4-21-2008

# Neurocircuitry and Molecular Basis of Conditioned Defeat in Male Syrian Hamsters

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# NEUROCIRCUITRY AND MOLECULAR BASIS OF CONDITIONED DEFEAT IN MALE SYRIAN HAMSTERS

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Under the Direction of Kim L. Huhman, Ph.D.

## ABSTRACT

Stress affects virtually all organisms and can result in both physiological and behavioral changes. Conditioned defeat in Syrian hamsters is a model of stress-induced behavioral plasticity that occurs in a social context. In this model, hamsters are defeated by a larger, more aggressive counterpart. Defeated hamsters subsequently fail to defend their own territory and show striking and long-lasting increases in submissive behavior even when paired with a non-threatening counterpart. The present series of experiments seeks to identify the brain regions and molecular mediators that contribute to this behavioral plasticity. One brain region that has been overlooked by our laboratory is the hippocampus. The results of the first study suggested that the ventral, but not dorsal, hippocampus is important for the acquisition of conditioned defeat as temporary inactivation of the ventral hippocampus prior to defeat training significantly reduced submissive and defensive behaviors when hamsters were tested with a non-aggressive intruder. Next, we sought to identify a potential molecular mediator of social stress-

induced behavioral plasticity in hamsters identified as winners or losers after a fight. Using in situ hybridization for brain-derived neurotrophic factor (BDNF) mRNA, we showed that winning and losing hamsters exhibited differences in BDNF mRNA in several regions including the basolateral and medial amygdala as well as the dentate gyrus of the dorsal hippocampus and CA1 of the ventral hippocampus. We next showed that neurotrophic activity in the basolateral amygdala is important for the acquisition of conditioned defeat because K252a infused into the basolateral amygdala prior to defeat training by an aggressive counterpart, significantly decreased submissive and defensive behavior during subsequent testing. Finally, existing data suggest that the amygdala and hippocampus interact to modulate the formation of emotional memories. To test the hypothesis that the basolateral amygdala and ventral hippocampus interact to mediate the behavioral plasticity observed in conditioned defeat, we simultaneously inactivated these regions either contralaterally or ipsilaterally prior to social defeat. Our results suggest that BLA and VHPC interact to mediate the acquisition of conditioned defeat, however, the nature of this interaction remains to be determined.

INDEX WORDS: Social stress, Behavioral plasticity, Hamster, Amygdala, Hippocampus, Brain-derived neurotrophic factor, Agonistic behavior, Learning and memory

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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

2008

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May 2008

## ACKNOWLEDGEMENTS

There are many people who have helped me both professionally and personally during my graduate career. First, I thank the members of my dissertation committee, Dr. Elliott Albers, Dr. Marise Parent, and Dr. Kerry Ressler for all of their scientific expertise and guidance. I also thank my dissertation advisor, Dr. Kim Huhman, who has been an amazing mentor and friend over the past six years. I am especially grateful for her unconditional support in my decision to follow a non-academic career path after graduation. I thank the members of the Huhman laboratory, both past and present: Dr. Matthew Cooper, Dr. Alicia Faruzzi, Dr. Matia Solomon, Dr. Chris Markham, Lauren Zelinski, and Jeris Israel. I sincerely appreciate all of the love and support from Alisa Norvelle, who has been my lab mom for three years and made me cups of tea and consoled me when my experiments did not go as planned.

I thank my fellow graduate students; many of whom I have formed close friendships. Pam Maras and Laura Been kept me laughing and entertained at school and conferences, ya'll. I also thank those students who supported me during my time at Georgia State University, Dr. Desiree Krebs-Kraft, Dr. Michelle Foster, Dr. Megan Dailey, Varenka Lorenzi, John Ryan, Kelli Duncan, Mahin Shabazi, and Stephanie Gutzler. I also thank Mary Karom for allowing me to use her quantification equipment and Dr. Chris Ehlen for teaching me how to use it. I greatly appreciate Dr. Lisa Stanek for teaching me everything I know about BDNF and allowing me to shadow her in Kerry Ressler's laboratory for a summer. I thank Dr. Kelly Lambert for sparking my interest in neuroscience as an undergraduate and encouraged me to never stop asking "why" and "how".

There is no question that I would not have made it out of graduate school alive without the support from my friends and family. I thank Claire Brown listening to me talk incessantly about my experiences in graduate school and making me laugh when life did not go as planned. I am also grateful for the constant support of Meagan Sexton, Amanda Robinson, Kate Aumann, and Christina Pappas during my graduate career. I thank my parents, Howard and Shirley Lin, for their unconditional love and support throughout my life. Finally and most importantly, I thank the most amazing man I have ever met, Tyl Taylor (who happens to be my husband), whose support for me and for this long journey indicates the sensitive, enlightened and extraordinary person he is. Thank you for never letting me feel like a defeated hamster.

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| | |
|---|---|
| ACSF | Artificial cerebrospinal fluid |
| ACTH | Adrenocorticotropin |
| AH | Anterior hypothalams |
| ANOVA | Analysis of variance |
| BDNF | Brain-derived neurotrophic factor |
| BLA | Basolateral amygdala |
| BNST | Bed nucleus of the stria terminalis |
| CD | Conditioned defeat |
| CNS | Central nervous system |
| CR | Conditioned response |
| CREB | cAMP responsive element-binding protein |
| CRF | Corticotropin-releasing factor |
| CS | Conditioned stimulus |
| DG | Dentate gyrus |
| DHPC | Dorsal hippocampus |
| DMSO | Dimethylsulfoxide |
| DNA | Deoxyribonucleic acid |
| FGF | Fibroblast growth factor |
| GABA | Gamma-aminobutyric acid |
| HPA | Hypothalamic-pituitary adrenal |
| LTP | Long-term potentiation |

| | |
|---|---|
| MAPK | Mitogen-activated protein kinase |
| MeA | Medial amygdala |
| mRNA | Messenger ribonucleic acid |
| NaC | Nucleus accumbens |
| NE | Norepinephrine |
| NGF | Nerve growth factor |
| NMDA | *N*-methyl-D-aspartic acid |
| NT-3 | Neurotrophin-3 |
| PTSD | Post-traumatic stress disorder |
| PVN | Paraventricular nucleus |
| SEM | Standard error of the mean |
| US | Unconditioned stimulus |
| UTP | Uridine 5’-triphosphate |
| VHPC | Ventral hippocampus |

# CHAPTER 1

# GENERAL INTRODUCTION

## Stress

All organisms experience stress. The study of stress physiology refers to the internal response of an organism to perturbations that disrupt homeostatic balance. A stressor is defined as an event or context that disrupts this balance. Stressors can be physical (e.g., injuries received after a physical altercation) or psychological (e.g., expectation of an altercation). The stress response refers to the processes by which the body attempts to regain homeostatic balance after disruption by a stressor. Thus, when an organism is exposed to a stressor, a stress response occurs that is thought to aide the organism in recovering from the stressor. For example, when a zebra is being hunted by a lion in the Serengeti, adrenaline is released into the zebra's bloodstream. This release has many effects on the zebra's body such as increasing the rate at which glycogen is converted into glucose, which is then available for the energy that is required for running away from and successfully evading the lion.

The physiological changes that occur in response to a stressor are thought to be adaptive for survival. However, continual activation of this response is oftentimes maladaptive and the effect of the experience can become deleterious to the organism. The study of stress physiology is particularly important because most humans are exposed to situations in which the stress response is continually activated. Importantly, stress has been linked to the development of stress-related mental disorders. For example, posttraumatic stress disorder, anxiety and depression as well as drug abuse relapse in humans has been linked to stress (Bohus, Koolhaas and Korte, 1990; Plotsky et al., 1998; Arborelius et al., 1999; de Kloet et al., 2005; Koob 2006 ). For these reasons, it is

important to explore the neurobiological changes that occur following a stressful experience. Such knowledge will be critical for the development of future treatments for stress-related disorders.

**The stress response**

As previously mentioned, exposure to a stressor causes an internal physiological response that attempts to reestablish homeostatic balance. This physiological response is frequently referred to as the stress response and involves the activation of the hypothalamic-pituitary adrenal (HPA) axis. Upon exposure to stress, corticotropin-releasing factor (CRF) is released from the paraventricular nucleus (PVN) of the hypothalamus into the median eminence whereupon it is then transported to the anterior pituitary via the hypophysioportal system. This triggers the release of adrenocorticotropin (ACTH) from anterior pituitary corticotroph cells, and ACTH travels through the bloodstream to reach the adrenal gland. The adrenal gland is comprised of two parts: the cortex and the medulla. ACTH stimulates the adrenal cortex to release the stress hormones cortisol and/or corticosterone, while the adrenal medulla is responsible for the release of epinephrine during the stress response.

**How is stress studied?**

Due to the growing awareness that stress contributes to a variety of psychopathologies, animal models have been developed to evaluate the effects of stress on the brain as well as behavior. The literature addressing the neurobiological and behavioral responses to stress is considerable, but the majority of the studies have used artificial means of inducing the stress response such as prolonged periods of immobilization or restraint, forced swim, and foot shock stress. These models offer the

benefit of being highly controllable, yet they bear little similarity to stressors encountered in the lives of humans or animals. Therefore, because much of the stress encountered by humans is social in nature, the use of animal models in which stress occurs in a *social* context has become increasingly attractive.

Some examples of models of social stress include the visible burrow system, over-crowding, and social defeat (Blanchard, Spencer, Weiss, Blanchard, McEwen and Sakai, 1995). In the visible burrow system and over-crowding models, animals are grouped and maintained in colonies. The habitats in these models are semi-naturalistic, and the amount of social stress to which an animal is exposed largely depends on the size of the habitat, number and sex of the inhabitants, as well as each inhabitant's access to resources.

Social defeat is a variation of the resident-intruder model in which two conspecifics are paired together in a neutral or non-neutral arena to elicit agonistic behavior. This interaction usually results in the formation of a dominant-subordinate relationship in which one animal is identified as the "winner" and the other as the "loser". The behavioral and neurobiological changes that occur following an agonistic encounter are oftentimes more pronounced in the losing animal than they are in the winning animal.

**What are the effects of social stress?**

*Physiological changes*

Exposure to a social stressor results in increased activity of the hypothalamic-pituitary adrenal (HPA) axis as evidenced by significant elevations in adrenocorticotropin (ACTH), β- endorphin, and cortisol/corticosterone (Huhman, Bunnell, Mougey, and Meyerhoff, 1990; Huhman, Moore, Ferris, Mougey, and Meyerhoff, 1991; Huhman,

Moore, Mougey, and Meyerhoff, 1992). The activity of the autonomic nervous system is also altered in response to social stress as norepinephrine (NE) and epinephrine are increased in defeated animals (Brain, 1980). Additionally, heart rate, blood pressure, and core body temperature are increased following social stress (Meehan, Tornatzky, and Miczek, 1995; Tornatzky & Miczek, 1993). Chronic stress including social defeat also suppresses immune function as measured by lymphatic organ size (Blanchard, Spencer, Weiss, Blanchard, McEwen, and Sakai, 1995) and humoral immune function (Bohus, Koolhaas, Heijnen, and de Boer, 1993; Fleshner, Laudenslager, Simons, and Maier, 1989; Jasnow & Huhman, 2001). Recently, Foster, Solomon, Huhman, and Bartness (2006) showed that adiposity is significantly increased in hamsters subjected to social defeat, possibly mimicking how exposure to stress contributes to obesity in some humans.

*Behavioral changes*

In addition to physiological changes that occur following stress, many social and nonsocial behaviors are changed following exposure to a social stressor. For example, following social defeat a significant decrease in overall locomotor activity and in social contact is observed (Blanchard and Blanchard, 1989; Meerlo, Overkamp, Daan, van den Hoofdakker, and Koolhaas, 1996; Meerlo, Overkamp, and Koolhaas, 1997; Shively, 1998). Animals that have been defeated also display increased submissive and defensive behaviors when in the presence of a conspecific, and they often fail to defend their own territory (Meerlo et al., 1996, Potegal et al., 1993, van de Poll et al., 1982). Following social stress, high levels of anxiety are observed when measured in rats using various models such as elevated plus maze, defensive withdrawal and defensive burying (Fendt, Koch, and Schnitzler, 1997; Heinrichs, Pich, Miczek, Britton, and Koob, 1992; Martins,

Marras, and Guimaraes, 1997; Smagin, Harris, and Ryan, 1996). Exposure to a social stressor also results in alterations in food and water consumption, and these alterations are largely dependent on the species and duration of the stressor (Meerlo, Overkamp, Daan, van den Hoofdakker, and Koolhaas, 1996; Foster et al., 2006). Finally, social stress has been shown to disrupt reproductive behaviors (Blanchard & Blanchard, 1989).

Learning and memory processes are also affected by exposure to stress. Interestingly, the effects of stress on learning and memory are dependent on the type and duration of the stressor as well as the sex of the organism. In general, exposure to acute stress can actually enhance performance on learning and memory tasks (Wood and Shors, 1998), while exposure to chronic stress can impair performance on these tasks (Luine, Villegas, Martinez, and McEwen, 1994). An interesting way to study chronic psychosocial stress and its effects on cognition is with dominance hierarchies in tree shrews (Ohl and Fuchs, 1999), wherein subordinate tree shrews display impairments in a spatial discrimination task such as holeboard learning. Relatedly, rats that experience prolonged periods of psychosocial stress demonstrate spatial learning deficits (Krugers, Douma, Andringa, Bohus, Korf, and Luiten,1997).

**Conditioned defeat**

Another way to assess stress-induced changes in the brain and on behavior is with a phenomenon called conditioned defeat in male Syrian hamsters. Conditioned defeat is a phenomenon described in 1993 by Potegal and colleagues. When male Syrian hamsters are briefly exposed to a larger, more aggressive counterpart and are defeated, they subsequently fail to display normal territorial aggression even when a non-aggressive, smaller hamster is introduced into the home cage of the defeated animal. The previously

defeated animal fails to defend its territory and, interestingly, exhibits striking submissive and defensive behaviors. This behavioral change is maintained for at least one month in many animals, even when the defeated animal is repeatedly paired with a non-aggressive opponent (Huhman, Solomon, Janicki, Harmon, Lin, Israel, and Jasnow, 2003).

In addition to the behavioral changes that occur following social defeat, physiological changes have also been noted in defeated animals. As stated before, "losing" animals demonstrate an increase in HPA activity following defeat. Interestingly, the increase in HPA activity observed in defeated hamsters does not appear to be dependent on physical contact between the animals because the hormonal response of previously defeated animals persists even when a physical barrier separates the dominant and subordinate animals (Huhman et al., 1992). Thus, conditioned defeat in male Syrian hamsters can be described as a biologically relevant, psychologically potent stressor.

In summary, it is clear that social defeat is capable of producing significant changes in an organism's physiology as well as its behavior. Thus, social defeat models such as conditioned defeat should be valuable models with which to study the neurobiological mechanisms that underlie social stress-induced behavioral plasticity.

**Brain areas important for stress-induced behavioral plasticity**

*The amygdala*

The amygdala, an almond shaped, multinuclear structure located in the temporal lobe of the mammalian brain, has long been implicated in emotional processing and integration of responses to stressful stimuli (Kluver & Bucy, 1937; LeDoux, 1992; Davis, 1992; Maren & Fanselow, 1996; Davis, 1997). The amygdala has been shown to be important for a well known form of stress-induced behavioral plasticity called Pavlovian

fear conditioning. Briefly, fear conditioning is a process by which an organism learns to fear new stimuli. In this form of learning, the organism learns to associate fear with a neutral context (e.g., room) or neutral stimulus (e.g., a tone). After several pairings of the neutral stimulus (e.g., tone; CS) with a fearful stimulus (e.g., shock; US), the organism will elicit the conditioned response (e.g., freezing; CR) to the CS alone. This type of learning is important for an organism's survival. Hence, the organism must learn what types of situations or contexts are associated with danger and subsequently adapt its behavior (e.g., freezing or avoiding) to increase its chances of survival.

The amygdala is critical for the acquisition and expression of conditioned fear responses, as well as for the behavioral changes that occur in response to exposure to stressful stimuli. This role can be demonstrated by electrolytic or chemical lesions as well as by chemical inactivation. Such manipulations of the amygdala result in a disturbance of conditioned fear responses in mammals. For example, pre- and post-training lesions of the central, lateral, or basolateral amygdala decrease freezing to shock or to a context paired with a shock (LeDoux, Cicchetti, Xagoraris, and Romanski, 1990; Roozendaal, Koolhaas, and Bohus, 1991a; Roozendaal, Koolhaas, and Bohus, 1991b; Phillips & LeDoux, 1992). Chemical or electrolytic lesions made prior to training (i.e., pre-training) of the basolateral or central nucleus of the amygdala block both acquisition and expression of fear-potentiated startle, a type of Pavlovian fear conditioning (Sananes and Davis, 1992; Kim and Davis, 1993). Likewise, post-training lesions of the central or basolateral amygdala block the expression of fear-potentiated startle (Hitchcock and Davis, 1986; Campeau and Davis, 1995; Lee, Walker, and Davis, 1996). Further support for the critical role of the amygdala in the expression of conditioned fear comes from data

showing that freezing and fear-potentiated startle can be decreased when lesions to the basolateral amygdala are made up to one month after training (Lee et al., 1996; Maren and Fanselow, 1996; Cousens and Otton, 1998). These data suggest that the amygdala may be critical for the long-term expression of fear conditioning and raise the possibility that the amygdala is a critical site for the storage of fear memories.

Another model that has been fruitful in identifying the role of the amygdala in stress-induced behavioral plasticity is inhibitory avoidance. Briefly, in inhibitory avoidance learning, animals are placed in a rectangular compartment that is divided into 2 sections: one is brightly lit, such that it is aversive to the animal and the other is darkened. Given that most laboratory rodents are nocturnal and may be better protected from predators in the dark, they generally gravitate to the darkened side; however, in this model the animal receives a footshock when the darkened chamber is entered. The subsequent latency for animals to return to the darkened chamber in which they received a mild footshock on the previous day is recorded. Increased latency to return to the chamber where the footshock occurred is an indication that learning has occurred. Several groups have suggested that the amygdala is also important in this type of learning as post-training lesions or functional inactivation of the amygdala blocks contextual fear and consolidation of inhibitory avoidance learning (Liang, McGaugh, Martinez, Jensen, Vasquez, and Messing, 1982; Parent and McGaugh, 1994; Muller, Corodimas, Fridal, and LeDoux, 1997; Wilensky, Schafe, and LeDoux, 2000).

The amygdala also appears to be important for behavioral and physiological responses to ecologically-relevant unconditioned stressors, including social defeat. Specifically, increased c-*fos* activation has been observed in several regions of the limbic

system including the amygdala following social defeat (Kollack-Walker and Newman, 1995; Kollack-Walker, Watson, and Akil, 1997). In addition, defeated hamsters exhibit decreased submissive and defensive behaviors as well as decreased avoidance of a dominant animal with amygdala lesions (Bunnell, Sodetz, and Shalloway, 1970; Agrawal et al., 2000). Such lesions also produce a decrement in the innate fear of cats characteristically demonstrated by rats (Blanchard and Blanchard, 1972). These data suggest that the amygdala is critical not only for the physiological and behavioral changes that occur in response to conditioned stimuli, but also for those that occur in response to unconditioned stimuli, such as those observed in rats exposed de novo to cat odor.

The initial studies investigating the neurobiology of conditioned defeat revealed that the amygdala is a critical brain region for regulating the behavioral changes observed after social defeat in male Syrian hamsters. Thus, decreasing excitatory neurotransmission (via blockade of *N*-methyl-D-aspartic acid (NMDA) receptors) or increasing inhibitory neurotransmission (via activation of gamma-aminobutyric acid ($GABA_A$) receptors) significantly reduced the acquisition and expression of conditioned defeat (Jasnow, Cooper, and Huhman, 2004; Jasnow et al., 2001, respectively).

More recent studies of conditioned defeat have begun to identify some potential substrates within the amygdala that may mediate the behavioral changes observed in conditioned defeat. cAMP-responsive binding element protein (CREB) is a transcription factor important for learning and memory as well as synaptic plasticity. Overexpression of CREB in the BLA enhances the memory of social defeat as evidenced by increased levels of submissive/defensive behaviors compared with control animals (Jasnow, Israel,

Davis and Huhman, 2005). As previously mentioned, activation of NMDA receptors is important for conditioned defeat. NMDA receptors are composed of several subunits, all of which are thought to have different functions. Blockade of the NR2B subunit blocks the acquisition but not expression of conditioned defeat. Such a finding is particularly relevant to this dissertation given that the NR2B subunit is important for long-term potentiation and synaptic plasticity but not synaptic transmission.

Another limbic brain region that has received considerable attention for its role in behavioral plasticity (i.e., spatial/contextual learning) is the hippocampus. Importantly, because this region controls negative feedback in the HPA-axis, it is well-studied for its role in stress responsivity.

*The hippocampus*

The hippocampus is located in the medial temporal lobe in humans and is most commonly associated with learning and memory processes. More specifically, the hippocampus is critical for declarative memory which is based on experience and requires conscious awareness. Damage to the hippocampus can cause anterograde amnesia (i.e., difficulties in forming new memories) and in some cases retrograde amnesia (i.e., difficulties in recalling memories formed prior to damage or injury).

The hippocampus is also known for its role in spatial memory and navigation. Considerable evidence from lesion and recording studies in rodents suggests that the hippocampus is critical for learning about spatial relationships. For example, rats with hippocampal lesions show impaired learning in a radial arm maze (Olton, Becker, and Handelman, 1979; Jarrard, 1983) and a T-maze (Bannerman, Rawlins, McHugh, Deacon, Yee, Bast, Zhang, Pothuizen, and Feldon., 2001). In a Morris water maze, rats with

hippocampal lesions show impairments in swimming to a hidden, but not to a visible, platform (Morris, Garrud, Rawlins, and O'Keefe, 1982). Electrical recordings from hippocampal neurons, sometimes referred to as "place cells", show that the activity of these cells confers information about the spatial organization of an animal's environment (Sharp, Blaire, Etkin and Tzanetos, 1995; Best, White, and Minai, 2001; de Araujo, Rolls, and Stringer, 2001). These data suggest that the hippocampus is a brain region that is critical for mediating the behavioral changes associated with spatial/contextual learning.

As previously mentioned, Pavlovian fear conditioning is a model that can be used to investigate the neural correlates of stress-induced behavioral changes. Considerable data suggest that the hippocampus plays an important role in some types of fear conditioning. As can be expected based on its role in spatial memory, the hippocampus plays a critical role in contextual, but not tone, fear conditioning. Animals with hippocampal lesions fail to successfully associate a neutral context with a shock, but fear conditioning to a tone remains intact (Phillips and LeDoux, 1992).

Studies investigating the role of the hippocampus in spatial/contextual learning and memory tasks tend to focus on the dorsal hippocampus as a critical region for this type of learning. Interestingly, several groups have gathered anatomical and behavioral evidence for functional differentiation between the dorsal (DHPC) and ventral (VHPC) portions of the hippocampus (Moser and Moser, 1998; Risold and Swanson, 1996; Bannerman et al., 2004). While the DHPC is critical for spatial navigation (Moser, Moser and Andersen, 1993), it appears that the VHPC is critical for aversive behaviors (e.g., avoidance, conditioned freezing, and anxiety) due to its dense anatomical

connections with structures involved in emotion such as the hypothalamus, amygdala and nucleus accumbens (Bast et al., 2001; Moser and Moser 1998; Petrovich, Canteras, and Swanson, 2001). For example, Trivedi and Coover (2004) found that animals with hippocampal lesions restricted to the ventral portion do not avoid the open arms of an elevated T-maze. Additionally, excitotoxic VHPC lesions reduce anxiety-like behaviors in the social interaction test (McHugh, Deacon, Rawlins, and Bannerman, 2004). Collectively, these data indicate that the DHPC is critical for spatial/contextual learning, while the VHPC appears to be more important for fear and anxiety-related behaviors.

A functional dissociation between the DHPC and VHPC is also notable when examining defensive behaviors in response to ethologically-relevant threat stimuli. For example, animals that received DHPC lesions exhibited normal defensive behavior such as freezing, crouching, and stretch-attend postures following presentation of cat odor or a live cat. On the contrary, VHPC lesions reduced measures of immobility and increased exploration and risk assessment following presentation of cat-odor (Pentkowski, Blanchard, Lever, Litvin, and Blanchard, 2006). The authors of this study suggested that the VHPC is part of a neural circuit that is important for mediating defensive responses in a more biologically-relevant environment.

As one would predict with this complex structure, the hippocampus is not limited to mediation of spatial/contextual learning and fear and anxiety-like behaviors. Studies are beginning to focus on the role of the hippocampus in social behavior, specifically social recognition. Social recognition refers to an animal's ability to successfully differentiate between a familiar or non-familiar conspecific. This type of social learning is demonstrated when the amount of time an animal spends investigating a familiar

animal decreases upon re-exposure. Social recognition in mice appears to be mediated by the hippocampus as ibotenic acid lesions of this area result in mice failing to decrease the time of investigation of a familiar animal (Kogan, Frankland, and Silva, 2000). Additionally, previously defeated hamsters that are exposed to a familiar winner demonstrate elevated *fos* and *erg*-1 immunohistochemistry in CA1 of the anterior dorsal hippocampus (Lai, Ramiro, Yu, and Johnston, 2005). This study also showed that temporary inactivation with lidocaine of the anterior dorsal hippocampus in subordinate hamsters eliminated avoidance of a familiar winner.

Finally, the birth and death of new neurons in the hippocampus is influenced by social status in species that form dominance hierarchies. For example, position in a dominance hierarchy can influence neurogenesis in adult rats (Kozorovitskiy and Gould, 2004). Likewise, Lucassen et al. (2001; 2004) studied dominance hierarchies in tree shrews and found that lower ranking animals tend to exhibit more apoptotic cells in the hippocampus when compared to higher ranking shrews. Furthermore, lower ranking shrews also demonstrate deficits in hippocampally-mediated tasks.

To date, no one has investigated the role of the hippocampus in conditioned defeat in male Syrian hamsters. Considering that conditioned defeat seems to incorporate aspects of the processes mentioned above (i.e., contextual fear conditioning, anxiety, social recognition, etc), it seems probable that this structure plays an important, if not critical, role in conditioned defeat.

*Other brain regions important for stress-induced behavioral plasticity*

Several other brain regions have been implicated in the behavioral responses to social stress and fearful stimuli. These include but are not limited to the nucleus

accumbens, bed nucleus of the stria terminalis (BNST) and infralimbic cortex. Dopaminergic projections from the ventral tegmental area to the nucleus accumbens comprise the mesolimbic dopaminergic system. This system has been studied for its involvement in drug addiction as well as in psychosocial behaviors such as cooperation, affiliation, pair bonding and maternal attachment (Rilling, Gutman, Zeh, Pagoni, Berns and Kilts, 2002; Ochsner, 2004; Insel and Fernanld, 2004; Young and Wang, 2004; Buwalda, Kole, Veenema, Huininga, Korte, and Koolhaas, 2005). Acute activation of the mesolimbic dopaminergic pathway occurs during aggression and subordination, which is particularly interesting for this dissertation (Tidy and Miczeck, 1996; Cabib, D'Amato, Puglisi-Allegra, and Maestripieri, 2000). As is the case with conditioned defeat, following daily bouts of social defeat, mice demonstrate aversive responses such as avoidance of a caged, unfamiliar animal (Berton, McClung, DiLeone, Krishnan, Renthal, Russo, Graham, Tsankova, Bolanos, Rios, Monteggia, Self, and Nestler, 2006). This social avoidance appears to be in part dependent on the mesolimbic dopaminergic pathway, specifically the release of brain-derived neurotrophic factor from the ventral tegmental area to the nucleus accumbens.

The BNST is another brain region that is important in regulating the behavioral responses to stressful stimuli, although it appears to be more involved in those of unconditioned rather than conditioned responses. Lesions of the BNST block unconditioned types of responses such as corticotrophin-releasing hormone (CRH)-enhanced startle and light-enhanced startle (Walker and Davis, 1997; Lee and Davis, 1997; Gerwitz et al., 1998) but do not block conditioned types of responses such as fear-potentiated startle (Hitchcock and Davis, 1991; Lee and Davis 1997; Gerwitz et al.,

1998). Increased c-*fos* activation is observed in the BNST following social defeat in Syrian hamsters (Kollack-Walker and Newman, 1995; Kollack-Walker et al., 1997). Corticotrophin-releasing factor (CRF) signaling in the BNST is important for conditioned defeat as infusion of the CRF receptor antagonist, D-Phe$_{12-41}$ reduces the duration of submissive and defensive behaviors following social defeat (Jasnow et al., 2004). Subsequent studies revealed that $CRF_2$ receptors but not $CRF_1$ receptors in the BNST are responsible for this modulation because a $CRF_2$ receptor antagonist, anti-sauvagine-30, but not a $CRF_1$ receptor antagonist, reduces the behavioral effects of social defeat (Cooper and Huhman, 2005).

The medial prefrontal cortex is thought to play a role in the physiological response to stressful or fear-inducing stimuli and includes the prelimbic, infralimbic, and the anterior cingulate subregions. This region modulates the activity of the HPA-axis, serving as a negative feedback site (Diorio et al., 1993). Anatomically speaking, the medial prefrontal cortex is well-connected with subcortical structures believed to be involved in fear and anxiety such as the amygdala, anterior BNST, hippocampus, nucleus accumbens, hypothalamus and periaqueductal grey (Hurley et al., 1991; Kita and Oomura, 1981; McDonald et al., 1996; Sesack et al., 1989; Swanson, 1981). Excitotoxic lesions of the medial prefrontal cortex attenuate fear-related responses in several fear and anxiety assays (Shah and Treit, 2003). In an elevated-plus maze, animals with lesions of the medial prefrontal cortex (including both infralimbic and prelimbic subregions) spent more time in the open arms and had more entries into the open arms compared with controls. In a shock-probe burying test, lesioned animals spent less time displaying defensive probe burying behavior. This study also examined fear and anxiety in a social

context in that animals with medial prefrontal cortex lesions spent more time in active social interaction with a conspecific under anxiety-provoking conditions. Increased duration of social interaction is believed to represent lower levels of fear and anxiety (File and Hyde, 1978). Finally, rats exposed to social defeat demonstrate elevated Fos expression and Fos-like immunoreactivity in the infralimbic cortex suggesting that cells in this region are involved in the physiological and/or behavioral responses to social stress (Nikulina et al., 2004).

**Molecular/Cellular mediators of behavioral plasticity**

In addition to investigating specific brain regions that contribute to stress-induced behavioral plasticity, studies have begun to reveal the molecular/cellular players that act in these regions to produce stress-induced behavioral changes. A neuropeptide that has received considerable attention for its role in behavioral and synaptic plasticity is brain-derived neurotrophic factor.

*Brain Derived Neurotrophic Factor (BDNF)*

BDNF is a neurotrophin that belongs to the nerve growth factor family of peptides. The neurotrophins (including nerve growth factor (NGF), neurotrophin-3 (NT-3), neurtrophin 4/5, acidic- fibroblast growth factor (FGF), basic FGF, and BDNF), are well-known for their role in the development and maintenance of the nervous system and Historically, BDNF was thought to be critical during the development of the central nervous system only; however, recent evidence indicates that it is also important in the adult central nervous system.

In the adult central nervous system, BDNF is critical for neural plasticity. Several lines of evidence have demonstrated that BDNF is a potent mediator of both

morphological and electrophysiological changes. Exogenous application of BDNF to hippocampal slices *in vitro* causes marked increases in dendritic spines and arborization (Cohen-Cory, Escandon, and Fraser 1995; McAllister, Katz, and Lo, 1999). In the dentate gyrus, an area capable of undergoing neurogenesis, application of BDNF significantly increases the number of new neurons (Scharfman, Goodman, Macleod, Phai, Antonelli and Croll, 2005)

The trophic actions of BDNF are mediated by a membrane bound receptor called TrkB which belongs to the tyrosine kinase family of receptors. Binding of BDNF to TrkB leads to dimerization and transphosphorylation of tyrosine residues in the intracellular domain of the receptor and subsequent activation of an array of cytoplasmic signaling pathways, such as the Ras/Raf/MAP kinase pathway (Kaplan and Miller, 1997). It has been shown that upon binding, BDNF depolarizes neurons as rapidly as does glutamate (Kafitz, Rose, Thoenen, and Konnerth, 1999), enhances glutamatergic synaptic transmission (Levine, Dreyfus, Black, and Plummer, 1995), and increases phosphorylation of subunits of NMDA receptors in hippocampus (Suen, Wu, Levine, Mount, Xu, Lin, and Black, 1997).

In addition to catalytic TrkB, a truncated form of this receptor exists which lacks the internal tyrosine kinase domain. Although the function of truncated TrkB receptors is unknown, it has been proposed that this truncated receptor could serve to inactivate BDNF released into the synapse or that it may act as a reservoir station of BDNF intended for later release (Lindsay, 1994). Following injury, truncated TrkB receptors are upregulated by CNS glial cells and are thought to sequester BDNF to reduce local

availability and to prevent interaction with full length TrkB, thereby selectively inhibiting neurite outgrowth on adjacent neurons (Fryer, Kaplan, and Kromer, 1997).

*BDNF/TrkB and the hippocampus*

Many of the electrophysiological changes that occur during BDNF/TrkB signaling have been observed in the hippocampus. Hippocampal long-term potentiation (LTP) is a process that is critical to various hippocampally mediated forms of learning and is considered to be important in synaptic plasticity. When applied directly to *in vivo* hippocampal slices, BDNF has been shown to induce late-phase LTP (Messaoudi, Ying, Kanhema, Croll, and Bramham, 2002; Ying, Futter, Rosenblum, Webber, Hunt, Bliss, and Bramham, 2002). Likewise, LTP is impaired when endogenous BDNF is sequestered (Mu, Li, Yao, and Zhou, 1999).

Recent experimental evidence has suggested that BDNF is necessary for learning and memory in several hippocampally-mediated tasks. For example, BDNF is rapidly upregulated in the hippocampus following contextual as well as spatial learning (Hall, Thomas, and Everitt, 2000; Mizuno, Yamada, Olariu, Nawa, and Nabeshima, 2000). Infusion of BDNF antibodies into the hippocampus prior to training results in impaired spatial learning and memory in rats as assessed by the Morris water maze (Mu et al., 1999). Furthermore, conditional TrkB knockout mice, in which the knockout of the trkB gene is restricted to the forebrain and occurs during postnatal development, failed to successfully learn the Morris water maze (Minichiello, Korte, Wolfer, Kuhn, Unsicker, Cestari, Rossi-Arnaud, Lipp, Bonhoeffer, and Klein, 1999).

*BDNF/TrkB and the amygdala*

Activation of the TrkB receptor by BDNF initiates signaling pathways that lead to the activation of MAP kinase, PI3-kinase, and CREB phosphorylation, all of which are known molecular mediators of fear conditioning (Schlessinger & Ulrich, 1992; Patapoutian & Reichardt, 2001; Lu, Walker and Davis 2001; Davis, 2002; Tyler, Alonso, Bramham, and Pozzo-Miller, 2002). Recently, it has been observed that BDNF is critical for amygdala-dependent fear conditioning (Rattiner, Davis, French, and Ressler, 2004). Temporary upregulation of BDNF mRNA during the period following fear conditioning in the BLA was observed and occurred independent of the modality of the conditioned stimulus. In contrast, other neurotrophins such as nerve growth factor (NGF), neurotrophin 4/5, acidic- fibroblast growth factor (FGF), and basic FGF did not increase following fear conditioning. To establish a functional role for BDNF in amygdala-dependent fear conditioning, Rattiner et al. (2004) used a lenti-virus (lenti-TrkB.t1) to over-express the truncated isoform of TrkB receptors in the amygdala, thus inhibiting BDNF/TrkB signaling therein. Infusion of lenti-TrkB.t1 into the basolateral amygdala blocked fear acquisition without disrupting other behaviors. Thus, this study established a functional role for BDNF/TrkB signaling in amygdala-dependent fear conditioning.

*BDNF/TrkB, stress, and the behavioral effects of social defeat*

Numerous studies have examined how exposure to stress alters BDNF mRNA levels in various brain regions. Much of the literature addressing the neural responses to stress have reported decreases in BDNF mRNA expression following repeated or chronic stress treatments such as immobilization or foot shock (Nibuya, Takahashi, Russell, and Duman 1999; Smith, Makino, Kvetnansky, and Post, 1995). Few studies have examined

how models of social stress, such as conditioned defeat, alter neurtrophin levels in the adult brain. Fiore et al. (2004) found differences between dominant and subordinate aged male mice in mRNA levels in nerve growth factor (NGF) and BDNF in that dominant animals had higher levels of BDNF mRNA in the subventricular zone and hippocampus than did subordinate animals. Conversely, subordinate animals exhibited higher levels of NGF compared with dominant animals in these regions. Pizarro et al. (2004) showed decreased BDNF mRNA in mice exposed to a 10-min social defeat when compared to non-defeated animals, however, this decrease was detected in all brain regions (e.g., cortical and subcortical).

The functional role of BDNF/TrkB signaling in brain regions that we know are important for the behavioral changes that occur following social defeat are still poorly understood. A recent paper, however, indicated that BDNF/TrkB signaling in the mesolimbic dopamine pathway is critical for the behavioral changes that occur following social defeat in mice (Berton et al., 2006). Specifically, a ventral tegmental area-specific deletion of BDNF blocked the development of social aversion after defeat stress suggesting that BDNF released from the ventral tegmental area to the nucleus accumbens is critical for a conspecific to acquire salience as a threatening stimulus. This study was among the first to describe a role for BDNF/TrkB signaling in a model of social stress-induced behavioral plasticity.

**Specific Aims**

Many human psychopathologies can be linked to stressful or traumatic experiences. Animal models of stress-related behavioral changes have yielded vital information regarding the brain regions, neural circuitry, and in some cases

cellular/molecular mechanisms that contribute to these behavioral modifications. However, studies are needed in which the type of stress to which an animal is exposed mirrors that to which a human might encounter. We propose that conditioned defeat in Syrian hamsters is a model with which we can study how social stress alters both the brain and behavior.

Given that conditioned defeat can be considered as a model of stress-induced behavioral plasticity, it is possible that mediators of synaptic and behavioral plasticity, such as BDNF, that have been identified in traditional learning studies are also critical for the behavioral changes exhibited by losing animals. Where in the brain would BDNF act to mediate the behavioral plasticity underlying conditioned defeat? Studies of fear conditioning and conditioned defeat indicate that the basolateral amygdala is critical for mediating the behavioral responses to fear and social defeat, respectively. Another area that has not been studied in conditioned defeat but which has received considerable attention for its potential roles in social recognition and in fear and anxiety is the hippocampus. The overarching goal of this thesis is to investigate the neural circuitry and molecular signals that contribute to the behavioral changes observed in conditioned defeat.

**Specific Aim 1**

The hippocampus is a brain region that is most commonly associated with learning and memory processes. Specifically, the DHPC has been implicated in several spatial navigation/contextual learning paradigms including the radial arm maze (Olton and Fuchs, 1979; Jarrad, 1983) as well as the Morris water maze (Morris et al., 1982). Recently, however, the hippocampus is beginning to receive attention for its role in

learning, such as social recognition, that occurs in a social context. Lesions of this area produce an impairment of social recognition in mice (Kogan et al., 2000) and defeated hamsters that are exposed to a familiar winner show elevated immediate early gene activity in CA1 of the anterior dorsal hippocampus (Lai et al., 2005). To our knowledge, very little is known about how this area contributes to the behavioral changes that occur following exposure to a social stressor in Syrian hamsters. Therefore, it is important to establish whether there is a role of the DHPC in mediating the behavioral responses to social defeat. The purpose of the Experiments 1 and 2 was to test the hypothesis that the DHPC is important for the acquisition and/or expression of conditioned defeat. To test this hypothesis, we activated $GABA_A$ receptors in the dorsal hippocampus (DHPC) using muscimol immediately before training (acquisition) or before testing (expression).

Relatedly, several groups have proposed that the hippocampus can be functionally divided along its dorsal and ventral poles (Moser and Moser, 1988; Risold and Swanson, 1996; Bannerman et al., 2004). Unlike the dorsal hippocampus, the ventral hippocampus (VHPC) has been implicated in aversive behaviors such as avoidance, freezing, and anxiety. Rats with VHPC lesions do not avoid the open arms of a T-maze (Trivedi and Cooper, 2004), and excitotoxic lesions to this area reduce anxiety-like behaviors in a social interaction test (McHugh et al., 2004). In a predator odor model, which measures the behavioral responses to a naturalistic stressor, rats with VHPC lesions are less immobile (i.e., freezing behavior is decreased) and show increased exploration compared with animals with DHPC lesions (Pentowski et al., 2006). Finally, the VHPC has dense anatomical connections with brain structures such as the hypothalamus, amygdala, and nucleus accumbens that are implicated in emotional behavior (Bast et al., 2001; Petrovich et al., 2001). The purpose of Experiments 3 and 4 was to test the

hypothesis that the VHPC is involved in the acquisition and/or expression of conditioned defeat. To test this hypothesis, we activated $GABA_A$ receptors in the VHPC before training (acquisition) or testing (expression) using infusions of muscimol to temporarily inactivate the VHPC.

**Specific Aim 2**

Many studies have shown that BDNF mRNA expression can be altered in response to exposure to a stressor such as prolonged periods of restraint (Smith et al., 1995, Smith et al., 1996). To our knowledge, very few studies exist that examine how exposure to a more naturalistic stressor, such as an agonistic encounter, can alter BDNF mRNA in specific brain regions that are important for stress responsivity, fear, learning and memory, and social behavior. These brain regions include the nucleus accumbens, infralimbic cortex, bed nucleus of the stria terminalis (anterior and posterior), medial amygdala, central amygdala, basolateral amygdala, dorsal hippocampus (CA1, CA3, and dentate gyrus), ventral hippocampus (CA1, CA2, and dentate gyrus), anterior hypothalamus and ventromedial hypothalamus. Therefore, the purpose of Experiment 1 was to test the hypothesis that following a fight, winning and losing hamsters would exhibit differences in BDNF mRNA in these areas. To test this hypothesis we paired hamsters for 15 min fight during which time we identified a winner and loser and then processed the brains for BDNF mRNA in situ. We focused on BDNF mRNA and not other neurotrophins because past studies of amygdala-dependent fear conditioning demonstrate that nerve growth factor (NGF), neurotrophin 4/5, acidic- fibroblast growth factor (FGF), and basic FGF do not increase following fear conditioning (Rattiner et al., 2004).

Recent data suggests that the neurotrophin family of peptides are involved in the behavioral responses to stress (Fiore, Amendola, Triaca, Tirassa, Alleva, and Aloe, 2003; Berton et al., 2006). Exposure to an ethologically-relevant stressor, such as social defeat, has been shown to alter neurotrophin production in the brain (including the amygdala and hippocampus), however the functional relevance of these changes remains unknown (Pizarro, Lumley, Medina, Robinson, Chang, Alagappan, Bah, Dawood, Shah, Mark, Kendall, Smith, Saviolakis and Meyerhoff, 2004; Fiore et al., 2003). Therefore, the role of neurotrophins in stress-related behavioral changes, such as conditioned defeat, remains to be determined. The purpose of Experiments 2 and 3 was to test the hypothesis that neurotrophin signaling is important for the acquisition and expression of conditioned defeat. To test this hypothesis, we infused a non-selective Trk receptor antagonist, K252a, into the BLA before conditioned defeat training (acquisition) or conditioned defeat testing (expression). We focused on the BLA because our laboratory has previously shown that this area is critical for the acquisition and expression of conditioned defeat. Similarly, studies using Pavlovian fear-conditioning suggest that the basolateral amygdala is a potential site in which plasticity occurs to support behavioral changes.

**Specific Aim 3**

Several groups have hypothesized that the amygdala and hippocampus interact in the formation of memories (Packard, Cahill and McGaugh, 1994; McGaugh, 2002 & 2004; Akirav and Richter-Levin, 2002; Richter-Levin, 2004; McIntyre, Miyashita, Setlow, Marjon, Steward, Guzowski, and McGaugh, 2005; Vouimba, Yaniv, and Richter-Levin, 2007). This idea is supported by anatomical, electrophysiological, and functional

evidence. Anatomically, the basomedial and basolateral nuclei of the amygdala project to the hippocampus with the heaviest projections occurring between the BLA and CA1, CA3, and entorhinal cortex of the VHPC (Amaral et al., 1992). These regions of the VHPC in turn project via the ventral angular bundle to the basomedial and basolateral nuclei of the amygdala. Electrophysiological studies have shown that amygdala activity influences LTP-induction in the hippocampus. Pharmacological stimulation of the amygdala activates the entorhinal cortex and hippocampus (Packard et al., 1995) and lesions of the BLA attenuates LTP at the perforant path-dentate gyrus granule cell synapses in the hippocampus (Abe, 2001). High frequency stimulation of the BLA combined with tetanic stimulation of the perforant path facilitates hippocampal LTP (Ikegaya et al., 1996). Likewise, stimulation of the hippocampus increases amygdala LTP (Maren & Fanselow, 1995).

A substantial number of studies have demonstrated a functional role for amygdala-hippocampal interactions. For example, Packard et al., (1994) hypothesized that the amygdala modulates memories in other brain regions such as the caudate nucleus and hippocampus, two regions thought to be important in two different memory tasks. Essentially, amphetamine was infused into the amygdala, hippocampus or caudate nucleus immediately after rats were trained on one of two water maze tasks, a spatial task (thought to be hippocampally-dependent) or a visually cued task (thought to be caudate nucleus-dependent). The hippocampal infusion selectively enhanced retention of the spatial task, and the caudate infusion selectively enhanced retention of the visually cued task. Interestingly, when amphetamine was infused into the amygdala, retention on both tasks was enhanced. Additional evidence suggesting that the amygdala and hippocampus

interact in memory formation comes from a study showing that amygdala lesions block the memory-enhancing effect of direct hippocampal stimulation (Roozendaal and McGaugh, 1997). Finally, amygdala-hippocampal interactions are critical for contextual fear conditioning as electrolytic lesions of selected subregions of the VHPC produce a deficit in the acquisition of fear to a contextual conditioned stimulus, and NMDA lesions of the BLA produce a nonselective deficit in the acquisition of fear to both contextual and acoustic conditioned stimuli (Maren & Fanselow, 1995).

Given that it is known that the amygdala and hippocampus interact in the formation of memories and that the BLA and VHPC are involved in conditioned defeat, it is possible that these two brain regions interact to produce the behavioral changes associated with this phenomenon. Therefore, the purpose of Experiment 1 was to test the hypothesis that the BLA and VHPC interact to mediate the acquisition of conditioned defeat. To test this hypothesis, we disrupted the BLA-VHPC circuit before defeat training using infusions of muscimol into each region contralaterally or ipsilaterally to simultaneously inactivate these two regions.

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# CHAPTER 2

# ACTIVATION OF $GABA_A$ RECEPTORS IN THE VENTRAL BUT NOT DORSAL HIPPOCAMPUS REDUCES THE ACQUISITION OF CONDITIONED DEFEAT IN MALE SYRIAN HAMSTERS

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## Abbreviations

| | |
|---|---|
| 1. ANOVA | analysis of variance |
| 2. BNST | bed nucleus of the stria terminalis |
| 3. CD | conditioned defeat |
| 4. CRF | corticotrophin-releasing factor |
| 5. CRH | corticotrophin-releasing hormone |
| 6. DHPC | dorsal hippocampus |
| 7. GABA | gamma-aminobutyric acid |
| 8. M | mean |
| 9. SEM | standard error of the mean |
| 10. VHPC | ventral hippocampus |

**Abstract**

Male Syrian hamsters that are exposed to social defeat exhibit long-lasting behavioral changes characterized by a lack of normal territorial aggression and an increase in submissive and defensive behavior even when they are paired with a non-aggressive intruder. This phenomenon has been termed conditioned defeat (CD). Our laboratory has demonstrated that several of the brain regions involved in fear, anxiety, and stress-responsivity, such as the amygdala and bed nucleus of the stria terminalis, are also involved in the behavioral changes associated with CD. One brain region that we have not examined for a role in CD is the hippocampus. The hippocampus can be functionally divided into the dorsal hippocampus (DHPC), which mediates spatial learning and memory and perhaps social recognition, and the ventral hippocampus (VHPC), which is thought to modulate fearful and anxious behaviors. Thus, it seems reasonable to hypothesize that the hippocampus may mediate, at least in part, the behavioral changes that occur in response to social defeat. The goal of the present study was to determine if activation of $GABA_A$ receptors in the DHPC or VHPC reduces the acquisition or expression of CD. Bilateral infusions of muscimol (1.1, 2.2, 2.7, 3.3 nmol, or vehicle) into the VHPC but not the DHPC immediately before the initial defeat significantly reduced the acquisition of CD as evidenced by a reduction in the display of submissive/defensive behaviors during the subsequent testing session. Conversely, bilateral infusions of muscimol (1.1, 2.2, 2.7 nmol or vehicle) into the VHPC or DHPC immediately before the testing session did not reduce the expression of CD. These results suggest that the VHPC but not the DHPC is part of a neural circuit that mediates behavioral changes that occur in response to social stress.

## Introduction

The ability to alter one's behavior based on experience is critical for survival. Social experience, in particular, can serve as a critical stimulus for behavioral change. Our laboratory examines the striking physiological and behavioral changes that occur following a single social defeat session in male Syrian hamsters. Syrian hamsters are solitary animals that will normally defend their territory against intruding conspecifics. If a hamster is paired with a larger, more aggressive animal and is defeated, however, it subsequently becomes highly submissive and fails to defend its home cage, even when paired with a smaller, non-aggressive animal. Instead of attacking the intruding animal, previously defeated hamsters avoid social interaction and readily submit to intruders. This behavioral change has been called conditioned defeat (CD; Potegal et al. 1993). Given the drastic behavioral changes observed in CD, we believe that it is an attractive model with which to study the behavioral plasticity following exposure to a biologically-relevant stressor.

Recent work from our laboratory suggests that several of the brain regions known to underlie fear, anxiety, and stress responsiveness also subserve CD. The amygdala is a brain region that is strongly implicated in fear and anxiety (Hitchcock and Davis, 1986; LeDoux et al. 1990; Phillips and LeDoux, 1992; Muller et al. 1997). Jasnow and Huhman (2001) demonstrated that the amygdala is critical for the acquisition and expression of CD. Infusion of muscimol into the central and basolateral nuclei of the amygdala either before the initial defeat or immediately before testing significantly reduces the duration of submissive/defensive behaviors displayed by defeated hamsters. The bed nucleus of the stria terminalis (BNST) is another brain region that is important in regulating the

behavioral responses to stressful stimuli. Lesions of the BNST block corticotrophin-releasing hormone (CRH)-enhanced startle and light-enhanced startle (Walker and Davis, 1997; Lee and Davis, 1997; Gewirtz et al. 1998) but not fear-potentiated startle (Walker and Davis, 1997) suggesting that the BNST may be more important in unconditioned (anxiety-like) responses than in conditioned (fearful) responses. The BNST appears to be involved in the behavioral responses to social stress because c-fos activation is increased in this region following social defeat in Syrian hamsters (Kollack-Walker and Newman, 1995; Kollack-Walker et al. 1997). We have suggested that the BNST is a component of the neural circuit that mediates CD because infusion of the CRF receptor antagonist, D-$Phe_{12-41}$ into the BNST reduces the duration of submissive and defensive behaviors following social defeat (Jasnow et al. 2004).

One brain region that has been largely overlooked by our laboratory, but which receives considerable attention for its role in spatial and contextual learning and memory as well as stress-responsivity, is the hippocampus. Interestingly, several groups have gathered anatomical and behavioral data demonstrating a functional differentiation between the dorsal (DHPC) and ventral (VHPC) regions of the hippocampus (Risold and Swanson, 1996; Moser and Moser, 1998; Bannerman et al. 2004). While the DHPC is critical for learning about spatial relationships and for navigation (Moser et al. 1993), it appears that the VHPC plays an important role in the production of behaviors emitted in response to a variety of aversive stimuli (e.g., avoidance, conditioned freezing, and anxiety-like behaviors). For example, Trivedi and Coover (2004) found that animals with hippocampal lesions restricted to the VHPC do not avoid the open arms of an elevated T-maze and excitotoxic VHPC lesions reduce anxiety-like behaviors in a social interaction

test (McHugh et al. 2004). Relatedly, animals that receive DHPC lesions exhibit normal defensive behavior such as freezing, crouching, and stretch-attend postures following presentation of cat odor or a live cat, while those receiving VHPC lesions exhibit reduced measures of immobility and increased exploration and risk assessment following presentation of cat-odor, behavioral changes that are thought to reflect decreased anxiety (Pentkowski et al. 2006).The findings from this latter study are particularly relevant to the experiments presented here because they are among the few that examine how hippocampus mediates the behavioral changes associated with a threat stimulus that an organism is likely to encounter in their natural habitat.

Recent studies have begun to focus on the role of the hippocampus in social behavior, specifically social recognition. Social recognition in mice appears to be mediated, at least in part, by the hippocampus because ibotenic acid lesions covering the full rostral-caudal extent of the hippocampus result in mice failing to decrease the time of olfactory investigation of a familiar animal (Kogan et al. 2000). Additionally, previously defeated hamsters that are exposed to a familiar winner demonstrate elevated *fos* and *erg*-1 immunohistochemistry in CA1 of the anterior DHPC and temporary inactivation of this area with lidocaine eliminates avoidance of a familiar winner (Lai et al. 2005). This finding is especially relevant to the current experiments because Lai et al. (2005) utilized a behavioral procedure that is very similar to that of CD in which experimental hamsters are socially defeated and then re-exposed to a conspecific.

In sum, the literature reviewed above suggests that the DHPC is important for spatial learning and memory as well as social recognition, while the VHPC is important for the production of fearful or anxious behaviors. CD is an attractive model because it

should involve neural circuits important in social behavior and recognition, as well as fear and anxiety. Therefore, it seems reasonable to hypothesize that the DHPC and the VHPC might each play distinct, yet important, roles in CD. To determine whether these brain regions appear to be a component of the neural circuit underlying CD, we tested the hypothesis that activation of $GABA_A$ receptors in the DHPC or VHPC with muscimol would reduce the acquisition and expression of conditioned defeat in Syrian hamsters

## Experimental Procedures

### Animals and Housing Conditions

Male Syrian hamsters (*Mesocricetus auratus*) weighing 120-140g were purchased from Charles River Laboratories and individually housed for 12-14 days prior to the start of each experiment. Older hamsters (> 6 months) that weighed 160-180 g were housed individually and used as resident aggressors during defeat training (see below). Younger hamsters (2 months) that weighed 100-110 g were group housed (5 hamsters per cage) and used as non-aggressive intruders during testing (see below). All hamsters were housed in polycarbonate cages (20 x 40 x 20 cm) with wire mesh tops, and food and water were available *ad libitum*. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee, and all methods were in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Every effort was made to minimize the number of subjects used as well as to minimize any suffering by the animals.

### Surgical procedures

Hamsters were deeply anesthetized with sodium pentobarbital (90mg/kg) and stereotaxically implanted with 4mm, 26-gauge guide cannulae (Plastics One, Roanoke,

Virginia). Lambda and bregma were leveled prior to placement of the guide cannulae. Guide cannulae were implanted bilaterally aimed at the DHPC (Experiments 1 and 2) or the VHPC (Experiments 3 and 4). Stereotaxic coordinates for the DHPC were 0.4mm posterior and ±1.8mm lateral to bregma and 2.0mm below dura. Infusions were made with a needle that projected 1.2mm beyond the bottom of the guide cannulae. Stereotaxic coordinates for the VHPC were 2.6mm posterior and ±3.7mm lateral to bregma and 1.9mm below dura. Infusions were made with a needle that projected 4.2mm beyond the bottom of the guide cannulae. After surgery, dummy stylets were placed in the guide cannulae to help prevent clogging. All hamsters were given 10-12 days to recover from surgery before the behavioral procedure. Hamsters were handled each day following surgery by gently restraining them and removing and replacing the dummy stylet.

**Social defeat and behavioral testing**

The conditioned defeat model has been described in detail elsewhere (Huhman et al., 2003). Prior to each experiment, hamsters were weight-matched and randomly assigned to groups. On the day of training, hamsters were transported to the behavior room. All training and testing occurred during the first 2 hr of the dark phase of the light: dark cycle to control for circadian rhythmicity of physiology and behavior. Training consisted of one 15-min exposure to a resident aggressor in the aggressor's home cage. Resident aggressors reliably attacked the experimental hamsters and all subjects displayed submissive behavior in response. Any hamster bitten such that it bled was removed from the study and examined by a veterinarian. During training, no-defeat controls were placed in a resident aggressor's empty cage for 15-min. The next day, all experimental hamsters and no-defeat controls were transported to the behavior room, and

a non-aggressive intruder was placed in their home cage for 5-min. The testing session was videotaped, transferred to CD-ROM, and later scored by an observer blind to the experimental conditions using behavioral scoring software (The Observer, Noldus Information Technology, Wageningen, the Netherlands). We recorded the total duration of four classes of behavior during the 5-min test: (a) submissive and defensive behavior (flee, avoidance, tail-up, upright and side defense, full submissive posture, stretch-attend, head flag, attempted escape from cage), (b) aggressive (upright and side offense, chase and attack, including bite), (c) social behavior (attend, approach, investigate, sniff, nose touching, and flank marking), and (d) non-social behavior (locomotion, exploration, self-grooming, nesting, feeding, and sleeping). A statistically significant reduction in the duration of submissive and defensive behaviors and/or the display of territorial aggression signified a reduction of conditioned defeat. For Experiments 1 and 3, the behavior of the resident aggressor was scored to ensure that the presence of a drugged subject during training did not alter the behavior of the resident aggressors and that all animals received similar defeats.

**Drug infusions and site verification**

Infusions into the DHPC or VHPC were administered to freely moving hamsters over 3 min with a Hamilton syringe mounted on a syringe pump (Harvard apparatus PHD 2000, South Natick, MA, USA) connected to a 33-gauge needle via polyethylene tubing (Fisher Scientific, Suwanee, GA). The needle was kept in place for an additional minute before being removed and the dummy stylet replaced. All hamsters were administered infusions of the $GABA_A$ antagonist, muscimol, (Sigma) or vehicle control (300nl saline). We selected muscimol because it is a reliable agent for temporarily inactivating the

amygdala (Helmstetter and Bellgowan, 1994; Muller et al. 1997) and the hippocampus (Mao and Robinson, 1998). At the conclusion of each experiment, hamsters were given a lethal dose of sodium pentobarbital and infused with 300nl of India ink to verify the placement of the needle. Brains were removed and placed in 10% buffered formalin. Brains were sliced on a cryostat and sections were stained with neutral red. Sections were coverslipped with DPX mountant (VWR International Ltd., Poole, England) and examined under a light microscope for evidence of ink in the DHPC or VHPC. Only hamsters with bilateral ink injections within 0.5mm of the DHPC or VHPC were included in the data analysis.

**Experiment 1: DHPC/acquisition of conditioned defeat**

The purpose of Experiment 1 was to determine whether infusion of muscimol into the DHPC would reduce the acquisition of conditioned defeat. Animals (n = 41) were matched by weight and randomly assigned to groups. Hamsters received infusions of either muscimol (1.1, 2.2, or 3.3 nmol in 300 nl saline) or vehicle immediately before being placed in the cage of a resident aggressor for 15 min. On the following day, animals were tested for 5 min in their own home cage in the presence of a non-aggressive intruder, as described above.

**Experiment 2: DHPC/expression of conditioned defeat**

The purpose of Experiment 2 was to determine whether infusion of muscimol into the DHPC would reduce the expression of conditioned defeat. Animals (n = 31) were matched by weight and randomly assigned to groups. All hamsters were placed into the cage of a resident aggressor for 15 min for conditioned defeat training. On the following day, animals received infusions of either muscimol (1.1 or 2.2 nmol in 300 nl saline) or

vehicle immediately before being tested in their own home cage for 5 min with a non-aggressive intruder. We did not include a 3.3 nmol group (as in Experiment 1) because we observed that this dose produced non-specific behavioral effects such as ataxia and repetitive licking and food pouching during testing. These effects were not observed in Experiment 1 when animals given this dose of muscimol were paired with a resident aggressor, thus hamsters given this dose of muscimol are capable of responding with normal submissive behaviors when attacked.

**Experiment 3: VHPC/acquisition of conditioned defeat**

The purpose of Experiment 3 was to determine whether infusion of muscimol into the VHPC would reduce the acquisition of conditioned defeat. Animals (n = 41) were matched by weight and randomly assigned to one of two conditions. Hamsters received infusions of either muscimol (1.1, 2.2, or 2.7 nmol in 300 nl saline) or vehicle immediately before being placed in the cage of a resident aggressor for 15 min. Twenty-four hours later, all hamsters were tested for 5 min in their own home cage in the presence of a non-aggressive intruder.

**Experiment 4: VHPC/expression of conditioned defeat**

The purpose of Experiment 4 was to determine whether infusion of muscimol into the VHPC would reduce the expression of conditioned defeat. Animals (n = 17) were matched by weight and randomly assigned to one of two conditions. All hamsters were placed into the cage of a resident aggressor for 15 min for conditioned defeat training. On the following day, animals received infusions of either muscimol (2.7 nmol in 300 nl saline) or vehicle immediately before being tested in their own home cage for 5 min with a non-aggressive intruder. In an effort to reduce the number of animals required, we

started with only one dose of muscimol to determine whether this relatively high dose would reduce the expression of CD. Further investigation using intermediate doses of muscimol were not warranted because infusion of a high dose of muscimol (2.7 nmol) had no effect on CD (See Results).

**Statistical analyses**

For all experiments, the total duration (seconds) of each behavior displayed (submissive and defensive, aggressive, social, and non-social) was determined. The mean total duration of each behavior was compared using a one-way analysis of variance (ANOVA). Significant differences for all analyses were ascribed at $P < 0.05$. Statistically significant differences were analyzed further using a Tukey-Kramer multiple comparison post-hoc tests to compare all pairwise differences among group means.

## Results

No animals had to be removed from any of the experiments in these studies due to a serious bite (i.e., one that caused bleeding) during training.

### Experiment 1: Muscimol infused into the DHPC does not reduce the acquisition of conditioned defeat

A total of 36 animals were used in the statistical analysis: vehicle (n = 9), 1.1 nmol (n = 7), 2.2 nmol (n = 12), 3.3 nmol (n = 8). Infusion of muscimol into the DHPC immediately before defeat did not reduce the display of submissive and defensive behaviors ($F_{(3,35)} = .072$; $P > 0.05$, Figure 1). In addition, there were no significant differences observed in aggressive ($F_{(3,35)} = 0.924$; $P > 0.05$), social ($F_{(3,35)} = 1.23$; $P > 0.05$), and non-social ($F_{(3,35)} = 0.177$; $P > .05$) behaviors (Figure 1). Histological analysis revealed that needle placements were mainly within the DHPC (Figure 2). Two animals

had bilateral placements in which the needles extended beyond the DHPC into the lateral posterior thalamic nucleus and one animal had bilateral placement in which the needles did not reach the DHPC and were instead placed into corpus callosum dorsal to the DHPC. The behaviors of these animals were statistically similar to those that received infusion of vehicle (duration of submissive behavior; M = 115, SEM = ± 23.41). Infusion sites for two animals were not able to be verified as a result of blocked cannulae at the time of dye infusion and these were omitted from the experiment.

**Experiment 1a: Muscimol infused into the DHPC reduces habituation to an open field**

The results of Experiment 1 indicated that inactivation of the DHPC prior to defeat training had no effect on the display of submissive and defensive behaviors during testing. In an effort to demonstrate that infusion of muscimol into the DHPC could alter hamster behavior in some way, we used habituation to an open field as a positive control (Vianna et al. 2000). In this model, animals are placed in an open field for five minutes on two consecutive days, and the total number of line crosses is recorded. A reduction in the number of line crosses on the second day indicates that habituation to the open field has occurred (i.e., the subject remembers the context). Following infusion of muscimol (1.1 and 2.2 nmol) hamsters exhibited significantly fewer line crosses on day 1 than did controls, and on day 2 muscimol-treated hamsters showed no habituation to the open field. By contrast, vehicle-treated hamsters crossed significantly fewer lines on day 2 compared to day 1 (data not shown).

**Experiment 2: Muscimol infused into the DHPC does not reduce the expression of conditioned defeat**

A total of 25 animals were used in the statistical analysis: vehicle (n = 11), 1.1 nmol (n = 5), 2.2 nmol (n = 9). Infusion of muscimol into the DHPC immediately before animals were tested with a non-aggressive intruder did not reduce the expression of submissive and defensive behaviors compared with animals that received vehicle control ($F_{(2,24)} = 0.755$; $P > 0.05$, Figure 3). Additionally no significant differences were observed in aggressive ($F_{(2, 24)} = 0.874$; $P > 0.05$), social ($F_{(2,24)} = 2.441$; $P > 0.05$), and nonsocial ($F_{(2, 24)} = 2.992$; $P > 0.05$) behaviors (Figure 3). Histological analysis revealed that needle placements were localized mainly within the DHPC (Figure 2). A total of six animals were removed from statistical analysis. Two animals received unilateral DHPC infusion on one side with external capsule placement on the other side. The infusion site for four animals could not be verified as a result of blockade in one or both cannulae at the time of dye infusion.

**Experiment 3: Muscimol infused into the VHPC reduces the acquisition of conditioned defeat**

A total of 32 animals were used in the statistical analysis: vehicle (n = 8), 1.1 nmol (n = 8), 2.2 nmol (n = 9), 2.7 nmol (n = 7). Infusion of muscimol immediately before training significantly reduced the display of submissive and defensive behaviors during subsequent testing ($F_{(3, 31)} = 4.096$; $P < 0.05$, Figure 4) without altering the behavior of either the resident aggressors or subjects during training. For example, the duration of aggressive behavior was similar for all resident aggressors (M = 495 sec, SEM = ± 41.1) and the duration of submissive behavior was similar for all experimental

hamsters (M = 548 sec, SEM = ± 39.34) during training regardless of the drug state of the experiment hamster. Post-hoc analysis revealed that infusion of muscimol into the VHPC reduced submissive and defensive behaviors at all doses when compared with animals receiving vehicle control ($P < 0.05$). No differences in submissive and defensive behaviors were observed among doses of muscimol. Infusion of muscimol also increased non-social behavior ($F_{(3, 31)} = 7.895$; $P < 0.05$, Figure 4) at all doses compared with animals receiving vehicle control ($P < 0.05$). Finally, there were no significant differences in aggressive ($F_{(3, 31)} = 0.768$; $P > 0.05$) and social ($F(3, 31) = 0.137$; $P > 0.05$) behaviors (Figure 4). Histological analysis revealed that needle placements were localized mainly in the VHPC (Figure 5). A total of five animals were excluded from the analysis. Two animals had bilateral needle placements that extended beyond the VHPC into the amygdalohippocampal area. One animal received unilateral VHPC infusion and the other infusion was placed in the lateral entorhinal cortex. The infusion site for one animal could not be verified as a result of blocked cannulae at the time of dye infusion

**Experiment 4: Muscimol infused into the VHPC does not reduce the expression of conditioned defeat**

A total of 17 animals were included in the analysis: vehicle (n = 8), 2.7nmol (n = 9). Infusion of muscimol immediately before testing with a non-aggressive intruder did not reduce the expression of submissive and defensive behaviors compared with animals that received vehicle control ($F_{(1,16)} = 0.81$; $P > 0.05$, Figure 6). No significant differences were observed in aggressive ($F_{(1, 16)} = 2.794$; $P > 0.05$), social ($F_{(1, 16)} = 0.012$; $P > 0.05$), and non-social behavior ($F_{(1,16)} = 0.216$; $P > 0.05$, Figure 6). Histological analysis revealed that the needle placements were mainly in the VHPC (Figure 5). The infusion

site for one animal could not be verified due to blocked cannulae at the time of dye infusion.

## Discussion

The present experiments indicate that infusion of the $GABA_A$ agonist, muscimol into the VHPC reduces the acquisition but not expression of conditioned defeat while infusion of muscimol into the DHPC has no effect on the acquisition or expression of CD. Temporary inactivation of the VHPC immediately prior to training reduced the duration of submissive and defensive behavior during testing 24 hr later. These data are the first to suggest that the VHPC is a part of the neural circuit whereby aversive social experience leads to changes in future social behavior. The results of these experiments also support the hypothesis that a functional dissociation exists between the dorsal versus ventral portions of the hippocampus (Risold and Swanson, 1996; Moser and Moser, 1998; Bannerman et al. 2004).

The finding that the VHPC plays an important role in the acquisition of CD provides further support for the hypothesis that this brain area is important in the acquisition of fear and anxiety-like behaviors. VHPC lesions reduce avoidance of the open arms of an elevated T-maze (Trivedi and Coover, 2004), anxiety-like behaviors in a social interaction test (McHugh et al. 2004) as well as anxiety-like responses to an ethologically-relevant threat stimulus (Pentowski et al., 2006). The VHPC has reciprocal connections to multiple brain regions that are important in fear, anxiety, and stress-responsivity (Bast et al., 2001; Moser and Moser, 1998; Petrovich et al. 2001) such as the amygdala, a region that our laboratory has shown to be critically important in the

acquisition and expression of conditioned defeat (Jasnow and Huhman, 2001). The fact that temporary inactivation of the VHPC reduces the acquisition but not expression of CD raises the possibility that the VHPC is more important for encoding information about the social environment and less important for the actual retrieval and subsequent expression of behavioral responses to social stress.

These experiments also showed that temporary inactivation of the DHPC has no effect on either the acquisition or expression of CD. These results are perhaps surprising given the findings of Lai et al. (2005) suggesting that the anterior DHPC is important in social recognition in Syrian hamsters following a previous social defeat. In their study, hamsters were exposed to two conspecifics with which they had different experiences (i.e., exposure across a wire-mesh barrier or a fight). When tested in a Y-maze, the defeated hamsters avoided the familiar winner and were attracted to the neutral stimulus male. Hamsters exposed to a familiar winner showed several regions with elevated c-Fos and Erg-1 immunohistochemistry staining, including CA1 of the anterior DHPC, when compared to those that were exposed to a neutral stimulus male. Further, temporary inactivation of this area with lidocaine eliminated avoidance of the familiar dominant opponent. One possible explanation for this discrepancy could be the differences in the behavioral procedures used. Lai et al. (2005) exposed experimental subjects to three, brief defeat sessions between which each subject was returned to their home cage. It is possible that three, separate exposures to a conspecific leads to a recruitment of the DHPC in order to encode information about individual identity whereas a single pairing does not.

In Experiment 3 it should be noted that in addition to exhibiting reduced duration of submissive/defensive behaviors, hamsters that received pre-training infusions of muscimol into the VHPC also demonstrated increases in the duration of non-social

behavior on the subsequent testing day. Muscimol, particularly at high doses, can produce ataxia and sedation, and one might argue that the increase in non-social behavior in Experiment 3 could be due to a carry-over effect of muscimol 24-hr following infusion. This is unlikely, however, because in Experiment 4 hamsters received muscimol infusions immediately before the testing session, and these hamsters did not show any changes in submissive/defensive, aggressive, social and non-social behavior when compared to hamsters that received vehicle. Thus, the increase in non-social behavior exhibited by hamsters that received muscimol may actually reflect a more subtle avoidance of the non-aggressive animal which we conservatively did not score as submissive/defensive behavior. In addition, the total duration of social behavior did not differ among groups in Experiment 3, further indicating that muscimol does not affect all behaviors non-specifically.

These studies are among to first to demonstrate a functional dissociation between the DHPC and VHPC in an ethologically-relevant form of social stress-induced behavioral plasticity. In addition, these studies suggest that the VHPC is a component of the neural circuit underlying the behavioral changes that occur following defeat. Interestingly, reciprocal connections exist between the VHPC and the amygdala, a region that is critical for conditioned defeat (Jasnow and Huhman, 2001). Ongoing studies in our laboratory are investigating the interaction between these two brain regions to determine how the interaction between the VHPC and the amygdala contributes to the acquisition of conditioned defeat.

**Acknowledgements**

This work was supported by Grant MH62044 to KLH, the Brains and Behavior Program at Georgia State University and in part by the Science and Technology Center Program (Center for Behavioral Neuroscience) of the National Science Foundation under agreement IBN-987675. We thank Alisa Norvelle and the Animal Care Staff at Georgia State University for their technical assistance.

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Figure Legends

*Figure 1*: Total duration (mean ± S.E.M.) of submissive/defensive (a), aggressive (b), social (c), and non-social (d) behavior displayed by defeated hamsters during a 5-min test with a non-aggressive intruder. Animals received bilateral infusion of 0, 1.1 nmol, 2.2 nmol, or 3.3 nmol of muscimol into the DHPC immediately before being defeated for 15 min. There was no effect of drug on any behavioral class.

*Figure 2*: Histological reconstructions of injection sites of animals receiving infusions into CA1 of the DHA in Experiment 1(A) and Experiment 2 (B). Shaded dots represent the site of injection in one or more animals. Shaded squares represent anatomical misses. Drawings are adapted from Morin and Wood (2001).

*Figure 3*: Total duration (mean ± S.E.M.) of submissive/defensive (a), aggressive (b), social (c), and nonsocial (d) behavior displayed by defeated hamsters during a 5-min test with a non-aggressive intruder. Animals received bilateral infusions of 0, 1.1 nmol, or 2.2 nmol of muscimol into the DHA immediately before being tested with a non-aggressive intruder for 5 min.

*Figure. 4*: Total duration (mean ± S.E.M.) of submissive/defensive (a), aggressive (b), social (c), and non-social (d) behavior displayed by defeated hamsters during a 5-min test with a non-aggressive intruder on Day 2. Animals received bilateral infusion of 0, 1.1 nmol, 2.2 nmol, or 2.7 nmol of muscimol into CA1 of the VHPC immediately before being defeated for 15 min on Day1. Significant differences are indicated by unshared letters ($P < 0.05$).

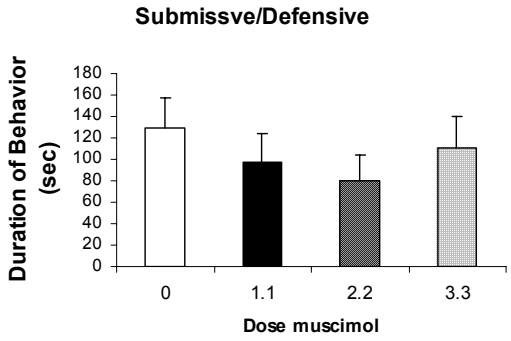
*Figure.5*: Histological reconstructions of injection sites for animals receiving infusions into CA1 of the VHPC in Experiment 3 (a) and Experiment 4 (b). Shaded dots represent

the site of injection in one of more animals. Shaded squares indicate anatomical misses. Drawings are adapted from Morin and Wood (2001).

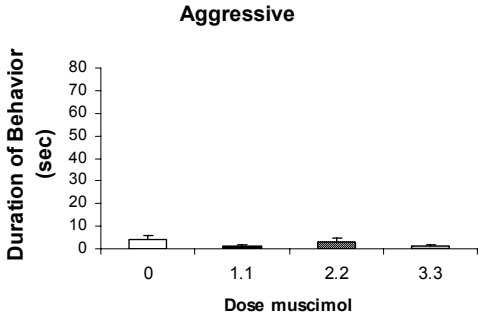
*Figure 6*: Total duration (mean ± S.E.M.) of submissive/defensive (a), aggressive (b), social (c), and non-social (d) behavior displayed by hamsters during a 5-min test with a non-aggressive intruder. Animals received bilateral infusion of 0 or 2.7 nmol of muscimol into CA1 of the VHPC immediately before being tested with a no-aggressive intruder for 5 min.

Figure 1

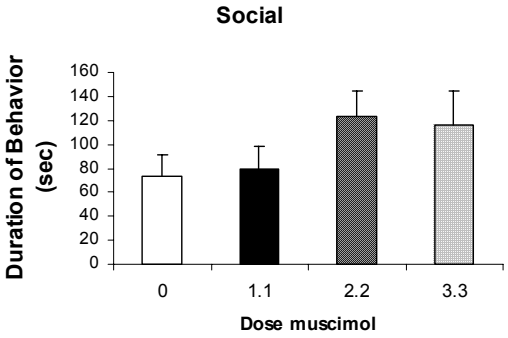
a)




b)




c)




d)

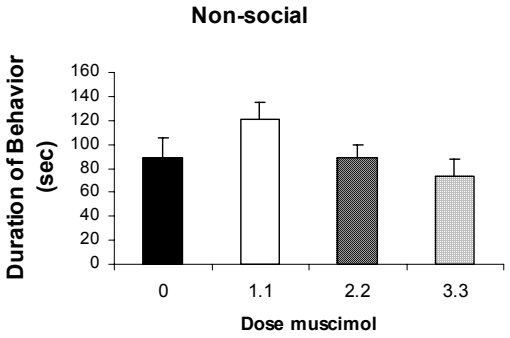
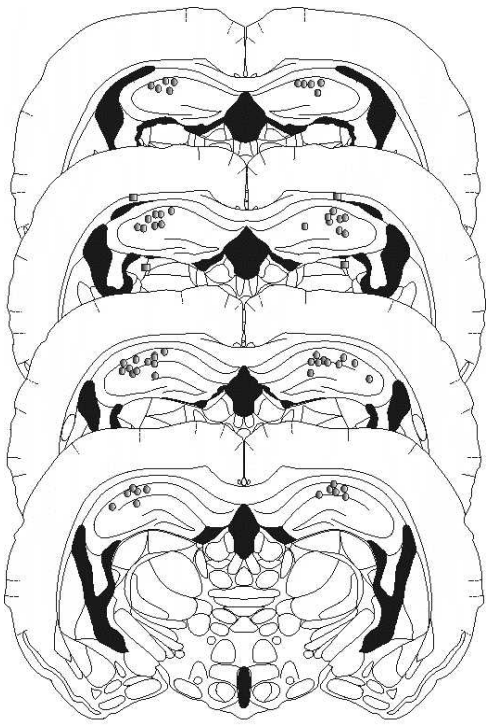


Figure 2

A)



B)

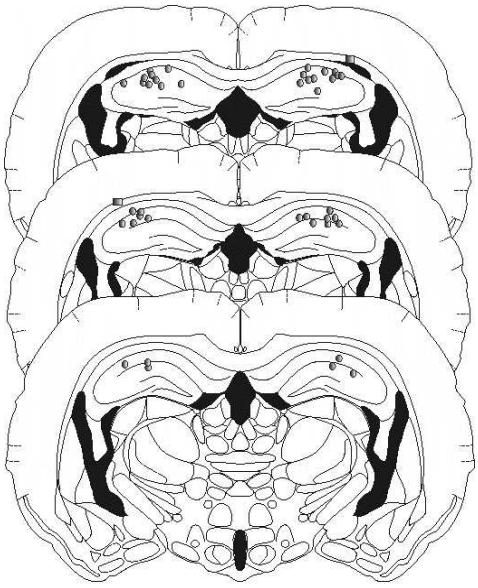


Figure 3

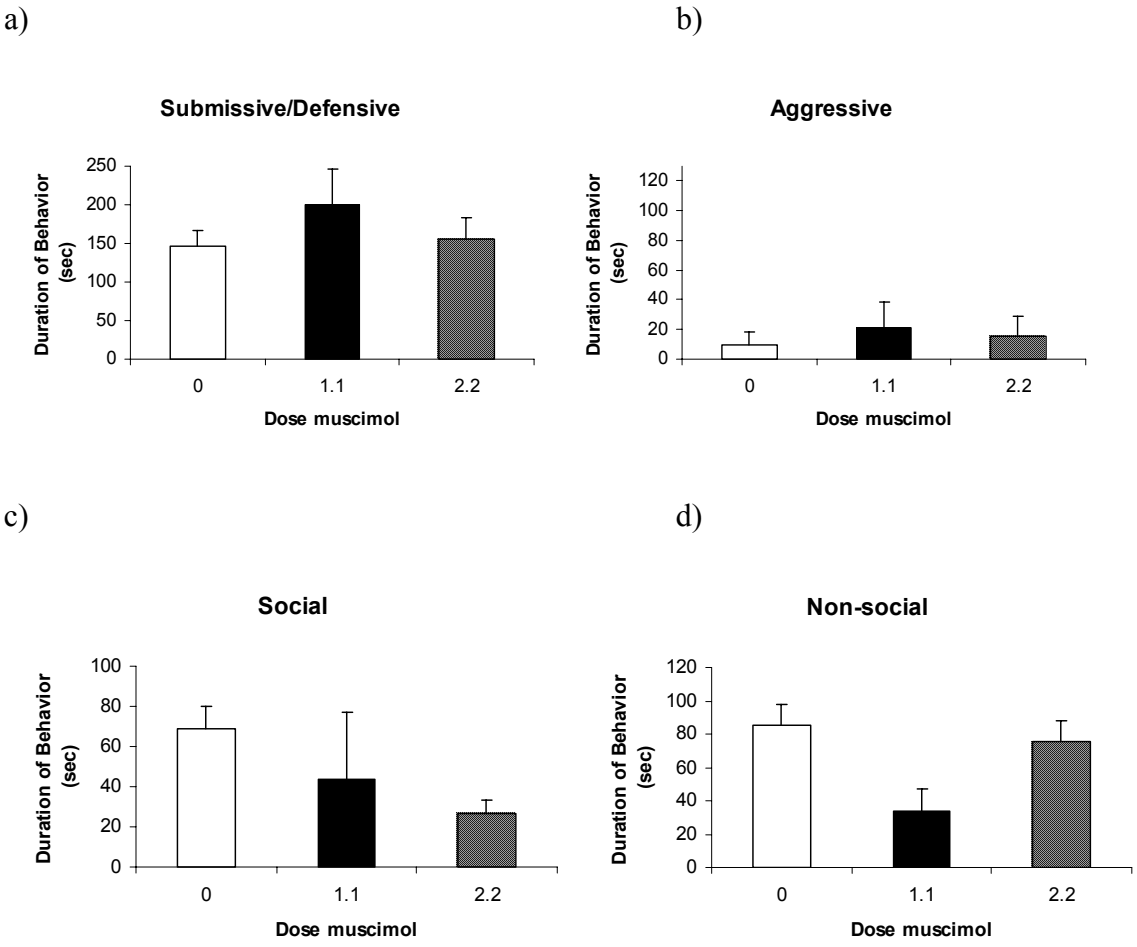
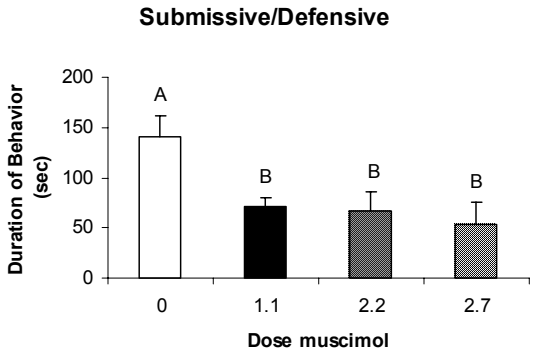

a)
Submissive/Defensive
Duration of Behavior (sec)
250
200
150
100
50
0
0
1.1
2.2
Dose muscimol
b)
Aggressive
Duration of Behavior (sec)
120
100
80
60
40
20
0
0
1.1
2.2
Dose muscimol
c)
Social
Duration of Behavior (sec)
100
80
60
40
20
0
0
1.1
2.2
Dose muscimol
d)
Non-social
Duration of Behavior (sec)
120
100
80
60
40
20
0
0
1.1
2.2
Dose muscimol

Figure 4

a)

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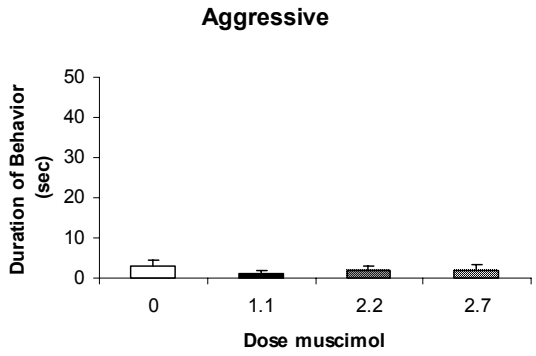


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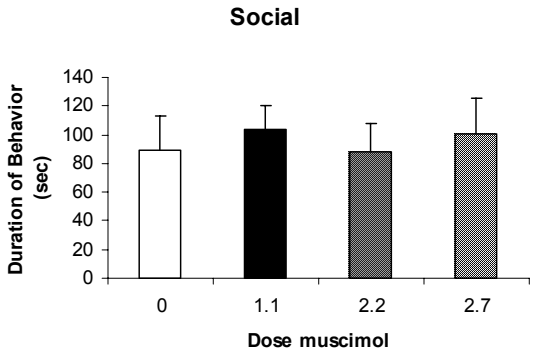


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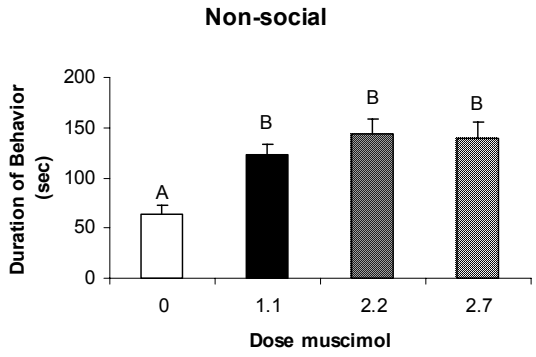
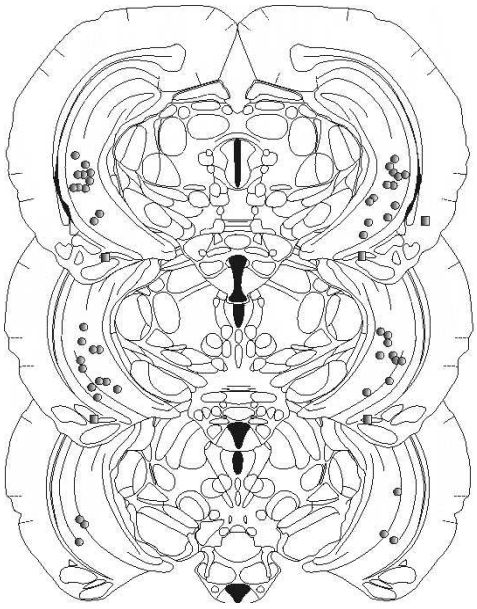


Figure 5

A)



B)

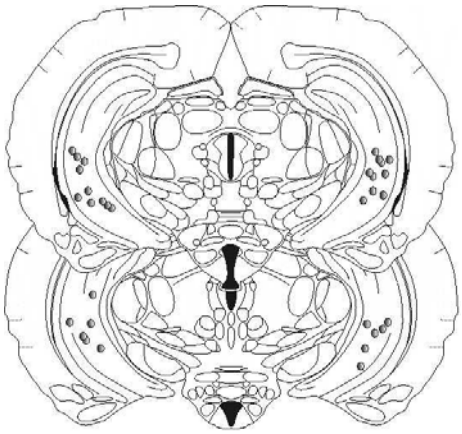
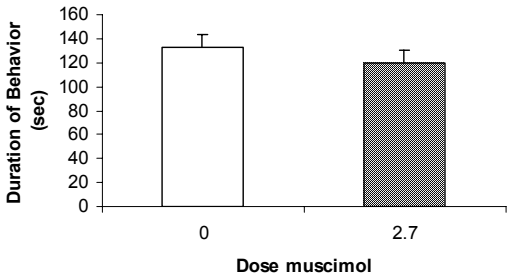


Figure 6

a)

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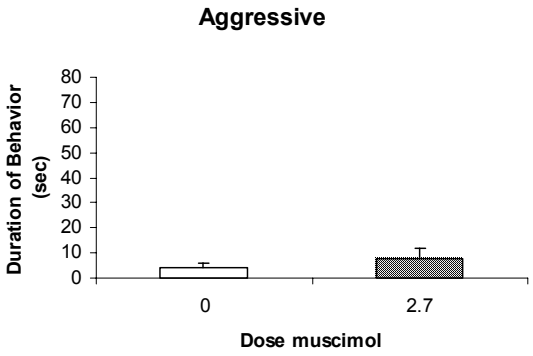


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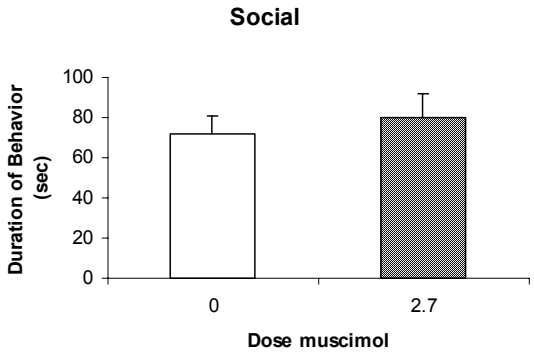
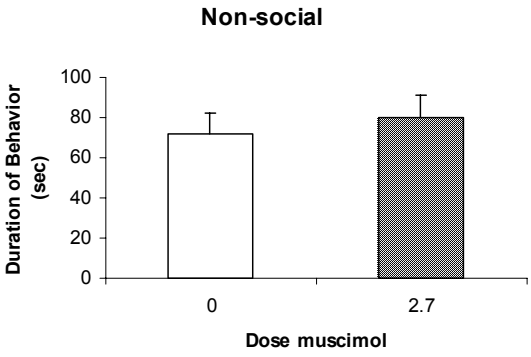



d)



# CHAPTER 3

# DIFFERENTIAL BDNF EXPRESSION IN LIMBIC BRAIN REGIONS FOLLOWING SOCIAL DEFEAT VS. TERRITORIAL AGGRESSION

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Running title: SOCIAL CONFLICT ALTERS BDNF IN SYRIAN HAMSTERS

Keywords: stress, plasticity, basolateral amygdala, hippocampus

## Abstract

Syrian hamsters are solitary animals that exhibit aggressive behavior and readily defend their home territories under laboratory conditions. Following social defeat, however, losing hamsters no longer defend their home territories but instead submit to intruding conspecifics even when the intruder is non-threatening. The mechanisms underlying this experience-induced behavioral plasticity and the neural differences between winning and losing animals in agonistic behavior are unclear. The present study tested the hypothesis that changes in brain-derived neurotrophic factor (BDNF) mediate this form of emotional plasticity. Male hamsters were paired for 15-min using a resident-intruder model, and individuals were identified as winners or losers on the basis of their behavior. Novel cage control animals were placed into another animal's empty cage for 15-min. BDNF was examined with *in situ* hybridization 2 hours after treatment during the consolidation period of emotional learning. Losing animals had significantly more BDNF mRNA in the basolateral (BLA) and medial (MeA) nuclei of the amygdala when compared to winning animals as well as novel cage and home cage controls. Interestingly, winning animals had significantly more BDNF mRNA in the dentate gyrus of the dorsal hippocampus (DHPC DG) and in CA1 of the ventral hippocampus compared to losing animals, novel and home cage controls. No conflict-related changes in BDNF mRNA were observed several other regions including the bed nucleus of the stria terminalis and central amygdala. Next, we demonstrated that K252a, a Trk receptor antagonist, significantly reduced the acquisition of conditioned defeat when administered within the BLA. These data suggest a model in which BDNF-mediated plasticity within

the BLA supports learning of social defeat in losing animals, whereas BDNF-mediated plasticity within the hippocampus may support learning of territory in winning animals.

## Introduction

Virtually all organisms are exposed to stress. An understanding of the behavioral and physiological responses to stress is important because many psychopathologies (i.e., depression and post-traumatic stress disorder) are stress-related. Stress physiology can be studied in the laboratory with a variety of models including foot-shock, forced swim, and prolonged periods of restraint. These stressors are reliable, but they may have little relevance to the daily lives of most organisms. Social stress reliably affects the brain and behavior and is experienced by a wide variety of organisms. Laboratory models of social stress include the visible burrow system, over-crowding, and social defeat. Social stress models are particularly useful because they are considered ethologically relevant and because much, if not most, of the stress encountered by humans occurs in a social context (Plotsky et al., 1998; Bjorkqvist, 2001; Buwalda et al, 2005).

Social stress in non-humans occurs largely in the form of social conflict. Such conflicts generally occur between two male conspecifics that are competing for access to resources such as food or territory as well as for potential mates. At the conclusion of the conflict, a "winner" (i.e., dominant) and "loser" (i.e., subordinate) are readily identifiable. Dominant and subordinate animals often display disparate physiological responses following social conflict with subordinate animals demonstrating increased activation of the hypothalamic-pituitary adrenal (HPA) axis when compared with dominant animals (Huhman et al., 1990; Huhman et al., 1991; Huhman et al., 1992; Blanchard et al., 1995; Spencer et al., 1996). Other physiological changes that occur in subordinate animals include increases in autonomic system activity (Brain, 1980) heart rate, blood pressure, core body temperature (Meehan et al., 1995; Tornatzky et al., 1993), and adiposity

(Foster et al., 2006). Chronic social defeat also suppresses immune function as measured by lymphatic organ size (Blanchard et al., 1995) and humoral immune function (Bohus et al., 1993, Fleshner et al., 1989, Jasnow et al., 2001).

Striking behavioral changes are often observed in subordinate animals following an agonistic encounter. For example, subordinates may display decreases in overall locomotor activity and social contact (Blanchard and Blanchard, 1989; Meerlo et al., 1996, Meerlo et al, 1997, Shively, 1998). Chronic social defeat also alters food and water consumption (Meerlo et al., 1996, Foster et al., 2006) and disrupts reproductive behaviors (Blanchard & Blanchard, 1989). Finally, subordinate animals display increased submissive and defensive behaviors when in the presence of a conspecific, and they often fail to defend their own territory (Meerlo et al., 1996, Potegal et al., 1993, van de Poll et al., 1982). An example of this occurs in male Syrian hamsters. After being briefly exposed to a larger, more aggressive counterpart, defeated male hamsters subsequently fail to display normal territorial aggression even when a non-aggressive, smaller hamster is introduced into the home cage of the defeated animal. This behavioral change is termed conditioned defeat (Potegal et al., 1993) and is maintained for at least one month in many animals (Huhman et al., 2003).

Because social stress is hypothesized to be important in the etiology of depression and a variety of anxiety disorders (Nemeroff, 1998; Arborelius et al., 1999; Patten, 1999), it is critical to study the neurobiological processes involved in the behavioral plasticity following social stress. An attractive candidate for mediating social stress-induced behavioral changes is brain-derived neurotrophic factor (BDNF).

BDNF is a neurotrophin that belongs to the nerve growth factor family of peptides. The neurotrophins (including nerve growth factor (NGF), neurotrophin-3 (NT-3), neurtrophin 4/5, acidic- fibroblast growth factor (FGF), basic FGF, and BDNF) are well-known for their role in the development and maintenance of the nervous system (Barde et al., 1982; Leibrock et al., 1989). BDNF and its receptor, TrkB, are critical for synaptic and behavioral plasticity (Lo, 1995; Thoenen, 1995; McAllister et al., 1999). BDNF is rapidly upregulated in the hippocampus following spatial and contextual learning (Hall et al., 2000; Mizuno et al., 2000) and in the amygdala following fear conditioning (Rattiner et al., 2004a)

BDNF mRNA expression is also altered in response to stress. Traditional models of stress such as immobilization, footshock, and forced swimming all decrease BDNF mRNA in the hippocampus (Smith et al., 1995; Ueyama et al., 1997). Only a few studies have examined how social stress alters BDNF in the brain. Fiore et al. (2004) found differences between dominant and subordinate aged male mice in NGF and BDNF in that dominant animals had higher levels of BDNF mRNA in the subventricular zone and hippocampus than did subordinate animals. Conversely, subordinate animals exhibited higher levels of NGF compared with dominant animals in these regions. Pizarro et al. (2004) found that BDNF mRNA in mice exposed to a 10-min social defeat was lower when compared to non-defeated animals, however, this decrease was detected in all brain regions studied (e.g., cortical and subcortical) which raises the possibility that this decrement was not region-specific. Interestingly, Berton et al. (2006) demonstrated that BDNF protein levels in the nucleus accumbens are upregulated following ten days of chronic social defeat. Together, these studies provide important information regarding the

effects of social defeat on BDNF in the brain, yet each has a potential limitation (e.g., aged animals, non-specific effects, prolonged and severe levels of stress) which might limit their generalizability. The goal of Experiment 1 was to examine the effects of exposure to an acute social conflict on BDNF mRNA in the brain of male Syrian hamsters. Experiment 1 tested the prediction that following social conflict, winning and losing animals will exhibit differences in BDNF mRNA in brain regions that are important in stress-responsivity and social behavior.

A functional role for BDNF has been demonstrated primarily in the hippocampus wherein BDNF enhances long-term potentiation (LTP; Figurov et al., 1996). In addition, BDNF-mutant mice show a deficit in LTP, an effect which can be reversed by exogenous application of BDNF (Patterson et al., 1996). Infusion of BDNF antibodies into the hippocampus prior to training results in impaired spatial learning and memory (Mu et al., 1999), and conditional TrkB knockout mice, in which the knockout of the TrkB gene is restricted to the forebrain and occurs during postnatal development, fail to successfully learn the Morris water maze (Minichiello et al., 1999).

Recently, a functional role of BDNF has also been demonstrated in regions outside of the hippocampus. For example, Rattiner et al. (2004a) showed that BDNF/TrkB signaling within the BLA is necessary for the acquisition of conditioned fear. Additionally, BDNF/TrkB signaling in the mesolimbic dopamine pathway (i.e., ventral tegmental area and nucleus accumbens) has been shown to be important for the development of social withdrawal/aversion in previously defeated mice (Berton et al., 2006).

Given that BDNF is upregulated in the BLA, a region that has been shown to be critical for fear conditioning as well as conditioned defeat (Jasnow and Huhman, 2001) the goal of Experiment 2 was to determine the functional significance of this increase. Therefore, Experiment 2 tested the hypothesis that neurotrophin signaling in the BLA is necessary for the acquisition of conditioned defeat. Unfortunately, a selective TrkB receptor antagonist does not exist so it is impossible to specifically assess, pharmacologically, the functional role of BDNF/TrkB signaling in conditioned defeat. Instead, we used K252a, a non-selective Trk receptor antagonist to evaluate the general role of neurotrophins in mediating the behavioral effects of social defeat as a first assessment of the broader hypothesis that neurotrophin receptor activation is necessary for the acquisition of conditioned defeat.

## Results

*Experiment 1*

One pair of animals did not exhibit any agonistic behavior and did not establish a strong winner/loser relationship. Thus, a total of 28 animals were used in the analysis: winners (n=7), losers (n=7), novel cage control (n=6), and home cage control (n=6). There was a significant difference in BDNF mRNA in the BLA among winners, losers, novel cage and home cage controls 2 hours after the social interaction ($F_{(3, 27)} = 34.38$, $P < 0.05$, Figure 7). Post-hoc analysis revealed that losers had more BDNF mRNA in the BLA than did winners, novel cage and home cage controls. In addition, winners had more BDNF mRNA in the BLA than did novel cage and home cage controls (Figure 10).

A significant difference in BDNF mRNA in the MeA among the groups was also detected ($F_{(3, 27)} = 30.85$, $P < 0.05$, Figure 7). Post-hoc analysis showed that losers had

higher BDNF mRNA levels in the MeA than did winners, novel and home cage controls. Winning animals had higher BDNF mRNA levels than did novel and home cage controls. Finally, novel cage controls had higher BDNF mRNA levels than did home cage controls (Figure 10).

There was also a significant difference among the groups in BDNF mRNA in the dentate gyrus of the dorsal hippocampus (DHPC DG, $F_{(3, 27)} = 8.47$, $P < 0.01$, Figure 7). Post-hoc tests revealed that winners had more BDNF mRNA in DHPC DG than did losers, novel, and home cage controls. Additionally, losers had more BDNF mRNA in DHPC DG than did novel and home cage controls (Figure 10).

A significant difference in BDNF mRNA was found among the groups in CA1 of the ventral hippocampus (VHPC CA1, $F_{(3, 27)} = 3.06$, $P < 0.05$, Figure 8). Further analysis showed that winners and losers did not differ from one another but both had more BDNF mRNA in VHPC CA1 than did novel and home cage controls (Figure 10).

No significant differences among the groups were detected in the following regions: infralimbic cortex ($F_{(3, 27)} = 0.50$, $P > 0.05$), anterior hypothalamus($F_{(3, 27)} = 0.1.77$, $P > 0.05$) , ventromedial hypothalamus ($F_{(3, 27)} = 0.11$, $P > 0.05$), nucleus accumbens ($F_{(3, 27)} = 0.85$, $P > 0.05$), anterior bed nucleus of the stria terminals ($F_{(3, 27)} = 0.96$, $P > 0.05$), posterior bed nucleus of the stria terminals ($F_{(3, 27)} = 0..75$, $P > 0.05$), DHPC CA1 ($F_{(3, 27)} = 0.42$, $P > 0.05$), DHPC CA3 ($F_{(3, 27)} = 0.61$, $P > 0.05$), VHPC CA3($F_{(3, 27)} = 0.11$, $P > 0.05$) , VHPC DG ($F_{(3, 27)} = 0.62$, $P > 0.05$), and central amygdala ($F_{(3, 27)} = 0.28$, $P > 0.05$, Figure 9) .

A significant, positive correlation was found between the duration of submissive behavior and BDNF mRNA in the MeA ($R^2 = 0.721$, $P < 0.05$). Significant, negative

correlations were detected between duration of aggressive behavior and BDNF mRNA in the MeA ($R^2$ = -0.618, $P < 0.05$) and BLA ($R^2$ = -0.742, $P < 0.05$) as well as the duration of non-social behavior and BDNF mRNA in the MeA ($R^2$ = -0.608, $P < 0.05$). Finally, in the DHPC DG a significant, negative correlation was found between BDNF mRNA and the duration of submissive behavior ($R^2$ = -0.542, $P < 0.05$, Table 1).

*Experiment 2: Acquisition of conditioned defeat*

*Effects of infusion of K252a into the BLA on the acquisition of conditioned defeat*

A total of 20 animals with bilateral cannula placements in the BLA were used in the statistical analysis: vehicle (n = 9), K252a (n = 11). The duration of submissive/defensive behavior was significantly reduced in animals that received infusion of K252a immediately before defeat training (M = 27.63, SEM = 9.03) when compared to animals that received vehicle (M = 61.22, SEM = 7.33), $t(18) = 2.789$, $p < 0.05$ (Figure 11). There were no significant differences between animals that received K252a or vehicle in aggressive, social, and non-social behavior. Of the 11 animals that were excluded, two lost their cannula during the recovery period and could not be used in the study. Seven animals had injections that were not localized to the BLA. Two of these animals had bilateral placements in the central amygdala (capsular) while one had unilateral placements in the central amygdala with BLA placement on the other side. Two animals had bilateral placements in the ventral endopiriform nucleus and one had unilateral placement in the ventral endopiriform nucleus on one side with BLA placement on the other side. One animal had unilateral placement in the posterior basomedial amygdalar nucleus on one side with BLA placement on the other side. Two animals did not receive injections as a result of obstructed cannulae (Figure 12).

Of the animals that were considered bilateral anatomical "misses", three received K252a infusion and one received vehicle infusion. The animals that received K252a showed levels of submissive and defensive (M = 55.04, SEM = 11.34) behaviors during the testing session that was comparable to that of vehicle controls. Of those animals considered unilateral anatomical "misses", four received K252a infusions and one received vehicle infusions. These animals also showed levels of submissive and defensive behaviors comparable to vehicle controls (M = 65.03, SEM = 8.1).

Because K252a infused prior to the training session reduced submissive and defensive behaviors during testing, one might argue that such a reduction could be due to the fact that the training session for animals that received vehicle was different from that experienced by animals that received K252a (i.e., the behavior of the resident aggressor was different depending on treatment). To address this issue, the behavior of the resident aggressor was scored during each training session to ensure that both groups of experimental hamsters experienced comparable levels of aggression. All experimental animals received high levels of aggression (Figure 13) and no significant differences in the behavior of the aggressors towards the two groups was noted during defeat training: submissive [$t(18) = -0.77$, $P > 0.05$] , aggressive [$t(18) = 6.26$, $P > 0.05$], social [$t(18) = 7.33$, $P > 0.05$] and non-social [$t(18) = 6.43$, $P > 0.05$]. All experimental animals, regardless of treatment, responded to the initial defeat training with high levels of submissive/defensive behaviors.

## Discussion

*Experiment 1: Agonistic behavior alters BDNF mRNA in the BLA, MeA, DHPC DG, and VHPC CA1*

The results of Experiment 1 indicate that exposure to an agonistic encounter is capable of producing specific changes in BDNF mRNA in a variety of brain regions. Losing, winning, novel and home cage control animals displayed differences in BDNF mRNA levels in the BLA, MeA, DHPC DG, and VHPC CA1 but not in the infralimbic cortex, anterior hypothalamus, ventromedial hypothalamus, nucleus accumbens, bed nucleus of the stria terminalis and central amygdala. In some cases, the duration of a particular behavior (i.e., submissive, aggressive, social, non-social) correlated either positively or negatively with the amount of BDNF mRNA in particular brain regions.

Our finding that social defeat stress increases BDNF mRNA in the BLA is not consistent with previous studies showing that non-social stressors (immobilization or restraint) and exposure to acute social defeat decrease BDNF mRNA (Smith et al., 1995; Xu et al., 2004; Pizarro et al., 2004). Pizarro et al. (2004) showed that mice exposed to social defeat exhibited decreased BDNF mRNA in several cortical and subcortical regions, including the BLA, 24 hours following defeat. There are several important methodological differences that might explain this inconsistency. First of all, it is possible that the BDNF mRNA response to a social stressor in which behavioral plasticity occurs (conditioned defeat) might be very different from the response to a non-social stressor. In addition, the differences between the social defeat methodology, particularly the 2hr versus the 24hr sampling period used in the Pizarro et al. study and the present study, respectively, might explain the opposite response of BDNF mRNA. A significant

literature now suggests that BDNF is rapidly upregulated during the consolidation period following various forms of learning including emotional fear learning (Jones et al., 2007, Rattiner, et al 2004a,b). Thus, it is quite possible that BDNF mRNA might increase immediately following a stressor as part of the synaptic plasticity mediating memory consolidation while it is reduced 24 hr later via a different mechanism mediating the effects of long-term stress. Further, hamsters in the current study underwent an acute social defeat by a single opponent while mice in the Pizarro et al. study were exposed to three defeat sessions by three different aggressors. It is possible that exposure to this more severe social stress results in decreased BDNF levels, while less intense forms of social stress such as an acute social defeat in hamsters result in an increase in BDNF.

Pizarro et al. (2004) demonstrated changes in BDNF mRNA in both cortical and subcortical regions in socially defeated mice. This finding may be problematic in that all regions examined showed similar changes in BDNF mRNA levels 24 hours following defeat. In the present study, we found that hamsters show very selective increases and/or decreases in specific brain regions. Furthermore, we included a novel cage control group in which hamsters were exposed to an empty aggressor's cage for 15 minutes. The inclusion of this group in addition to a home cage control allowed us to demonstrate that the changes we observed in both winning and losing hamsters were specific to social interaction and fight outcome and were not due simply to exposure to a novel environment.

It is important to note that the nature of agonistic interactions also varies among species. During exposure to an aggressor, defeated hamsters emit behavioral signals (e.g., submissive postures) that are thought to decrease the likelihood of a subsequent attack.

Thus, it is important for losing hamsters to be able to alter their behavior (i.e., increase submission and defense) when confronted with an aggressive counterpart. Increased BDNF may be important in mediating the social learning that occurs in losing hamsters. Defeated mice also produce submissive and defensive behaviors when attacked by an aggressor; however; it is not known if these behaviors alter the course of the fight in the same way that they appear to do in hamsters.

Recent data from our laboratory suggest that at least some of the plasticity underlying conditioned defeat occurs in the BLA. For example, over-expression of CREB in the BLA enhances the memory of social defeat (Jasnow et al., 2005), while pretraining BLA infusions of ifenprodil (an NMDA NR2B subunit antagonist, D.E. Day and K.L. Huhman, SFN abstract) or anisomycin (a protein synthesis inhibitor, Markham and Huhman, 2008) both reduce the behavioral effects of social defeat. The results of this experiment are consistent with the hypothesis that plasticity in the BLA mediates, at least in part, the behavioral changes observed following defeat and suggest that BDNF may be an important molecular mediator of these changes.

The finding that losing animals had higher levels of BDNF mRNA in the MeA than did winning animals, novel and home cage controls is also interesting given the role of the MeA in processing chemosensory information. This increase suggests that the MeA may also be an important site of plasticity following social interactions, however Markham and Huhman (2008) recently found that pre-training infusion of anisomycin into the MeA does not reduce the behavioral effects of social defeat. This finding is interesting because, although we observed an increase in BDNF mRNA in losing animals, protein synthesis inhibition in this region has no effect on social-stress induced

behavioral changes. Hamsters rely heavily upon olfactory functioning to avoid predation, identify mating partners and in some cases to recognize a conspecific (Petrulis et al., 2004). Interestingly, novel cage control animals exhibited higher levels of BDNF mRNA in the MeA than did home cage controls, suggesting that exposure to a novel environment induces changes in BDNF. In addition, BDNF mRNA levels were higher in animals that engaged in a fight (i.e., winners and losers) than they were in both control groups. It may be the case that social contact involving exposure to novel odor stimuli induces some degree of plasticity in the MeA to effectively encode chemosensory information relevant to social interaction but that this plasticity is not critical for conditioned defeat.

Our results also indicated that specific subregions of the DHPC and VHPC showed differences in BDNF mRNA among losers, winners, novel and home cage controls. Most studies that examine changes in BDNF mRNA levels following stress, including social defeat, report decreases in BDNF mRNA in the hippocampus (Smith et al., 1995, Nibuya et al., 1995, Pizarro et al., 2004). Again, most of these studies involve chronic stress or examination of BDNF following a prolonged period, in contrast to our current study where we examined BDNF during the consolidation period immediately following an acute emotional learning event. We found that winning animals had significantly higher BDNF mRNA levels in DHPC DG than did losers, novel and home cage controls. This finding suggests that the behaviors associated with aggression and winning a fight may also involve plastic mechanisms. In other words, the finding that DHPC DG BDNF levels were greater in winners and BLA BDNF levels were greater in losers may be consistent with the notion that winners are encoding spatial representations

involved in defending their potential new territory, whereas losers may be primarily activating their fear and flight circuitry.

In VHPC CA1, losers and winners demonstrated higher levels of BDNF mRNA than did novel and home cage controls, but losers and winners did not differ significantly from one another. To date, few studies have examined how stress, either social or non-social, affects BDNF in the ventral portions of the hippocampus. Our data are interesting given recent finding from our laboratory suggesting that the VHPC is important for mediating the behavioral changes that occur in both losing and winning animals following an agonistic encounter (S.L. Taylor and K.L. Huhman, submitted). It is possible that BDNF signaling within the VHPC is a critical player in mediating these changes.

Finally, we applied several correlation analyses to detect a relationship between the duration of a particular behavior class and the level of BDNF mRNA in specific brain regions. Shorter durations of submissive behaviors as well as high levels of aggressive behaviors correlated with lower levels of BDNF mRNA in the MeA. In the BLA, longer durations of aggressive behaviors were correlated with lower levels of BDNF mRNA. While no relationship was detected between duration of submissive behavior and BDNF mRNA levels in the BLA, it is possible that engaging in submissive and defensive behaviors upregulated BDNF to support the behavioral changes associated with losing. Interestingly, a positive correlation was detected between duration of submissive behavior and levels of BDNF mRNA in the MeA. A negative correlation was detected between duration of submissive behavior and BDNF mRNA in DHPC DG.

Overall, these findings may be most consistent with a model in which: 1) BDNF is actively regulated at a transcriptional level during the memory consolidation period following social conflict in brain regions involved in emotional learning, 2) emotional learning-induced increases in BDNF in amygdala regions (BLA and MeA) in animals when social conflict resulted in losing and submissive behaviors, and 3) emotional learning-induced increases in BDNF in hippocampal regions (DHPC DG and VHPC CA1) in animals when social conflict resulted in winning and territorial aggressive behaviors.

*Experiment 2: Infusion of K252a reduces the acquisition of conditioned defeat*

The results of Experiment 2 indicate that neurotrophin activity in the BLA is important for the acquisition of conditioned defeat because blockade of Trk receptors in the BLA during the initial social defeat training resulted in a significant reduction in conditioned defeat.

The finding that blockade of Trk receptors during the initial social defeat session reduces the display of submissive and defensive behaviors 24-hours later during the testing session suggests that neurotrophic activity in the BLA is important for learning or encoding information about losing a fight. An alternative explanation is that the treatment altered the levels of agonistic behavior during the training session such that animals receiving vehicle were defeated differently than those that received K252a. In other words, it is possible that aggressors behaved differently towards drug animals. We maintain that this is not the case, however, because all animals received similar defeats regardless of treatment group (See Figure 13).

This study is among the first to demonstrate a functional role for neurotrophic factors in the behavioral changes that occur following social defeat. Although K252a is a non-selective neurotrophin receptor antagonist, it is very possible that BDNF in the BLA mediates at least some of the behavioral responses to social stress. Future studies should focus on selectively targeting TrkB receptors to directly assess the role of BDNF in conditioned defeat. It is also possible that other neurotrophins, such as nerve growth factor, play a role in these behavioral changes.

## Conclusions

Exposure to social and non-social stressors has been shown to alter both the brain and behavior. The study of how biologically-relevant stressors, such as social defeat, effect the brain and behavior is important for understanding the pathology that underlies fear and anxiety disorders such as post-traumatic stress disorder (PTSD). Here, we show that agonistic encounters in hamsters can alter BDNF mRNA in specific brain regions that are important in stress-responsivity, fear, social behavior, and learning. Furthermore, we demonstrate that neurotrophic activity in the BLA is important for the acquisition of conditioned defeat.

In conclusion, studies of BDNF show promise in elucidating the mechanisms by which social stress produces alterations in the brain and subsequent behavior. Ongoing studies in our laboratory are focusing on the plastic mechanisms that occur within the neural circuit underlying conditioned defeat.

## Materials and Methods

### Experiment 1

#### Animals and Housing Conditions

Thirty adult male Syrian hamsters *(Mesocricetus auratus*) were obtained from Charles River Laboratories. Subjects weighed 120-140g at the beginning of each experiment. One week after arrival, animals were individually housed in polycarbonate cages (20 x 40 x 20cm) with wire mesh tops, corn cob bedding and cotton nesting materials in temperature-controlled (20°C ±2°) colony rooms on a 14:10 hr light/dark cycle with lights off at 1100. Food and water were available *ad libitum*. All behavioral procedures were conducted during the first two hours of the light/dark cycle. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee, and all methods were in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Every effort was made to minimize the number of subjects used as well as to minimize any suffering by the animals.

#### Social defeat

All animals were housed individually for two weeks and handled daily for one week prior to the start of the experiment. Animals were weight-matched and assigned to one of three groups. Sixteen animals (i.e., 8 pairs) were assigned to undergo a single, 15 min social interaction. These social conflict sessions were conducted under dim red illumination and videotaped. The total duration of the following behaviors was recorded: submissive-defensive (see below for description), aggressive (see below for description),

social (attend, approach, sniff, nose-touching, and flank-marking) and non-social (locomotion, exploration, self-grooming, nesting, feeding, and sleeping). No animals were wounded during the procedure. Based on their behavior during a 15-min social defeat, which occurred in one of the animals' home cage, the subjects were characterized as either winners (Group 1) or losers (Group 2). Animals that were designated winners produced aggressive behaviors such as chasing, lunging attacks and frequent displays of upright and side offense postures. Animals that were designated losers produced submissive behaviors such as flight, tail lift, cage escape attempts, upright and side defense, and full submissive posture. No animals produced both submissive and aggressive behaviors. Animals in Group 3 served as novel cage controls and were placed in an empty animal's cage for 15-min. Animals in Group 4 were home cage controls. All animals were sacrificed 2hr following their respective treatments. This time point was selected because this is when the greatest changes in BDNF mRNA are observed following fear conditioning (Rattiner et al., 2004a)

***In situ* hybridization**

A partial BDNF clone, containing the entire exon V coding sequence but no significant portion of the 5'-exon was subcloned from rat genomic DNA based on the NIH database sequence. This BDNF exon V clone has previously been sequenced and extensively tested (Jones et al., 2007; Rattiner et al., 2004a,b). In situ hybridization was performed as follows. Hamsters were lightly anesthetized with isoflourane gas, decapitated and their brains were removed and rapidly frozen on dry ice. Prior to sectioning, the brains were stored at -80°C. Brains were sectioned at 20μm thickness on a Leica Cryostat at -20°C onto Superfrost Plus (Fisher) slides. Sections containing

anatomical areas of interest (infralimbic cortex, anterior hypothalamus, ventromedial hypothalamus, nucleus accumbens, anterior bed nucleus of the stria terminals, posterior bed nucleus of the stria terminals, CA1, CA3 and dentate gyrus of dorsal and ventral hippocampus, central, medial, and basolateral amygdala) were placed on 28 consecutive slides to produce four identical sets of slides for each animal. In situ hybridization was performed as previously described (Sassoon et al., 1988; Rattiner et al., 2004a,b). [$^{35}$S] UTP (1250Ci/ml; DuPont NEN, Boston, MA)- labeled riboprobes were prepared from linearized clones using T7 polymerase at high specific activity by only using radioactive UTP in the polymerase reaction, with ~30% incorporation. After preparation of full-length antisense RNA strands, the RNA was base hydrolyzed to average lengths of 50-100bp and isolated using a Riboprobe spin column. Hybridizations were performed using parafilm at 52°C overnight. The slides were then stringently washed, air-dried and placed against Kodak (Rochester, NY) magnetic resonance autoradiography film for 14 days at room temperature. Optical density values of autoradiographs were obtained using MCID Basic for Windows (Imaging Research, Ontario, Canada) and calibrated using a density-step wedge (Kodak). For each section, optical density values were determined bilaterally for the anatomical regions of interest. Background optical density values were measured in neighboring regions that lacked hybridization and subtracted from the region of interest values to produce a normalized optical density value. Normalized optical density values were calculated for two different cryostat sections for each anatomical region of interest and averaged to produce the optical density for each animal per region of interest.

**Statistical analysis**

The optical density data for each region of interest for Experiment 1 violated the assumption of homogeneity of variance (Levene's Test for Equality of Variances) therefore nonparametric statistical tests were used. A Kruskal-Wallis test was used to compare optical density values for each anatomical region of interest between winners, losers, novel cage, and home cage control animals. The Spearman rho correlation was used to determine an association between the duration of submissive and aggressive behavior and BDNF mRNA in regions of interest. For all comparisons, the alpha level was set at $p < 0.05$

**Experiment 2**

**Animals and Housing Conditions**

Syrian hamsters weighing 120-140g were purchased from Charles River Laboratories and individually housed for 12-14 days prior to the start of each experiment. Older hamsters (> 6 months) that weighed 160-180 g were housed individually and used as resident aggressors during the defeat phase (see below). Younger hamsters (2 months) that weighed 100-110 g were group housed (5 hamsters per cage) and used as non-aggressive intruders during the testing phase (see below). The cage, bedding, nesting materials, and food availability were the same as those described in Experiment 1.

**Stereotaxic surgery**

Subjects were deeply anesthetized with sodium pentobarbital (90mg/kg) and were then bilaterally implanted with 4mm, 26-gauge guide cannulae aimed at the BLA. Lambda and bregma were leveled prior to placement of the guide cannulae. Stereotaxic coordinates were 0.4mm posterior and ±3.9mm lateral to bregma and 2.1mm below dura.

Infusions were made with a needle that projected 4.2mm beyond the bottom of the guide cannulae. After surgery, dummy stylets were placed in the guide cannulae to help prevent clogging. All hamsters were given 10-12 days to recover from surgery before the behavioral procedure. Hamsters were handled each day following surgery by gently restraining them and removing and replacing the dummy stylet.

**Social defeat and behavioral testing**

The conditioned defeat model has been described elsewhere (Huhman et al., 2003). Briefly, prior to each experiment hamsters were weight-matched and randomly assigned to groups. On the training day, hamsters were transported to the behavior room. All training and testing occurred during the first 2 hr of the dark phase of the light:dark cycle. Training consisted of one, 15-min exposure to a resident aggressor in the aggressor's home cage. Resident aggressors reliably attacked the experimental hamsters and all hamsters displayed submissive behaviors. Any hamster bitten such that it bled was removed from the study and examined by a veterinarian. The next day, a non-aggressive intruder was placed in their home cage for 5-min. The training and testing sessions were videotaped, transferred to CD-ROM, and later scored by an observer blind to the experimental conditions using behavioral scoring software (The Observer, Noldus Information Technology, Wageningen, the Netherlands). We recorded the total duration of four classes of behavior during the 5-min test: (a) submissive and defensive behavior (flee, avoidance, tail-up, upright and side defense, full submissive posture, stretch-attend, head flag, attempted escape from cage), (b) aggressive (upright and side offense, chase and attack, including bite), (c) social behavior (attend, approach, investigate, sniff, nose touching, and flank marking), and (d) non-social behavior (locomotion, exploration, self-

grooming, nesting, feeding, and sleeping). A reduction in conditioned defeat was indicated by a statistically significant reduction in submissive and defensive behaviors or by the return of normal territorial aggression.

**Site verification**

At the conclusion of the experiment, hamsters were given a lethal dose of sodium pentobarbital and infused with 300nl of India ink to verify the placement of the needle. Brains were removed and placed in 10% buffered formalin. Brains were sliced on a Leica cryostat and sections were stained with neutral red. Sections were coverslipped with DPX mountant and examined under a light microscope for evidence of ink in the BLA. Only hamsters with bilateral ink injections within 0.5mm of the BLA were included in the data analysis (See Figure 12 ).

**Experiment 2: Acquisition of conditioned defeat**

The purpose of Experiment 2 was to test the hypothesis that injection of a Trk receptor antagonist, K252a, into the BLA would reduce the acquisition of conditioned defeat. Thirty-one hamsters were matched by weight and randomly assigned to one of two conditions. Hamsters received infusions of K252a (25µg, 50µM) in 0.5µl artificial CSF (ACSF)/50% DMSO or 0.5µl vehicle control (ACSF/50%DMSO) immediately before being placed into the cage of a resident aggressor for 15-min for conditioned defeat acquisition. Pre-training infusions of K252a at this dose into the BLA impairs fear conditioning in rats (Rattiner et al., 2004a). Infusions were administered at a rate of 0.25µl per minute with a syringe pump (Harvard apparatus PHD 2000, Natick, MA) and a Hamilton syringe connected to a 33-gauge needle via polyethylene tubing. The drug or vehicle was kept separate from the water in the tubing by a 1µl air bubble. Movement of

the air bubble was monitored to assess the success of the injection procedure. Following the infusion, the needle was left in place for 2-min, then removed and replaced with the dummy stylet. On the day following defeat training, animals were tested in their own home cage against a non-aggressive intruder for 5-min.

**Statistical analysis**

The total durations of submissive and defensive, aggressive, social and non-social behavior emitted during the testing session were individually analyzed using an independent samples t-test. The criterion for significance was $p < 0.05$.

## Acknowledgments

This work was supported by Grant MH62044 to KLH, Grant NIH DA019624 to KJR, the Burroughs Wellcome Fund, Yerkes National Primate Research Center (RR-00165) the Brains and Behavior Program at Georgia State University and in part by the Science and Technology Center Program (Center for Behavioral Neuroscience) of the National Science Foundation under agreement IBN-987675. We thank Alisa Norvelle, Dr. Chris Ehlen, and the Animal Care Staff at Georgia State University for their technical assistance.

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Figure 7. BDNF mRNA in the BLA, MeA, and DHPC DG 2hr after exposure to an agonistic encounter at low and high power magnification.

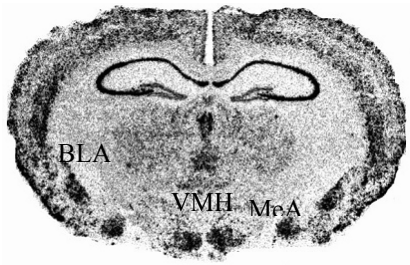
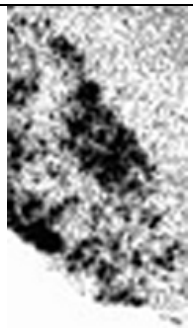
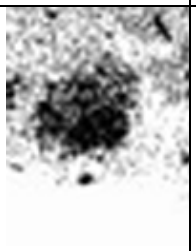
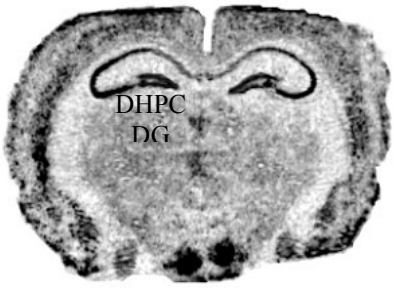
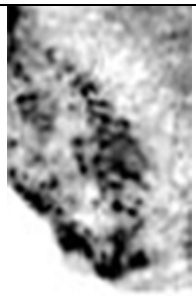
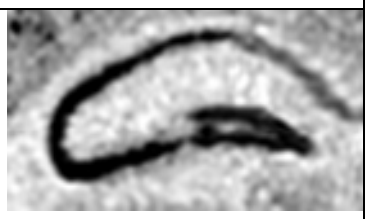
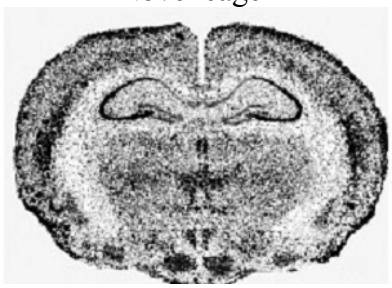
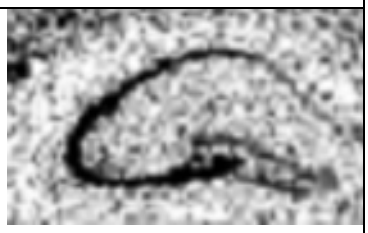
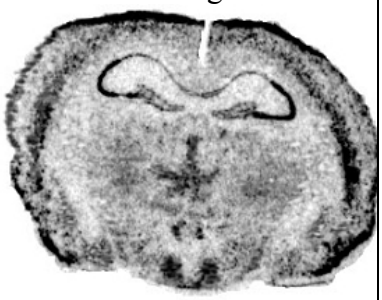
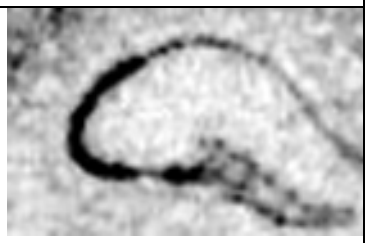
| | BLA | MeA | DHPC DG |
|---|---|---|---|
| Loser   |  |  |  |
| Winner   |  |  |  |
| Novel cage  |  |  |  |
| Home cage  |  |  |  |

Figure 8. BDNF mRNA in the VHPC CA1 2 hr following exposure to an agonistic encounter at high and lower power magnification.

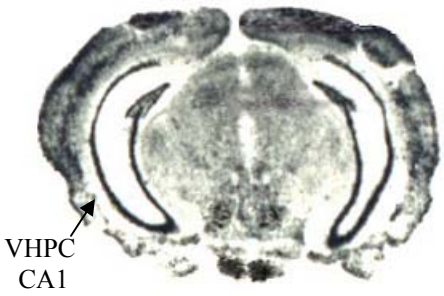
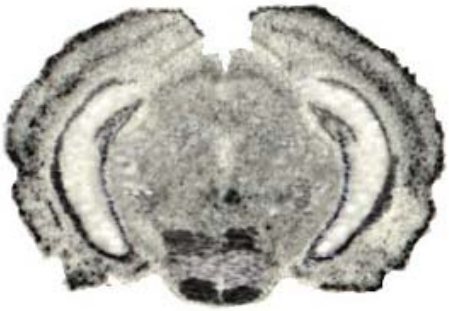
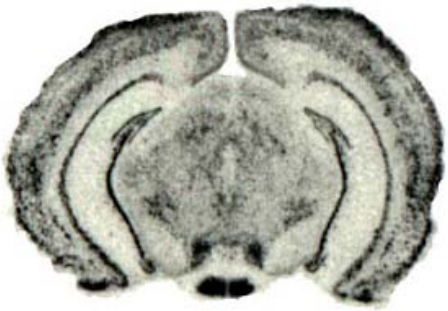
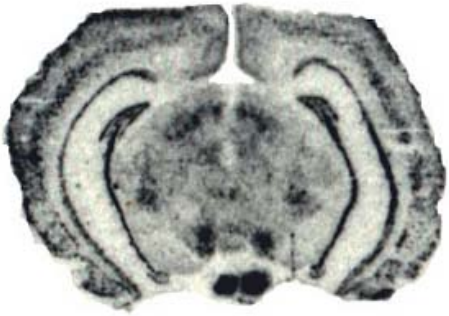
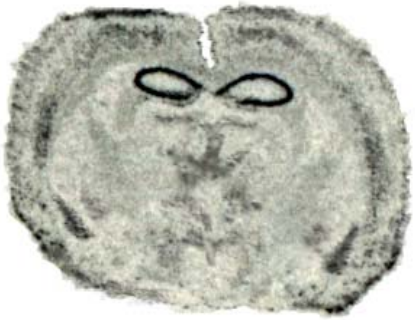
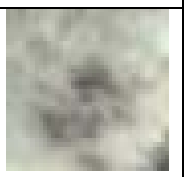
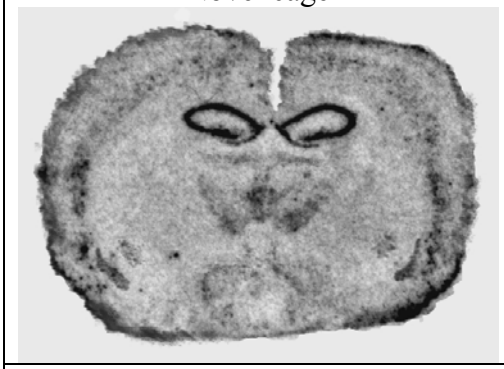
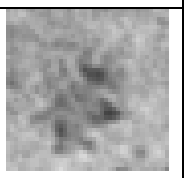
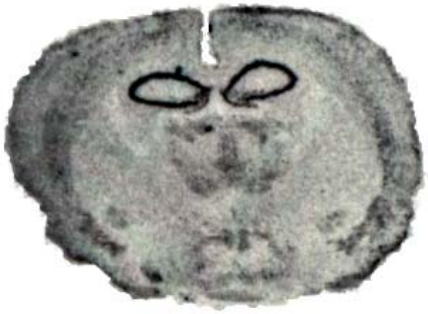
| | VHPC CA1 |
|---|---|
| Loser   |  |
| Winner  |  |
| Novel cage  |  |
| Home cage  |  |

Figure 9. BDNF mRNA in the CeA 2 hr following an agonistic encounter at low and high power magnification. There were no differences in BDNF mRNA in the CeA in losers, winners, novel cage controls, and home cage controls.

| | CeA |
|---|---|
| Loser   |  |
| Winner  |  |
| Novel cage  |  |
| Home cage  |  |

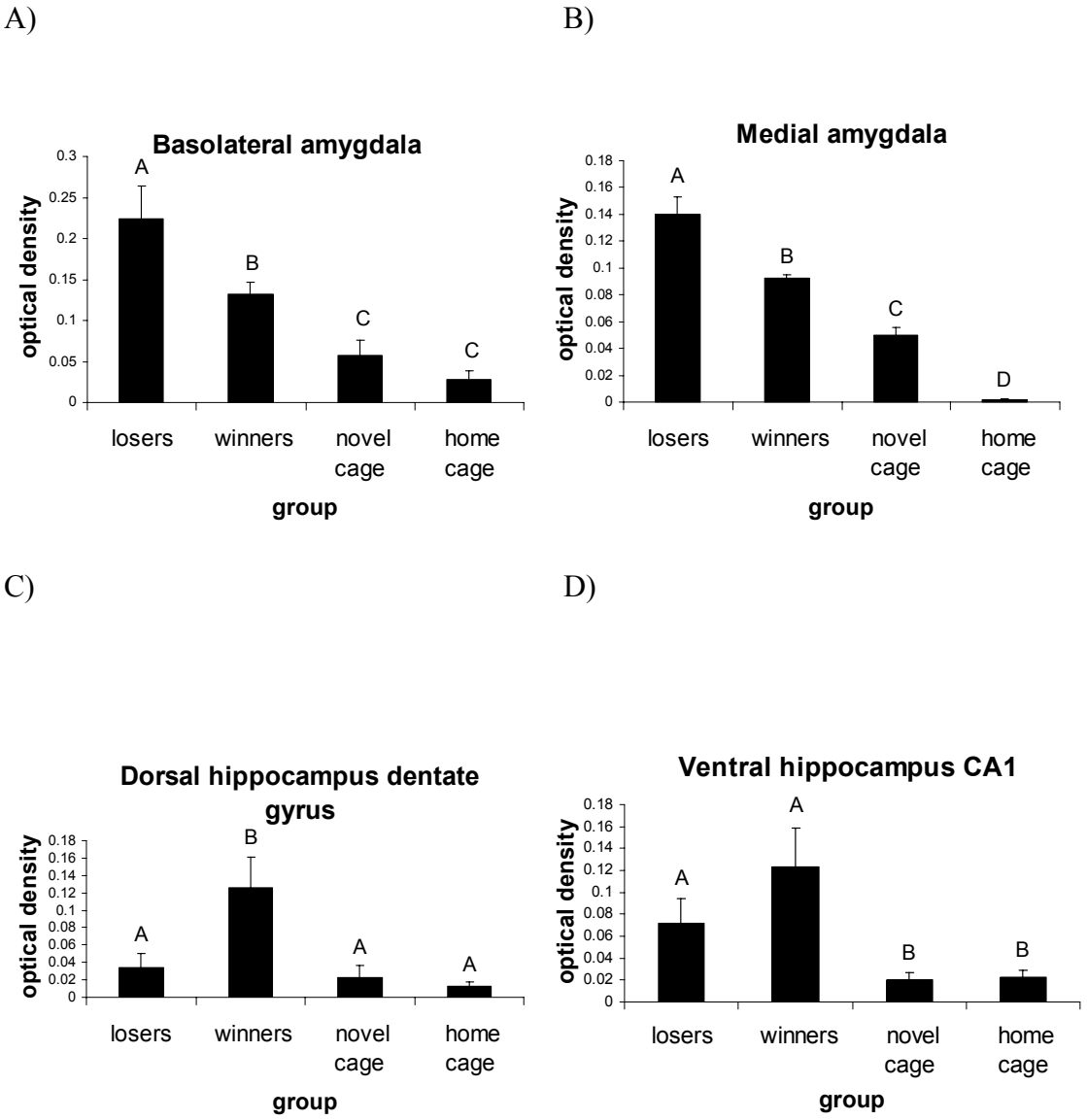



Figure 10. Mean (± standard error of the mean) of BDNF mRNA in the BLA, MeA, DHPC DG, and VHPC CA1 in losers, winners, and novel and home cage controls. Unshared letters indicate a significant difference among groups ($P < 0.05$).

TABLE 1. Correlations between duration of agonistic behavior and BDNF mRNA in medial amygdala (MeA), basolateral amygdala (BLA), dentate gyrus of dorsal hippocampus (DHPC DG) and CA1 of ventral hippocampus (VHPC CA1). Significant correlations are denoted with an asterisk (*). $P < 0.05$.

| | BDNF mRNA | | | |
|---|---|---|---|---|
| | MeA | BLA | DHPC DG | VHPC CA1 |
| Submissive | 0.721* | 0.428 | -0.542* | -0.369 |
| Aggressive | -0.608* | -0.728* | 0.467 | 0.434 |
| Social | 0.169 | 0.033 | 0.037 | -0.157 |
| Non-social | -0.691* | -0.266 | 0.301 | 0.121 |

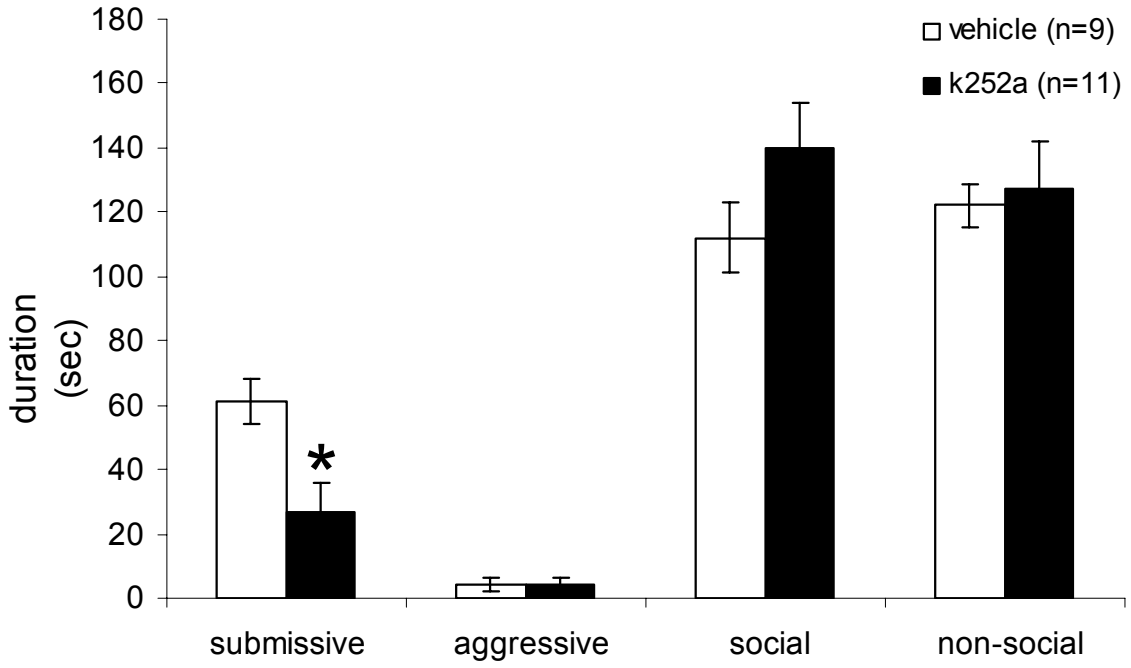



Figure 11. Acquisition of conditioned defeat. Total duration (mean ± S.E.M.) of submissive/defensive, aggressive, social, and nonsocial behavior displayed by defeated hamsters during a 5-min test with a non-aggressive intruder. Animals received bilateral infusions of vehicle or K252a into the BLA immediately before being defeated by a resident aggressor for 15 min on the previous day. Asterisk indicates a significant difference from vehicle ($p < 0.05$).

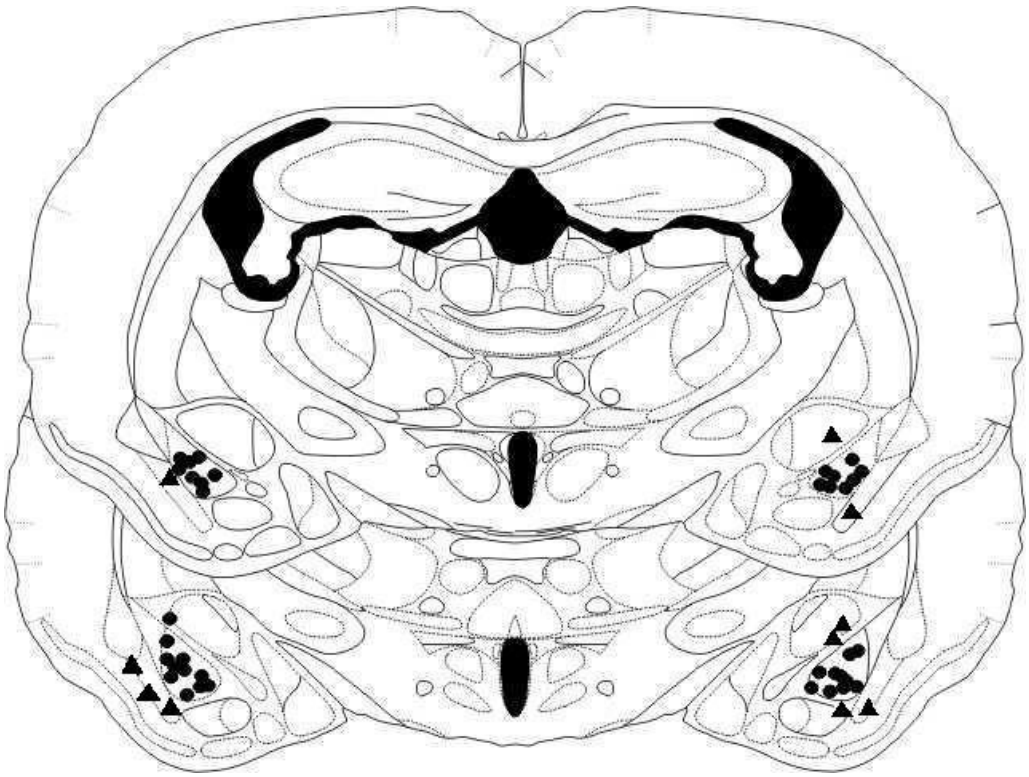


Figure 12. Histological reconstructions of injection sites of animals receiving infusions into the BLA in Experiment 2. Black dots represent the site of injection in one or more animals. Black triangles represent anatomical misses. Drawings are adapted from Morin and Wood (2001).

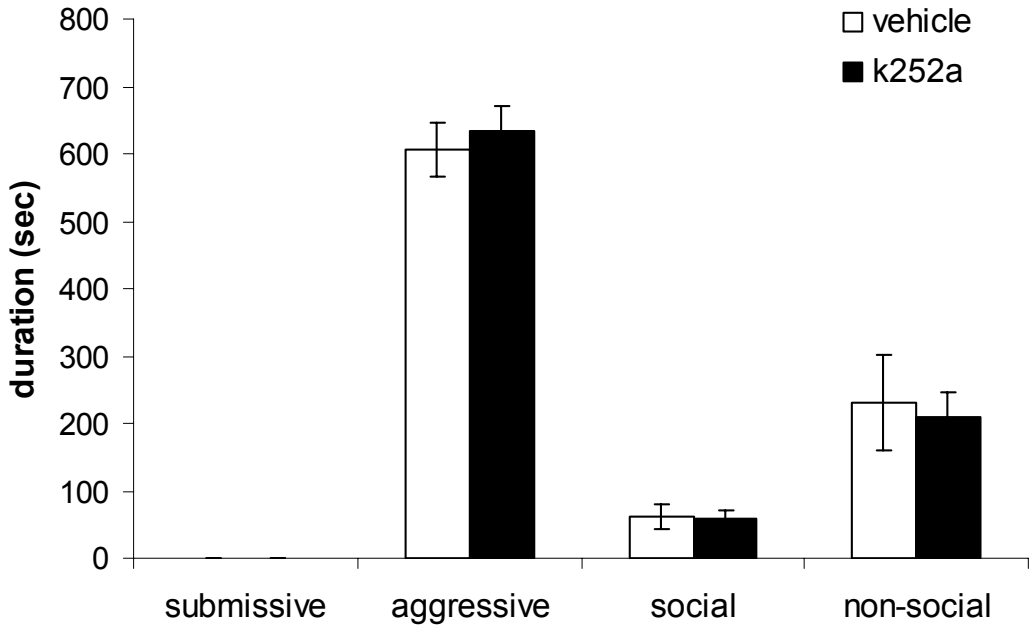



Figure 13. Total duration (mean ± S.E.M.) of submissive, aggressive, social and non-social behavior displayed by resident aggressors toward experimental animals during a 15-min training session. These data indicate that the defeat training by the resident aggressor was not altered by treatment received by the subject.

# CHAPTER 4

# DO THE BASOLATERAL AMYGDALA AND VENTRAL HIPPOCAMPUS INTERACT TO MEDIATE THE ACQUISITION OF CONDITIONED DEFEAT?

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## Abstract

A number of studies suggest that the hippocampus and amygdala interact in the formation of emotionally relevant memories. Our laboratory employs a biologically-relevant model of emotional learning termed conditioned defeat. In this model, experimental hamsters are defeated by an aggressive counterpart and are then exposed to a non-aggressive intruder. Instead of defending its own territory, as it normally would have prior to social defeat, the defeated hamster readily submits to the non-threatening intruder. Our laboratory has shown that the basolateral amygdala (BLA) and ventral hippocampus (VHPC) are two regions that are important in the acquisition of conditioned defeat. The present experiment tested the hypothesis that the BLA and ipsilateral VHPC interact to modulate the acquisition of conditioned defeat. Subjects were randomly assigned to one of three groups. In Group 1, vehicle was infused into the right BLA and left VHPC; in Group 2 muscimol was infused into the right BLA and left VHPC, and in Group 3 muscimol was infused into the right BLA and right VHPC. Infusion of muscimol into the right BLA and left VHPC as well as into the right BLA and right VHPC prior to defeat training significantly reduced the display of submissive and defensive behavior during subsequent testing. These results are consistent with the hypothesis that the BLA and VHPC are a part of the neural circuit mediating the formation of emotionally relevant memories; however, they do not rule out the possibility that the observed reduction in submissive and defensive behavior is dependent primarily on the temporary inactivation of the right BLA, alone, and/or that contralateral connections between the BLA and VHPC exist which causes the contralateral injections to also disrupt acquisition of conditioned defeat.

## Introduction

Several groups have hypothesized that the amygdala and hippocampus interact to modulate memory formation (Packard, Cahill and McGaugh, 1994; McGaugh, 2002 & 2004; Akirav and Richter-Levin, 2002; Richter-Levin, 2004; McIntyre, Miyashita, Setlow, Marjon, Steward, Guzowski, and McGaugh, 2005; Vouimba, Yaniv, and Richter-Levin, 2007). This idea is supported by anatomical, electrophysiological, and functional evidence. Anatomically, the basomedial and basolateral nuclei of the amygdala project to the hippocampus with the heaviest projections occurring between the BLA and CA1, CA3, and entorhinal cortex of the ventral hippocampus (VHPC; Amaral et al., 1992). These regions of the VHPC in turn project to the BLA via the ventral angular bundle to the BLA. Electrophysiological studies have shown that amygdala activity influences LTP-induction in the hippocampus. Pharmacological stimulation of the amygdala activates the entorhinal cortex and hippocampus (Packard et al., 1995), and lesions of the BLA attenuate LTP at the perforant path-dentate gyrus granule cell synapses in the hippocampus (Abe, 2001). High frequency stimulation of the BLA combined with tetanic stimulation of the perforant path facilitates hippocampal LTP (Ikegaya et al., 1996). Likewise, stimulation of the hippocampus increases amygdala LTP (Maren & Fanselow, 1995). Very little is known about whether the connections between the BLA and VHPC are mainly ipsilateral, contralateral, or both; however, the studies mentioned above examined only ipsilateral connections, with no mention of contralateral connections between the amygdala and hippocampus. A strong piece of evidence suggesting that the connections between the amygdala and hippocampus are solely ipsilateral comes from a study in which the excitatory amino acid, NMDA, was injected into the left amygdala and

vehicle was injected into the right amygdala. When Fos immunoreactivity was examined on the left and right sides of the hippocampus, high levels of Fos activation were observed in the left hippocampus while little or no Fos activation was observed in the right hippocampus (Packard et al., 1993).

A substantial number of studies have demonstrated a functional role for amygdala-hippocampal interactions. For example, Packard et al., (1994) hypothesized that the amygdala modulates memories in other brain regions such as the caudate nucleus and hippocampus, two regions thought to be important in different memory tasks. In the Packard study, amphetamine was infused into the amygdala, hippocampus or caudate nucleus immediately after rats were trained on one of two water maze tasks, a spatial task (thought to be hippocampally-dependent) or a visually cued task (thought to be caudate nucleus-dependent). The hippocampal infusion selectively enhanced retention of the spatial task while the caudate infusion selectively enhanced retention of the visually cued task. Interestingly, when amphetamine was infused into the amygdala, retention on both tasks was enhanced. Additional evidence suggesting that the amygdala and hippocampus interact in memory formation comes from a study showing that amygdala lesions block the memory-enhancing effect of direct hippocampal stimulation (Roozendaal and McGaugh, 1997).

Amygdala-hippocampal interactions are also important for another learning and memory task, namely, fear conditioning. Electrolytic lesions of selected subregions of the VHPC produce a deficit in the acquisition of fear to a contextual conditioned stimulus, and NMDA lesions of the BLA produce a nonselective deficit in the acquisition of fear to both contextual and acoustic conditioned stimuli (Maren & Fanselow, 1995).

Conditioned defeat in male Syrian hamsters is a biologically-relevant form of emotional learning. In this model, experimental hamsters are defeated by a larger, more aggressive hamster and are then exposed to a non-threatening intruder. Instead of readily defending its own territory, defeated hamsters show many behavioral changes, including a profound and long-lasting increase in submissive and defensive behaviors. Our laboratory has demonstrated that both the BLA and VHPC are important components of the neural circuitry mediating the acquisition of conditioned defeat. Temporary inactivation of either the BLA or VHPC immediately before the initial social defeat significantly reduces the duration and submissive and defensive behaviors when defeated hamsters are subsequently tested with a non-aggressive animal (Jasnow and Huhman, 2001; Taylor and Huhman; submitted).

Given that it is known that the amygdala and hippocampus interact in the formation of emotional memories and that the BLA and VHPC are both involved in the memory of social defeat, it is possible that these two brain regions interact to produce the behavioral changes observed in conditioned defeat. Therefore, the purpose of this experiment was to test the hypothesis that the BLA and VHPC interact to mediate the acquisition of conditioned defeat.

## Experimental Procedures

### Animals and Housing Conditions

Male Syrian hamsters (*Mesocricetus auratus*) weighing 120-140g were purchased from Charles River Laboratories and individually housed for 12-14 days prior to the start of each experiment. Older hamsters (> 6 months) that weighed 160-180 g were housed individually and used as resident aggressors during defeat training (see below). Younger

hamsters (2 months) that weighed 100-110 g were group housed (5 hamsters per cage) and used as non-aggressive intruders during testing (see below). All hamsters were housed in polycarbonate cages (20 x 40 x 20 cm) with wire mesh tops, and food and water were available *ad libitum*. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee, and all methods were in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Every effort was made to minimize the number of subjects used as well as to minimize any suffering by the animals.

**Surgical procedures**

Hamsters were deeply anesthetized with sodium pentobarbital (90mg/kg) and stereotaxically implanted with 4mm, 26-gauge guide cannulae (Plastics One, Roanoke, Virginia). Lambda and bregma were leveled prior to placement of the guide cannulae. Guide cannulae were implanted in the BLA and VHPC. Animals in Groups 1 and 2 received BLA implantation on the right side and VHPC implantation on the left side. If BLA-VHPC projections are mainly ipsilateral, as hypothesized, this treatment should inactivate the BLA-VHPC circuit bilaterally, resulting in a significant reduction in the acquisition of conditioned defeat. Stereotaxic coordinates for the BLA were 0.4mm posterior and -3.8mm lateral to bregma and 1.9mm below dura. Stereotaxic coordinates for the VHPC were 2.4mm posterior and +3.7mm lateral to bregma and -2.9mm below dura. Animals in Group 3 received ipsilateral BLA and VHPC implantations on the right side. Stereotaxic coordinates for the BLA are the same as those mentioned above. In order to fit two cannulae on one side of the skull, VHPC coordinates in Group 3 were altered such that the cannlua was implanted at a 25º angle and the coordinates were -

2.8mm posterior and +4.5mm lateral to bregma and 1-.6mm below dura. The BLA-VHPC circuit should be intact on the contralateral side of the brain from the injections, thus we hypothesized that these hamsters would produce normal or intermediate levels of conditioned defeat. BLA infusions were made with a needle that projected 4.2mm beyond the bottom of the guide cannulae. VHPC infusions were made with a needle that projected 1.2mm beyond the bottom of the cannulae. After surgery, dummy stylets were placed in the guide cannulae to help prevent clogging. All hamsters were given 10-12 days to recover from surgery before the behavioral procedure. Hamsters were handled each day following surgery by gently restraining them and removing and replacing the dummy stylet.

**Social defeat and behavioral testing**

The conditioned defeat model has been described in detail elsewhere (Huhman et al., 2003). Hamsters were weight-matched and assigned to one of three groups described above. On the day of training, hamsters were transported to the behavior room. All training and testing occurred during the first 2 hr of the dark phase of the light: dark cycle to control for circadian rhythmicity of physiology and behavior. Training consisted of one 15-min exposure to a resident aggressor in the aggressor's home cage. Resident aggressors reliably attacked the experimental hamsters, and all subjects displayed submissive behavior in response. Any hamster bitten such that it bled was removed from the study and examined by a veterinarian. The next day, all experimental hamsters were transported to the behavior room, and a non-aggressive intruder was placed in their home cage for 5-min. The testing session was videotaped, transferred to CD-ROM, and later scored by an observer blind to the experimental conditions using behavioral scoring

software (The Observer, Noldus Information Technology, Wageningen, the Netherlands). We recorded the total duration of four classes of behavior during the 5-min test: (a) submissive and defensive behavior (flee, avoidance, tail-up, upright and side defense, full submissive posture, stretch-attend, head flag, attempted escape from cage), (b) aggressive (upright and side offense, chase and attack, including bite), (c) social behavior (attend, approach, investigate, sniff, nose touching, and flank marking), and (d) non-social behavior (locomotion, exploration, self-grooming, nesting, feeding, and sleeping). A statistically significant reduction in the duration of submissive and defensive behaviors and/or the display of territorial aggression signified a reduction of conditioned defeat. The behavior of the resident aggressor during training was scored to ensure that the presence of a drugged subject during training did not alter the behavior of the resident aggressors and that all animals received similar defeats.

**Drug infusions and site verification**

Infusions into the BLA and VHPC were administered to freely moving hamsters over 2 min with a Hamilton syringe mounted on a syringe pump (Harvard apparatus PHD 2000, South Natick, MA, USA) connected to a 33-gauge needle via polyethylene tubing (Fisher Scientific, Suwanee, GA). The needle was kept in place for an additional minute before being removed and the dummy stylet replaced. Hamsters received infusions of either muscimol (1.1nmol in the BLA and 2.7nmol in the VHPC in 200 nl saline) or vehicle immediately before being placed in the cage of a resident aggressor for 15 min. We selected muscimol because it is a reliable agent for temporarily inactivating the amygdala (Helmstetter and Bellgowan, 1994; Muller et al. 1997) and the hippocampus (Mao and Robinson, 1998). We selected these doses because previous work from our

laboratory indicates that they are effective at reducing the acquisition of conditioned defeat (Markham and Huhman, 2008; Taylor and Huhman, submitted) without producing non-specific or undesirable behavioral effects. At the conclusion of each experiment, hamsters were given a lethal dose of sodium pentobarbital and infused with 300nl of India ink to verify the placement of the needle. Brains were removed and placed in 10% buffered formalin. Brains were sliced on a cryostat and sections were stained with neutral red. Sections were coverslipped with DPX mountant (VWR International Ltd., Poole, England) and examined under a light microscope for evidence of ink in the BLA or VHPC. Only hamsters with bilateral ink injections within 0.5mm of the BLA or VHPC were included in the data analysis.

**Statistical analyses**

The total duration (seconds) of each behavior displayed (submissive and defensive, aggressive, social, and non-social) was determined. The mean total duration of each behavior was compared using a one-way analysis of variance (ANOVA). Significant differences for all analyses were ascribed at $p < 0.05$. Statistically significant differences were further analyzed using a Tukey-Kramer multiple comparison post-hoc test to compare all pairwise differences among group means.

## Results

No animals had to be removed from this experiment due to a serious bite (i.e., one that caused bleeding) during training. A total of 20 animals were used in the statistical analysis: right BLA-left VHPC vehicle (n = 6; Group 1), right BLA-left VHPC muscimol (n = 7; Group 2), right BLA-right VHPC muscimol (n = 7; Group 3). ANOVA revealed a significant effect of treatment on the display of submissive and defensive behaviors

during subsequent testing ($F_{(2, 19)} = 5.703$; $p < 0.05$, Figure 14). Treatment did not affect the initial defeat experience as indicated by the fact that the durations of aggressive, social and non-social behavior of the resident aggressor toward the experimental animal were similar among the three groups regardless of the drug state of the experimental hamster (Figure 15). Post-hoc analysis revealed that simultaneous inactivation of the right BLA and left VHPC as well as simultaneous inactivation of the right BLA and right VHPC significantly reduced the duration of submissive and defensive behavior when compared to vehicle controls; however, these two groups did not differ significantly from each other ($p < 0.05$). There were no significant differences in aggressive ($F_{(2, 19)} = 0.907$; $p > 0.05$), social ($F_{(2, 19)} = 0.70$; $p > 0.05$) and non-social behaviors ($F_{(2, 19)} = 1.472$; $p > 0.05$, Figure 14) among groups.

Histological analysis revealed that needle placements were localized mainly in the BLA and VHPC (Figure 16). A total of 10 animals were excluded from the analysis. Five animals lost a cannlua during the recovery period and could not be used in the study. Five animals had injections that were not localized into the BLA or VHPC. Of the placements that were aimed at the right BLA and right VHPC, one animal had placement into the granular insular cortex and VHPC, while another had placement into the lateral amygdala and VHPC. Of the placements that were aimed at the right BLA and left VHPC, one animal had placement into the posterior BLA on the right side and VHPC on the left side. The infusion sites for two animals could not be verified as a result of blocked cannulae at the time of dye infusion.

All animals that were considered anatomical “misses” received infusions of muscimol. These animals showed levels of submissive and defensive behaviors (M =

115 sec, SEM = ± 15.23) during the testing session that were comparable to that of vehicle controls.

## Discussion

The results of this experiment indicate that simultaneous inactivation of the right BLA and left VHPC as well as the right BLA and right VHPC reduce the acquisition of conditioned defeat. When muscimol is infused into these areas immediately prior to defeat training, the duration of submissive and defensive behaviors is reduced during subsequent testing. These results are interesting given the current notion that the amygdala and hippocampus interact to modulate the formation of emotionally based memories; however, a few alternative interpretations exist which are discussed below.

The finding that Groups 2 and 3 both showed reduced levels of conditioned defeat was rather surprising. We initially hypothesized that while Group 1 would show control (i.e., high) levels of conditioned defeat, Group 2 would show low levels and Group 3 would exhibit either high or intermediate levels of conditioned defeat. Theoretically, animals in Group 3 would have only one side of the BLA-VHPC circuit disrupted, while the contralateral side remained intact (i.e., functioning), which would result in normal or intermediate levels of conditioned defeat. This was not the case, however, because Groups 2 and 3 showed surprisingly similar reductions in submissive and defensive behavior. One possible explanation for this effect could be that contralateral connections between the BLA and VHPC exist. If this is the case, it would not be surprising that the manipulations applied to Groups 2 and 3 only moderately reduced conditioned defeat because only a portion of the circuit was disrupted in each case (See Figure 17). In other words, Group 3 animals had the circuit on the right side (BLA-VHPC) disrupted,

however, the circuit on the left side remained fully functional. Likewise, Group 2 animals had only one right BLA-left VHPC circuit disrupted, while the left BLA-right VHPC remained functionally intact. A follow-up experiment could use tract tracing to elucidate the nature of BLA-VHPC connections in hamsters.

An alternative explanation for the similar reduction in submissive and defensive behaviors in Groups 2 and 3 could be that the effect is mediated specifically by the right BLA only. Research in both humans and non-human animals suggests that some level of laterality exists in terms of emotional processing and expression and fear conditioning in that the right amygdala may be of greater importance than the left (Adolphs, Damasio, Tranel, and Damasio, 1996; Cahill et al., 2000; Coleman-Mesches and McGaugh, 1995, Scicli, Petrovich, Swanson and Thompson, 2004; Goosens and Maren, 2001, Baker and Kim, 2004). Our laboratory has not yet tested the hypothesis that the right BLA is more important than the left BLA in the acquisition of conditioned defeat; however, ongoing experiments are investigating this possibility with the use of muscimol infusions into the right BLA and vehicle infusions into the left VHPC.

In sum, the results from the present experiment must be interpreted with caution because additional studies are needed to clarify whether or not the BLA and VHPC interact to mediate the acquisition of conditioned defeat.

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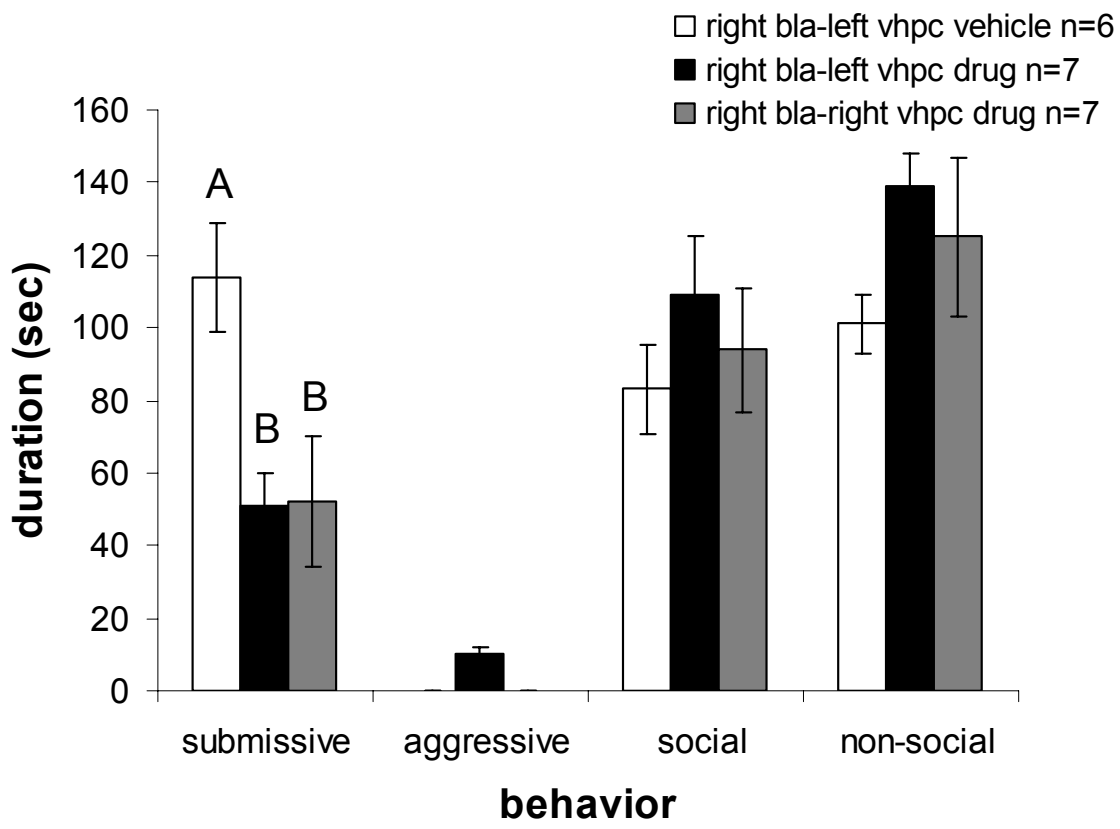


Figure 14. Acquisition of conditioned defeat. Total duration (mean ± S.E.M.) of submissive/defensive, aggressive, social, and non-social behavior exhibited by defeated hamsters during a 5-min test with a non-aggressive intruder. Animals received infusions of vehicle or muscimol immediately before being defeated by a resident aggressor for 15 min on the previous day. Non-shared letters indicates a significant difference ($P < 0.05$).

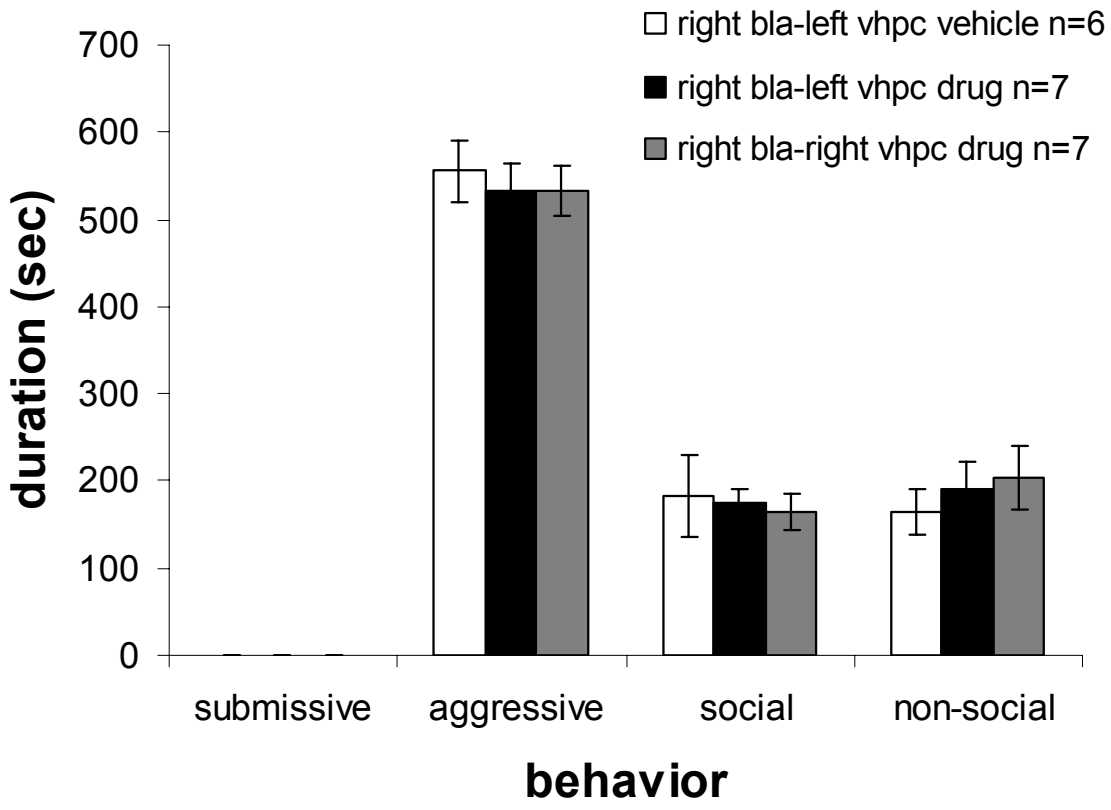


Figure 15. Total duration (mean ± S.E.M.) of submissive/defensive, aggressive, social and non-social behavior displayed by resident aggressors toward experimental animals during a 15-min training session. These data indicate that the defeat training by the resident aggressor was not altered by the treatment received by the subject.

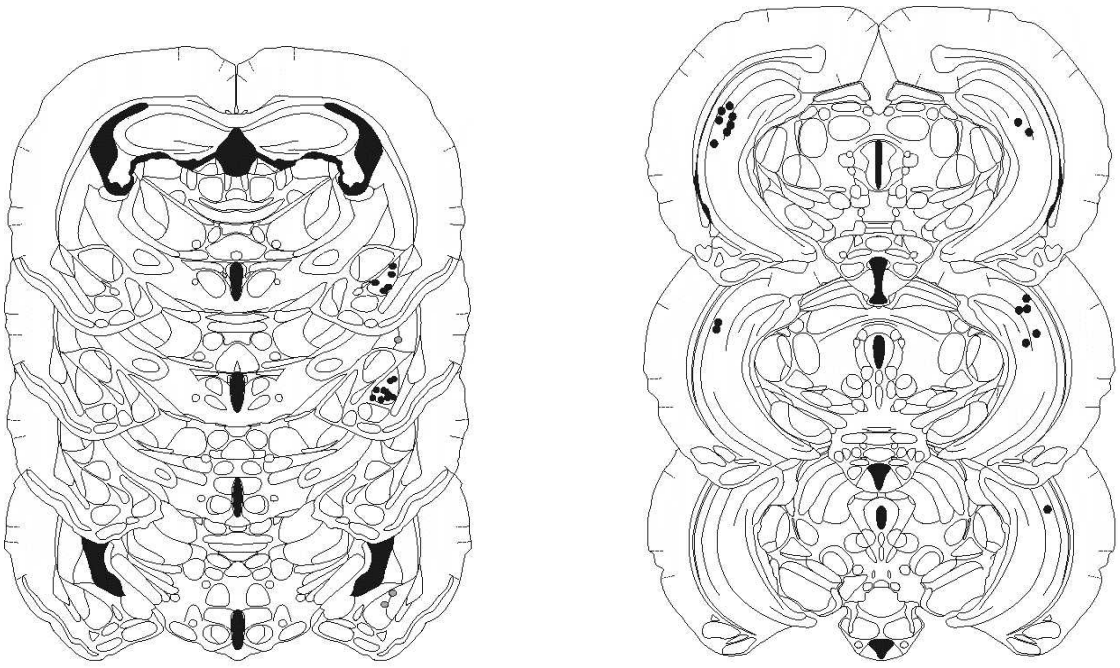

Figure 16. Histological reconstructions of injection sites of animals receiving infusions into the right BLA and left or right VHPC. Black dots represent site of injection in one or more animals. Grey dots represent anatomical misses. Drawings adapted from Morin and Wood (2001).

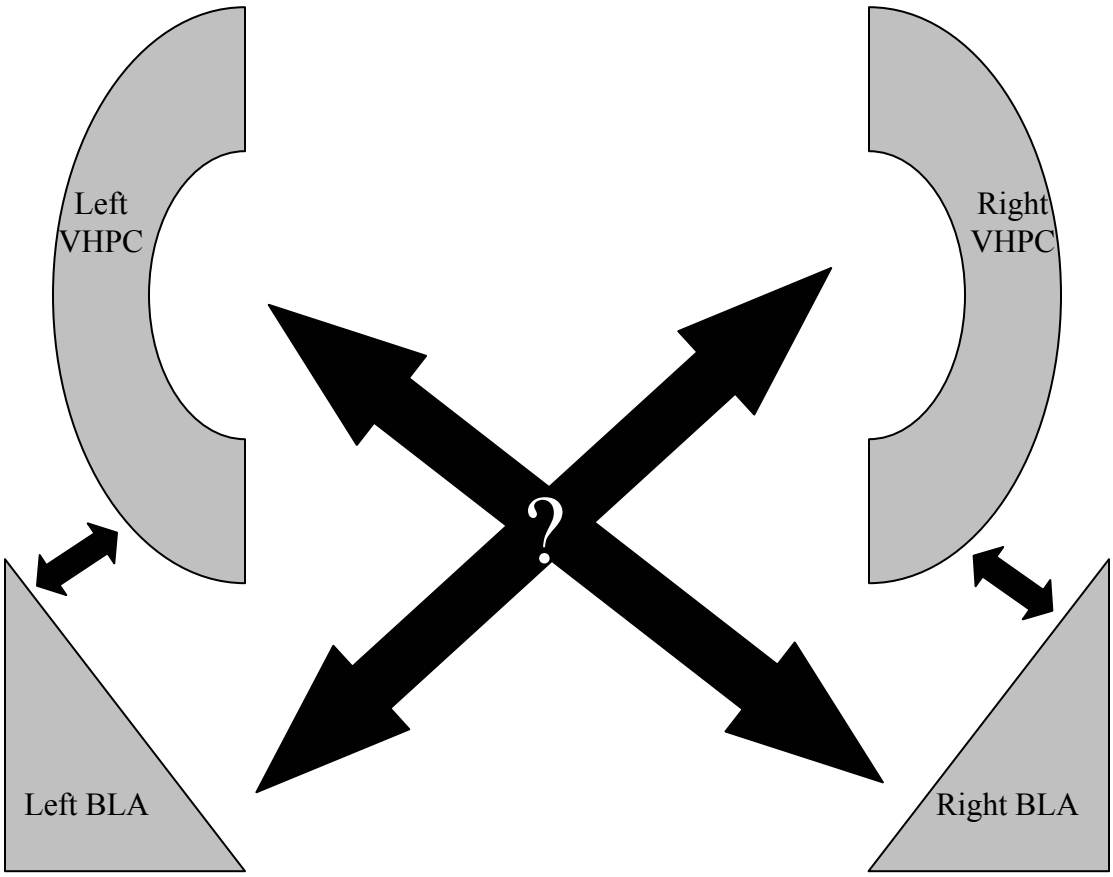


Figure 17 . Schematic illustration of connections between the BLA and VHPC. This figure demonstrates that although it is known that ipsilateral connections exist between the BLA and VHPC, less is known about the presence and/or function of contralateral connections.

# CHAPTER 5

## Conclusions

*Summary of basic findings*

The study of how stress alters both the brain and behavior has become one of the most interesting issues in neuroscience today because virtually all organisms, including humans, are exposed to stress and that exposure in humans is thought to play a critical role in the etiology of a number of psychopathologies. Since the majority of stress encountered by humans occurs in a social context, the use of a model of social stress-induced behavioral plasticity is important in order to identify the brain regions and molecular mediators that underlie the behavioral changes that occur following social stress. To this end, we employed a model of social stress-induced behavioral change in Syrian hamsters that we have termed conditioned defeat to study the underlying biological processes. Traditional models of the behavioral responses to stressful or fearful stimuli have been critical to our understanding of these processes, however, one drawback is that these models use artificial, unimodal stimuli (i.e., shock) to elicit fear and stress responses. Because conditioned defeat in Syrian hamsters occurs in a seminatural environment and involves the processing of multimodal cues, it gives us a unique opportunity to study the broader neural components important in stress-induced changes in behavior than is possible with other models.

An important goal regarding the neurobiology of stress-induced behavioral change is to identify brain regions that mediate these changes. The hippocampus is a large and complex brain structure that subserves numerous functions and is most often noted for its role in learning and memory. The hippocampus also plays a role in stress

responsivity and emotional behaviors. Past research regarding the role of the hippocampus in experience-induced changes in behavior has generally treated this structure as homogenous and has focused primarily on the anterior dorsal region. Recently, it has been suggested that the hippocampus is functionally divided along its dorsal and ventral poles, with the dorsal portion being important for spatial and contextual learning and the ventral portion being important for emotion including fear and anxiety-like behaviors. Our current data illustrate that the VHPC and not the DHPC is involved in the acquisition of conditioned defeat because temporary inactivation of the VHPC using a $GABA_A$ agonist, muscimol, prior to training reduced levels of submissive and defensive behaviors during testing. Inactivation of the DHPC either before training (acquisition) or before testing (expression) did not reduce submissive and defensive behaviors during testing. These results suggest that the VHPC, but not the DHPC, may be a part of the neural circuit mediating the social stress-induced behavioral changes observed following social defeat in Syrian hamsters.

Research on the molecular mediators of experience-induced changes in behavior has suggested that brain-derived neurotrophic factor (BDNF) is an important player in regulating the synaptic plasticity that underlies these changes. In the next set of experiments, we found that following exposure to an agonistic encounter there were differences between animals that won a fight versus animals that lost a fight in BDNF mRNA levels in several brain regions including the BLA, MeA, DHPC DG, and VHPC CA1 but not in other regions including the CeA, NaC, BNST, AH, and VMH. Losers exhibited higher levels of BDNF mRNA in the BLA and MeA while winners had higher levels of BDNF mRNA in the DHPC DG and VHPC CA1. One of the most interesting

findings was that the losers had higher levels of BDNF mRNA in the BLA, a region that is critical for conditioned defeat. These data suggest that BDNF in the BLA, and possibly other sites, is an important molecular mediator of conditioned defeat. Unfortunately, it is impossible to pharmacologically evaluate the mechanistic role of BDNF in the BLA because a specific receptor antagonist for BDNF (i.e., TrkB) receptors is not available. Because of this limitation we infused K252a, a non-specific neurotrophin (i.e., Trk) receptor antagonist, into the BLA prior to defeat training or testing. Infusion of K252a prior to defeat training reduced submissive and defensive behaviors during testing, supporting an important role for neurotrophins in the BLA in the acquisition of conditioned defeat.

Finally, while it is important to identify the brain regions important in mediating social-stress induced changes in behavior, it is equally important to begin to build a functional neural circuit subserving conditioned defeat that begins to define how involved brain regions interact with one another to support this behavioral plasticity. Given that we know that the BLA and VHPC are both important for the acquisition of conditioned defeat, we sought to determine whether these two regions act in concert to mediate its acquisition. Because the BLA-VHPC connections were thought to be ipsilateral, we gave unilateral injections of muscimol either in both areas on the same side of the brain or both areas on contralateral sides. We hypothesized that if these brain areas act together to mediate the acquisition of conditioned defeat, then conditioned defeat would be significantly reduced only in the group receiving the contralateral injections. We found, however, that conditioned defeat was significantly reduced in both drug groups as compared to hamsters receiving vehicle injections in the BLA-VHPC. Thus, the data

may indicate either 1) that the right BLA, alone, controls the acquisition of conditioned defeat or 2) that contralateral connections between the BLA and VHPC exist such that ipsilateral injections of muscimol also disrupt the functional BLA-VHPC circuit, but not enough to significantly reduce the acquisition of conditioned defeat due to the activity of these contralateral connections which may be compensating for the ipsilateral disruption.

*Contributions of the findings to field and clinical implications*

Conditioned defeat is a phenomenon that is thought to involve brain regions important for the behavioral responses to stressful or fearful stimuli. Our finding that the VHPC, but not the DHPC, is important for the acquisition of conditioned defeat supports the current hypothesis that hippocampus is functionally differentiated along its dorsal and ventral poles and extends this idea into more ethologically-relevant models of emotional learning such as conditioned defeat.

Abundant existing data show how artificial stressors, such as footshock or immobilization, affect BDNF levels in the central nervous system with most, if not all, reporting decreases in this neuropeptide in brain regions important in stress responsivity. There are a limited number of studies that examine how biologically-relevant stressors, such as social defeat, affect BDNF in the central nervous system. Further, the data from the existing studies are correlational and do not directly assess whether there is a functional role for an increase or decrease in BDNF. The present experiments are among the first to show that there are selective increases or decreases in BDNF mRNA in not only animals exposed to social defeat (i.e., losers) but also animals that display aggression (i.e., winners). Our finding that infusion of K252a into the BLA reduces the acquisition of conditioned defeat suggests that neurotrophin signaling, at least in part,

plays an important functional role in mediating the behavioral effects of social defeat. Our data are among the first to suggest that BDNF may also play an important role in behavioral and brain changes that may underlie an animal learning that it is dominant, as well.

Conditioned defeat in Syrian hamsters has similarities to several other models of fear, anxiety and social avoidance that are mediated, at least in part, by BDNF. BDNF/TrkB signaling in the BLA is critical for emotional learning in rats as assessed by fear-potentiated startle (Rattiner et al., 2004). In addition, BDNF activity in the mesolimbic dopaminergic system is important for the development of social avoidance and withdrawal in defeated mice (Berton et al., 2006). These data, coupled with the results of the current studies, suggest that BDNF is important in mediating the synaptic plasticity that underlies a variety of fear- and anxiety-induced changes. Dysfunction in synaptic plasticity is thought to underlie some mood disorders, such as depression, and it is possible that BDNF plays a role in this dysfunction. It has been reported that synaptic plasticity, as well as BDNF, is reduced in depression and is normalized following treatment with antidepressant medication, and it is important to note that many pharmacological treatments for these disorders can effect neural plasticity.

An emerging hypothesis regarding the neurobiology of mood and anxiety disorders suggests that a deficit in plasticity in particular neural circuits underlies many mental illnesses (Castren et al., 2005). A very brief review of the literature regarding anxiety-related disorders and BDNF may lead one to assume that BDNF has general antidepressant-like effects. However, the pro- versus antidepressant-like effects of BDNF depends on the brain region of interest. For example, antidepressants increase

hippocampal BDNF (Nibuya et al., 1995; Nibuya et al., 1996, Coppell et al., 2003) and infusion of BDNF into the hippocampus produces antidepressant-like behaviors in a forced swim test as well as in the learned helplessness model (Shirayama et al., 2002). On the other hand, BDNF may have pro-depressant effects in the mesolimbic dopaminergic system. Inactivation of the BDNF/TrkB system produces antidepressant like behaviors in a social defeat/avoidance model in mice (Berton et al., 2006). In addition, a distinction exists between anxiety-like behaviors and fear conditioning in their effects on BDNF levels in the DHPC and amygdala. Anxiety-like behaviors as measured with an elevated plus maze positively correlate with BDNF levels in the DHPC, while fear-conditioning positively correlates with BDNF levels in the amygdala (Yee et al., 2006). Thus, the role of BDNF in mood and anxiety disorders is complex. Castren et al., (2007) suggests that BDNF is a critical tool for activity-dependent changes in the structure of neural networks. Furthermore, whether or not BDNF produces a pro- versus anti-depressant like effect depends on the function of particular neural networks. In the case of conditioned defeat and other scenarios in which exposure to stress or trauma causes long-lasting changes in behavior, it may be that application of BDNF to particular areas in the brain (i.e., the BLA) would have pro-depressant like effects, while in others (i.e., the hippocampus) it may have anti-depressant like effects.

Finally, we show that disruption of the BLA-VHPC circuit reduces the acquisition of conditioned defeat. It has been proposed that memory is not a single entity, but rather it is the result of an interaction of several systems and processes (Squire et al., 1996). Receiving a vast amount of information requires an organism to decide what is important and what is less relevant. Remembering an event that may compromise an organism's

survival (i.e., being attacked by predator) is certainly more important than remembering events that have no relevance to survival or reproduction. This idea is supported by studies showing that emotionally-arousing events are better remembered than non-arousing events (Loftus, 1979; McGaugh, 1992). The amygdala plays an essential role in the behavioral and physiological reactions to events with emotional significance and in the formation of emotion-related memories (Cahill and McGaugh, 1998; Davis, 1992; LeDoux, 2000). An interesting hypothesis has recently emerged which suggests that the amygdala interacts with other brain regions important in memory formation to induce or strengthen neuroplasticity in those areas, a phenomenon termed Emotional tagging (Richter-Levin and Akirav, 2003). In this model, the amygdala "tags" an emotionally arousing experience as important by strengthening the synapses located on neurons in another brain region that are simultaneously engaged in the learning situation.

It is possible that emotional tagging is occurring in the BLA-hippocampal circuit. Anatomical, electrophysiological, and functional evidence suggests that these two regions interact in the formation of emotionally-arousing events (Akirav and Richter-Levin, 1999; Ikegaya et al., 1995; Pitkanen at el., 2000). Emotional tagging within this system likely involves synaptic plasticity, and it is important to identify the molecules and processes that may contribute to the strengthening of existing synapses or development of new contacts between the BLA and VHPC. Several potential candidate molecules and processes for emotional tagging include the immediate early gene Arc, the neural cell adhesion molecule (NCAM), and interestingly, BDNF/TrkB signaling (Martin and Kosik, 2002). In the case of conditioned defeat, it may be that BDNF/TrkB signaling both within and between the BLA and VHPC mediates the memory of social defeat. BDNF mediated

plasticity could be occurring in the BLA and BDNF release from the BLA to regions important in the acquisition of conditioned defeat, such as the VHPC, could be potential mechanisms by which social defeat causes behavioral plasticity.

*Remaining questions and future directions*

The data from the present study improve our understanding of social stress-induced behavioral plasticity; however, more work is needed to elucidate the mechanisms of this behavioral plasticity. We have shown that a non-selective Trk receptor antagonist infused into the BLA reduces the acquisition of conditioned defeat. To conclude that BDNF/TrkB signaling within the BLA is critically important for conditioned defeat, specific blockade of TrkB receptors during defeat training is necessary. One way to accomplish this would be to use a lenti-viral vector (TrkB.t1) that overexpresses the dominant-negative (i.e., truncated) isoform of TrkB in the BLA. While the full-length, high affinity Trk B receptor is active and thought to mediate the biological effects of BDNF, truncated TrkB receptors have short cytoplasmic domains that lack the internal kinase region and are thought to inhibit neurotrophin signaling mediated by full-length TrkB receptors. Overexpression of truncated TrkB receptors in the BLA would therefore reduce BDNF/TrkB signaling specifically. Thus, if BDNF/TrkB signaling in the BLA is important for conditioned defeat, then overexpression of truncated TrkB receptors in the BLA during training should reduce submissive and defensive behaviors during testing.

A second issue involves deciphering the exact location of plasticity within the conditioned defeat circuit. Does plasticity occur solely within the BLA? Or does it also occur elsewhere, such as the VHPC? One way to answer this question would be to first assess whether BDNF/TrkB signaling in the VHPC is important in the acquisition of

conditioned defeat. Another way this could be tested would be to infuse a protein synthesis inhibitor, such as anisomycin, into the VHPC prior to defeat training. Since neural plasticity and learning and memory are known to involve the production of new proteins, then inhibiting protein synthesis in the VHPC during defeat training should reduce or block the memory of defeat when subsequently tested. If neither of these manipulations in the VHPC reduces the acquisition of conditioned defeat, one may conclude that although this region is an important part of the conditioned defeat neural circuit, plasticity within this region is not critical in mediating the behavioral effects of social defeat. Alternatively, if inhibition of BDNF/TrkB signaling or protein synthesis inhibition within the VHPC does reduce the acquisition of conditioned defeat, then one may conclude that plasticity is occurring there and consider the fact that encoding information about social defeat occurs in regions outside of the BLA. This latter finding would support the idea that emotional tagging is occurring between the BLA and VHPC via BDNF to mediate the behavioral changes observed following social defeat.

Finally, an interesting finding from this study is that animals that won a fight have higher levels of BDNF mRNA in two hippocampal subregions. This raises the possibility that losing a fight is not the only event that induces plastic neural events. Winning is also a situation that may involve neural plasticity. Very little is known about aggression, winning and plasticity; however, winning may be an interesting new model of hippocampal plasticity. It could be that BDNF-mediated plasticity in the hippocampus contributes to the behaviors emitted by aggressive hamsters that have won a fight. Winning/dominant hamsters typically exhibit decreased latencies to attack intruders and increased frequencies of attacks towards intruders. It is possible that BDNF in the

hippocampus mediates these behaviors in winning animals. One possible way to test this hypothesis would be to inhibit BDNF/TrkB signaling in the VHPC of winning hamsters during their first fight and then pair them pair them with another hamster 24hr later during which time the latency to first attack and frequency of attacks would be recorded. If BDNF/TrkB signaling in the VHPC is important for encoding or learning about "winning" status, then inhibition of this BDNF should decrease the number of attacks and increase the latency to first attack when paired with another animal 24hr following the first fight.

In general, a better understanding of how ethologically-relevant models of stress-induced changes in behavior, such as conditioned defeat, is needed to increase our appreciation of how the central nervous system changes following stressful events. The data presented in this series of experiments describe some of the molecular mechanisms and some of the anatomical components of the neural circuit that mediate social-stress induced changes in behavior in hamsters. As previously mentioned, neural plasticity is diminished in many mood and anxiety disorders, and exposure to social stress in humans is known to contribute to the development these disorders. Given that we have now have evidence that conditioned defeat involves neural plasticity, it will be important for future research to investigate how molecular mediators of plasticity, such as BDNF, work within the conditioned defeat neural circuit. Such research will enhance our understanding of how neural plasticity contributes to social-stress induced changes in behavior and will suggest ways to engineer pharmacological treatments that target the systems and mechanisms involved in the behavioral responses to social stress.

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