brain-derived neurotrophic factor: Implications in brain-related diseases. *World journal of biological chemistry* 5: 409-28
- Almeida RC, Souza DG, Soletti RC, Lopez MG, Rodrigues AL, Gabilan NH. 2006. Involvement of PKA, MAPK/ERK and CaMKII, but not PKC in the acute antidepressant-like effect of memantine in mice. *Neuroscience letters* 395: 93-7
- Altar CA. 1999. Neurotrophins and depression. *Trends in pharmacological sciences* 20: 59-61
- Andero R, Daviu N, Escorihuela RM, Nadal R, Armario A. 2012. 7,8-dihydroxyflavone, a TrkB receptor agonist, blocks long-term spatial memory impairment caused by immobilization stress in rats. *Hippocampus* 22: 399-408
- Andero R, Heldt SA, Ye K, Liu X, Armario A, Ressler KJ. 2011. Effect of 7,8-dihydroxyflavone, a small-molecule TrkB agonist, on emotional learning. *The American journal of psychiatry* 168: 163-72
- Arthur DB, Akassoglou K, Insel PA. 2005. P2Y2 receptor activates nerve growth factor/TrkA signaling to enhance neuronal differentiation. *Proceedings of the National Academy of Sciences of the United States of America* 102: 19138-43
- Autry AE, Monteggia LM. 2012. Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacological reviews* 64: 238-58
- Berg MM, Sternberg DW, Parada LF, Chao MV. 1992. K-252a inhibits nerve growth factor-induced trk proto-oncogene tyrosine phosphorylation and kinase activity. *The Journal of biological chemistry* 267: 13-6
- Berretta S, Pantazopoulos H, Caldera M, Pantazopoulos P, Pare D. 2005. Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience* 132: 943-53
- Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, et al. 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* 311: 864-8
- Boyle LM. 2013. A neuroplasticity hypothesis of chronic stress in the basolateral amygdala. *The Yale journal of biology and medicine* 86: 117-25
- Brunig I, Penschuck S, Berninger B, Benson J, Fritschy JM. 2001. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA(A) receptor surface expression. *The European journal of neuroscience* 13: 1320-8
- Casarotto PC, de Bortoli VC, Correa FM, Resstel LB, Zangrossi H, Jr. 2010. Panicolytic-like effect of BDNF in the rat dorsal periaqueductal grey matter: the role of 5-HT and GABA. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* 13: 573-82
- Castren E, Rantamaki T. 2010. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Developmental neurobiology* 70: 289-97
- Cazorla M, Premont J, Mann A, Girard N, Kellendonk C, Rognan D. 2011. Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. *The Journal of clinical investigation* 121: 1846-57

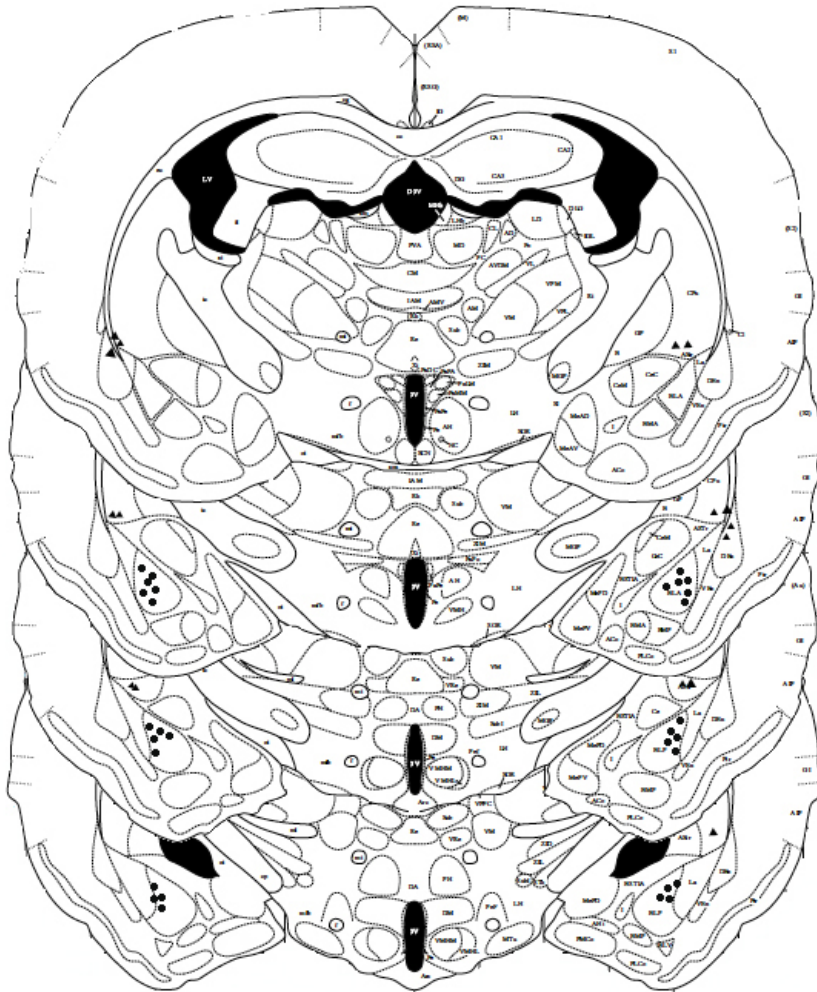
- Chao MV, Rajagopal R, Lee FS. 2006. Neurotrophin signalling in health and disease. *Clinical science* 110: 167-73
- Choi DC, Gourley SL, Ressler KJ. 2012. Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning. *Translational psychiatry* 2: e205
- Choi DC, Maguschak KA, Ye K, Jang SW, Myers KM, Ressler KJ. 2010. Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2675-80
- Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S. 1997. Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17: 2295-313
- Cunha C, Brambilla R, Thomas KL. 2010. A simple role for BDNF in learning and memory? *Frontiers in molecular neuroscience* 3: 1
- Dell'Osso L, Carmassi C, Del Debbio A, Catena Dell'Osso M, Bianchi C, et al. 2009. Brain-derived neurotrophic factor plasma levels in patients suffering from post-traumatic stress disorder. *Progress in neuro-psychopharmacology & biological psychiatry* 33: 899-902
- Duman RS. 2004. Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular medicine* 5: 11-25
- Duman RS, Monteggia LM. 2006. A neurotrophic model for stress-related mood disorders. *Biological psychiatry* 59: 1116-27
- Dwivedi Y. 2010. Brain-derived neurotrophic factor and suicide pathogenesis. *Annals of medicine* 42: 87-96
- Elmariah SB, Crumling MA, Parsons TD, Balice-Gordon RJ. 2004. Postsynaptic TrkB-mediated signaling modulates excitatory and inhibitory neurotransmitter receptor clustering at hippocampal synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24: 2380-93
- Fanous S, Hammer RP, Jr., Nikulina EM. 2010. Short- and long-term effects of intermittent social defeat stress on brain-derived neurotrophic factor expression in mesocorticolimbic brain regions. *Neuroscience* 167: 598-607
- Fukuchi M, Kirikoshi Y, Mori A, Eda R, Ihara D, et al. 2014. Excitatory GABA induces BDNF transcription via CRT1 and phosphorylated CREB-related pathways in immature cortical cells. *Journal of neurochemistry*
- Gray JD, Milner TA, McEwen BS. 2013. Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. *Neuroscience* 239: 214-27
- Guilloux JP, Douillard-Guilloux G, Kota R, Wang X, Gardier AM, et al. 2012. Molecular evidence for BDNF- and GABA-related dysfunctions in the amygdala of female subjects with major depression. *Molecular psychiatry* 17: 1130-42
- Jasnow AM, Huhman KL. 2001. Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain research* 920: 142-50
- Karatsoreos IN, McEwen BS. 2011. Psychobiological allostasis: resistance, resilience and vulnerability. *Trends in cognitive sciences* 15: 576-84



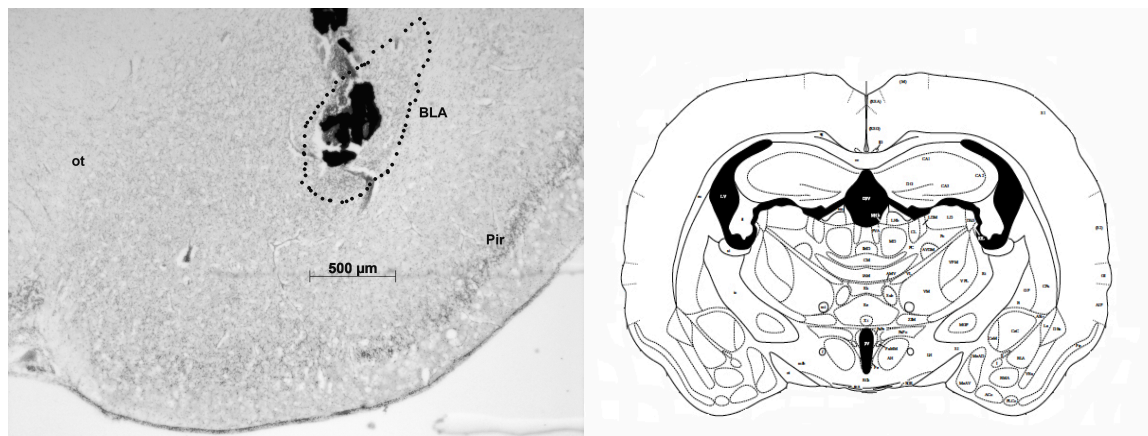
- Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. 2002. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry research* 109: 143-8
- Koizumi S, Contreras ML, Matsuda Y, Hama T, Lazarovici P, Guroff G. 1988. K-252a: a specific inhibitor of the action of nerve growth factor on PC 12 cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 8: 715-21
- Kuczewski N, Porcher C, Gaiarsa JL. 2010. Activity-dependent dendritic secretion of brain-derived neurotrophic factor modulates synaptic plasticity. *The European journal of neuroscience* 32: 1239-44
- Lakshminarasimhan H, Chattarji S. 2012. Stress leads to contrasting effects on the levels of brain derived neurotrophic factor in the hippocampus and amygdala. *PloS one* 7: e30481
- Mahan AL, Ressler KJ. 2012. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends in neurosciences* 35: 24-35
- Markham CM, Huhman KL. 2008. Is the medial amygdala part of the neural circuit modulating conditioned defeat in Syrian hamsters? *Learning & memory* 15: 6-12
- Minichiello L. 2009. TrkB signalling pathways in LTP and learning. *Nature reviews. Neuroscience* 10: 850-60
- Mou L, Heldt SA, Ressler KJ. 2011. Rapid brain-derived neurotrophic factor-dependent sequestration of amygdala and hippocampal GABA(A) receptors via different tyrosine receptor kinase B-mediated phosphorylation pathways. *Neuroscience* 176: 72-85
- Pittenger C, Duman RS. 2008. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 33: 88-109
- Pizarro JM, Lumley LA, Medina W, Robison CL, Chang WE, et al. 2004. Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain research* 1025: 10-20
- Rakofsky JJ, Ressler KJ, Dunlop BW. 2012. BDNF function as a potential mediator of bipolar disorder and post-traumatic stress disorder comorbidity. *Molecular psychiatry* 17: 22-35
- Rattiner LM, Davis M, French CT, Ressler KJ. 2004. Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24: 4796-806
- Rothman SM, Mattson MP. 2013. Activity-dependent, stress-responsive BDNF signaling and the quest for optimal brain health and resilience throughout the lifespan. *Neuroscience* 239: 228-40
- Russo N, Russo M, Daino D, Freschi L, Fiore L, et al. 2012. Evaluation of brain-derived neurotrophic factor in menstrual blood and its identification in human endometrium. *Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology* 28: 492-5
- Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, et al. 2003. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23: 349-57

- Sakata K, Martinowich K, Woo NH, Schloesser RJ, Jimenez DV, et al. 2013. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. *Proceedings of the National Academy of Sciences of the United States of America* 110: 15103-8
- Seil FJ, Drake-Baumann R. 2000. TrkB receptor ligands promote activity-dependent inhibitory synaptogenesis. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 20: 5367-73
- Sofroniew MV, Howe CL, Mobley WC. 2001. Nerve growth factor signaling, neuroprotection, and neural repair. *Annual review of neuroscience* 24: 1217-81
- Suliman S, Hemmings SM, Seedat S. 2013. Brain-Derived Neurotrophic Factor (BDNF) protein levels in anxiety disorders: systematic review and meta-regression analysis. *Frontiers in integrative neuroscience* 7: 55
- Taliaz D, Nagaraj V, Haramati S, Chen A, Zangen A. 2013. Altered brain-derived neurotrophic factor expression in the ventral tegmental area, but not in the hippocampus, is essential for antidepressant-like effects of electroconvulsive therapy. *Biological psychiatry* 74: 305-12
- Taylor SL, Stanek LM, Ressler KJ, Huhman KL. 2011. Differential brain-derived neurotrophic factor expression in limbic brain regions following social defeat or territorial aggression. *Behavioral neuroscience* 125: 911-20
- Wuchter J, Beuter S, Treindl F, Herrmann T, Zeck G, et al. 2012. A comprehensive small interfering RNA screen identifies signaling pathways required for gephyrin clustering. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 32: 14821-34
- Yang C, Liu JF, Chai BS, Fang Q, Chai N, et al. 2013. Stress within a restricted time window selectively affects the persistence of long-term memory. *PloS one* 8: e59075

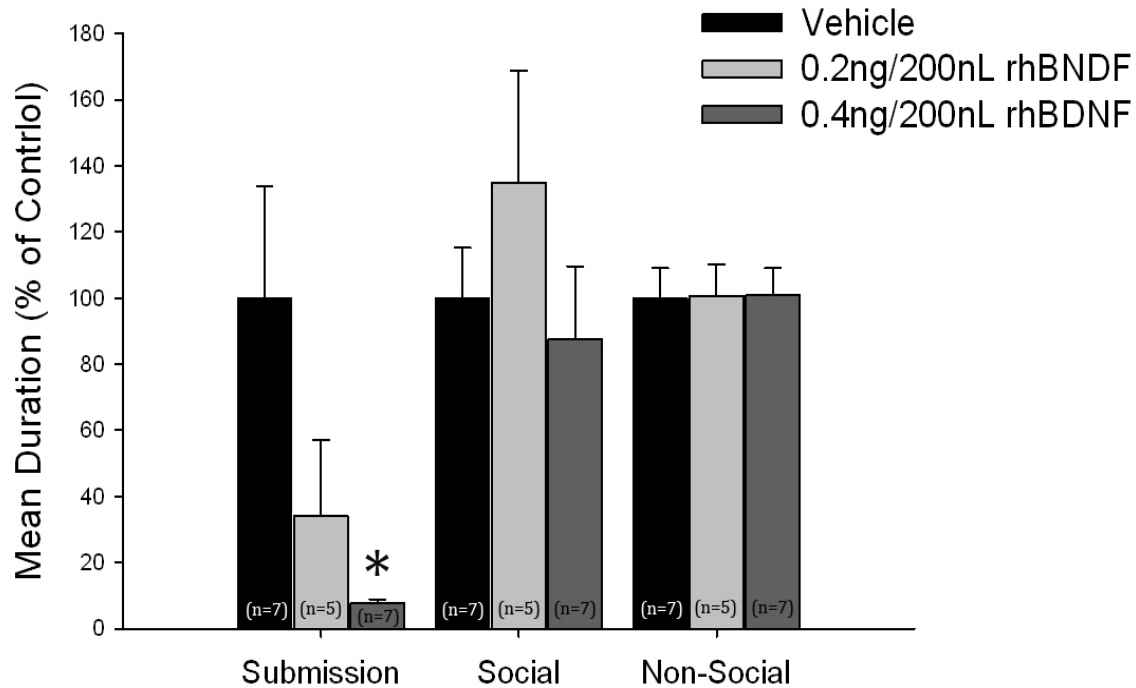
## 2.7 Figures



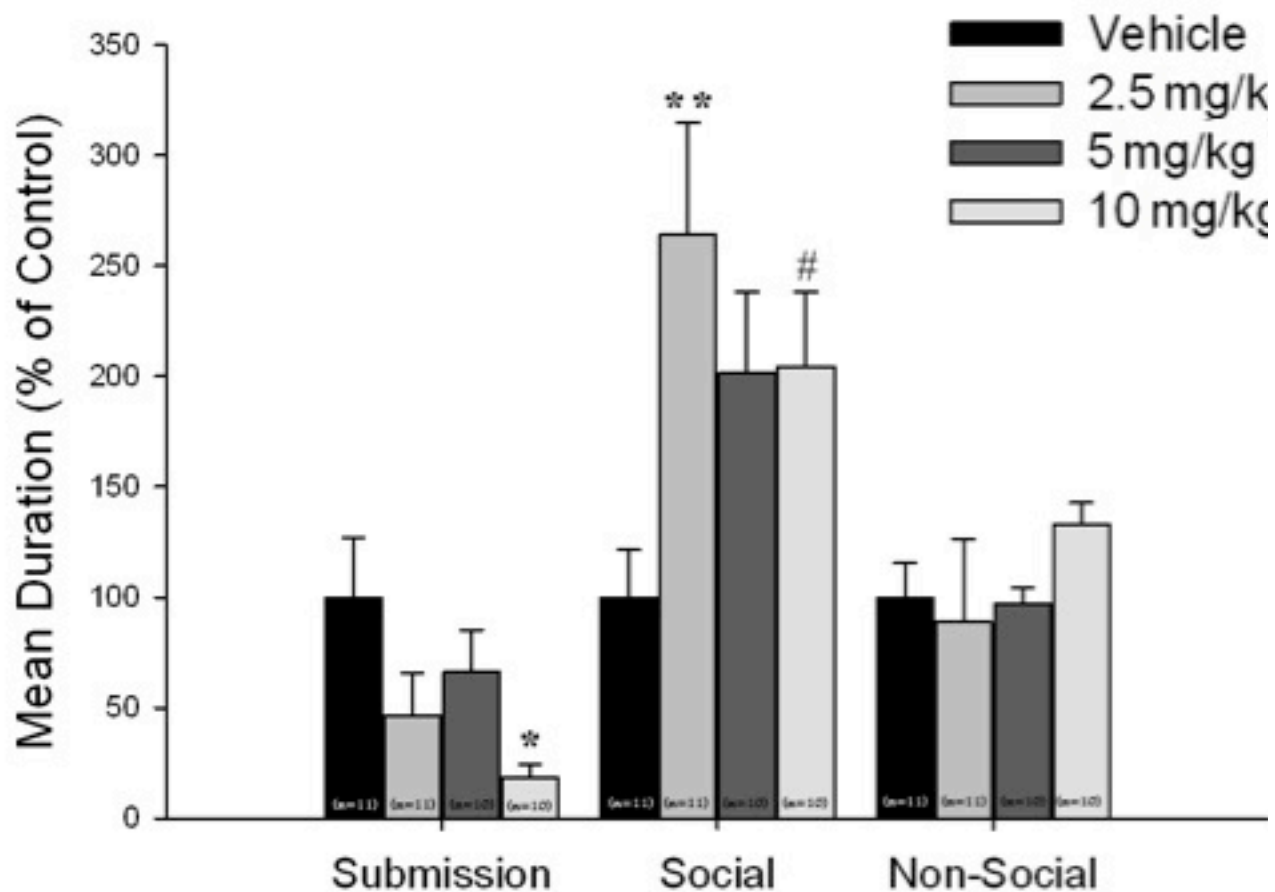
**Figure 2.1:** Histological recreation of injection sites of animals receiving infusions in the BLA. Each symbol represents the injection site in one or more animals: Dots represent one or more hits and (triangles) represent misplaced injection sites. Drawings are adapted from Morin and Wood (2001).



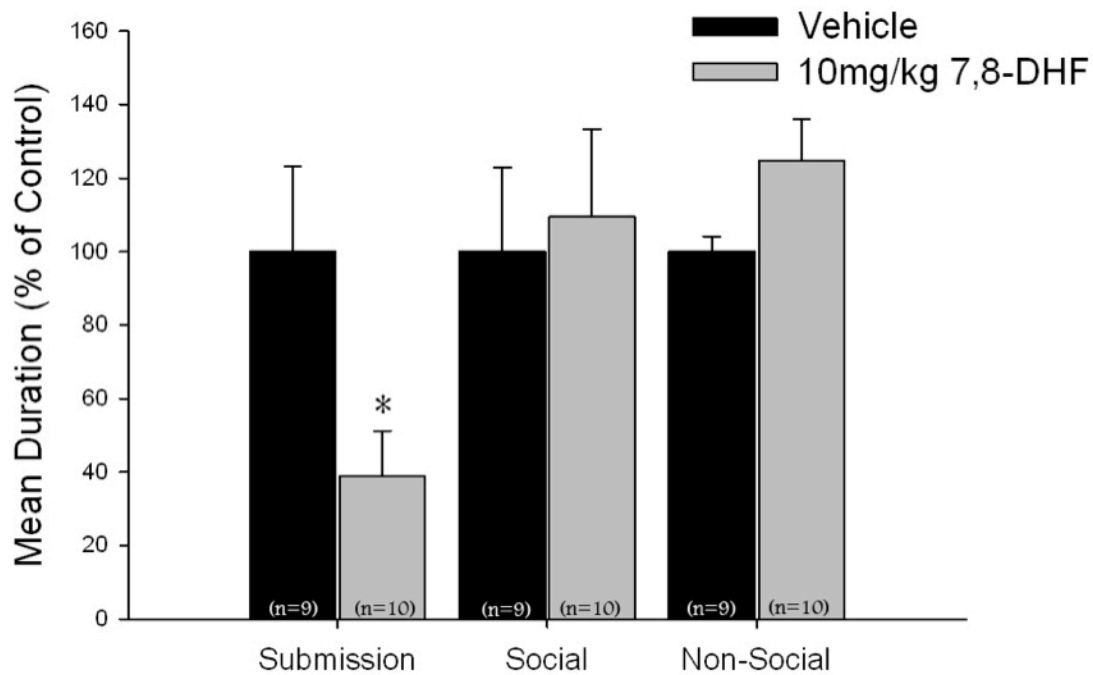
**Figure 2.2** Representative photomicrograph is shown of a coronal brain section of the BLA. The needle tract and ink injection are clearly visible. Injection volume (200 nl) of the ink was identical to the drug/vehicle volume used in Experiment 1. *Abbreviations* BLA-basolateral amygdala, Pir-piriform cortex, ot-optic tract.



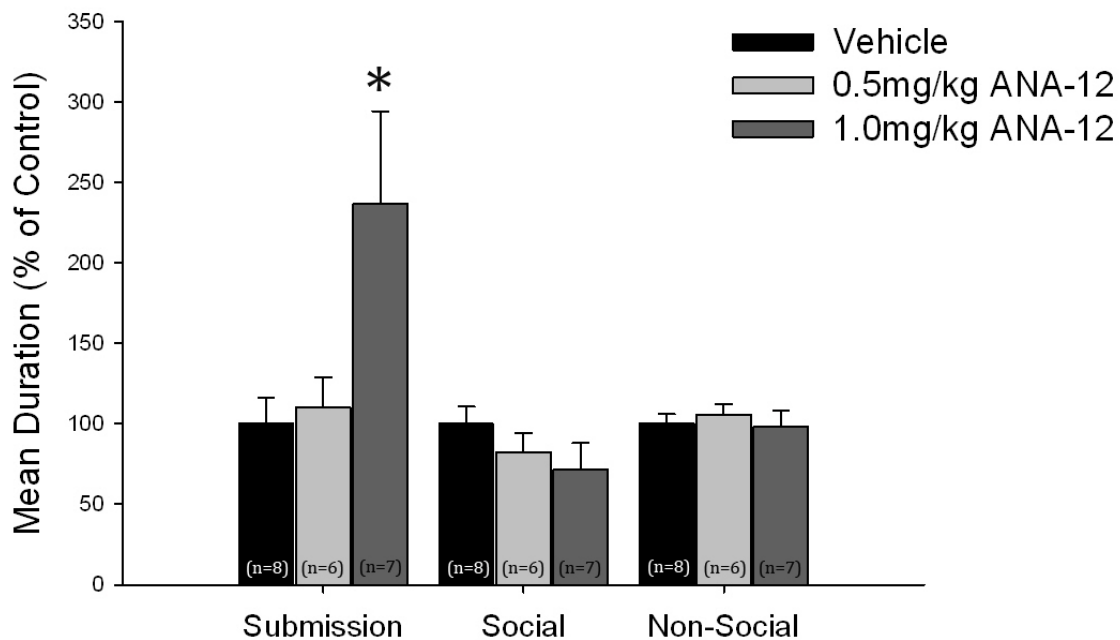
**Figure 2.3**BDNF in the BLA reduces the acquisition of social defeat. Behaviors exhibited by previously defeated hamsters during a 5min test with an NAI are shown as percent of vehicle ( $\pm$  standard error of the mean). Hamsters received a bilateral microinjection of rhBDNF or vehicle immediately prior to a 15 min social defeat with an RA. Symbols indicate differences for which  $p < 0.05$ . \* Indicates a significant difference ( $p < 0.05$ ) between defeated animals treated with rhBDNF or vehicle.



**Figure 2.4** 7,8-DHF reduces the acquisition of social defeat. Behaviors exhibited by previously defeated hamsters during a 5min test with an NAI are shown as percent of vehicle ( $\pm$  standard error of the mean). Hamsters received an IP injection of 7,8-DHF or vehicle immediately prior to a 5 min social defeat with an RA. Symbols indicate differences for which  $p < 0.05$ . \* Indicates a significant difference ( $p < 0.05$ ) in submission between defeated animals treated with 10mg/kg rhBDNF or vehicle. \*\* Indicates a significant difference ( $p < 0.05$ ) in social behavior between defeated animals treated with 2.5mg/kg rhBDNF or vehicle. # Indicates a significant difference ( $p < 0.05$ ) in social behavior between between defeated animals treated with 10mg/kg rhBDNF or vehicle.



**Figure 2.5** 7, 8-DHF reduces the consolidation of social defeat. Behaviors exhibited by previously defeated hamsters during a 5min test with an NAI are shown as percent of vehicle ( $\pm$  standard error of the mean). Hamsters received an IP injection of 7, 8-DHF or vehicle immediately after a 5 min social defeat with an RA. \* Indicates a significant difference ( $p < 0.05$ ) between defeated animals treated with 7, 8-DHF or vehicle.



**Figure 2.6** ANA-12 enhances the consolidation of social defeat. Behaviors exhibited by previously defeated hamsters during a 5min test with an NAI are shown as percent of vehicle ( $\pm$  standard error of the mean). Hamsters received an IP injection of ANA-12 or vehicle immediately after a 5 min social defeat with an RA. \* Indicates a significant difference ( $p < 0.05$ ) between defeated animals treated with ANA-12 or vehicle.



**Table 2.1 Mean Duration of Behavior (in sec) for No-Defeat animals in Consolidation Studies.**

<b>Behavior</b>	<b>Vehicle</b>	<b>Drug</b>
<b>Submission</b>	7,8-DHF (10mg/kg) (TrkB agonist)	5.22 ± 2.59      1.36 ± 1.00
	ANA-12 (1.0mg/kg) (TrkB antagonist)	2.9 ± 2.34      10.22 ± 7.40
<b>Social</b>	7,8-DHF (10mg/kg) (TrkB agonist)	204.30 ± 20.50      222.34 ± 18.85
	ANA-12 (1.0mg/kg) (TrkB antagonist)	144.45 ± 14.71      128.51 ± 15.88
<b>Non-Social</b>	7,8-DHF (10mg/kg) (TrkB agonist)	97.80 ± 11.89      87.33 ± 12.20
	ANA-12 (1.0mg/kg) (TrkB antagonist)	74.19 ± 7.61      79.15 ± 11.43

### **3 DOES 7,8-DIHYDROXYFLAVONE ALTER DEFEAT-INDUCED NEURAL ACTIVATION IN THE NEURAL CIRCUIT MEDIATING CONDITIONED DEFEAT**

#### **3.1 Abstract**

Brain derived neurotrophic factor (BDNF) has numerous actions within the nervous system. This molecule promotes fear learning and is increased and decreased in a nucleus-dependent fashion by stress. Our lab recently found that 7,8-dihydroxyflavone (7,8-DHF), a novel tyrosine kinase B (TrkB) receptor agonist, administered peripherally reduces submissive behavior in Syrian hamsters following social stress. It is not currently clear where in the brain this molecule is acting to alter the response to social stress. The aim of this project was to determine if 7,8-DHF alters defeat-induced neural activity in a nucleus-dependent fashion. Specifically, we measured social defeat-induced FOS-immunoreactivity (FOS-IR) as a proxy for neural activation in specific nodes of the neural circuit that mediates conditioned defeat and compared FOS-IR in animals administered 7,8-DHF to that observed following administration of vehicle or saline controls. A serious confound arose because 7,8-DHF must be dissolved in dimethyl-sulfoxide (DMSO), a solvent that has a variety of effects on both neurons and glia. Unfortunately, DMSO, alone, appears to induce FOS-IR in many of the nodes of the neural circuit mediating conditioned defeat as compared to vehicle controls. As a result, we are unable to address our original question. We were able to replicate previous work from our lab and others that defeat increases FOS-IR in the BLA and other nodes as compared to no defeat controls. We also found that 7,8-DHF reduces FOS-IR in the BLA and CeA as compared to the DMSO vehicle regardless of defeat experience. These data are discussed briefly.

#### **3.2 Introduction**

Brain derived neurotrophic factor (BDNF) has gained increasing attention for its role in stress responding, learning, and memory (Chao et al., 2006; Minichiello 2009). This protein

binds to tyrosine kinase receptors (TrkB) receptors in both brain and periphery and is known to be involved in neuron growth, plasticity, and synapse development (Chao et al., 2006; Minichiello, 2009). Therefore, it is not surprising that novel compounds have recently been developed to target TrkB, the cognate receptor for BDNF. 7, 8-dihydroxyflavone (7, 8-DHF), for example, is a novel TrkB receptor agonist that can be administered peripherally and that crosses the blood brain barrier (BBB) (Jang et al., 2010). This route of administration provides a clear translational advantage, given that BDNF, itself, does not appear to cross the BBB (Pardridge, 2007), which limits its efficacy when administered peripherally.

7, 8-DHF administered peripherally in Syrian hamsters immediately after social defeat reduces submissive behaviors during subsequent testing 24h later (Jeffress et al., in preparation). As 7, 8-DHF administered peripherally prevents defeat-induced behavioral changes, the purpose of this project was to determine where in the brain 7, 8-DHF may be regulating these changes. We recently found that BDNF administered into the basolateral amygdala (BLA) reduces submissive behavior (Jeffress et al., in preparation), suggesting that BDNF promotes resiliency to stress and that this behavioral change is mediated, at least in part, by the BLA. BDNF in the BLA is of particular interest given that this nucleus is necessary for the dramatic behavioral change after social defeat known as conditioned defeat (CD) (Jasnow, 2005; Markham et al., 2010).

Recent work on BDNF and social stress has generated controversy as to whether BDNF promotes or prevents behavioral responses to stress. It has recently been suggested that the effects of this molecule depend upon where in a neural circuit BDNF is examined because BDNF may site-specifically increase or decrease with stress exposure and the behavioral output is going to reflect the net activity of the overall circuit (reviewed in Sakata, 2013). Repeated social defeat stress, for example, decreases BDNF protein in the amygdala of rats (Fanous et al., 2010), whereas repeated social stress in mice increases BDNF protein in the amygdala (reviewed by

Boyle, 2013). Given such conflicting literature about BDNF in the amygdala, it is not surprising that several labs continue to examine this protein in models of stress and fear learning.

The BLA is of particular interest for our lab because this nucleus plays a central role in the putative neural circuit of CD (Figure 1). The BLA is necessary for both the acquisition and expression of CD (Jasnow, 2005; Markham et al., 2010; Day et al., 2011), and synaptic plasticity in the BLA is required for CD learning (Jasnow, 2005; Markham et al., 2010). Beyond CD, the BLA has a clear and critical role in fear learning (reviewed in Martijena & Molina, 2012; Roozendaal, McEwen & Chattarji, 2009; McGaugh, 2000), and changes in neuronal activation are found in the BLA following stress. For example, the expression of the immediate early gene product FOS increases in the BLA following social defeat in Syrian hamsters (Markham et al., 2010) and in mice (Bourne et al., 2013; Nikulina et al., 2008), indicating that defeat stress induces neural activity in the BLA. Chronic stress also increases spine density and dendritic growth in the BLA (reviewed by Roozendaal, McEwen & Chattarji, 2009). Because activation of the BLA is critical for CD it seems likely that a molecule that promotes resiliency to social stress such as BDNF may act in the BLA to reduce defeat-induced neural activation.

It is also possible that activation of the BLA in response to social defeat is modulated by numerous other nodes within the circuit mediating conditioned defeat such as the medial prefrontal cortex (mPFC). The mPFC in rodents is composed of the prelimbic cortex (PL) and the infralimbic cortex (IL) (Milad et al., 2007; Myers-Schulz & Koenigs, 2012; Quirk & Beer, 2006; Slattery, Neumann, & Cryan, 2011). We have previously reported that the mPFC is necessary for the acquisition of CD (Markham et al., 2012), yet this brain region is also known to promote resiliency (Bader et al., 2014; Amat et al., 2014). Therefore, it is possible that BDNF reduces CD by promoting activation of the mPFC which in turn suppresses neural activity in the BLA.

The goal of the project was to determine where within the brain a peripherally administered TrkB receptor agonist might act to reduce the behavioral response to social defeat.

FOS-immunoreactivity (FOS-IR) was used as an indirect marker of neural activation to determine which brain nuclei might be involved. Specifically, FOS-IR in the PL, IL, CeA, BLA, medial amygdala (MeA), and the caudate putamen (CPu) were examined. We predicted that systemic 7, 8-DHF would reduce FOS-IR in the BLA and possibly other nodes within the conditioned defeat circuit.

### **3.3 Methods & Materials**

#### ***3.3.1 Animals and Housing Conditions***

Thirty-one male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories, New York, NY) that weighed 120-140 g, and were between 9-10 weeks old at the time of testing, were used in this experiment. Upon arrival, animals were individually housed for one week prior to any manipulation (quarantine measure), and all hamsters were handled for five days prior to behavioral procedures to acclimate them to experimenter handling. Hamsters were housed in polycarbonate cages (20x 40 x 20cm) with corncob bedding and wire mesh tops in a temperature ( $20 \pm 2^\circ$  C) and humidity-controlled room. The room was maintained on 14:10 light/dark cycle with lights off at 10:00 h. Food and water were available *ad libitum*. Older hamsters (>6 months) individually housed (>1 month) weighing at least 180g served as resident aggressors (RA) in defeat training for all experiments. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

#### ***3.3.2 Social Defeat***

Prior to training, hamsters were weight-matched and randomly assigned to groups. All animals were transported in their home cage to the behavioral testing suite within the animal vivarium 30 mins prior to all behavioral manipulations. All behavioral procedures were

performed in the first 3 h of the light dark cycle to minimize any circadian effects. Social defeat training occurred in the home cage of the RA for 15 min. No-defeat animals were placed into an empty RA cage for 15 min to expose animals to odors but no physical interaction.

### **3.3.3 Drug Administration**

Animals received a 10mg/kg intraperitoneal (IP) injection of the TrkB receptor agonist, 7,8-DHF, dissolved in 40% Dimethylsulfoxide (DMSO) and 60% saline (TCI America; Montgomeryville, PA), the respective vehicle (40% DMSO/60% saline), or 100% physiological saline immediately after defeat. This dosage of 7,8-DHF has been used previously and was found to significantly reduce CD. We chose to add a group of animals receiving a saline control treatment as a positive control for our drug vehicle (40% DMSO/60% saline) given that DMSO is reported to have several wide-reaching and negative effects on cells that are often ignored (reviewed by Santos et al., 2003).

### **3.3.4 Immunohistochemistry**

Animals were administered an anesthetizing dose of sodium pentobarbital 60 min after the peripheral injection. Subjects were then perfused transcardially with 0.1 M potassium phosphate-buffered saline (KPBS) followed by 4% paraformaldehyde. Brains were post-fixed in 4% paraformaldehyde overnight and switched to a 30% sucrose-paraformaldehyde solution and kept at 4°C for an additional 48 h. Brains were blocked on the coronal plane and sectioned on a cryostat to a thickness of 30  $\mu$ m. Consecutive sections were placed sequentially across 4 wells filled with cryoprotectant. The sections included the PL and the IL, and continued through the BLA. Sections were labeled for *FOS* using primary antisera directed against the protein product of the immediate early gene *c-fos* (rabbit anti-*c-fos* polyclonal antibody, 1:10,000; Santa Cruz Biotechnology).

All washes, rinses, and incubations were performed in 25-well tissue culture plates, which were gently shaken on an orbital shaker throughout the procedure. Sections were rinsed

in 0.1 M KPBS and incubated in a 0.3% hydrogen peroxide solution for 15 min, followed by a KPBS wash. Sections were then incubated for 18 h in the primary antibody at room temperature in 1% solution of Triton X-100 in 0.1 M KPBS (1% KPBS-T) with normal goat serum (NGS). Following incubation with the primary antibody, the sections were rinsed in KPBS and incubated for 90 min at room temperature in 1% KPBS-T containing the secondary antibody (biotintylated horse anti-rabbit IgG polyclonal antibody, 1:200; Jackson ImmunoResearch). They were then rinsed with KPBS, followed by incubation for 1 h with an avidinbiotin complex reagent (Vectastain Elite ABC kit: Vector Laboratories). After rinsing with KPBS, sections were incubated in nickel-3, 39-diaminobenzidene (DAB) Kit (Vector) for 2-4 min in order to produce a blue-black-stained product and then washed again in KPBS in order to halt the DAB reaction. Finally, sections were mounted on gelatin-coated slides, dehydrated, and cover slipped with DPX (VWR International).

### ***3.3.5 Cell Counting***

An observer blind to the treatment conditions performed all cell counts using bright field microscopy at 10X magnification. For the quantification of FOS-IR labeling, areas of interest were first delineated using adjoining Neutral Red-stained sections. FOS-labeled cells were photographed using a Zeiss Camera (Jena, Germany) within the region of interest established by the Neutral Red templates. Cells were counted using Image J Software (NIH) in the PL, IL, CeA, BLA, MeA, and the CPu. Threshold levels were set, background levels were controlled, and cells were counted by this program within a computer-generated template placed over the region of interest on each photograph. See Figure 2, for a representative photomicrograph.

### ***3.3.6 Statistical Methods***

Independent samples t-tests were used to compare saline-defeat and saline-no defeat in regions of interest. 2x2 ANOVAs were used to compare the effects of defeat (defeat or no defeat)

and drug (7,8-DHF or 40%DMSO/60%DMSO) on FOS-IR in each region of interest. Analyses were performed with IBM SPSS software (20.0.0). Criterion for significance was  $p < 0.05$ .

### **3.4 Results**

#### ***3.4.1 Basolateral amygdala***

Animals that received IP saline immediately after a 15 min defeat ( $n=7$ ) demonstrated a significant increase in FOS-IR in the BLA as compared to animals that received saline but were not exposed to defeat ( $n=3$ ) ( $t(8)=2.77$ ,  $p < 0.05$ ) (see Figure 3).

IP administration of 7,8-DHF reduced the expression of FOS-IR in the BLA of hamsters ( $n=11$ ) as compared to FOS-IR in the BLA of hamsters ( $n=10$ ) that received an IP injection of vehicle (40% DMSO/ 60% saline) ( $F(1)=4.912$ ,  $p < 0.05$ ) (see Figure 4). No main effect of defeat and no defeat  $\times$  drug interaction was found in the BLA.

#### ***3.4.2 Central Amygdala***

Animals that received IP saline immediately after a 15 min defeat ( $n=7$ ) appeared to demonstrate an increase in FOS-IR in the CeA as compared to animals that received saline but were not exposed to defeat ( $n=3$ ). This apparent increase was not significant, but there was a strong trend to suggest that defeat enhances FOS-IR in this nucleus ( $t(8) = -2.191$ ,  $p = 0.06$ ) (Figure 3).

IP administration of 7,8-DHF reduced the expression of FOS-IR in the CeA of hamsters ( $n=11$ ) as compared to FOS-IR in the CeA of hamsters ( $n=10$ ) that received an IP injection of vehicle (40% DMSO/60% saline) ( $F(1)=8.462$ ,  $p < 0.05$ ) (Figure 5). No main effect of defeat and no interaction was found.

#### ***3.4.3 Medial Amygdala***

FOS-IR was examined in the MeA of animals that received saline immediately after a 15 min defeat ( $n=7$ ) or after 15 min of no defeat ( $n=3$ ). There were no differences among groups in this nucleus ( $t(8) = -1.184$ ,  $p > 0.05$ ) (see Figure 3).



No main effect of defeat ( $F(1,17)=0.730, p>0.05$ ) or drug ( $F(1,17)=0.678, p>0.05$ ) was found in the MeA, and there was no drug x defeat interaction ( $F(1,17)=0.012, p>0.05$ ) (see Figure 6).

#### **3.4.4 Prelimbic Cortex**

FOS-IR was examined in the PL of animals that had received saline immediately after a 15 min defeat ( $n=7$ ) or after 15 min of no defeat ( $n=3$ ). There were no differences in this nucleus ( $t(8)=0.490, p>0.05$ ) (see Figure 3).

No main effect of defeat ( $F(1,17)=0.316, p>0.05$ ) or drug ( $F(1,17)=0.722, p>0.05$ ) was found in the PL. There was also no interaction in this nucleus ( $F(1,17)=2.135, p>0.05$ ) (see Figure 7).

#### **3.4.5 Infralimbic Cortex**

FOS-IR was examined in the IL of animals that had received saline immediately after a 15 min defeat ( $n=7$ ) or after 15 min of no defeat ( $n=3$ ). There were no differences in this nucleus ( $t(8)=0.570, p>0.05$ ) (see Figure 3).

No main effect of defeat ( $F(1,17)=0.054, p>0.05$ ) or drug ( $F(1,17)=0.050, p>0.05$ ) was found in the IL, and there was no drug x defeat interaction ( $F(1,17)=2.962, p>0.05$ ) (see Figure 7).

#### **3.4.6 Caudate Putamen**

In order to ensure that 7,8-DHF or the vehicle (40%DMSO/60%saline), alone, did not cause global changes in neural activity throughout the brain, FOS-positive cells were also counted in the CPu, a brain region that is near both the mPFC and the amygdala, but that is not included in the putative neural circuit controlling CD.

FOS-IR was examined in the CPu of animals that had received saline immediately after a 15 min defeat (n=7) or after 15 min of no defeat (n=3). There were no differences in this nucleus ( $t(8) = 0.676, p > 0.05$ ) (Figure 3).

No main effect of defeat ( $F(1,17) = 0.837, p > 0.05$ ) or drug ( $F(1,17) = 0.276, p > 0.05$ ) was found in the CPu. There was also no interaction in this nucleus ( $F(1,17) = 0.029, p > 0.05$ ) (see Figure 8).

### 3.5 Discussion

Unfortunately, the current project did not produce interpretable data due to the distinct FOS response that resulted from the vehicle injection. As a result of this confound, we were prevented from addressing our original hypothesis. There are, nonetheless, some important points that can be gleaned from this work. First, our findings strongly suggest that using a 40% DMSO/60% saline vehicle is contraindicated, as this mixture independently enhances neural activation as measured by FOS-IR in some brain nuclei regardless of defeat experience. Fortunately, we had included a separate group of animals that received only saline injections because there have been sparse reports that DMSO is not an ideal solvent for pharmacological manipulations (Santos et al., 2003). These saline-treated animals also served as a positive control wherein we were able to demonstrate that exposure to social defeat stimulates FOS-IR in the BLA, a finding that our lab and others had reported previously (Bourne et al., 2013; Nikulina et al., 2008; Markham et al., 2010).

We also found a strong trend suggesting that defeat increases FOS-IR in the CeA. A similar pattern was observed in the MeA and the IL. The statistical power in the current study was limited due to the small group size in the saline-defeat condition and to the unequal group numbers in defeat and no-defeat conditions. Previous studies have found that 15 min defeats increased *fos* mRNA in the CeA of defeated hamsters as compared to handled controls (Kollack-

Walker et al., 1997; Kollack-Walker et al., 1999), so it is likely that increasing our group numbers would move this trend to statistical significance. Because demonstrating this difference was not the aim of this study, however, we elected not to use additional animals for this purpose.

Similarly, we did not observe significant changes in FOS-IR in the MeA possibly again because of statistical reasons (i.e., sample size) as increases in *c-fos* mRNA in the MeA have been reported previously in fighting hamsters as compared to handled controls (Kollack-Walker et al., 1997).

We were somewhat surprised that defeat did not significantly alter FOS-IR in the PL, and IL given that others have reported neural activation in these 2 brain areas. For example, repeated social defeat stress increases delta Fos B expression in the IL and the PL (Nikulina et al., 2012), and Morrison and colleagues also reported that FOS-IR is increased in the IL of dominant as compared to subordinate Syrian hamsters (Morrison et al., 2012). Our failure to demonstrate a change in FOS-IR could be due to the fact that 1) our animals were not subjected to repeated defeat, and 2) we were not comparing dominant and subordinate animals.

As expected, we did not observe changes in FOS-IR in the CPu. The CPu was selected to serve as a control nucleus near our regions of interest to ensure that 7,8-DHF and the vehicle were not inducing global neural activity. The finding that there were no changes in FOS-IR in the CPu further supports the contention that the changes in neural activation observed were nucleus specific.

Unfortunately, to date, all commercially available compounds that bind specifically to TrkB receptors are not soluble in physiological saline but must be dissolved in vehicles such as DMSO. DMSO is an extremely effective solvent that is widely used in behavioral pharmacological studies, but it has some important limitations that are largely overlooked. For example, 0.2% DMSO is reported to induce *c-fos* mRNA in cell culture in Northern analysis (Stewart, Herrera, & Nordheim, 1990). DMSO also decreases cell viability of hippocampal cells in culture (Lu & Mattson, 2001), increases EPSP amplitudes in NMDA and non-NMDA receptors (Tsvyetynska et al., 2005), induces apoptosis in the developing CNS (P0-P30)

(Hanslick et al., 2009), and inhibits glutamate activity in hippocampal neurons (Lu & Mattson, 2001).

In our study, DMSO appeared to have very variable effects on FOS-IR within the different nuclei within the conditioned defeat neural circuit, rendering the data largely uninterpretable. 7, 8-DHF, however, significantly reduces the DMSO-induced increase in FOS-IR seen in the BLA and CeA. We are uncertain as to why TrkB receptor agonism reduces DMSO-induced FOS-IR, and we can only speculate on why this occurred. A possible explanation is that BDNF is purported to have a restorative effect, given that it is known to reduce apoptosis, stress, and inflammation (see Duman, 2009 for review). Thus, the TrkB receptor agonist might have had these same effects within the BLA and CeA, which then reduced the DMSO-induced cellular activation. Despite our findings that DMSO alters FOS-IR, we have separately tested whether DMSO, alone, alters behavior in agonistic interactions and have shown that it does not (data not shown).

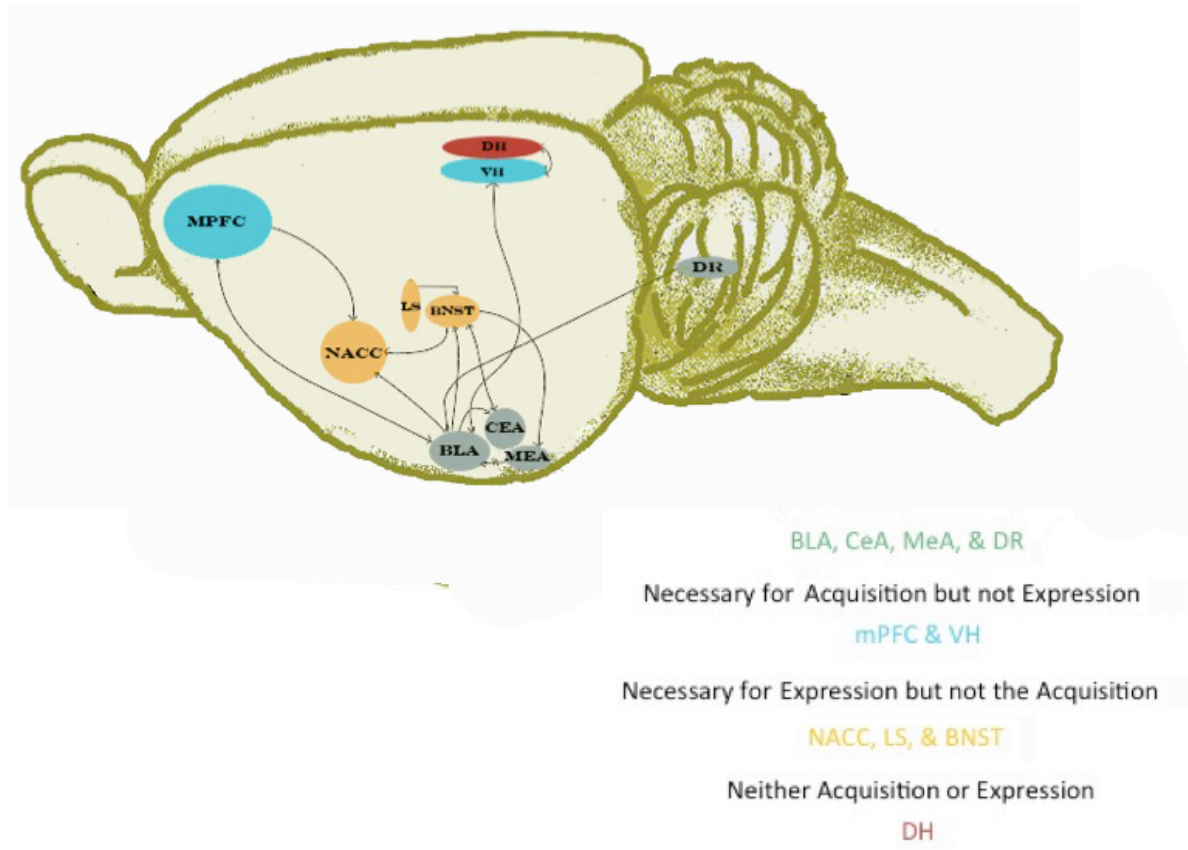
In summary, the current findings are largely uninterpretable because of the unfortunate effect of the DMSO vehicle on FOS-IR in both the BLA and the CeA. These findings highlight the importance of positive controls such as saline controls in experimental design and also underline the importance of developing novel compounds that are readily soluble in physiological saline or other vehicles that do not have biobehavioral effects.

### 3.6 References

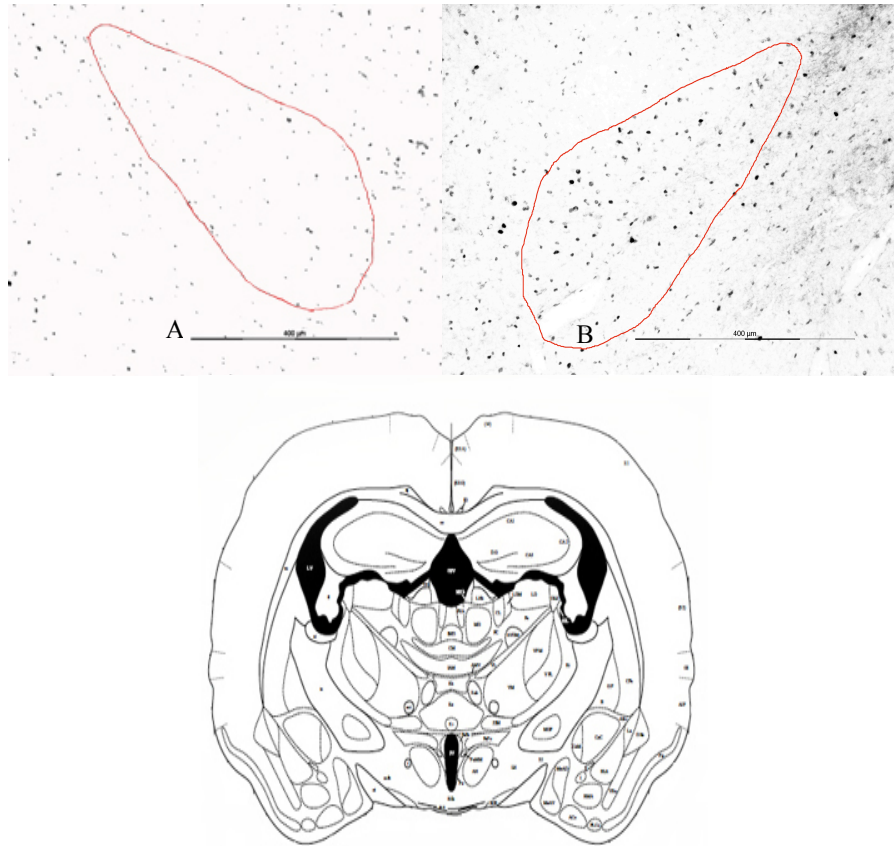
- Boyle LM. 2013. A neuroplasticity hypothesis of chronic stress in the basolateral amygdala. *The Yale journal of biology and medicine* 86: 117-25
- Chao MV, Rajagopal R, Lee FS. 2006. Neurotrophin signalling in health and disease. *Clinical science* 110: 167-73
- Day DE, Cooper MA, Markham CM, Huhman KL. 2011. NR2B subunit of the NMDA receptor in the basolateral amygdala is necessary for the acquisition of conditioned defeat in Syrian hamsters. *Behavioural brain research* 217: 55-9
- Duman RS. 2009. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. *Dialogues in clinical neuroscience* 11: 239-55
- Fanous S, Hammer RP, Jr., Nikulina EM. 2010. Short- and long-term effects of intermittent social defeat stress on brain-derived neurotrophic factor expression in mesocorticolimbic brain regions. *Neuroscience* 167: 598-607
- Guo XJ, Tian XS, Ruan Z, Chen YT, Wu L, et al. 2014. Dysregulation of neurotrophic and inflammatory systems accompanied by decreased CREB signaling in ischemic rat retina. *Experimental eye research* 125: 156-63
- Hanslick JL, Lau K, Noguchi KK, Olney JW, Zorumski CF, et al. 2009. Dimethyl sulfoxide (DMSO) produces widespread apoptosis in the developing central nervous system. *Neurobiology of disease* 34: 1-10
- Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, et al. 2010. A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2687-92
- Jasnow AM, Huhman KL. 2001. Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain research* 920: 142-50
- Jeffress EC, Thompson, BM, Markham, C, Huhman KL. BDNF reduces behavioral responses to social stress in Syrian hamsters. In preparation
- Kollack-Walker S, Don C, Watson SJ, Akil H. 1999. Differential expression of c-fos mRNA within neurocircuits of male hamsters exposed to acute or chronic defeat. *Journal of neuroendocrinology* 11: 547-59
- Kollack-Walker S, Watson SJ, Akil H. 1997. Social stress in hamsters: defeat activates specific neurocircuits within the brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17: 8842-55
- Lu C, Mattson MP. 2001. Dimethyl sulfoxide suppresses NMDA- and AMPA-induced ion currents and calcium influx and protects against excitotoxic death in hippocampal neurons. *Exp Neurol* 170: 180-5
- Markham CM, Luckett CA, Huhman KL. 2012. The medial prefrontal cortex is both necessary and sufficient for the acquisition of conditioned defeat. *Neuropharmacology* 62: 933-9
- Markham CM, Norvelle A, Huhman KL. 2009. Role of the bed nucleus of the stria terminalis in the acquisition and expression of conditioned defeat in Syrian hamsters. *Behavioural brain research* 198: 69-73

- Markham CM, Taylor SL, Huhman KL. 2010. Role of amygdala and hippocampus in the neural circuit subserving conditioned defeat in Syrian hamsters. *Learning & memory* 17: 109-16
- Martijena ID, Molina VA. 2012. The influence of stress on fear memory processes. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.]* 45: 308-13
- McGaugh JL. 2000. Memory--a century of consolidation. *Science* 287: 248-51
- Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL. 2007. A role for the human dorsal anterior cingulate cortex in fear expression. *Biological psychiatry* 62: 1191-4
- Minichiello L. 2009. TrkB signalling pathways in LTP and learning. *Nature reviews. Neuroscience* 10: 850-60
- Morrison KE, Curry DW, Cooper MA. 2012. Social status alters defeat-induced neural activation in Syrian hamsters. *Neuroscience* 210: 168-78
- Myers-Schulz B, Koenigs M. 2012. Functional anatomy of ventromedial prefrontal cortex: implications for mood and anxiety disorders. *Molecular psychiatry* 17: 132-41
- Nikulina EM, Arrillaga-Romany I, Miczek KA, Hammer RP, Jr. 2008. Long-lasting alteration in mesocorticolimbic structures after repeated social defeat stress in rats: time course of mu-opioid receptor mRNA and FosB/DeltaFosB immunoreactivity. *The European journal of neuroscience* 27: 2272-84
- Nikulina EM, Lacagnina MJ, Fanous S, Wang J, Hammer RP, Jr. 2012. Intermittent social defeat stress enhances mesocorticolimbic DeltaFosB/BDNF co-expression and persistently activates corticotegmental neurons: implication for vulnerability to psychostimulants. *Neuroscience* 212: 38-48
- Quirk GJ, Beer JS. 2006. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Current opinion in neurobiology* 16: 723-7
- Roosendaal B, McEwen BS, Chattarji S. 2009. Stress, memory and the amygdala. *Nature reviews. Neuroscience* 10: 423-33
- Sakata K, Martinowich K, Woo NH, Schloesser RJ, Jimenez DV, et al. 2013. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. *Proceedings of the National Academy of Sciences of the United States of America* 110: 15103-8
- Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. 2003. Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. *Biochemical pharmacology* 65: 1035-41
- Slattery DA, Neumann ID, Cryan JF. 2011. Transient inactivation of the infralimbic cortex induces antidepressant-like effects in the rat. *Journal of psychopharmacology* 25: 1295-303
- Stewart AF, Herrera RE, Nordheim A. 1990. Rapid induction of c-fos transcription reveals quantitative linkage of RNA polymerase II and DNA topoisomerase I enzyme activities. *Cell* 60: 141-9
- Tsvyetylnska NA, Hill RH, Grillner S. 2005. Role of AMPA receptor desensitization and the side effects of a DMSO vehicle on reticulospinal EPSPs and locomotor activity. *Journal of neurophysiology* 94: 3951-60

## 3.7 Figures

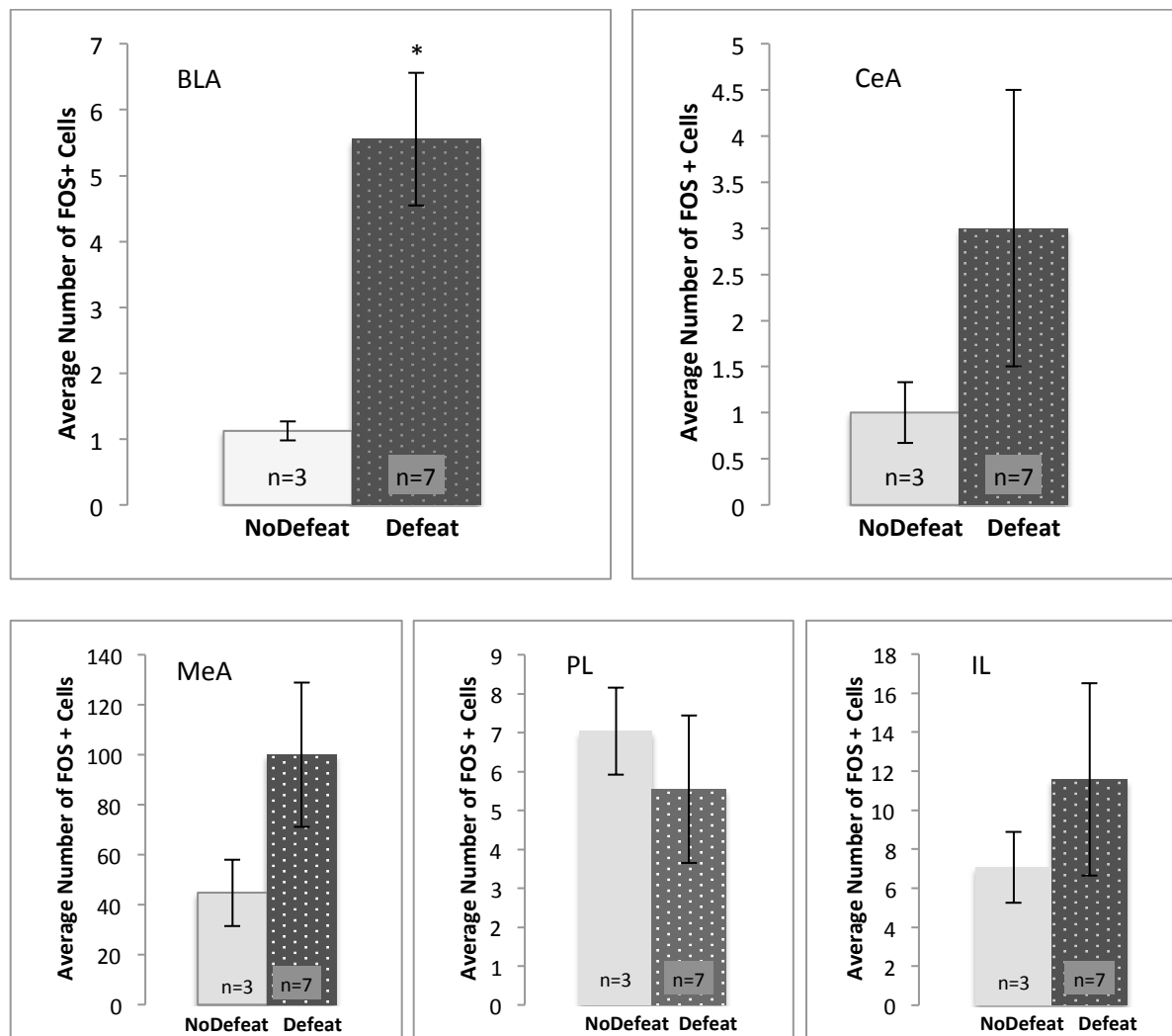


**Figure 3.1.** Putative Circuit Mediating the Acquisition and Expression of Conditioned Defeat

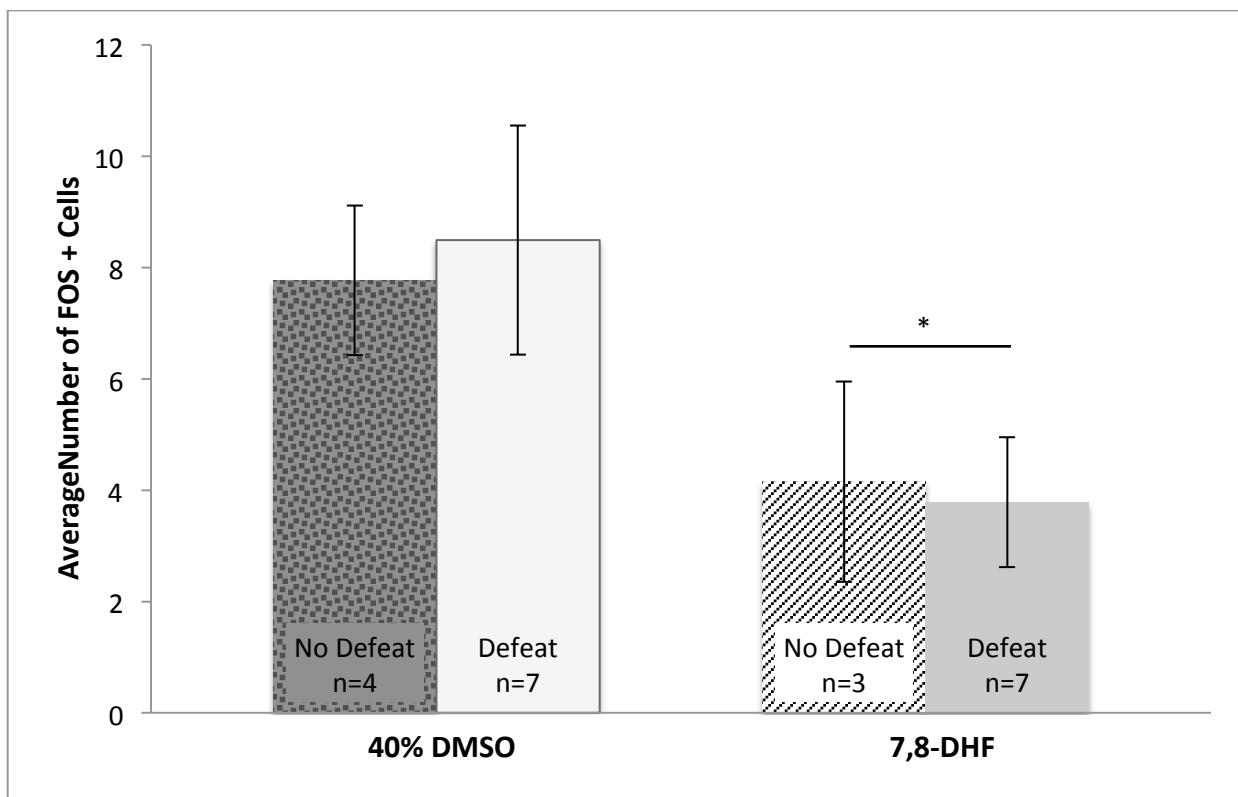


**Figure 3.2.** Representative photomicrograph (10X magnification) of defeat-induced Fos-IR in the BLA in saline no defeat (A) and saline defeat (B). (outlined in red). Black dots indicate FOS immunopositive nuclei. Sample photomicrograph and the adjoining drawing of the BLA are approximately 1.2 mm posterior to bregma (adapted from Morin & Wood, 2001).

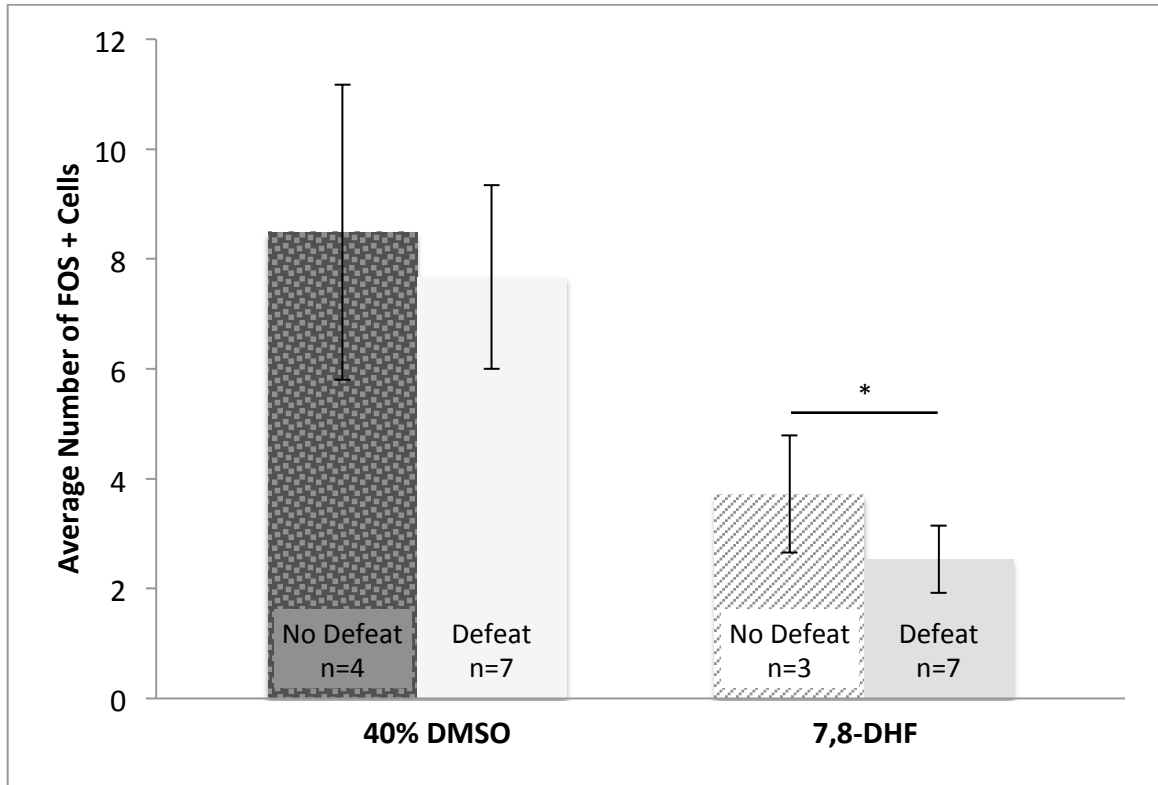




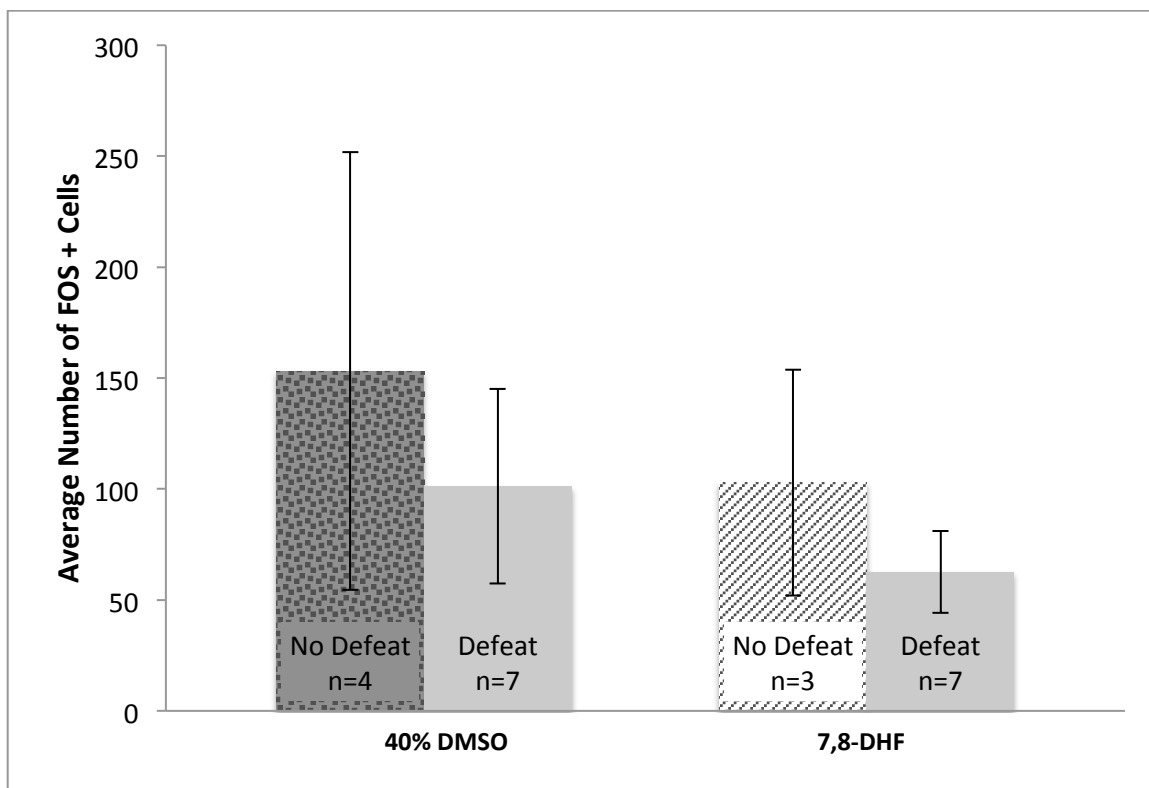
**Figure 3.3.** Defeat and FOS-IR in saline controls. Defeat significantly enhances FOS-IR in the BLA. A strong trend ( $p=0.06$ ) is also observed in the CeA. Defeat, however, does not significantly alter FOS-IR in the additional nuclei within our putative CD circuit including the MeA, the PL, or the IL. \* indicates a significant difference ( $p<0.05$ ) between FOS-IR in the BLA of defeat and no defeat groups.



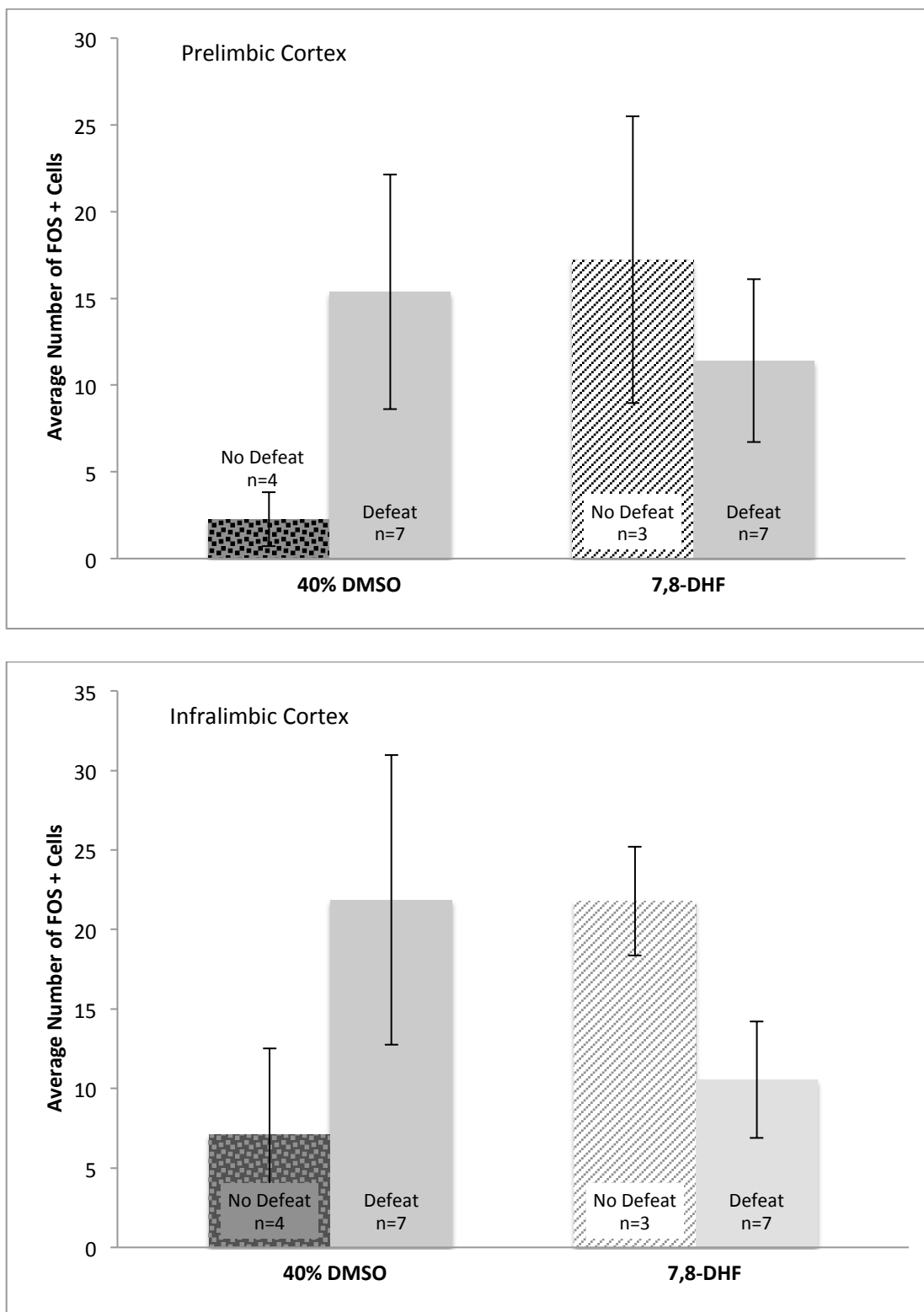
**Figure 3.4.** 7,8DHF decreases vehicle induced FOS-IR in the BLA. 7,8-DHF significantly reduces vehicle induced FOS-IR in the BLA as compared to hamsters treated with vehicle (40% DMSO/ 60% saline) regardless of defeat experience (defeat or no defeat). There is no main effect of defeat on FOS-IR and no significant interactions between drug x defeat on FOS-IR were found in the BLA.\* indicates a significant difference ( $p < 0.05$ ) between FOS-IR in the BLA of animals treated with 7,8-DHF or vehicle (40% DMSO/60% Saline).



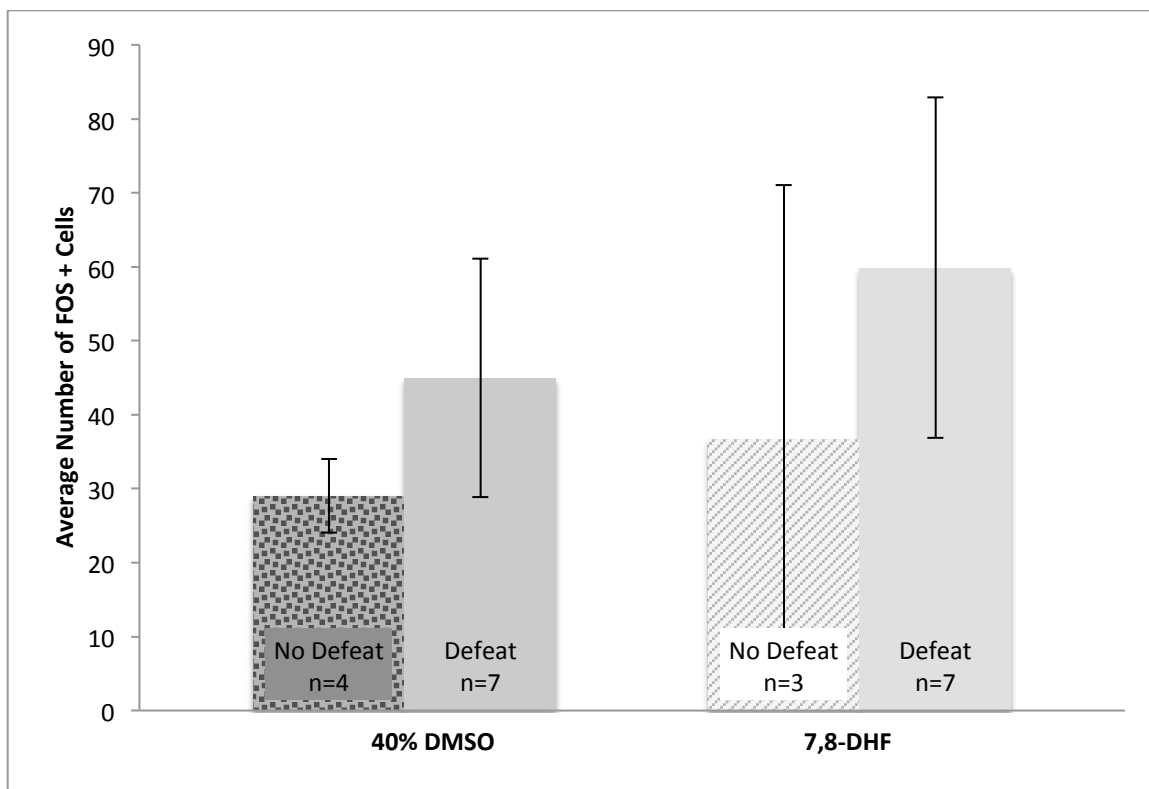
**Figure 3.5.** 7,8DHF decreases vehicle induced FOS-IR in the CeA. 7,8-DHF significantly reduces vehicle induced FOS-IR in the CeA as compared to hamsters treated with vehicle (40% DMSO/ 60% saline) regardless of defeat experience (defeat or no defeat). There is no main effect of defeat on FOS-IR and no significant interactions between drug x defeat on FOS-IR were found in the CeA.\* indicates a significant difference ( $p < 0.05$ ) between FOS-IR in the CeA of animals treated with 7,8-DHF or vehicle (40% DMSO/60% Saline).



**Figure 3.6 .** No significant differences in FOS-IR were found in the MeA. No main effects of defeat or drug were found in the MeA. There are also no interactions between defeat x drug.



**Figure 3.7.** No significant differences in FOS-IR were found in the mPFC (PL or IL). No main effects of defeat or drug were found in the mPFC (PL or IL). There are also no interactions between defeat x drug.



**Figure 3.8** . No significant differences in FOS-IR were found in the CPu. The CPu was selected as control nucleus outside of the putative CD circuit. No main effects of defeat or drug were found in the CPu. There are also no interactions between defeat x drug. FOS-IR is highly variable within this nucleus across the groups.

## **4 BDNF IN THE MEDIAL PREFRONTAL CORTEX ALTERS DEFEAT INDUCED NEURAL ACTIVITY IN SYRIAN HAMSTERS**

### **4.1 Abstract**

Brain derived neurotrophic factor (BDNF) has numerous actions within the nervous system. This molecule, which binds to the tyrosine kinase B (TrkB) receptor, is necessary for fear learning and alters behavioral responses to stress. It appears that BDNF may increase in some brain nuclei and decrease in others following exposure to stress. Our lab has recently demonstrated that 7,8-dihydroxyflavone (7,8-DHF), a novel TrkB receptor agonist, administered peripherally reduces submissive behaviors in Syrian hamsters following social stress, a finding which suggests that BDNF may have stress-reducing or resilience-promoting effects. This work, however, did not identify where in the brain BDNF might act to alter the response to social defeat stress. One circuit of interest in terms of stress responding and resilience is the medial prefrontal cortex (mPFC), which is reciprocally connected with the basolateral amygdala (BLA), the key site of plasticity in conditioned defeat (CD). Interestingly, disruption of this circuit is associated with neuropsychiatric disorders including posttraumatic stress disorder (PTSD). This project was designed to test the hypothesis that BDNF alters defeat-induced neural activation in the BLA at least in part by acting within the mPFC. Interestingly, we found that BDNF microinjected site-specifically into the mPFC after social defeat increases defeat-induced neural activation of the BLA, as measured via expression FOS-immunoreactivity (FOS-IR). These results suggest that BDNF may alter social stress-induced behavioral responses at least in part by acting in the mPFC, which in turn modulates neural activity in the BLA.

## 4.2 Introduction

Stress is a critical factor that places individuals at an increased risk for developing neuropsychiatric disorders (Sapolsky, 2000; Karatsoreos & McEwen, 2011) including depression and posttraumatic stress disorder (PTSD) (Gilbert & Allan 1998; Heim & Nemeroff 2001; McEwen & Stellar 1993; Pêgo et al., 2010; Shin & Liberzon, 2010). The amygdala and the medial prefrontal cortex (mPFC) are altered by stress and modulate fear memory and stress responding (reviewed by Roozendaal, McEwen & Chattarji, 2009). It is therefore not surprising that these same projections between the amygdala and the mPFC appear to have a role in neuropsychiatric illnesses such as PTSD and depression. Specifically, disease states such as PTSD and depression are often characterized by an over-active amygdala and decreased inhibitory control of the amygdala by the mPFC (Rabinak et al., 2014; Rive et al., 2013; Erk et al., 2010; Hamilton et al., 2008; Jovanovic et al., 2013).

In addition, work in animal models has demonstrated that reciprocal projections between the amygdala and the mPFC are involved in fear learning and in threat identification (Ishikawa & Nakamura 2003; Marek et al., 2013; Ongur & Price 2000). The mPFC is composed of the infralimbic cortex (IL) and the prelimbic cortex (PL) in rodents, which is analogous to Brodmann's Area (BA) 25 and BA 32, respectively, in humans (Milad et al., 2007; Myers-Schulz & Koenigs, 2012; Quirk & Beer, 2006; Slattery, Neumann, & Cryan, 2011). Tract tracing studies in animals have found that the IL and the PL send projections to sub-nuclei within the amygdala, including the basolateral amygdala (BLA) and the central amygdala (CeA) (Vertes, 2004; Gabbott, 2005). These 2 sub-nuclei in the mPFC appear to act in opposition, given that stimulation of the PL enhances freezing (Vidal-Gonzalez et al., 2006), whereas stimulation of the IL decreases freezing (Milad & Quirk, 2002; Milad et al., 2004). Accordingly, the PL is necessary in fear acquisition and expression, whereas the IL is necessary for fear extinction (Sierra-Mercado et al., 2011). It has previously been suggested that the projections from the PL to the BLA are excitatory, which in turn would increase activity of the BLA and could result in



excitation of the CeA and downstream symptoms of fear and anxiety (i.e., increase stress-induced freezing) (Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011). In contrast, projections of the IL synapse on the intercalated cells, clusters of GABAergic neurons that exert feed-forward inhibition of the CeA, resulting in reduced freezing behavior (Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011). Overall, the preponderance of evidence suggests that the PL and IL have opposite effects on the amygdala and that dysregulation of either might play a role in a variety of neuropsychiatric disorders.

The PL and IL, not surprisingly, display altered neuronal activity following exposure to stress. Specifically, both the PL and IL are active following stress, as measured by immediate early genes. Repeated social defeat, for example, increases delta FOS B, a truncated splice variant of FosB, in the IL and PL (Nikulina et al., 2012). Studies examining the IL and PL individually have found region-specific differences in neural activation. Morrison and colleagues found that FOS-immunoreactivity (FOS-IR) is increased in the IL of dominant as compared to subordinate Syrian hamsters (Morrison et al., 2012). Muscimol administered site-specifically in the IL of dominant hamsters increases CD (Morrison et al., 2013), suggesting that the IL is important for resistance to a social defeat stressor. Similarly, lesions of the IL eliminate the stress-reducing effects of enriched housing environments on social defeat in mice (Lehmann and Herkenham, 2011). Furthermore, recent work suggests that the PL underlies susceptibility to social defeat. Specifically, over expression of delta FOS B in the PL and infusion of a cholecystokinin (CCK) agonist into the PL promotes depression-like and anxiety-like behaviors after social defeat (Vialou et al., 2014). These studies suggest that both the PL and the IL are differentially altered by stress and that they may play opposite roles in modulating brain regions that control behavioral responses to stress.

Stress also alters BDNF expression in the mPFC. BDNF mRNA in both the PL and IL increase immediately following inescapable stress in rats (Bland et al., 2005), suggesting that BDNF is involved in stress-induced changes in both of these nuclei. Site-specific changes in

BDNF expression is of particular interest given that BDNF is altered by stress in the neural circuit implicated in PTSD (e.g., the mPFC and the amygdala). Interestingly, recent work using BDNF knockout mice has found that BDNF in the PL is necessary for the consolidation of fear learning (Choi et al., 2010; Choi et al., 2012), whereas BDNF in the IL is necessary for extinction of fear (Peters et al., 2010). This finding provides additional evidence that these 2 nuclei act in opposition and that BDNF may also play an important role in this process.

One animal model that may help to shed light on the role that BDNF plays in the PL and IL is conditioned defeat (CD), a dramatic behavioral change that occurs following exposure to a brief social defeat stressor (Potegal et al., 1993; Huhman et al., 1990; Huhman et al., 1991a; Huhman et al., 1991b; Huhman et al., 1992; Huhman et al., 2003). The putative neural circuit that underlies CD in hamsters has been characterized (Markham & Huhman, 2008; Markham et al., 2009; Markham et al., 2010; McDonald et al., 2012), and the BLA and the mPFC have been shown to modulate the acquisition and/or expression of CD (Jasnow, 2005; Markham et al., 2010; Day et al., 2011; Markham et al., 2012). We have recently demonstrated that BDNF administered peripherally or site-specifically into the BLA reduces CD (Jeffress et al., in preparation); however, it is unclear if the BLA is the only nuclei wherein BDNF acts to alter neuronal activity and in turn mediate behavioral output. Given that the BLA and the mPFC are reciprocally connected and are both altered by stress, and that BDNF within in these nuclei is involved in conditioned learning, it seems reasonable to propose that BDNF may act to modulate CD at least in part via its actions in the mPFC. Thus, this project was designed to gain a better understanding of the circuitry underlying CD and the role of BDNF within that circuit. We expected that BDNF administered into the PL would enhance defeat-induced FOS-IR in the BLA and CeA, whereas BDNF administered into the IL would suppress defeat-induced FOS-IR in the BLA and CeA. The predictions are illustrated in Figure 1 (adapted from Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011),

### **4.3 Methods & Materials**

#### ***4.3.1 Animals and Housing Conditions***

Subjects in all experiments were male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories, New York, NY) that weighed 120-140 g and were between 9-10 weeks old at the time of testing. Upon arrival, animals were group housed with 5-6 animals per cage for one week prior to any manipulation (quarantine measure). All hamsters were handled for five days prior to behavioral procedures to acclimate them to experimenter handling, and singly housed in polycarbonate cages (20x 40 x 20cm) with corncob bedding and wire mesh tops in a temperature ( $20 \pm 2^\circ$  C) and humidity controlled room. The room was maintained on 14:10 light dark cycle with lights off at 10:00 h. Food and water were available *ad libitum*. Older hamsters (>6 months) individually housed (>1 month) weighing at least 180g served as resident aggressors (RA) in defeat training. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

#### ***4.3.2 Surgical Procedures***

Subjects were deeply anesthetized for the duration of the surgery (3.75% isoflurane at a flow rate of 1 lpm), and breathing and body temperature were monitored while animals were under anesthesia. A unilateral stainless steel guide cannula (26-gauge, Plastics One, Roanoke, VA) was stereotaxically implanted at a  $20^\circ$  angle toward the midline in either the left or right hemisphere (0.2mm posterior and  $\pm 3.2$ mm lateral to bregma and 2.2 mm below dura for IL; 0.2mm posterior and  $\pm 2.9$ mm lateral to bregma and 1.9 mm below dura for PL). Following surgery, dummy stylets were placed in the guide cannula to help maintain patency and 1 cc of physiological saline and 0.1 cc of ketoprofen were injected subcutaneously in order to aid in recovery. In order to minimize tissue damage to the area of interest, a smaller, 33-gauge

injection needle (Plastics One, Roanoke, VA) that projected 1.2 mm below the guide cannula was used on injection day in order to reach a final depth of 3.1 or 3.4 mm below dura. Prior to behavioral testing, hamsters were monitored daily to ensure that there were no adverse outcomes from the surgery. Hamsters were allowed 5 days to recover.

#### ***4.3.3 Social Defeat***

The CD model has been described in detail elsewhere (Huhman et al., 2003). Prior to training, hamsters were weight matched and randomly assigned to groups. All animals were transported in their home cage to the behavioral testing suite within the animal vivarium 30 min prior to all behavioral manipulations in order to acclimate. All behavioral procedures were performed in the first 3 h of the light dark cycle to minimize any circadian effects. Social defeat training occurred in the home cage of the RA for 15 min. No animals were injured during training. No-defeat animals were placed into an empty RA homecage for 15 min to expose animals to odors but no physical interaction.

#### ***4.3.4 Drug Administration***

Animals received a unilateral infusion of rhBDNF (Sigma; St. Louis, MO) into the PL or IL. Animals (n=45) were randomly assigned to groups (defeat or no defeat) and within these 2 conditions; animals received either 0.4ng/50nL rhBDNF in physiological saline or vehicle (saline). The very small injection volume (50nL) was selected to attempt to limit diffusion of the microinjection of rhBDNF between adjacent nuclei (i.e., PL and IL). Dosages were selected based on previous work using site-specific microinjections of rhBDNF in rats in the periaqueductal grey (Casarotto et al., 2010). All injections were administered immediately following a 15 min defeat or a no-defeat control exposure to the RA cage.

#### **4.3.5 Site Verification & Immunohistochemistry**

Sixty minutes after the behavioral manipulation and microinjection, all experimental animals received an overdose of sodium pentobarbital and were unilaterally infused with 50nl of India ink to verify needle placement. Subjects were then perfused transcardially with 0.1 M potassium phosphate-buffered saline (KPBS) and 4% paraformaldehyde. Brains were post-fixed in 4% paraformaldehyde overnight and switched to a 30% sucrose-paraformaldehyde solution and kept at 4°C for an additional 48 h. Brains were blocked on the coronal plane and sectioned on a cryostat (Leica CM 3050 S) to a thickness of 30 um. Consecutive sections were placed sequentially across 4 wells filled with cyroprotectant. The sections covered the BLA in its entirety. One series of sections containing the PL and the IL were collected and then stained with Neutral red; cover slipped with Permount mountant (Fisher), and then examined using a light microscope for ink in the PL/IL. Only brains with a clear unilateral ink injection within 0.3mm of the PL or the IL was included in the final analysis as determined by 2 blind observers. Injections that were outside of the PL or IL (i.e., greater than 0.3mm) or that had ink diffusion within both the IL and PL were analyzed separately as an anatomical miss control group and injections that did not result in visible ink were excluded from analysis.

Sections were labeled for FOS using primary antisera directed against the protein product of the immediate early gene *c-fos* (rabbit anti-*c-fos* polyclonal antibody, 1:10000; Santa Cruz Biotechnology). All washes, rinses, and incubations were performed in 25-well tissue culture plates, which were gently shaken on an orbital shaker throughout the procedure. Sections were rinsed in 0.1 M KPBS and incubated in a 0.3% hydrogen peroxide solution for 15 min, followed by a KPBS wash. Sections were then incubated for 18 h in the primary antibody at room temperature in 1% solution of Triton X-100 in 0.1 M KPBS (1% KPBS-T) with normal goat serum (NGS). Following incubation with the primary antibody, the sections were rinsed in KPBS and incubated for 90 min at room temperature in 1% KPBS-T containing the secondary antibody (biotintylated horse anti-rabbit IgG polyclonal antibody, 1:200; Jackson ImmunoResearch).

They were then rinsed with KPBS, followed by incubation for 1 h with an avidinbiotin complex reagent (Vectastain Elite ABC kit: Vector Laboratories). After rinsing with KPBS, sections were incubated in nickel-3, 39-diaminobenzidine (DAB) Kit (Vector) for 2-4 min in order to produce a blue-black-stained product and then washed again in KPBS in order to halt the DAB reaction. Finally, sections were mounted on gelatin-coated slides, dehydrated, and cover slipped with Permount (Fisher).

#### ***4.3.6 Cell Counting***

Two observers that were blind to the treatment conditions performed all cell counts by hand using bright field microscopy at 10X magnification. For the quantification of FOS-IR labeling, areas of interest were first delineated using adjoining Neutral Red stained sections. FOS-labeled cells were photographed using a Zeiss Camera (Jena, Germany) within the region of interest established by the templates determined from nissl-stained alternate sections.

#### ***4.3.7 Statistical Methods***

FOS values were expressed as mean  $\pm$  SEM. A 2x2 ANOVA was used to compare the effects of defeat and drug on FOS-IR when data did not violate assumptions. Non-parametric tests (Kruskal-Wallis using Mann Whitney U for post-hoc analysis) were used when our behavioral data violated assumptions of homogeneity (Levene's Test,  $P > 0.05$ ) or normality (examined using the Kolmogorov Smirnov test). Analyses were performed with IBM SPSS software (20.0.0). Criterion for significance was set to  $p < 0.05$ .

### **4.4 Results**

Statistical analysis revealed that there were no differences between injections in the PL and the IL (Table 1), thus data from PL and the IL injections were collapsed for statistical analysis. A 2x2 ANOVA was used to compare the effects of defeat and drug on FOS-IR in the

CeA, whereas Kruskal Wallis and Mann-Whitney U tests was used to compare the effects of defeat and drug on FOS-IR in the BLA, as the BLA data were non-normal.

Figure 2 shows the injections sites for all animals. Figure 3 is a representative photomicrograph of FOS-IR in the BLA indicating where FOS-IR was analyzed following mPFC administration of BDNF or saline in defeated and non-defeated animals.

#### **4.4.1 Basolateral amygdala**

As shown in Figure 4, there is a statistically significant main effect of defeat on FOS-IR in the BLA ( $U=74.00, p<0.05$ ). Defeated animals ( $n=30; \text{Mdn}=7$ ) had increased FOS-IR as compared to no defeat animals ( $n=15; \text{Mdn}=1$ ). Furthermore, there is a statistically significant main effect of drug on FOS-IR in the BLA ( $U=143.50, p<0.05$ ). Animals receiving a microinjection of BDNF ( $n=22; \text{Mdn}=7$ ) had increased FOS-IR as compared to animals receiving a microinjection of saline ( $n=23; \text{Mdn}=0$ ). Further analysis revealed that defeated animals that received BDNF ( $n=15$ ) have a significant increase in FOS-IR in the BLA as compared to defeated animals receiving saline ( $n=15$ ) ( $U=62.50, p<0.05$ ). Similarly, no-defeat animals treated with BDNF ( $n=8$ ) also demonstrated a significant increase in FOS-IR in the BLA as compared to no-defeat animals receiving saline ( $n=8$ ) ( $U=6.00, p<0.05$ ).

#### **4.4.2 Central Amygdala**

As shown in Figure 5, there is a statistically significant group difference in FOS-IR in the CeA between defeat ( $n=30$ ) and no-defeat animals ( $n=15$ ) ( $F(1,41)=0.182, p<0.05$ ). There were no main effects for drug administered via microinjection (BDNF or saline) ( $F(1,41)=15.022, p>0.05$ ). Furthermore, no interactions were found between drug and defeat ( $F(1,41)=0.570, p>0.05$ ).

## 4.5 Discussion

Our current results demonstrate that BDNF administered directly into the mPFC after social defeat stimulates neural activity in the BLA, as measured via FOS-IR. These data demonstrate that BDNF in the mPFC can alter BLA activation, but it is not clear that this would alter behavioral output given that the BLA activation is not associated with a concurrent activation of its major output pathway, the CeA. We originally expected that BDNF administered into the PL would enhance defeat-induced FOS-IR in the BLA and CeA, whereas BDNF administered into the IL would suppress defeat-induced FOS-IR in the BLA and CeA. This result clearly was not found, and there are a number of factors that could explain this. It is important to note, however that we did observe the expected increases FOS-IR in the BLA and the CeA of defeated as compared to no-defeat animals, which replicates previous findings of our laboratory and others (Bourne et al., 2013; Nikulina et al., 2008; Markham et al., 2010; Kollack-Walker et al., 1997; Kollack-Walker et al., 1999) and serves as a positive control to demonstrate that our immunohistochemical procedures were adequate.

As stated above, BDNF administered into the mPFC following defeat increases FOS-IR in the BLA. Thus, it is possible that BDNF-active drugs alter behavioral responses to social defeat via the connections between the mPFC and BLA. An important caveat to this possibility, however, is that we also found that BDNF administered into the mPFC does not alter FOS-IR in the CeA, the output center of the BLA. This suggests that the administration of BDNF in the mPFC activated neurons in the BLA that do not in turn excite or promote neural activity in the CeA. The BLA is composed of 2 major types of neurons: inhibitory GABAergic (gamma-amino butyric acid) interneurons and excitatory glutamatergic pyramidal neurons (reviewed in Pape and Pare, 2010), both of which express FOS-IR when activated (Hale et al., 2010; Lukkes et al., 2012). In fact, GABAergic interneurons outnumber glutamatergic projection neurons as identified using electrophysiological recording techniques (Rainnie, Asprodini, & Shinnick-Gallagher, 1993; Likhtik et al., 2006). In order to determine the cell types activated in the BLA



following the administration of BDNF in the mPFC, double-labeled immunohistochemistry studies are needed. Our current results only examine FOS-IR independently. We are therefore unable to comment on what types of neurons are activated in the BLA following the administration of BDNF in the mPFC, but the lack of concurrent stimulation in the CeA and the previous finding that BDNF and TrkB agonists reduce CD suggests that the FOS activation is occurring in cells other than glutamatergic projection neurons.

Our current findings may suggest that BDNF administered site specifically into the mPFC enhances neural activity in GABAergic interneurons in the BLA, as other laboratories have found that FOS-IR can be measured in GABAergic interneurons in the BLA (Hale et al., 2010; Lukkes et al., 2012). If this is the case in the current study, enhanced activity in interneurons would suggest an increase in the inhibitory tone in the BLA, which should result in a decrease in submissive and defensive behavior in Syrian hamsters after social defeat. As FOS-IR in the CeA did not change, we speculate that the BLA is not sending excitatory impulses to the CeA. An increase in GABAergic activity in the interneurons, as measured by FOS-IR in the BLA, would be consistent with recent findings from our lab that CD is reduced following peripheral administration of a TrkB agonist (Jeffress et al., in preparation). Thus, one possibility is that BDNF in the mPFC may reduce submissive behavior in Syrian hamsters via enhanced GABAergic signaling in the BLA. However, without double-label immunohistochemistry studies and behavioral studies with BDNF microinjections in the mPFC, this is only speculation.

An alternative explanation for the current findings is that BDNF in the mPFC did stimulate FOS in glutamatergic project neurons, which is what is most commonly expected, but that this drug treatment also stimulated other neurons that were able to dampen the downstream response in the CeA. This could occur if neurons in the intercalated cells between the BLA and CeA were also stimulated by mPFC BDNF injections. This is a reasonable possibility given that the mPFC projects to these cells (Vertes, 2004; Gabbott, 2005; also reviewed in Pape & Pare, 2010), and they are known to inhibit fear-like responses and to

promote extinction of fear memories (reviewed in Pape & Pare, 2010). Future studies should examine the possible role of these cells in this pathway.

Originally, we expected that BDNF administered into the PL would enhance defeat-induced FOS-IR in the BLA and CeA, whereas BDNF administered into the IL would suppress defeat-induced FOS-IR in the BLA and CeA. It is possible that the volume of BDNF used for our current microinjections in the PL and IL was too large and thus BDNF may have diffused between the 2 adjacent nuclei. However this is unlikely, given that the injection volume used was minimized in attempts to reduce this concern. It is more likely that reciprocal projections between the PL and IL are altered by defeat and BDNF injections (Hoover & Vertes, 2007), and thus activity between the 2 cortical nuclei is altered independently prior to altering downstream activity in the BLA. To date, the projections between the 2 cortical nuclei have not been neurochemically identified (Sangha et al., 2012), thus without additional studies it is unclear how BDNF may mediate or alter these projections to mediate a net response from the mPFC leading to increased activity in the BLA.

In conclusion we have found that BDNF administered into the mPFC alters FOS-IR in the BLA, a down stream target of the mPFC, following social defeat. These results suggest that BDNF in the mPFC may act to reduce CD in Syrian hamsters by altering activity of the BLA. Additional work, however, is necessary to clarify how changes in neuronal activity in specific cell types within this circuit mediate behavioral changes. Our findings, in turn, suggest that this circuit is one of the ways in which BDNF may mediate behavioral changes following social defeat in hamsters and this possibility should be tested directly in future studies.

## 4.6 References

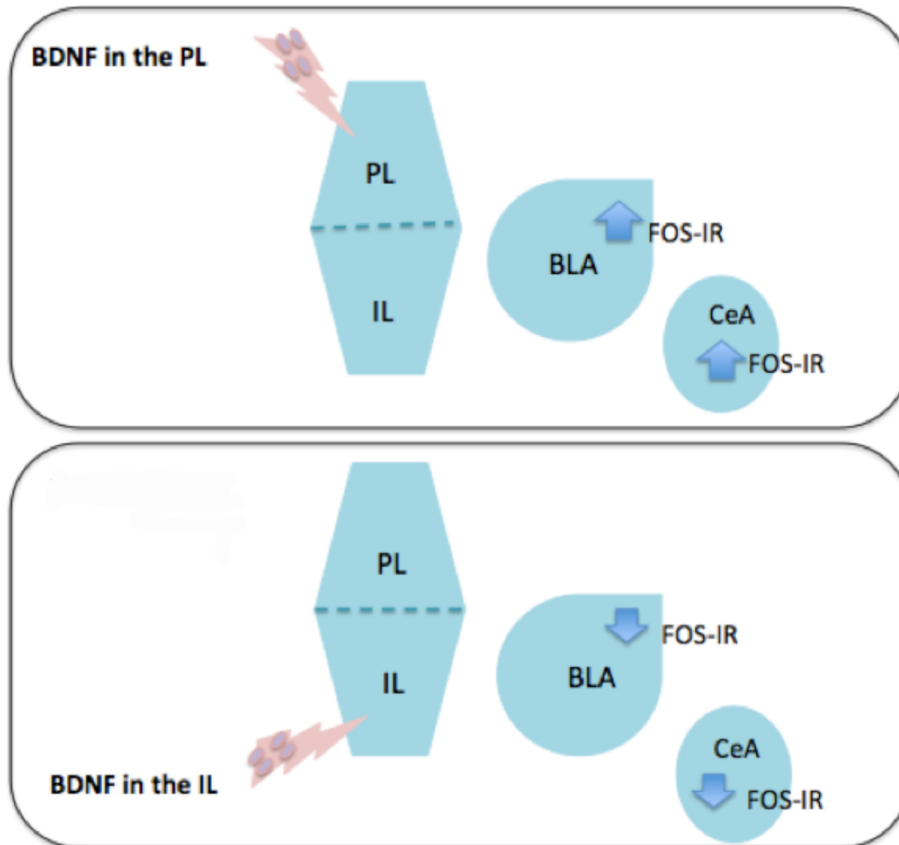
- Bland ST, Schmid MJ, Der-Avakian A, Watkins LR, Spencer RL, Maier SF. 2005. Expression of c-fos and BDNF mRNA in subregions of the prefrontal cortex of male and female rats after acute uncontrollable stress. *Brain research* 1051: 90-9
- Bourne AR, Mohan G, Stone MF, Pham MQ, Schultz CR, et al. 2013. Olfactory cues increase avoidance behavior and induce Fos expression in the amygdala, hippocampus and prefrontal cortex of socially defeated mice. *Behavioural brain research* 256: 188-96
- Casarotto PC, de Bortoli VC, Correa FM, Resstel LB, Zangrossi H, Jr. 2010. Panicolytic-like effect of BDNF in the rat dorsal periaqueductal grey matter: the role of 5-HT and GABA. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* 13: 573-82
- Choi DC, Gourley SL, Ressler KJ. 2012. Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning. *Translational psychiatry* 2: e205
- Choi DC, Maguschak KA, Ye K, Jang SW, Myers KM, Ressler KJ. 2010. Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2675-80
- Day DE, Cooper MA, Markham CM, Huhman KL. 2011. NR2B subunit of the NMDA receptor in the basolateral amygdala is necessary for the acquisition of conditioned defeat in Syrian hamsters. *Behavioural brain research* 217: 55-9
- Erk S, Mikschl A, Stier S, Ciaramidaro A, Gapp V, et al. 2010. Acute and sustained effects of cognitive emotion regulation in major depression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30: 15726-34
- Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ. 2005. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. *The Journal of comparative neurology* 492: 145-77
- Gilbert P, Allan S. 1998. The role of defeat and entrapment (arrested flight) in depression: an exploration of an evolutionary view. *Psychological medicine* 28: 585-98
- Gilboa A, Shalev AY, Laor L, Lester H, Louzoun Y, et al. 2004. Functional connectivity of the prefrontal cortex and the amygdala in posttraumatic stress disorder. *Biological psychiatry* 55: 263-72
- Hale MW, Johnson PL, Westerman AM, Abrams JK, Shekhar A, Lowry CA. 2010. Multiple anxiogenic drugs recruit a parvalbumin-containing subpopulation of GABAergic interneurons in the basolateral amygdala. *Progress in neuro-psychopharmacology & biological psychiatry* 34: 1285-93
- Hamilton JP, Gotlib IH. 2008. Neural substrates of increased memory sensitivity for negative stimuli in major depression. *Biological psychiatry* 63: 1155-62
- Heim C, Nemeroff CB. 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological psychiatry* 49: 1023-39
- Hoover WB, Vertes RP. 2007. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain structure & function* 212: 149-79

- Huhman KL, Bunnell BN, Mougey EH, Meyerhoff JL. 1990. Effects of social conflict on POMC-derived peptides and glucocorticoids in male golden hamsters. *Physiology & behavior* 47: 949-56
- Huhman KL, Hebert MA, Meyerhoff JL, Bunnell BN. 1991a. Plasma cyclic AMP increases in hamsters following exposure to a graded footshock stressor. *Psychoneuroendocrinology* 16: 559-63
- Huhman KL, Moore TO, Ferris CF, Mougey EH, Meyerhoff JL. 1991b. Acute and repeated exposure to social conflict in male golden hamsters: increases in plasma POMC-peptides and cortisol and decreases in plasma testosterone. *Hormones and behavior* 25: 206-16
- Huhman KL, Moore TO, Mougey EH, Meyerhoff JL. 1992. Hormonal responses to fighting in hamsters: separation of physical and psychological causes. *Physiology & behavior* 51: 1083-6
- Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, et al. 2003. Conditioned defeat in male and female Syrian hamsters. *Hormones and behavior* 44: 293-9
- Ishikawa A, Nakamura S. 2003. Convergence and interaction of hippocampal and amygdalar projections within the prefrontal cortex in the rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23: 9987-95
- Jasnow AM, Huhman KL. 2001. Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain research* 920: 142-50
- Jeffress EC, Thompson, BM, Markham, C, Huhman KL. BDNF reduces behavioral responses to social stress in Syrian hamsters. In preparation
- Jovanovic T, Ely T, Fani N, Glover EM, Gutman D, et al. 2013. Reduced neural activation during an inhibition task is associated with impaired fear inhibition in a traumatized civilian sample. *Cortex; a journal devoted to the study of the nervous system and behavior* 49: 1884-91
- Kollack-Walker S, Don C, Watson SJ, Akil H. 1999. Differential expression of c-fos mRNA within neurocircuits of male hamsters exposed to acute or chronic defeat. *Journal of neuroendocrinology* 11: 547-59
- Kollack-Walker S, Watson SJ, Akil H. 1997. Social stress in hamsters: defeat activates specific neurocircuits within the brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17: 8842-55
- Lehmann ML, Herkenham M. 2011. Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31: 6159-73
- Likhtik E, Pelletier JG, Popescu AT, Pare D. 2006. Identification of basolateral amygdala projection cells and interneurons using extracellular recordings. *Journal of neurophysiology* 96: 3257-65
- Lukkes JL, Burke AR, Zelin NS, Hale MW, Lowry CA. 2012. Post-weaning social isolation attenuates c-Fos expression in GABAergic interneurons in the basolateral amygdala of adult female rats. *Physiology & behavior* 107: 719-25
- Marek R, Strobel C, Bredy TW, Sah P. 2013. The amygdala and medial prefrontal cortex: partners in the fear circuit. *The Journal of physiology* 591: 2381-91

- Markham CM, Luckett CA, Huhman KL. 2012. The medial prefrontal cortex is both necessary and sufficient for the acquisition of conditioned defeat. *Neuropharmacology* 62: 933-9
- Markham CM, Norvelle A, Huhman KL. 2009. Role of the bed nucleus of the stria terminalis in the acquisition and expression of conditioned defeat in Syrian hamsters. *Behavioural brain research* 198: 69-73
- Markham CM, Taylor SL, Huhman KL. 2010. Role of amygdala and hippocampus in the neural circuit subserving conditioned defeat in Syrian hamsters. *Learning & memory* 17: 109-16
- McDonald MM, Markham CM, Norvelle A, Albers HE, Huhman KL. 2012. GABAA receptor activation in the lateral septum reduces the expression of conditioned defeat and increases aggression in Syrian hamsters. *Brain research* 1439: 27-33
- McEwen BS, Stellar E. 1993. Stress and the individual. Mechanisms leading to disease. *Archives of internal medicine* 153: 2093-101
- Milad MR, Quirk GJ. 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420: 70-4
- Milad MR, Vidal-Gonzalez I, Quirk GJ. 2004. Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behavioral neuroscience* 118: 389-94
- Morrison KE, Bader LR, McLaughlin CN, Cooper MA. 2013. Defeat-induced activation of the ventral medial prefrontal cortex is necessary for resistance to conditioned defeat. *Behavioural brain research* 243: 158-64
- Morrison KE, Curry DW, Cooper MA. 2012. Social status alters defeat-induced neural activation in Syrian hamsters. *Neuroscience* 210: 168-78
- Nikulina EM, Arrillaga-Romany I, Miczek KA, Hammer RP, Jr. 2008. Long-lasting alteration in mesocorticolimbic structures after repeated social defeat stress in rats: time course of mu-opioid receptor mRNA and FosB/DeltaFosB immunoreactivity. *The European journal of neuroscience* 27: 2272-84
- Nikulina EM, Lacagnina MJ, Fanous S, Wang J, Hammer RP, Jr. 2012. Intermittent social defeat stress enhances mesocorticolimbic DeltaFosB/BDNF co-expression and persistently activates corticotegmental neurons: implication for vulnerability to psychostimulants. *Neuroscience* 212: 38-48
- Ongur D, Price JL. 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral cortex* 10: 206-19
- Pape HC, Pare D. 2010. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiological reviews* 90: 419-63
- Pego JM, Sousa JC, Almeida OF, Sousa N. 2010. Stress and the neuroendocrinology of anxiety disorders. *Current topics in behavioral neurosciences* 2: 97-117
- Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. 2010. Induction of fear extinction with hippocampal-infralimbic BDNF. *Science* 328: 1288-90
- Potegal M, Huhman K, Moore T, Meyerhoff J. 1993. Conditioned defeat in the Syrian golden hamster (*Mesocricetus auratus*). *Behavioral and neural biology* 60: 93-102
- Rainnie DG, Asprodini EK, Shinnick-Gallagher P. 1993. Intracellular recordings from morphologically identified neurons of the basolateral amygdala. *Journal of neurophysiology* 69: 1350-62

- Rive MM, van Rooijen G, Veltman DJ, Phillips ML, Schene AH, Ruhe HG. 2013. Neural correlates of dysfunctional emotion regulation in major depressive disorder. A systematic review of neuroimaging studies. *Neuroscience and biobehavioral reviews* 37: 2529-53
- Roosendaal B, McEwen BS, Chattarji S. 2009. Stress, memory and the amygdala. *Nature reviews. Neuroscience* 10: 423-33
- Sakata K, Martinowich K, Woo NH, Schloesser RJ, Jimenez DV, et al. 2013. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. *Proceedings of the National Academy of Sciences of the United States of America* 110: 15103-8
- Sangha S, Ilenseer J, Sosulina L, Lesting J, Pape HC. 2012. Differential regulation of glutamic acid decarboxylase gene expression after extinction of a recent memory vs. intermediate memory. *Learning & memory* 19: 194-200
- Shin LM, Liberzon I. 2010. The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 35: 169-91
- Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. 2011. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 36: 529-38
- Vertes RP. 2004. Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse* 51: 32-58
- Vialou V, Bagot RC, Cahill ME, Ferguson D, Robison AJ, et al. 2014. Prefrontal cortical circuit for depression- and anxiety-related behaviors mediated by cholecystokinin: role of DeltaFosB. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34: 3878-87
- Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ. 2006. Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learning & memory* 13: 728-33

## 4.7 Figures

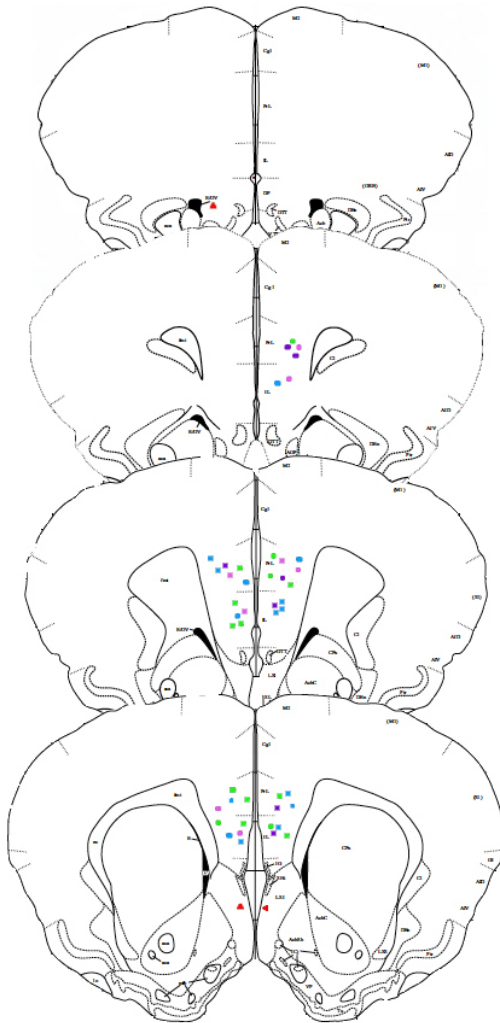


**Figure 4.1.** Expected neuronal activation following rhBDNF microinjections. The pattern shown in the upper panel would be expected to be associated with enhanced CD and the pattern in the lower panel would be expected to be associated with reduced CD (adapted from Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011).

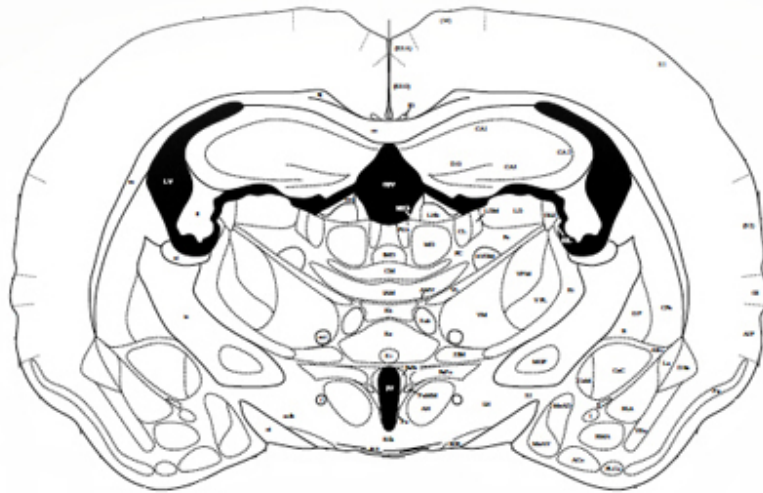
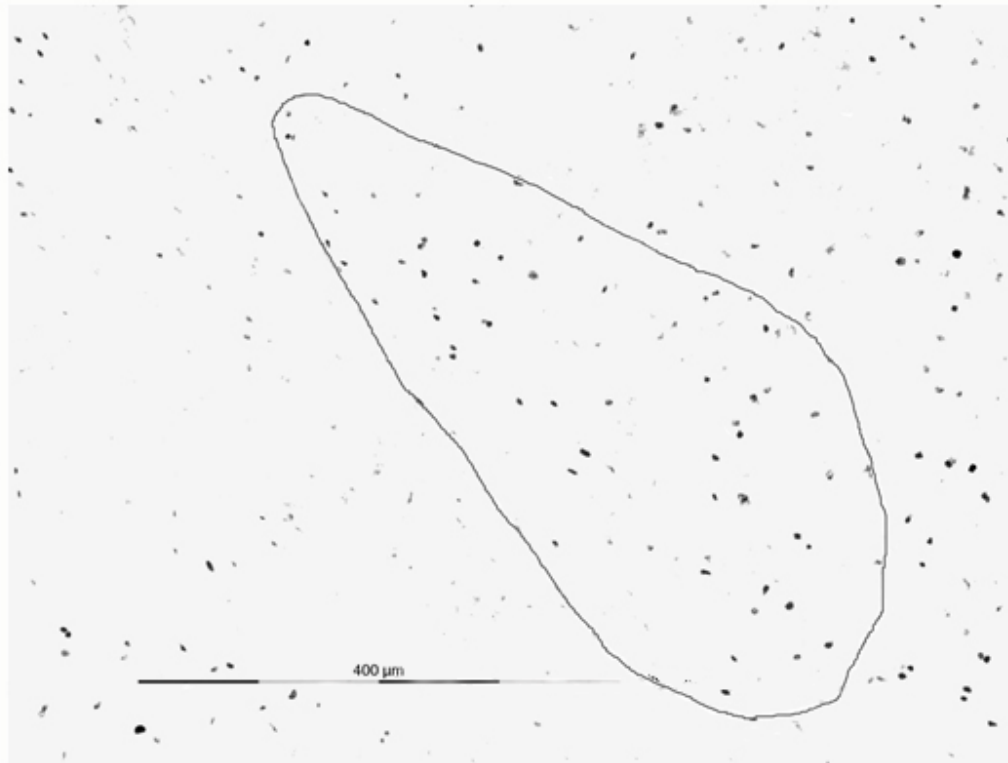
**Table 4.1.** Average number of FOS-IR positive cells in the BLA following administration of saline or rhBDNF into the PL or IL. PL and IL were collapsed into the mPFC for statistical analyses.

	<b>PL-Saline</b>	<b>IL-Saline</b>	<b>PL-rhBDNF</b>	<b>IL-rhBDNF</b>
BLA FOS-IR IN DEFEATED ANIMALS (AVG ± SEM)	7.30±1.06	6.65±0.95	9.31±1.25	9.04±0.44
BLA FOS-IR IN NO-DEFEAT ANIMALS (AVG ± SEM)	3.51±0.26	3.94±0.40	5.08±0.38	6.03±0.88

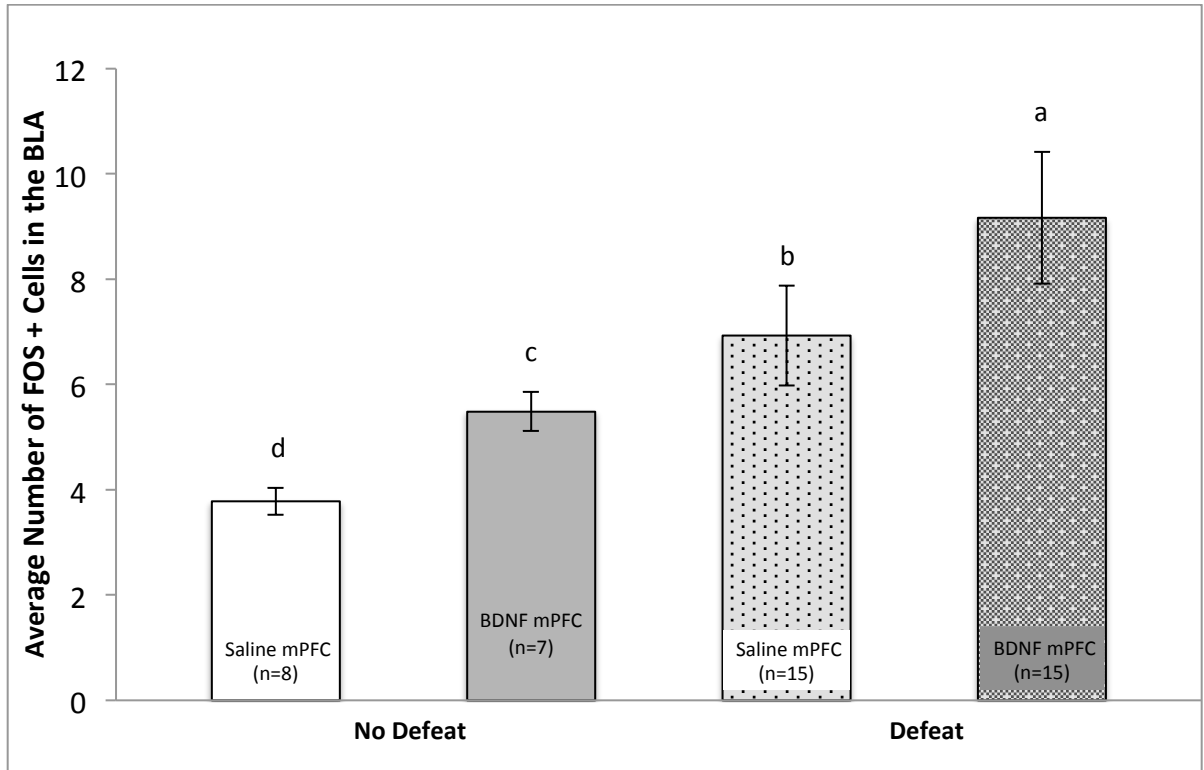




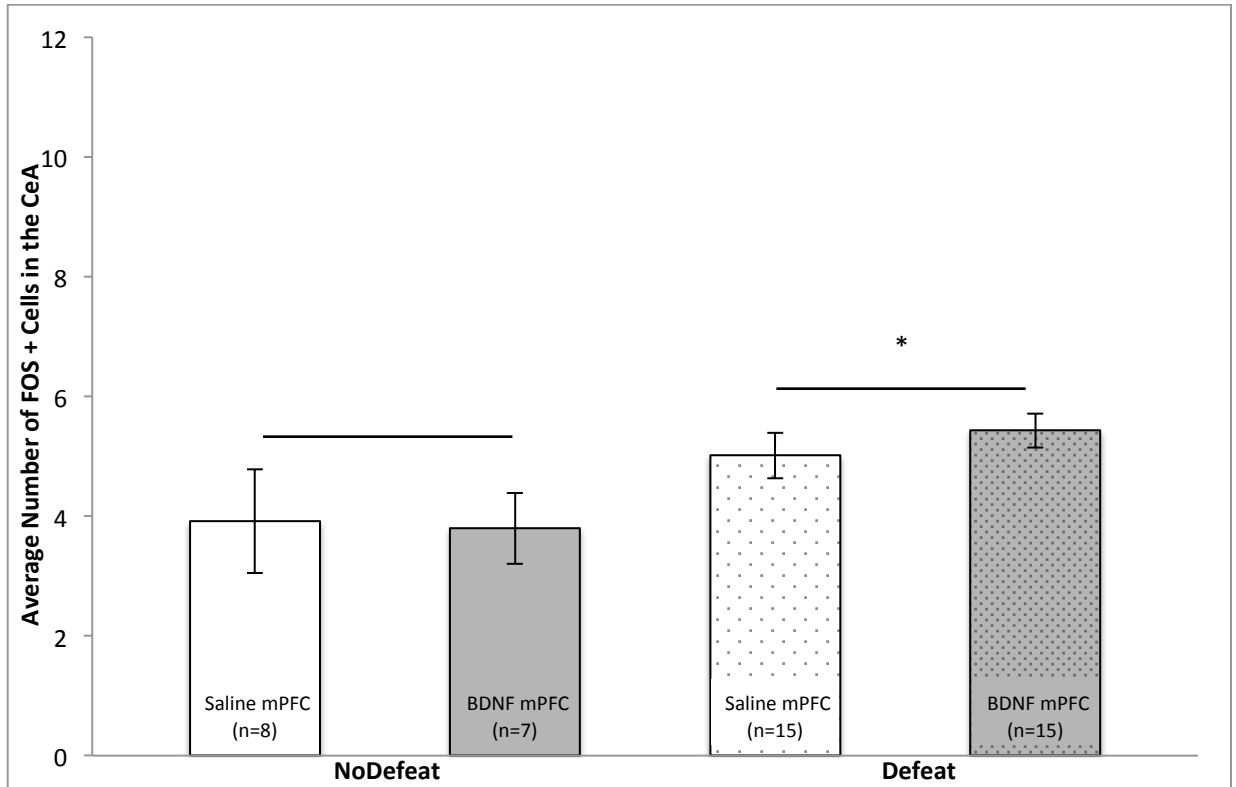
**Figure 4.2.** Histological re-creation of injection sites of animals receiving unilateral infusions of rhBDNF or saline in the mPFC (approximately 3.2mm-2.4mm anterior to bregma). (Dots) Each symbol represents the site of injection in one or more animal (pink: no-defeat saline; purple: defeat saline; green: no defeat BDNF; blue: defeat BDNF). Red triangles represent misplaced injection sites. Drawings are adapted from Morin and Wood (2001).



**Figure 4.3.** A representative photomicrograph of FOS-IR in the BLA. Photomicrograph taken at 10X magnification approximately 1.2 mm posterior to bregma. The BLA is outlined in the photomicrograph) and the drawing adapted from Morin and Wood (2001) indicates where FOS-IR was counted in the BLA. Black dots indicate FOS immunopositive nuclei.



**Figure 4.4** FOS-IR in the BLA following microinjections of BDNF into the mPFC after a defeat experience. Animals experiencing social defeat had significantly more FOS-IR in the BLA as compared to no-defeat animals. BDNF administered via microinjection into the mPFC (PL or IL) further increases defeat-induced FOS-IR in the BLA. Individual letters represent a significant difference ( $p < 0.05$ ) between groups.



**Figure 4.5.** Main effect of defeat in the CeA. Defeated animals had significantly more FOS-IR in the CeA as compared to no-defeat animals. BDNF administered into the mPFC, however, does not alter defeat-induced FOS-IR in the CeA. There are also no interactions between defeat x BDNF administration. \* Indicates a significant main effect ( $p < 0.05$ ) of defeat on FOS, which was significantly higher in defeated animals..

## 5 CONCLUSIONS

### 5.1 Summary of Basic Findings, Caveats and Some Future Directions

Humans, like most social species, continually interact with conspecifics, and social stress-related symptoms are often a common outcome of these interactions. Given that we as a society are faced with pervasive social stress and that this exposure can exacerbate psychiatric disorders, it is not surprising that stress is a pertinent topic within our society and within the field of neuroscience. One of the most common ways for our field to study the neurobiology of this unavoidable and important relationship is by using animal models. Animal models provide an ethically acceptable and ethologically relevant way to explore the neurobiological concomitants of social stress. Conditioned defeat (CD) is one model that allows us to examine the natural effects of social stress in the form of social defeat in Syrian hamsters, and this model, unlike other resident-intruder models, does not require special arrangements (e.g., complex housing conditions) to stimulate aggression but instead takes advantage of the natural territorial and aggressive tendencies of Syrian hamsters. Brief agonistic encounters in hamsters are also not associated with tissue damage from bites, unlike that found in widely used mouse models of defeat, so the response to defeat in this species most likely is not confounded by an inflammatory response. Additional advantages of this model include 1) our established knowledge of the putative circuit underlying CD and 2) our understanding of some of the cellular and molecular mediators that may also be involved in this social stress-induced behavioral change.

One neurotropic factor that has garnered attention as an important modulator of stress responding, learning, and neuronal plasticity is brain derived neurotrophic factor (BDNF). Unfortunately, it is still unclear how BDNF is involved in stress responses because some studies suggest that it is important for fear learning and that it amplifies stress responding, whereas other studies indicate that BDNF reduces the effects of stress, promotes resilience, and underlies

the effectiveness of antidepressants. The overarching goal of the current project was to more clearly define the role of BDNF in CD and to begin to define where in the neural circuit mediating CD BDNF has its effects. Given previous work from our lab and others using k252a, a nonspecific Trk receptor antagonist, we originally hypothesized that BDNF, via activation of tyrosine kinase B (TrkB) receptors, is necessary for fear learning (Rattiner et al., 2004; Taylor et al., 2011; reviewed in Mahan & Ressler, 2012). We demonstrated that activating TrkB receptors via microinjections of rhBDNF into the basolateral amygdala (BLA), however, supports the alternative hypothesis that BDNF protects against the effects of social defeat stress as measured by a reduction in subsequent submissive behaviors in previously defeated hamsters. Thus, BDNF in the BLA appears to be sufficient to produce a reduction in CD, a response that can be interpreted as increased resilience to the social defeat stressor.

As this finding was counter to what we expected, we hypothesized that it was possible that BDNF could promote CD by acting in other nuclei within the CD circuit and that potentially opposing actions of BDNF could result in a different net effect if the pharmacological agents were given systemically. Thus, we initially set out to characterize the effect of global manipulation of TrkB receptors with systemic injections. This approach seemed a good first step because of our concerns about the vehicle in which these drugs were dissolved, and it had the added benefit of having potential translational implications because pharmacological manipulations in humans would almost certainly be systemic. We demonstrated that the specific TrkB receptor agonist, 7,8- dihydroxyflavone (7,8-DHF), administered peripherally *reduces* submissive behavior when administered immediately prior to or immediately after social defeat. This is consistent with the hypothesis that BDNF reduces CD via binding at Trk B receptors. In addition, systemic administration of a TrkB receptor antagonist, ANA-12 ([N2-2-2-Oxoazepan-3-yl amino] carbonyl phenyl benzo (b) thiophene-2-carboxamide), immediately after social defeat *increases* submissive behavior. Together, the current findings strongly suggest that BDNF does not promote CD learning but, instead, that it reduces behavioral

responses to social stress in Syrian hamsters. Finally, this study suggests that these effects are mediated at least in part in the BLA.

Earlier studies indirectly suggested that BDNF was necessary for CD when a nonspecific Trk antagonist, k252a was used, but they also measured BDNF mRNA, to assess the potential response of BDNF systems to social defeat. The findings suggested that BDNF was stimulated by defeat in the BLA because the mRNA was highest in defeated hamsters. In order to better clarify how social stress alters BDNF, we examined BDNF protein because changes in mRNA are not always associated with changes in the corresponding protein, and it is the protein that underlies the biological processes. It is possible, given our current findings, that the increase in BDNF was not associated with a concomitant increase in the protein product or that it increased pro-BDNF, which is known to have an opposite effect to BDNF (Lu, Pang, & Woo, 2005). We did examine BDNF protein in serum and brain following social defeat, but did not find any significant changes in the protein (see Appendix A). Although these data are not definitive, they do suggest that the changes in BDNF mRNA following defeat are not necessarily reflected in concomitant changes in BDNF, at least when examined in serum or in dissected brain regions of interest.

It also appears that k252a reduced the acquisition of CD in our earlier study via an alternative mechanism besides BDNF/ TrkB signaling. It is important to note that k252a, which has often been promoted in the literature as a BDNF receptor antagonist, actually blocks numerous kinases in the Trk receptor family (Arthur et al., 2005, Berg et al., 1992, Koizumi et al., 1988, Sofroniew et al., 2001) including TrkA receptors, the receptors for nerve growth factor (NGF). Thus, one possible alternative is that a blockade of NGF signaling caused the reduction in CD following k252a administration (Taylor et al., 2011) and that it is, instead, NGF that promotes CD learning. We have completed a study to examine this possibility, however, and we found that administration of gambogic amide, a specific TrkA agonist, immediately after social defeat does not alter submissive behavior (see Appendix B). Although TrkA receptors do not

appear to play a role in CD, it is likely that the behavioral effects of k252a are mediated via another kinase(s) in signaling pathways of BDNF (e.g., mitogen activated protein kinase) or via another protein kinase altogether. Future studies should address this question.

Next, it was of primary interest to determine other sites in our putative CD circuit that a systemic TrkB agonist might be acting to induce the observed behavioral changes (i.e., the increase in submission and the decrease in territorial aggression). The activation of the immediate early gene *c-fos* was used as a marker to begin to determine in which of these nuclei the systemically administered drugs might act. Because BDNF microinjected into the BLA reduces submission in defeated hamsters, we anticipated that neural activity would change at least in the BLA and perhaps in other nuclei, as well, following the peripheral administration of a TrkB receptor agonist. Unfortunately, we could not address our original hypothesis because the vehicle in which 7,8-DHF must be dissolved contained 40% dimethyl-sulfoxide (DMSO), which appeared to stimulate FOS activation in a subset of sites within the CD circuit and therefore confounded our FOS-immunoreactivity (FOS-IR) data. In addition, it appeared that 7,8-DHF reduces this DMSO-induced FOS-IR in the BLA and the central amygdala (CeA), regardless of the behavioral manipulation (i.e., defeat or no defeat). Fortunately, we had included a saline-treated group as a positive control and were able to replicate that defeated hamsters displayed significant increases in FOS-IR in the BLA as compared to no-defeat animals suggesting that the anomalous findings were due to confounds introduced by the drug or vehicle and not by overall problems with our immunohistochemical procedures.

Finally, the third aim examined whether BDNF administered in the mPFC (i.e., PL or IL) following a behavioral manipulation (i.e., defeat or no-defeat) would alter FOS-IR in the BLA. Overall, the results demonstrated that this BDNF may act to modulate responses to social stress via this pathway. BDNF in the mPFC was effective in altering defeat-induced FOS-IR in the BLA, although the stimulation of FOS in the BLA may not be the first prediction made based on the finding that BDNF in the BLA suppresses CD. Drug administration in the mPFC did not alter



FOS-IR in the CeA, however, which suggests that the BLA activation is not associated with a stimulatory output to the CeA. The most parsimonious explanation for these findings are that the FOS activation found in the BLA is in inhibitory interneurons rather than excitatory projection neurons. Again, we were able to demonstrate that defeat significantly increases FOS-IR in the BLA and the CeA as compared to no-defeat animals, which replicates previous findings of our laboratory and suggests that our immunohistochemical procedures were again sound. A disappointing finding in this study was that we were unable to demonstrate that the IL and PL within the mPFC have opposing effects on the BLA as has been demonstrated previously in the literature using traditional fear conditioning procedures. It may be that there is an important species difference in the structure and role of this brain area in hamsters or that these brain areas do not have opposing effects on brain and behavior following social defeat stress. It is also reasonable to speculate that our methodology was unable to tease apart the separate roles of these contiguous nuclei because of their very close proximity. Future studies should reexamine this question to elucidate the roles of the IL and PL in brain and behavioral responses to social defeat in hamsters.

## **5.2 Contributions of the Findings to the Field and Clinical Implications**

Despite our results that specific TrkB agonists reduce submission being opposite to what we had initially predicted, they are consistent with an alternative hypothesis that BDNF promotes resiliency to stress (reviewed by Russo et al., 2012; Karatsoreos & McEwen, 2011). Interestingly, BDNF is suppressed following exposure to stress in humans, and reduced BDNF protein in serum is observed in patients with numerous psychiatric illnesses, as discussed previously in this document (Karege et al., 2002; reviewed by Duman and Monteggia, 2006; Castren & Rantamaki, 2010; Dell'osso et al., 2009; review by Rakofsky et al., 2012; Suliman, Hemmings, & Seedat, 2013; Dwivedi, 2010). Furthermore, it is thought that diminished BDNF signaling following stress might be an underlying factor in the development of psychiatric

disorders. Restoring BDNF signaling also plays a critical role in the treatment of these disorders, as BDNF levels are normalized after antidepressant treatment, and blocking BDNF abolishes the effectiveness of antidepressant treatment in rodent models (Karege et al., 2002; reviewed by Duman and Monteggia, 2006; Castren & Rantamaki, 2010; Suliman, Hemmings, & Seedat, 2013; Saarelinen et al., 2003). Furthermore, BDNF in the mPFC and the hippocampus has antidepressant actions (reviewed in Duman, 2014), suggesting that when we administer a TrkB receptor agonist peripherally it may be acting to promote the activation of these brain regions, which, in turn, inhibits the effects of stress (i.e., defeat). Given the potential translational applications of BDNF as an antidepressant or as a molecule that reduces the effects of stress (e.g., promotes resiliency), it is of particular interest to note that our peripheral injection of a TrkB receptor agonist produced a pro-resilient response (e.g., a reduction in the behavioral response to social stress). This finding supports the possibility that BDNF-active drugs could be an effective treatment for those suffering from several stress-related psychopathologies, including PTSD and depression (Altar 1999, Duman 2004, Duman & Monteggia 2006).

The findings discussed above illustrate that peripheral administration of DMSO as a vehicle in rodents is not ideal. Visual observations of hamsters following IP administration of DMSO reveals changes in behavioral responses including unusual behavioral posturing such as stretching and hopping (personal observations). Weiczner et al. (2008) also report changes in animal behavior following IP administration of DMSO in rats. DMSO, itself, is also reported to have numerous effects on neurons (Stewart, Herrerra, & Nordheim, 1990; Lu & Mattson, 2001; Tsvyetynska et al., 2005). The current work reinforces the wide range of effects of DMSO that are often ignored but that should be considered in the future as potential confounds.

Overall, the current project only begins to address our overarching question about the role of BDNF in CD. Additional work is clearly needed to address the remaining questions that we have introduced throughout this document. At this time, however, our data do appear to indicate that BDNF acting via TrkB receptors reduces the effects of stress typically observed in

Syrian hamsters after social defeat stress. This change in behavioral responses to stress appears to be mediated at least in part via the BLA and its connections with the mPFC.

### **5.3 Remaining Questions and Additional Future Directions**

The findings in the current project have begun to clarify the complex role of BDNF in CD, but additional research is needed to better characterize the role of this molecule in conditioned defeat. First and foremost, studies should examine how BDNF is modulating numerous other neurotransmitter systems including GABAergic and glutamatergic neurons at both a behavioral and a cellular level. GABAergic interneurons and glutamatergic pyramidal neurons are 2 types of neurons found in the BLA (reviewed in Pape and Pare, 2010), both of which express FOS-IR when activated (Hale et al., 2010; Lukkes et al., 2012). In order to determine the cell types activated in the BLA following the administration of BDNF in the mPFC, double-labeled immunohistochemistry studies are needed. Our current study only examined FOS-IR; we are therefore unable to comment on the phenotype of the neurons that are activated in the BLA following the administration of BDNF in the mPFC. Given that a recent review examined how reducing BDNF signaling alters neuronal transmission at both glutamatergic and GABAergic synapses (Ninan, 2014) suggests that BDNF has quite complex effects on synaptic plasticity and neuronal transmission within the putative CD circuit that may mediate behavioral responses in highly nuanced ways depending on the animal's particular experience.

Without additional double label immunohistochemistry studies, we must speculate on what our current findings suggest regarding the role of BDNF in the mPFC in our CD model. The most parsimonious explanations for our current findings, however, is that BDNF administered site specifically into the mPFC enhances neural activity in GABAergic interneurons in the BLA. This is consistent with the finding that FOS-IR can be measured in GABAergic interneurons in the BLA (Hale et al., 2010; Lukkes et al., 2012). If this is the case in the current study, enhanced

activity in GABAergic interneurons would increase the inhibitory tone in the BLA that would in turn lead to a decrease in submissive and defensive behavior in Syrian hamsters after social defeat. FOS-IR in the CeA did not change following the current manipulation, which is consistent with the hypothesis that the BLA is not sending excitatory impulses to the CeA. It is also possible that BDNF in the mPFC suppresses cellular activation in the CeA either via a direct projection to the CeA (Vertes, 2004) or via an indirect projection to the intercalated cell groups between the BLA and the CeA (Berretta et al., 2005; Royer, Martina, & Pare, 1999), either of which would result in less activation of the CeA.

An alternative explanation for the above immunohistochemical data is that the FOS-IR is primarily reflecting activation within glutamatergic cells, the more typical role that FOS is supposed to play. Numerous studies examining FOS-IR in the BLA following stress report that glutamatergic projection neurons are activated, which is then reflected in increased excitatory output of the BLA (Vyas et al., 2002; reviewed in Roozendaal et al., 2009). Increased FOS-IR in glutamatergic neurons in the BLA following administration of BDNF into the mPFC would presumably cause an increase in submissive behavior in Syrian hamsters, however. Clearly, additional studies are needed to tease apart these possibilities. One of the first steps should be to determine if submission is decreased when BDNF is administered in the mPFC and to determine which cells in the BLA are activated by the microinjections of BDNF in the mPFC following social defeat.

Additional studies could consider the role that proBDNF, the pre-cleaved form of BDNF, may play in CD when binding to p75 receptors, as this could explain the increased BDNF mRNA observed in defeated hamsters (Taylor et al., 2010). The novel TrkB compounds used in the current studies specifically target TrkB receptors and therefore purportedly do not interact with p75 receptors (Jang et al., 2010; Carloza et al., 2011). However, as pro-BDNF, binds to p75 receptors and often has the opposite effect of BDNF/TrkB signaling, such as inducing LTD and apoptosis (Lu, Pang, & Woo, 2005; Martinowich et al., 2012), it could be interesting to examine

how p75 receptors specifically in the amygdala or hippocampus may alter behavioral responses to social defeat in Syrian hamsters. To date limited work has been completed to examine this possibility.

Additionally, future studies should begin to investigate the role of BDNF in other nuclei within our putative circuit of CD. Examining BDNF within individual nuclei may help to elucidate where in the brain BDNF acts to promote or inhibit behavioral responses to social stress. Studies should be completed to examine the actions of BDNF in the mPFC on behavior as suggested above, given that the mPFC is known to have connections with downstream nuclei including the amygdala (Berglind et al., 2007; Choi et al., 2010; Gourley et al., 2009; Choi et al., 2012) and that our current findings using FOS-IR also demonstrate connectivity that could be important for behavioral changes. Furthermore, the work of other labs suggests that BDNF in the mPFC may be one molecular mechanism that alters emotional responses and fear learning. Another nucleus to focus on is the nucleus accumbens (NAc) and the hippocampus, as a recent review suggests that BDNF has pro-depressive actions in the NAc, but antidepressant actions in the hippocampus (see Duman, 2014 for review). Specifically, BDNF protein is increased in the NAc after 10 days of social defeat (Berton et al., 2006), and altered levels of BDNF in the NAc, may, therefore, explain how some animals are susceptible to the negative effects of social stress whereas other animals are resilient (Krishnan et al., 2007). The NAC is thus an intriguing nucleus that should be examined given our own findings about the role of BDNF in CD. Alternatively, stress and the administration of corticosterone into the hippocampus decreases BDNF mRNA and protein (Kozlovsky et al., 2007; Jacobsen et al., 2006). Nevertheless, repeated social stress reduces BDNF mRNA in the hippocampus (Nibuya et al., 1999), and given the role of the hippocampus in learning and memory, it is also an interesting nucleus in which to examine the effects of BDNF on CD. It is critical to remember that all of the nuclei examined in this project and the additional nuclei we propose for future examination are part of an interactive neural network. Recent work in rats, for example, found that BDNF protein levels

increase in the hippocampus, simultaneously decrease in the ventral tegmental area (VTA), and interestingly, not all of these changes are critical for antidepressant-like effects of electroconvulsive therapy (ECT) (Taliaz et al., 2013). Thus, the potential changes within the nuclei we examine are only a “snapshot” of activity within a single nucleus that is part of our dynamic circuit, and furthermore may not even be critical for observed behavioral changes. Recent work also suggests that the environment plays a large part in the regulation of BDNF, therefore suggesting that the environment even beyond the stressor should also be considered in future studies examining BDNF in the proposed nuclei of interest (reviewed in Karpova, 2014).

It is critical that all future experiments consider limitations we have discovered surrounding BDNF. First, investigators should be aware of the vehicle in which novel compounds for specific receptors are dissolved in for administration. BDNF, itself, cannot cross the blood brain barrier (BBB) (Pardridge, 2007), so it is extremely advantageous that novel compounds have been developed that can be administered peripherally and that specifically target one type of Trk receptor. The only constraint is that most of these compounds must be dissolved in solvents such as DMSO. Our initial concerns about this solvent are highlighted in FOS-IR studies wherein we found that DMSO increases FOS-IR independently of any other manipulation. It is therefore important as scientists strive to develop novel compounds to treat neurological disorders, that drug developers continue to be cognizant of effects that some drug vehicles, alone, may have on behavior and on cells.

A second limitation that should be considered is the timing of manipulations. We may not have seen altered FOS-IR, for example, given the timing of our manipulations, as animals were initially defeated and then administered a TrkB receptor agonist immediately after defeat before being sacrificed 1 h later. As a result there may not have been enough time for BDNF to completely reduce or increase neural activity (i.e., FOS-IR) that was already turned on by defeat. Timing should also be considered if additional studies are conducted to examine protein expression, given that differences may not be observed between defeat and no-defeat conditions

in the current work because we missed a critical time point and translation was not complete when we harvested the tissue.

Changes in endogenous BDNF are observed following a stressor in numerous animal models. In order to better clarify how social stress alters BDNF, we examined BDNF protein in hamsters following an acute social defeat. Measuring protein is preferable to measuring mRNA expression as changes in mRNA are not always associated with changes in the corresponding protein, and it is the protein that underlies the biological processes. Furthermore, the post-translational modification of BDNF is not well understood (Nawa et al., 1995). Interestingly, we found that there are no differences in BDNF protein expression in the serum, the BLA, the PL, or the IL following defeat in hamsters (Appendix B). These findings suggest that defeat does not alter BDNF protein levels in Syrian hamsters as compared to no-defeat animals, at least when examined 2.5 h after defeat, which is during the consolidation period. Given that we have shown that BDNF administered into the BLA immediately after defeat reduces the consolidation of CD, and others in our lab found that BDNF mRNA is increased in the BLA 2 h after defeat, we were surprised that there were no differences in protein expression. Differences in BDNF protein have been reported at this time point previously (Duclot & Kabbaj 2013). Thus, additional time points may be helpful to determine if BDNF is altered by defeat in Syrian hamsters. These 2 limitations of current studies should be considered in the design of future studies examining the role of neurotrophins in CD.

Brain derived neurotrophic factor is an extremely important protein in our nervous system as it regulates synaptic growth, plasticity, and neuron development (Adachi et al., 2014; Chao et al., 2006). In addition, research in the last several years has indicated that this molecule is involved in fear learning and stress, which, in turn, has clinical implications in disorders such as depression and PTSD. As BDNF has such wide-ranging effects, it is imperative that we continue to determine ways in which to isolate this protein's role in behavioral responses and in neuronal activity following social defeat in Syrian hamsters. Our data suggest that BDNF is a

molecule that prevents social stress-induced changes in Syrian hamsters at least in part via the BLA and perhaps the MPFC. Furthermore, this work suggests that BDNF/TrkB active compounds may be a potential treatment for stress-related neuropsychiatric disorders in humans.



## 5.4 References

- Adachi N, Numakawa T, Richards M, Nakajima S, Kunugi H. 2014. New insight in expression, transport, and secretion of brain-derived neurotrophic factor: Implications in brain-related diseases. *World journal of biological chemistry* 5: 409-28
- Altar CA. 1999. Neurotrophins and depression. *Trends in pharmacological sciences* 20: 59-61
- Amat J, Christianson JP, Aleksejev RM, Kim J, Richeson KR, et al. 2014. Control over a stressor involves the posterior dorsal striatum and the act/outcome circuit. *The European journal of neuroscience* 40: 2352-8
- Arthur DB, Akassoglou K, Insel PA. 2005. P2Y2 receptor activates nerve growth factor/TrkA signaling to enhance neuronal differentiation. *Proceedings of the National Academy of Sciences of the United States of America* 102: 19138-43
- Bader LR, Carboni JD, Burleson CA, Cooper MA. 2014. 5-HT1A receptor activation reduces fear-related behavior following social defeat in Syrian hamsters. *Pharmacology, biochemistry, and behavior* 122: 182-90
- Berg MM, Sternberg DW, Parada LF, Chao MV. 1992. K-252a inhibits nerve growth factor-induced trk proto-oncogene tyrosine phosphorylation and kinase activity. *The Journal of biological chemistry* 267: 13-6
- Berglind WJ, See RE, Fuchs RA, Ghee SM, Whitfield TW, Jr., et al. 2007. A BDNF infusion into the medial prefrontal cortex suppresses cocaine seeking in rats. *The European journal of neuroscience* 26: 757-66
- Berretta S, Pantazopoulos H, Caldera M, Pantazopoulos P, Pare D. 2005. Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience* 132: 943-53
- Bersani G, Iannitelli A, Fiore M, Angelucci F, Aloe L. 2000. Data and hypotheses on the role of nerve growth factor and other neurotrophins in psychiatric disorders. *Medical hypotheses* 55: 199-207
- Bourne AR, Mohan G, Stone MF, Pham MQ, Schultz CR, et al. 2013. Olfactory cues increase avoidance behavior and induce Fos expression in the amygdala, hippocampus and prefrontal cortex of socially defeated mice. *Behavioural brain research* 256: 188-96
- Castren E, Rantamaki T. 2010. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Developmental neurobiology* 70: 289-97
- Chao MV, Rajagopal R, Lee FS. 2006. Neurotrophin signalling in health and disease. *Clinical science* 110: 167-73
- Choi DC, Gourley SL, Ressler KJ. 2012. Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning. *Translational psychiatry* 2: e205
- Choi DC, Maguschak KA, Ye K, Jang SW, Myers KM, Ressler KJ. 2010. Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2675-80
- Dell'Osso L, Carmassi C, Del Debbio A, Catena Dell'Osso M, Bianchi C, et al. 2009. Brain-derived neurotrophic factor plasma levels in patients suffering from post-traumatic

- stress disorder. *Progress in neuro-psychopharmacology & biological psychiatry* 33: 899-902
- Duclot F, Kabbaj M. 2013. Individual differences in novelty seeking predict subsequent vulnerability to social defeat through a differential epigenetic regulation of brain-derived neurotrophic factor expression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33: 11048-60
- Duman RS. 2004. Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular medicine* 5: 11-25
- Duman RS. 2009. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. *Dialogues in clinical neuroscience* 11: 239-55
- Duman RS. 2014. Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. *Dialogues in clinical neuroscience* 16: 11-27
- Duman RS, Monteggia LM. 2006. A neurotrophic model for stress-related mood disorders. *Biological psychiatry* 59: 1116-27
- Dwivedi Y. 2010. Brain-derived neurotrophic factor and suicide pathogenesis. *Annals of medicine* 42: 87-96
- Elmariah SB, Crumling MA, Parsons TD, Balice-Gordon RJ. 2004. Postsynaptic TrkB-mediated signaling modulates excitatory and inhibitory neurotransmitter receptor clustering at hippocampal synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24: 2380-93
- Gourley SL, Kedves AT, Olausson P, Taylor JR. 2009. A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 34: 707-16
- Hale MW, Johnson PL, Westerman AM, Abrams JK, Shekhar A, Lowry CA. 2010. Multiple anxiogenic drugs recruit a parvalbumin-containing subpopulation of GABAergic interneurons in the basolateral amygdala. *Progress in neuro-psychopharmacology & biological psychiatry* 34: 1285-93
- Hanslick JL, Lau K, Noguchi KK, Olney JW, Zorumski CF, et al. 2009. Dimethyl sulfoxide (DMSO) produces widespread apoptosis in the developing central nervous system. *Neurobiology of disease* 34: 1-10
- Jacobsen JP, Mork A. 2006. Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. *Brain research* 1110: 221-5
- Jasnow AM, Huhman KL. 2001. Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain research* 920: 142-50
- Jasnow AM, Shi C, Israel JE, Davis M, Huhman KL. 2005. Memory of social defeat is facilitated by cAMP response element-binding protein overexpression in the amygdala. *Behavioral neuroscience* 119: 1125-30
- Karatsoreos IN, McEwen BS. 2011. Psychobiological allostasis: resistance, resilience and vulnerability. *Trends in cognitive sciences* 15: 576-84
- Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. 2002. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry research* 109: 143-8

- Karpova NN. 2014. Role of BDNF epigenetics in activity-dependent neuronal plasticity. *Neuropharmacology* 76 Pt C: 709-18
- Koizumi S, Contreras ML, Matsuda Y, Hama T, Lazarovici P, Guroff G. 1988. K-252a: a specific inhibitor of the action of nerve growth factor on PC 12 cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 8: 715-21
- Kozlovsky N, Matar MA, Kaplan Z, Kotler M, Zohar J, Cohen H. 2007. Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* 10: 741-58
- Lessmann V, Gottmann K, Malcangio M. 2003. Neurotrophin secretion: current facts and future prospects. *Progress in neurobiology* 69: 341-74
- Lu C, Mattson MP. 2001. Dimethyl sulfoxide suppresses NMDA- and AMPA-induced ion currents and calcium influx and protects against excitotoxic death in hippocampal neurons. *Exp Neurol* 170: 180-5
- Lu B, Pang PT, Woo NH. 2005. The yin and yang of neurotrophin action. *Nature reviews. Neuroscience* 6: 603-14
- Lukkes JL, Burke AR, Zelin NS, Hale MW, Lowry CA. 2012. Post-weaning social isolation attenuates c-Fos expression in GABAergic interneurons in the basolateral amygdala of adult female rats. *Physiology & behavior* 107: 719-25
- Maestripieri D, De Simone R, Aloe L, Alleva E. 1990. Social status and nerve growth factor serum levels after agonistic encounters in mice. *Physiology & behavior* 47: 161-4
- Mahan AL, Ressler KJ. 2012. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends in neurosciences* 35: 24-35
- Markham CM, Luckett CA, Huhman KL. 2012. The medial prefrontal cortex is both necessary and sufficient for the acquisition of conditioned defeat. *Neuropharmacology* 62: 933-9
- Markham CM, Taylor SL, Huhman KL. 2010. Role of amygdala and hippocampus in the neural circuit subserving conditioned defeat in Syrian hamsters. *Learning & memory* 17: 109-16
- Martinowich K, Schloesser RJ, Lu Y, Jimenez DV, Paredes D, et al. 2012. Roles of p75(NTR), long-term depression, and cholinergic transmission in anxiety and acute stress coping. *Biological psychiatry* 71: 75-83
- Minichiello L. 2009. TrkB signalling pathways in LTP and learning. *Nature reviews. Neuroscience* 10: 850-60
- Nawa H, Carnahan J, Gall C. 1995. BDNF protein measured by a novel enzyme immunoassay in normal brain and after seizure: partial disagreement with mRNA levels. *The European journal of neuroscience* 7: 1527-35
- Nibuya M, Takahashi M, Russell DS, Duman RS. 1999. Repeated stress increases catalytic TrkB mRNA in rat hippocampus. *Neuroscience letters* 267: 81-4
- Nikulina EM, Arrillaga-Romany I, Miczek KA, Hammer RP, Jr. 2008. Long-lasting alteration in mesocorticolimbic structures after repeated social defeat stress in rats: time course of mu-opioid receptor mRNA and FosB/DeltaFosB immunoreactivity. *The European journal of neuroscience* 27: 2272-84
- Ninan I. 2014. Synaptic regulation of affective behaviors; role of BDNF. *Neuropharmacology* 76 Pt C: 684-95

- Pape HC, Pare D. 2010. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiological reviews* 90: 419-63
- Pardridge WM. 2007. Blood-brain barrier delivery. *Drug discovery today* 12: 54-61
- Rakofsky JJ, Ressler KJ, Dunlop BW. 2012. BDNF function as a potential mediator of bipolar disorder and post-traumatic stress disorder comorbidity. *Molecular psychiatry* 17: 22-35
- Rattiner LM, Davis M, French CT, Ressler KJ. 2004. Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24: 4796-806
- Roosendaal B, McEwen BS, Chattarji S. 2009. Stress, memory and the amygdala. *Nature reviews. Neuroscience* 10: 423-33
- Rothman SM, Mattson MP. 2013. Activity-dependent, stress-responsive BDNF signaling and the quest for optimal brain health and resilience throughout the lifespan. *Neuroscience* 239: 228-40
- Royer S, Martina M, Pare D. 1999. An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 19: 10575-83
- Russo N, Russo M, Daino D, Freschi L, Fiore L, et al. 2012. Evaluation of brain-derived neurotrophic factor in menstrual blood and its identification in human endometrium. *Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology* 28: 492-5
- Sakata K, Martinowich K, Woo NH, Schloesser RJ, Jimenez DV, et al. 2013. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. *Proceedings of the National Academy of Sciences of the United States of America* 110: 15103-8
- Sofroniew MV, Howe CL, Mobley WC. 2001. Nerve growth factor signaling, neuroprotection, and neural repair. *Annual review of neuroscience* 24: 1217-81
- Stewart AF, Herrera RE, Nordheim A. 1990. Rapid induction of c-fos transcription reveals quantitative linkage of RNA polymerase II and DNA topoisomerase I enzyme activities. *Cell* 60: 141-9
- Taliaz D, Nagaraj V, Haramati S, Chen A, Zangen A. 2013. Altered brain-derived neurotrophic factor expression in the ventral tegmental area, but not in the hippocampus, is essential for antidepressant-like effects of electroconvulsive therapy. *Biological psychiatry* 74: 305-12
- Taylor SL, Stanek LM, Ressler KJ, Huhman KL. 2011. Differential brain-derived neurotrophic factor expression in limbic brain regions following social defeat or territorial aggression. *Behavioral neuroscience* 125: 911-20
- Tsvyetylnska NA, Hill RH, Grillner S. 2005. Role of AMPA receptor desensitization and the side effects of a DMSO vehicle on reticulospinal EPSPs and locomotor activity. *Journal of neurophysiology* 94: 3951-60
- Vertes RP. 2004. Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse* 51: 32-58
- Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. 2002. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid

neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22: 6810-8

## APPENDICES

### **Appendix A: Defeat does not change BDNF protein expression in serum, the BLA, the PL or the IL**

#### ***Appendix A.1: Introduction***

Previous work from our lab indicates that BDNF mRNA was increased in the BLA of defeated hamsters (Taylor et al., 2011). Whereas, others have reported findings in mice that BDNF mRNA is reduced 24 h after social defeat stress in the hippocampus, BLA, and parts of the cortex (Pizarro et al., 2004) however, many labs have examined BDNF protein levels in these same nuclei. Measuring protein is preferable to measuring mRNA expression as, the post-translational modification of BDNF is not well understood (Nawa et al., 1995), and changes in mRNA are not always associated with changes in the corresponding protein. Several labs have examined BDNF protein levels in several different brain nuclei involved in stress and fear learning, and interestingly, the changes in BDNF appear to be nucleus-dependent (reviewed in Sakata, 2013) and, as reported previously, are inconsistent. BDNF protein expression, for example, is increased in the mPFC by repeated social stress in mice (Nikulina et al., 2012) and in the amygdala following chronic stress (reviewed by Boyle, 2013) while others, however, report that social stress reduces BDNF protein in the amygdala, the hypothalamus, and the hippocampus (Fanous et al., 2010; Pizarro et al., 2004).

In attempts to clarify how defeat alters BDNF, and in turn reduces CD, we examined BDNF protein in hamsters, specifically in the in serum, the BLA, the PL, and the IL of defeated and no defeated hamsters.

#### ***Appendix A.2: Materials & Methods***

##### **Animals and Housing Conditions**

Subjects were male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories, New York, NY) that weighed 120-140 g and were between 9-10 weeks old at the time of testing.

All hamsters were handled for five days prior to behavioral procedures to acclimate them to experimenter handling, and singly housed in polycarbonate cages (20x 40 x 20cm) with corncob bedding and wire mesh tops in a temperature ( $20 \pm 2^\circ \text{C}$ ) and humidity-controlled room. The room was maintained on a 14:10 light dark cycle with lights off at 10:00 am. Food and water were available *ad libitum*. Older hamsters (>6 months), which were individually housed (>1 month) and weighed at least 180g served as resident aggressors (RA) in defeat training for all experiments. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### **Social Defeat**

The CD model has been described elsewhere (Huhman et al., 2003). Prior to training, hamsters were matched by weight and then randomly assigned to groups. All animals were transported in their home cage to the behavioral testing suite within the animal vivarium 30 min prior to all behavioral manipulations in order to acclimate. All behavioral procedures were performed in the first 3 h of the dark phase of the daily light: dark cycle to minimize any circadian effects. Social defeat training occurred in the home cage of the RA for 15 min. In all experiments, no-defeat controls were placed into an empty RA cage.

### **Sample Collection and Preparation**

Hamsters were rapidly decapitated 2.5 h after a 15 min social defeat. Brains were removed on dry ice, and then stored at  $-80^\circ \text{C}$  until dissection and homogenization. Frozen brains are slowly brought to  $-20^\circ \text{C}$  and sectioned in a cryostat. From these sections, 1-2mm punches were taken of the BLA and mPFC. Trunk blood was collected in centrifuge tubes, and spun at  $4^\circ \text{C}$  at 3000rpm for 30 mins. Serum was then pulled off and then stored at  $-20^\circ \text{C}$  until use.

### **Assay Validation**

All assay kits were validated using tissue and serum samples. We determined linearity of sample dilution by serially diluting tissue and serum samples to 1:2, 1:4, 1:8, and 1:16 in sample buffer. The intra-assay precision was determined as the mean coefficient of variation (CV), and test reproducibility was determined as the mean CV of analyses from 5 different assays.

### **Enzyme-linked immunosorbent assay for Serum BDNF**

Tissue and serum samples were processed using BDNF Emax Immunoassay kit (Promega; Madison, WI) as described previously (Haenisch et al., 2008; Rost et al., 2005) with modifications for use with Syrian hamsters (Ma et al., 1998). Serum samples were diluted in 1:8 buffer and Tissue samples were diluted in 1:2 buffer (Ma et al., 1998). According to the manufacturer, this kit detects a minimum of 15.6 pg/ml of BDNF and demonstrates less than 3% cross-reactivity with the other neurotrophins (e.g., NGF).

### **Total Protein Quantification**

BDNF concentration in each serum and tissue sample was calculated not only by the amount of protein per milliliter sample, but also by the amount per milligram of total sample protein. Total protein in each sample was measured using Lowry Protein Assay (Bio-Rad; Hercules, CA). Serum samples were diluted to 1:128 and tissue samples were also diluted 1:32 for protein analysis.

### **Statistical Analysis**

The total volume of BDNF (pg/ug) in each sample was calculated (i.e., accounting for dilution factors and dividing BDNF expression by total protein expression) and compared



between defeat and no-defeat conditions. All data were analyzed using independent t-tests. Criterion for significance was  $p < 0.05$ .

### ***Appendix A.3: Results***

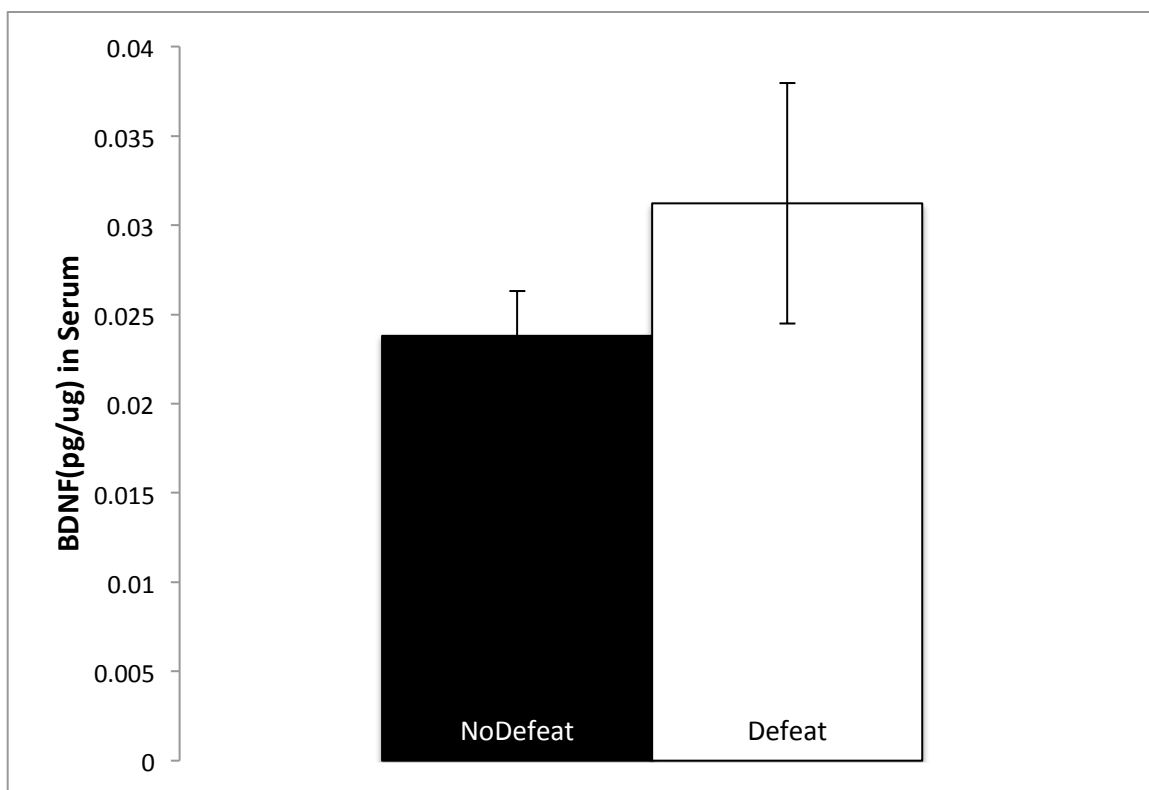
We found no differences in BDNF expression in serum ( $t(22)=1.840, p > 0.05$ ) (Figure 1), the BLA ( $t(22)=0.002, p > 0.05$ ) (Figure 2), the PL ( $t(22)=0.375, p > 0.05$ ) (Figure 3), or the IL ( $t(22)=4.949, p > 0.05$ ) (Figure 4), which suggested that defeat does not alter BDNF protein levels in Syrian hamsters 2.5 h after defeat.

### ***Appendix A.4: Discussion***

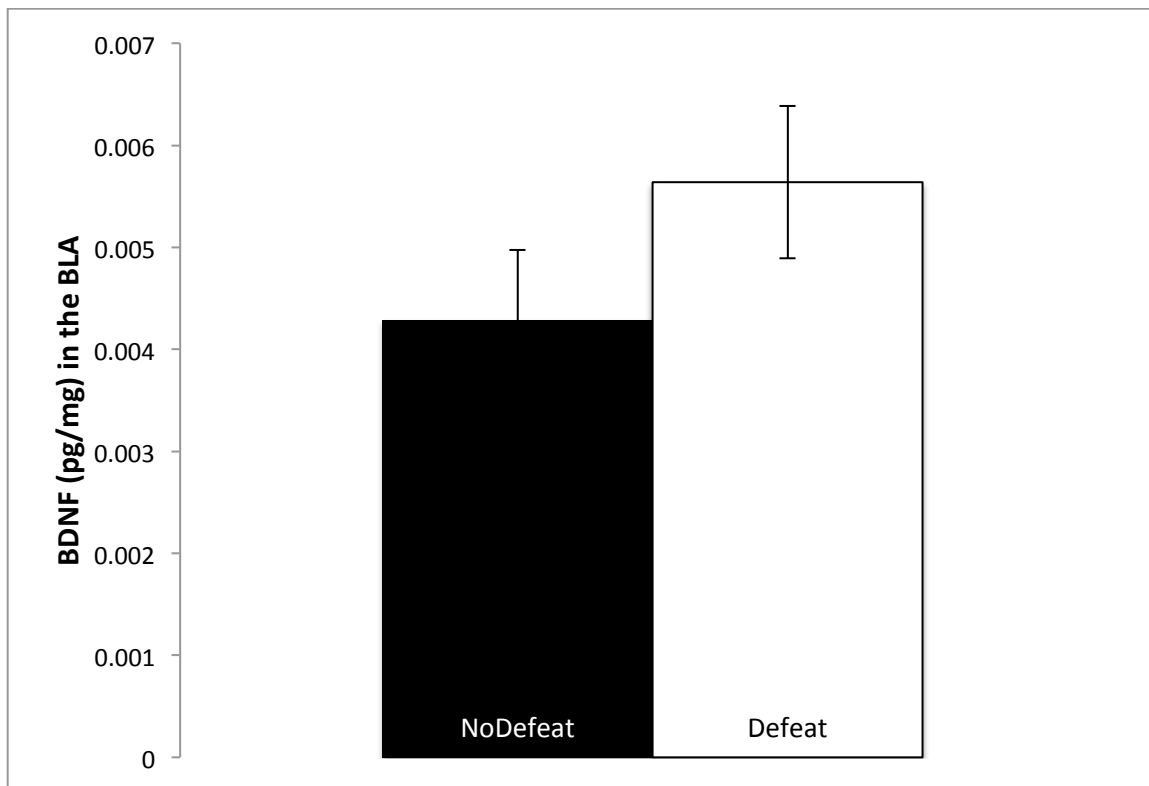
We found no differences in BDNF protein in the periphery (as measured in serum) or in 3 brain nuclei of interest: the BLA, the PL and the IL. Given we have shown that BDNF administered into the BLA reduces the consolidation of CD (Jeffress et al., in preparation) and that defeat increases BDNF mRNA in a nucleus dependent fashion (Taylor et al., 2011), we were surprised that there were no differences in BDNF protein expression. These findings may suggest that BDNF binds to TrkB receptors and in turn activates another transmitter system (e.g., GABA) or second messenger cascade (i.e. PI3K and ERK) that is ultimately responsible for the observed behavioral changes without up changing BDNF protein, 2.5 h after defeat. Future studies should examine BDNF protein expression at additional time points after defeat.

**Appendix A.5: References**

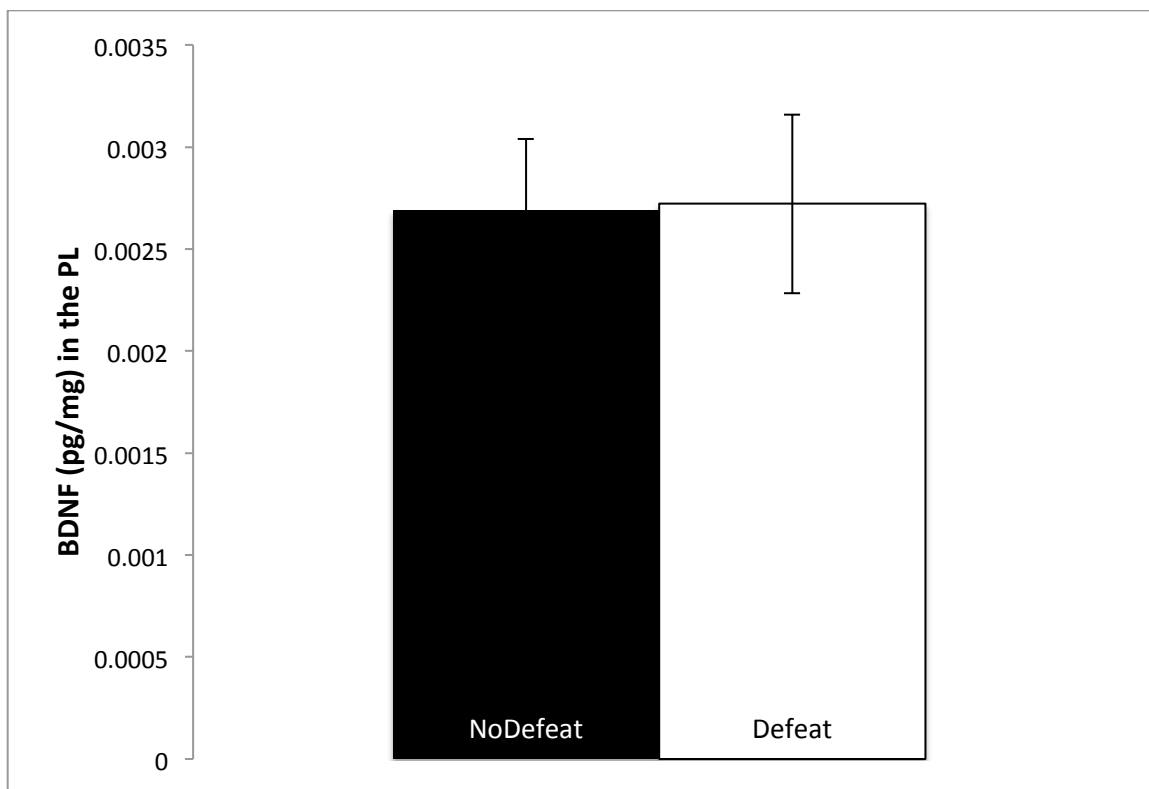
- Boyle LM. 2013. A neuroplasticity hypothesis of chronic stress in the basolateral amygdala. *The Yale journal of biology and medicine* 86: 117-25
- Fanou S, Hammer RP, Jr., Nikulina EM. 2010. Short- and long-term effects of intermittent social defeat stress on brain-derived neurotrophic factor expression in mesocorticolimbic brain regions. *Neuroscience* 167: 598-607
- Haenisch B, Gilsbach R, Bonisch H. 2008. Neurotrophin and neuropeptide expression in mouse brain is regulated by knockout of the norepinephrine transporter. *Journal of neural transmission* 115: 973-82
- Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, et al. 2003. Conditioned defeat in male and female Syrian hamsters. *Hormones and behavior* 44: 293-9
- Ma YT, Hsieh T, Forbes ME, Johnson JE, Frost DO. 1998. BDNF injected into the superior colliculus reduces developmental retinal ganglion cell death. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 18: 2097-107
- Nawa H, Carnahan J, Gall C. 1995. BDNF protein measured by a novel enzyme immunoassay in normal brain and after seizure: partial disagreement with mRNA levels. *The European journal of neuroscience* 7: 1527-35
- Nikulina EM, Lacagnina MJ, Fanous S, Wang J, Hammer RP, Jr. 2012. Intermittent social defeat stress enhances mesocorticolimbic DeltaFosB/BDNF co-expression and persistently activates corticotegmental neurons: implication for vulnerability to psychostimulants. *Neuroscience* 212: 38-48
- Pizarro JM, Lumley LA, Medina W, Robison CL, Chang WE, et al. 2004. Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain research* 1025: 10-20
- Rost B, Hanf G, Ohnemus U, Otto-Knapp R, Groneberg DA, et al. 2005. Monocytes of allergics and non-allergics produce, store and release the neurotrophins NGF, BDNF and NT-3. *Regulatory peptides* 124: 19-25
- Taylor SL, Stanek LM, Ressler KJ, Huhman KL. 2011. Differential brain-derived neurotrophic factor expression in limbic brain regions following social defeat or territorial aggression. *Behavioral neuroscience* 125: 911-20

**Appendix A.6: Figures**

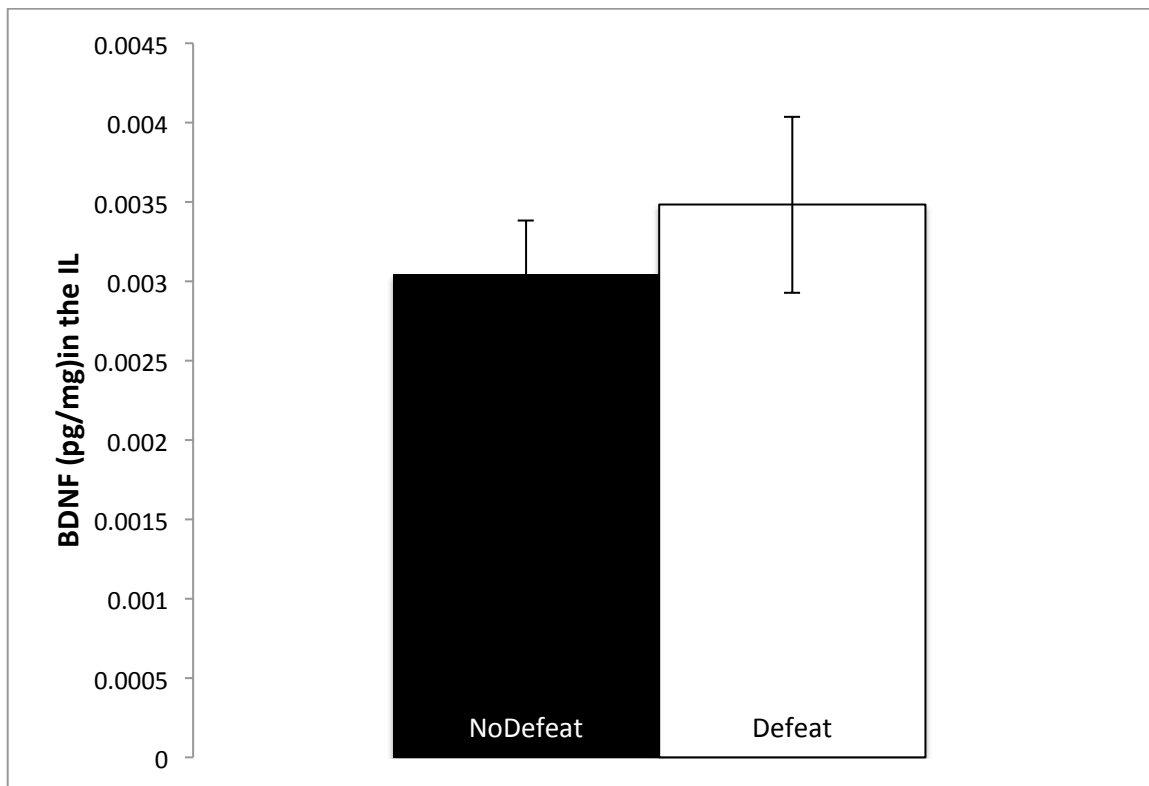
**Figure A.6.1** BDNF protein expression was examined in the serum of Syrian hamsters following a brief defeat or no defeat experience. No differences were found in BDNF expression (pg/ug) in the serum of defeated hamsters as compared to no defeat hamsters 2.5 h after a brief social defeat using an Emax Immunoassay kit.



**Figure A.6.2** BDNF protein expression was examined in the basolateral amygdala of Syrian hamsters following a brief defeat or no defeat experience. No differences were found in BDNF expression (pg/ug) in the basolateral amygdala of defeated hamsters as compared to no defeat hamsters 2.5 h after a brief social defeat using an Emax Immunoassay kit.



**Figure A.6.3** BDNF protein expression was examined in the prelimbic cortex of Syrian hamsters following a brief defeat or no defeat experience. No differences were found in BDNF expression (pg/ug) in the prelimbic cortex of defeated hamsters as compared to no defeat hamsters 2.5 h after a brief social defeat using an Emax Immunoassay kit.



**Figure A.6.4** BDNF protein expression was examined in the infralimbic cortex of Syrian hamsters following a brief defeat or no defeat experience. No differences were found in BDNF expression (pg/ug) in the prelimbic cortex of defeated hamsters as compared to no defeat hamsters 2.5 h after a brief social defeat using an Emax Immunoassay kit.

## **Appendix B: Gambogic Amide administered peripherally *does not alter* the consolidation of CD**

### ***Appendix B.1: Introduction***

Our lab has previously demonstrated that k252a, a nonspecific tyrosine kinase (Trk) receptor antagonist, reduces submissive behavior in defeated hamsters (Taylor et al., 2011). k252a, which has often been used as a BDNF receptor antagonist, actually blocks numerous protein kinases including members of the Trk receptor family (Arthur et al., 2005, Berg et al., 1992, Koizumi et al., 1988, Sofroniew et al., 2001) such as TrkA receptors, the receptors for nerve growth factor (NGF). Therefore, use of k252a is not ideal as it leaves unanswered the question of which neurotrophin mediates subsequent changes. Thus, one possible alternative is that a blockade of NGF signaling caused the reduction in CD following k252a and that it is instead NGF that promotes CD learning. To date, there are no TrkA antagonists commercially available, however, a TrkA receptor agonist, Gambogic Amide has been developed and can be administered peripherally (Jang et al., 2007). This experiment, therefore, aims to test whether TrkA receptor activation enhances consolidation of CD.

### ***Appendix B.2: Materials & Methods***

#### **Animals and Housing Conditions**

Subjects were male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories, New York, NY) that weighed 120-140 g and were between 9-10 weeks old at the time of testing. All hamsters were handled for five days prior to behavioral procedures to acclimate them to experimenter handling, and singly housed in polycarbonate cages (20x 40 x 20cm) with corncob bedding and wire mesh tops in a temperature ( $20 \pm 2^\circ$  C) and humidity controlled room. The room was maintained on a 14:10 light dark cycle with lights off at 10:00 am. Food and water were available *ad libitum*. Older hamsters (>6 months), which were individually housed (>1 month) and weighed at least 180g served as resident aggressors (RA) in defeat training for all

experiments, whereas smaller (110g-120g), younger (~ 7 weeks) hamsters were group housed and served as non-aggressive intruders (NAI) during CD testing. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### **Social Defeat and Behavioral Testing**

The CD model has been described elsewhere (Huhman et al., 2003). Prior to training, hamsters were matched by weight and then randomly assigned to groups. All animals were transported in their home cage to the behavioral testing suite within the animal vivarium 30 min prior to all behavioral manipulations in order to acclimate. All behavioral procedures were performed in the first 3 h of the dark phase of the daily light: dark cycle to minimize any circadian effects. Social defeat training occurred in the home cage of the RA for 5 min to reduce potential ceiling effect. No-defeat animals were placed into an empty RA cage for 5 min to expose animals to odors but no physical interaction. Behavioral testing occurred 24 h after training for a 5 min period with an NAI in the home cage of the experimental animal.

### **Behavioral Analysis**

All social defeat training and behavioral testing was recorded via video camera, transferred to an external hard drive, and later scored by a observer blind to experimental condition using Observer for Windows (Noldus Information Technology B.V., Wageningen, The Netherlands; version 7.0). Total durations for 4 classes of behavior were scored during testing including: (1) Social (stretch, approach, sniff, nose touching, and flank marking); (2) Non-social (locomotion, exploration, grooming, nesting, feeding and sleeping); (3) Submissive (flight, avoidance, tail up, upright and side defense, full submissive posture, stretch attend, attempted escape from cage); (4) Aggressive (upright and side offense, chase and attack, including bite).



### **Drug Administration**

Animals (n=69) were randomly assigned to groups (defeat or no defeat) and within these 2 conditions, animals received either 0.2 mg/kg of Gambogic Amide (Enzo Life Sciences; Farmingdale, NY) dissolved in 100% Dimethylsulfoxide (DMSO), used previously (Jang et al., 2007) or vehicle (100% DMSO) immediately after a 5min defeat. Post-training drug administration was used because the TrkA drugs were only soluble in DMSO solutions, and this vehicle solution caused the subjects to have an odor that was discernable by the investigators; therefore, post-training injections were used so that the subjects would have normal odors during defeat training. As described above, animals were tested drug free 24 h later in their home cage with an NAI for 5 min.

### **Statistical Analysis**

The total duration (seconds) of submission, aggression, social and non-social behaviors were determined. All data were analyzed using 2X2 ANOVAs. Criterion for significance was  $p < 0.05$ .

### ***Appendix B.3: Results***

#### **Gambogic Amide administered peripherally does not alter the consolidation of CD**

As shown in Figure 1, hamsters receiving a 0.2mg/kg IP injection of Gambogic Amide after defeat did not alter submissive and defensive behavior ( $F(1,63)=1.627$ ,  $p > 0.05$ ) when tested 24 h later. No significant differences were observed for non-social, social, or aggressive behavior.

Gambogic Amide did not have an effect on behavior independently in no-defeat controls, as there was no interaction ( $F(1,63)=0.807$ ,  $p > 0.05$ ; data not shown). Furthermore, all defeated

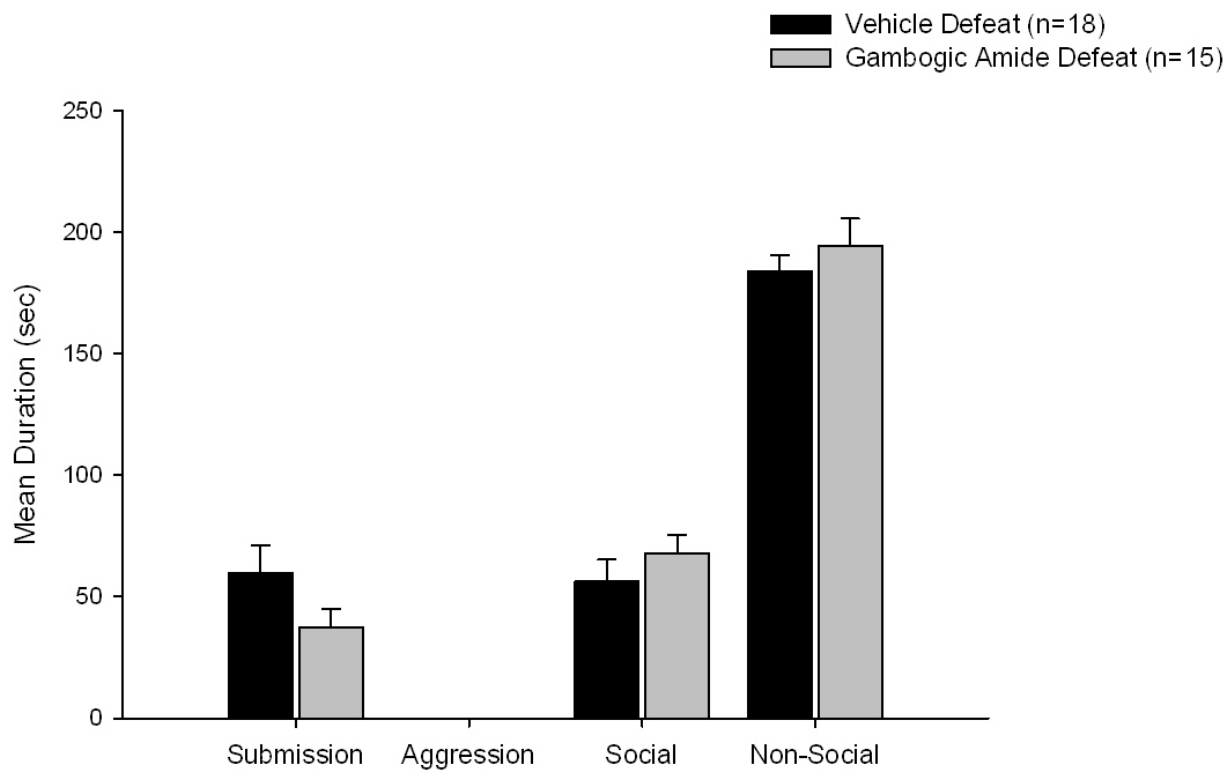
hamsters showed a significant increase in submissive behavior as compared to the appropriate no-defeat hamsters indicating that exposure to social defeat resulted in conditioned defeat as evidenced by a significant increase in the duration of submissive/defensive behavior following defeat ( $F(1,63)=38.318, p<0.05$ ; Table 1).

#### ***Appendix B.4: Discussion***

Gambogic Amide, a TrkA receptor agonist administered immediately after social defeat did not alter submissive behavior in Syrian hamsters (Figure 1). There appears to be a trend suggesting that Gambogic Amide reduces submission behavior, which is interesting considering that K252a, a non-specific Trk antagonist reduces submissive behavior in Syrian hamsters when administered immediately prior to social defeat. As K252 is reported to act on numerous protein kinases, and NGF is also understood to mediate neuron survival, growth, and development as well as in learning and memory (Chao et al., 2006; Minichiello, 2009; Autry & Monteggia, 2012; Gray et al., 2013) we expected that Gambogic Amide would enhance fear learning. The current results that Gambogic Amide does not alter submissive behaviors in Syrian hamsters, however, indicate that NGF is not involved in CD.

**Appendix B.5: References**

- Arthur DB, Akassoglou K, Insel PA. 2005. P2Y2 receptor activates nerve growth factor/TrkA signaling to enhance neuronal differentiation. *Proceedings of the National Academy of Sciences of the United States of America* 102: 19138-43
- Autry AE, Monteggia LM. 2012. Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacological reviews* 64: 238-58
- Berg MM, Sternberg DW, Parada LF, Chao MV. 1992. K-252a inhibits nerve growth factor-induced trk proto-oncogene tyrosine phosphorylation and kinase activity. *The Journal of biological chemistry* 267: 13-6
- Chao MV, Rajagopal R, Lee FS. 2006. Neurotrophin signalling in health and disease. *Clinical science* 110: 167-73
- Gray JD, Milner TA, McEwen BS. 2013. Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. *Neuroscience* 239: 214-27
- Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, et al. 2003. Conditioned defeat in male and female Syrian hamsters. *Hormones and behavior* 44: 293-9
- Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, et al. 2010. A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2687-92
- Koizumi S, Contreras ML, Matsuda Y, Hama T, Lazarovici P, Guroff G. 1988. K-252a: a specific inhibitor of the action of nerve growth factor on PC 12 cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 8: 715-21
- Minichiello L. 2009. TrkB signalling pathways in LTP and learning. *Nature reviews. Neuroscience* 10: 850-60
- Sofroniew MV, Howe CL, Mobley WC. 2001. Nerve growth factor signaling, neuroprotection, and neural repair. *Annual review of neuroscience* 24: 1217-81
- Taylor SL, Stanek LM, Ressler KJ, Huhman KL. 2011. Differential brain-derived neurotrophic factor expression in limbic brain regions following social defeat or territorial aggression. *Behavioral neuroscience* 125: 911-20

**Appendix B.6: Figures**

**Figure B.6.1** Gambogic amide does not significantly alter the consolidation of social defeat. Behaviors exhibited by previously defeated hamsters during a 5min test with an NAI are shown as mean duration ( $\pm$  standard error of the mean). Hamsters received an IP injection of Gambogic amide or vehicle immediately after a 5 min social defeat with an RA.

**Table B.6.1 Mean Duration of Behavior represented as percent of vehicle for No-Defeat animals in Consolidation studies.**

<b>Behavior</b>	<b>Vehicle</b>	<b>Gambogic Amide</b>
<b>Submission</b>	2.76 $\pm$ 1.65	0.21 $\pm$ 0.15
<b>Social</b>	146.23 $\pm$ 10.34	168.35 $\pm$ 12.87
<b>Non-Social</b>	125.38 $\pm$ 10.76	102.64 $\pm$ 14.12

## Appendix C: Curriculum Vitae

**Elizabeth Carney Jeffress Thorsteinson**  
 Georgia State University, Neuroscience Institute  
 100 Piedmont Ave. SE, Atlanta, GA, 30302  
 Laboratory: 404-413-6337  
 Fax: 404-413-5446  
 Cell: 804-426-3697  
 e-mail: ejeffress1@gsu.edu

### |Education|

B.S. Neuroscience with Honors, 2009

**Dickinson College, Carlisle, PA**

Study Abroad: University of East Anglia, Norwich, England

M.S. Neuroscience, 2012

**Georgia State University, Atlanta, GA**

Ph.D. Neuroscience with Certificate in Teaching Excellence, Expected May 2015

**Georgia State University, Atlanta, GA**

### |Professional Positions|

**2005-2007 University of Richmond, Department of Psychology**

Undergraduate Assistant with Craig Kinsley, Ph.D.

Maternal Behavior, postpartum aggression and hunting abilities in mom and virgin rats.

**2007-2009 Dickinson College, Department of Biology**

Undergraduate Assistant with Mary Melissa Niblock, Ph.D.

Central Chemoreception in the Mouse Cerebellum

Honors Thesis: C-fos expression increases significantly in the cerebellar Fastigial Nucleus Following exposure to 10% Carbon Dioxide in FTL+ mice.

**2009-2010 Georgia State University, Department of Biology**

Graduate Assistant with Anne Z. Murphy, Ph.D.

Impact of Aging on Chronic Pain in rats.

**2010-present Georgia State University, Neuroscience Institute**

Graduate Assistant with Kim L. Huhman, Ph.D.

Conditioned Defeat in the Syrian Hamster, an Ethologically Relevant Model of Social Stress

Thesis: The Role of Neurotrophic Factors in Conditioned Defeat

### |Science Education Community

**Outreach|**

2009, Dickinson College Science Night: Instructor  
 2009, Dickinson College Community Science Open House: Instructor  
 2009, Atlanta Society for Neuroscience Chapter Brain Bee: Logistics  
 Coordinator  
 2009- present, Atlanta Society for Neuroscience Brain Awareness Outreach:  
 Instructor  
 2010, BRAINS RULE! Neuroscience Exposition at Zoo Atlanta: Volunteer  
 2012, Science at Hand Day at Fernbank Natural History Museum: Presenter for  
 Georgia State University Neuroscience Institute  
 2011 - 2012, Atlanta Society for Neuroscience Chapter Brain Bee: Publicity  
 Coordinator  
 2013, Brains at the Museum! Imagine It! Children's Museum, Organizer and  
 Presenter  
 2013, Atlanta Science Festival: Informal Education Consultant  
 2013, Discovery Day! GSU signature event for the Atlanta Science Festival: Co-  
 Chair

**|Peer  
 Reviewed  
 Publications|**

M.M. Niblock, H. G. Gao, **E. C. Jeffress**, M. Murphy, E. E. Nattie. (2010). Fos-tau-lacZ mice reveal sex differences in brainstem c-fos activation in response to mild carbon dioxide exposure. *Brain Research*, 1311, 51-63.

**E.C. Jeffress** and K.L. Huhman. (2013). Copulatory and Agonistic Behavior Following Social Defeat. *Aggressive Behavior*, 39, 239-245.

**|Posters and  
 Presentations|**

M. M. Niblock, **E. C. Jeffress**, H. G. Gao, J. G. S. Clem, A. Li, M. Murphy, E. E. Nattie. Fos-tau-lacZ mice reveal potential sex differences in the neuroanatomy underlying central chemoreception. Neuroscience 2008, Washington DC, 2008.

**E.C. Jeffress** and K.L. Huhman, Interaction of sex and agonistic behavior in the conditioned defeat model? Brains & Behavior Spring Retreat 2011, Atlanta, GA, 2011

**E.C. Jeffress**, A. Norvelle, and K.L. Huhman. 7,8-Dihydroxyflavone, a TrkB receptor agonist, decreases submission levels following social defeat in Syrian hamsters. Neuroscience 2011, Washington DC, 2011.

**E.C. Jeffress** and K.L. Huhman. Social defeat increases acoustic startle responding and decreases sucrose preference in Syrian hamsters. Society for Behavioral Neuroendocrinology, Atlanta, GA, 2013

**E.C. Jeffress**, A. Norvelle, and K.L. Huhman. ANA-12, a Trk B receptor antagonist, increases submission following social defeat in Syrian hamsters. Society for Social Neuroscience, San Diego, CA, 2013

**E.C. Jeffress**, A. Norvelle, and K.L. Huhman. ANA-12, a Trk B receptor antagonist, increases submission following social defeat in Syrian hamsters. Neuroscience 2013, San Diego, CA, 2013

**|Invited Talks|**

**E.C. Jeffress**, K.E. McCann, and C. A. Lockett. Conditioned Defeat: The model, neural circuitry and future directions. Neuroscience Institute Breakfast Symposium, Atlanta GA, October 25<sup>th</sup>, 2011

**|Teaching  
& Related  
Experience|**

**Private Tutoring**

2005-2007, Middle School Science

2002-2004, Elementary School Reading & Comprehension

**Dickinson College**

2008- 2009, Teaching Assistant: Brain and Behavior (laboratory)

2008, Teaching Assistant: Research Methods in Biological Psychology

**Georgia State University, Department of Psychology and Department of Biology**

2011, Teaching Assistant: Introduction to Psychology

2011-2012, Teaching Assistant: Introduction to Biological Aspects of Psychology

2012, Teaching Assistant: Introduction to Research Design and Analysis

2012, Teaching Assistant: Introduction to Drugs and Behavior

2013, Lab Instructor & Teaching Assistant: Advanced Research Design and Analysis–Honors: 15 students, 15 weeks

2013, Guest Lecturer: Kyle Frantz’s Collaborative Internships in Biology

2013-present, Instructor: Introduction to Biological Aspects of Psychology: 100 students, 15 weeks

**Georgia State University, Summer Programs**

2012-present, Orientation Teaching Assistant: Behavioral Research Advancements In Neuroscience (BRAIN) Summer Research Program for Undergraduates

**Georgia State University, Supervisory Responsibilities**

2012, Simone Carrey, Institute on Neuroscience (ION) Scholar

2012, Kaitlin Murdoch, Wheeler High School Senior Project

2013, Emma Goldman, Institute on Neuroscience (ION) Scholar

2014, Aminah Matthews, Institute on Neuroscience (ION) Scholar



**|Invited  
Science  
Education  
Workshops &  
Panels|**

2012, GT-Neuro Brain Awareness Volunteer Workshop, Georgia Institute of Technology, March 5<sup>th</sup>: Workshop on education outreach activities for undergraduates to perform in the k-12 classroom

2012, Molecular Mechanisms of Learning and Memory in Aplysia, June 2<sup>nd</sup>: Workshop Consultant for the Behavioral Research Advancements In Neuroscience (BRAIN) Summer Research Program for Undergraduates.

2012, Graduate Student Panel, Georgia Institute of Technology, October 31<sup>st</sup>: Educating Undergraduates in an Introduction to Neuroscience Course about Graduate School.

**|Conference  
Coordination|**

2013, 17<sup>th</sup> Annual Society for Behavioral Neuroendocrinology Conference: Conference Organizer and Host, Held at Loews Midtown, Atlanta, June 2013.

**|Scientific  
Societies &  
University  
Committees|**

2008-2009, Neuroscience Club, President  
 2008-present, Society for Neuroscience  
 2011-present, Neuroscience Graduate Student Association, Undergraduate Placement Program Coordinator, Co-Chair for Keynote Speaker 2013-2014  
 2012-present, Atlanta Chapter of Society for Neuroscience, Council Member  
 2012-present, Society for Behavioral Neuroendocrinology  
 2012-present, Society for Social Neuroscience  
 2013-present, Georgia State University Committee for the Atlanta Science Festival: Discovery Day Sub-Committee  
 2013-2014, Neuroscience Graduate Student Association, Co-Chair & Co-Host for Keynote Speaker 2013-2014

**|Honors &  
Awards|**

2003, American Field Service Exchange & Scholarship  
 2009, Wheel & Chain  
 2009, Center for Neuromics Award for Research

**|Additional &  
Relevant  
Experience|**

2002-2004, Young Explorers Summer Experience: Assistant Teacher  
 2011-2013, American Field Service Exchange Programs: Atlanta Area Cluster Support Coordinator