

Effects of 5-HT_{1A} Receptors on the Development of Stress-Induced Changes in Behavior

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DEDICATION

To my grandfathers

Kenneth Bader

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ABSTRACT

Social defeat leads to both increased anxiety-like behavior and the formation of a fear memory for a specific opponent. We have shown that serotonin (5-HT) signaling, particularly in the basolateral amygdala (BLA), alters the acquisition of the conditioned defeat response in Syrian hamsters. While activation of 5-HT_{1A} receptors impairs the acquisition of conditioned defeat, it is unclear whether these receptors alter the development of anxiety-like behavior or formation of fear memories. One method for investigating the formation of defeat-induced fear memories is to measure avoidance of former opponents, and many researchers have reliably used the open field as a measure of anxiety in rodents. In this study, we investigated whether activation of 5-HT_{1A} receptors prior to social defeat would reduce subsequent avoidance of a former opponent but not alter anxiety-like behavior. We also investigated whether activation of 5-HT_{1A} receptors prior to social defeat would reduce the expression of Arc immunoreactivity in the BLA. We administered 8-OH-DPAT (0.0, 0.25, 0.5 mg/kg), a 5-HT_{1A} receptor agonist, prior to 3, 5-minute social defeats and 24- hours later exposed hamsters (*Mesocricetus auratus*) to a social interaction test to measure the conditioned defeat response immediately followed by a Y-maze test to measure avoidance of a former opponent and an open field test to measure anxiety. In a separate experiment, we administered 8-OH-DPAT (0.0, 0.25 mg/kg) prior to 3, 5-minute social defeats and 2- hours later brains were perfused and removed for Arc immunohistochemistry. We found that administration of 8-OH-DPAT prior to social defeat reduced the acquisition of conditioned defeat compared to vehicle controls. We also found that administration of 8-OH-DPAT prior to social defeat

reduced subsequent avoidance of former opponents but did not alter the classic measures of anxiety in the open field. 8-OH-DPAT administration prior to defeat also impaired Arc immunoreactivity in the BLA. These results suggest that 5-HT_{1A} receptors modulate the fear memory associated with the social defeat experience. Furthermore, these results raise the possibility that 5-HT_{1A} receptor activation disrupts Arc expression in the BLA and thereby impairs the development of a fear memory that is essential for the conditioned defeat response.

TABLE OF CONTENTS

Introduction.....	1
Chapter I Methods.....	6
Chapter II Results.....	14
Discussion.....	17
List of References.....	23
Appendix.....	29
Vita.....	36

LIST OF TABLES

Table 1 The frequencies of flee, stretch attend, and attack (mean $\pm$ SE) during conditioned defeat testing are shown.....	29
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LIST OF FIGURES

Figure 1 Photograph (2X magnification) of the BLA from a defeated hamster used in Arc quantification.....	30
Figure 2 Representative photomicrograph (10X magnification) of the BLA from a defeated hamster used in Arc quantification.....	31
Figure 3 Durations (mean $\pm$) of submissive, aggressive, non-agonistic social, and nonsocial behavior are shown for a 5-minute test with a novel, non-aggressive opponent.....	32
Figure 4 Durations (mean $\pm$) of time spent in proximity to former opponent, sniffing the former opponent, and sniffing plus proximity to the former opponent, and latency to sniff the former opponent are shown for a 3-minute test in the Y-maze.....	33
Figure 5 Number (mean $\pm$) of line crosses, center entries, and the latency to exit the hut are shown for a 5-minute test in an open field.....	34
Figure 6 Number (mean $\pm$ SE) of Arc immunopositive cells in the BLA measured following social defeat training.....	35

INTRODUCTION

Psychosocial stress is a common type of stress experienced by humans and is a contributing factor in the development of affective disorders, such as major depression, generalized anxiety disorder, and post-traumatic stress disorder (Anisman and Zacharko, 1992, Arborelius et al., 1999, Davidson, 2003). Animal models such as learned helplessness and fear conditioning have been used to investigate the neurobiological mechanisms underlying these stress-related psychopathologies. These animal models use artificial stressors which generate changes in behavior and neural signaling, however the effects may be dissimilar from those produced by social stress (Lopez et al., 1999). Because psychosocial stress is relevant to the etiology of depression and anxiety in humans (Cryan and Slattery, 2007), some research has emphasized the use of ethologically relevant models of animal social stress (Potegal et al., 1993, Blanchard et al., 1995, Fuchs and Flugge, 2003).

Social defeat is a robust stressor that leads to heightened HPA-axis activity (Blanchard et al., 1995) as well as changes in behavior (Ruis et al., 1999, Watt et al., 2009). These stress-induced behavioral changes include increased anxiety-like behavior demonstrated in the elevated plus maze (Heinrichs et al., 1992, Berton et al., 1998). Social defeat also leads to alterations in circadian rhythmicity (Tornatzky and Miczek, 1993, Meerlo et al., 1996a), feeding behavior (Bartolomucci et al., 2004, Iio et al., 2012), and locomotor activity (Rygula et al., 2005, Calvo et al., 2011).

Syrian hamsters have been used in social defeat models because of their natural territoriality and their reliability to attack an intruder in a laboratory setting (Nowak and Paradiso, 1983). Male hamsters also fail to defend their territory following social defeat

when a smaller, non-aggressive (NAI) hamster is placed into their cage (Potegal et al., 1993, Huhman et al., 2003). The hamster's inability to display aggression and defend its territory following acute social defeat is called conditioned defeat (CD) and is also characterized by increased submissive and defensive behaviors in subsequent social interactions (Huhman et al., 2003). While social defeat in hamsters leads to changes in agonistic behavior, the behavioral changes could result from the development of a fear memory, heightened anxiety following a stressful experience, or both.

One advantage of the CD model is that because behavioral changes occur following an acute social defeat, neural mechanisms controlling defeat-induced memories may be investigated. Our lab has previously shown that blockade of NMDA receptors in the BLA prior to social defeat impairs the acquisition of the CD response (Day et al., 2011). Others have found that overexpression of CREB, a transcription factor involved in memory-dependent synaptic plasticity, in the BLA prior to social defeat increases the acquisition of conditioned defeat (Jasnow et al., 2005). BDNF mRNA has also been found to increase in the BLA following social defeat compared to non-defeated animals (Taylor et al., 2011). A TrkB receptor antagonist administered into the BLA prior to social defeat also reduces the acquisition of CD (Taylor et al., 2011). Similarly, previous research has shown that NMDA receptors, CREB, and BDNF in the BLA are critical targets controlling the formation of fear-related memories (Rodrigues et al., 2001, Rattiner et al., 2005, Izumi et al., 2011). Altogether, these findings suggest that CD is controlled by neural circuitry in the BLA known to regulate fear memories.

To investigate the cellular mechanisms that control the development of fear memories, we used the immediate early gene *Arc* as a marker within the BLA. *Arc*

expression is associated with experience-dependent plasticity and the acquisition of auditory fear conditioning (Maddox and Schafe, 2011). Deletion of Arc also leads to memory impairment on amygdala-dependent tasks, such as fear conditioning (Ploski et al., 2008). Furthermore, Arc expression has also been found to increase in the prefrontal cortex, hippocampus, and nucleus accumbens following social defeat (Coppens et al., 2011).

Serotonin (5-HT) is a neurochemical known to be involved in the development of fear and anxiety (Owens and Nemeroff, 1998). In human studies, the 5-HT_{1A/2C} receptor antagonist ritanserin, has been shown to reduce anxiety (Ceulemans et al., 1985). Data from several animal models indicate that serotonin signaling in limbic and cortical brain regions can increase the development of anxiety-like behavior and fear memories (Graeff et al., 1997, Gardner et al., 2005, Maier and Watkins, 2005). Several studies have indicated a role for 5-HT_{1A} receptors in the development of both anxiety-like behavior and fear memories (Lehner et al., 2009, Kimura et al., 2011). Other studies have attempted to distinguish the role of 5-HT_{1A} receptors in fear and anxiety. For example, pharmacological activation of 5-HT_{1A} receptors in the hippocampus has been shown to impair the acquisition of contextual freezing (Stiedl et al., 2000). Whereas Stiedl et al. (2000) demonstrates a role for 5-HT_{1A} receptors in the development of fear memories, other studies have linked this receptor to the development of anxiety-like behavior. For example, low anxiety rats have been found to have a lower concentration of 5-HT_{1A} receptor protein, particularly in the dentate gyrus of the hippocampus (Lehner et al., 2009).

Several studies indicate that 5-HT_{1A} receptors in the amygdala modulate anxiety-like behavior, although the results are mixed with some finding an anxiogenic effect while others indicate an anxiolytic effect. For example, Graeff et al. (1993) found anxiogenic effects of 5-HT_{1A} receptor agonist administration into the amygdala prior to conditioned fear. In contrast, Li et al. (2012) demonstrated an anxiogenic effect on the elevated plus maze in mice with a reduction in 5-HT_{1A} receptor expression in the amygdala. Similarly, Gross et al. (2000) found that 5-HT_{1A} receptor knockout mice exhibited increased anxiety-like behavior in a number of behavioral tests, including open field locomotion and novelty-suppressed feeding. We have found that the activation of 5-HT_{1A} receptors in the BLA prior to social defeat training impairs the acquisition of the conditioned defeat response in Syrian hamsters (Morrison and Cooper, 2012). Also, social defeat reduces 5-HT_{1A} receptor mRNA in the dorsal raphe nucleus and blocking 5-HT_{1A} autoreceptors in the dorsal raphe reduces both the acquisition and expression of conditioned defeat (Cooper et al., 2008, Cooper et al., 2009). However, it is unclear whether these receptors alter the development of anxiety-like behavior or the formation of fear memories.

In Syrian hamsters, memory of social defeat has been studied using a Y-maze test. Following social defeat, hamsters show greater avoidance of a familiar winner in a Y-maze test compared to an unfamiliar winner (Lai and Johnston, 2002, Lai et al., 2005). These studies suggest that hamsters recognize former opponents and specifically avoid them following social defeat. Social avoidance in the Y-maze is characterized by reduced olfactory investigations in proximity of the stimulus animal as well reduced time spent in the stimulus arm (Bastida et al., 2009, Huang et al., 2011).

The open field has often been used as a test for anxiety-like behavior, especially following social defeat in rodents (Raab et al., 1986, Meerlo et al., 1996b, Solomon et al., 2007, Gannon et al., 2011). Studies have characterized anxiety-like behavior in the open field as reduced locomotion and decreased time spent in the center of the open field (Solomon et al., 2007). Similarly, in the defensive withdrawal test the latency to leave a small hut and enter an open field arena has been used as a measure of anxiety (Roman and Arborelius, 2009, Stiller et al., 2011).

The purpose of this study was to investigate the mechanisms by which 5-HT1A receptors modulate the development of CD. We hypothesized that activating 5-HT1A receptors prior to social defeat would impair the acquisition of CD by disrupting memory for the defeat experience and decrease defeat-induced Arc expression in the BLA.

CHAPTER 1 METHODS

Animals

We used adult male Syrian hamsters (*Mesocricetus auratus*) that weighed 130-170g (3-4months of age) at the start of the study, and were individually housed for 10-14 days prior to testing. Older hamsters that weighed 180-200 g (>6 months) were individually housed and used as resident aggressors for social-defeat training. Younger hamsters that weighed 90-120 g (2 months) were group-housed (4 per cage) and used as nonaggressive intruders for conditioned defeat testing. All animals were housed in polycarbonate cages (12 cm × 27 cm × 16 cm) with corncob bedding, cotton nesting materials, and wire-mesh tops. Animal cages were not changed for at least 1 week prior to testing to allow individuals to scent mark their territory. Animals were housed in a temperature-controlled colony room (20 ± 2 °C) and maintained on a 14:10 h light: dark cycle with food and water available *ad libitum*. Subjects were handled for 7-10 days prior to social defeat training and all behavioral testing occurred in the first three hours of the dark cycle. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee and follow the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Social Defeat Stress and Conditioned Defeat

Social defeat training consisted of three, 5-minute aggressive encounters in the home cage of a larger resident aggressor (RA). To ensure that every subject received similar amounts of aggression from the RA, timing of the defeat did not begin until the

first attack, which usually occurred within the first 60 seconds of the encounter. When drug treatments were administered prior to social defeat training, defeats were digitally recorded to ensure the absence of any nonspecific effects of the drug treatments. The number of attacks and total duration of aggressive behavior by the RA were later quantified. In experiments where drug treatments altered the duration of conditioned defeat behavior, no defeat controls were used to assess whether the treatments altered agonistic behavior in the absence of a social defeat experience. No defeat control animals were exposed to three different empty RA cages for 5 minutes each.

Conditioned defeat testing occurred 24 hours following social defeat training and consisted of one 5-minute encounter with a novel, non-aggressive intruder (NAI) in the home cage of the subject. Testing was recorded and later scored by a researcher blind to the experimental conditions using Noldus Observer. A second researcher scored a subset of testing sessions, and inter-observer reliability was greater than 90%. We quantified the duration of four categories of behavior: submissive/defensive (flee, avoid, upright and side defensive postures, tail-up, stretch-attend, head flag); aggressive (chase, attack, upright and side offensive postures); social (nose touching, sniff, approach); and nonsocial (locomotion, grooming, nesting, feeding) (Albers et al., 2002). We also quantified the number of flees, attacks, and stretch-attend postures displayed by the subject.

Apparatuses

Y-maze

In order to evaluate memory for the RA, a Y-shaped acrylic maze was used. The Y-maze is divided into eight rectangular boxes (10 cm wide × 10 cm high). The base of the Y (89 cm long) is divided into start box (20 cm) and stem (69 cm), and the two arms of the maze are 70 cm long and are divided into 3 sections. The sections closest to the stem are the basal parts (25 cm) of the arm while the compartments farther from the base are the distal parts (25 cm) of the arm. Subjects have access to all compartments of the Y-maze except for the most distal parts of the Y, which are the stimulus boxes (20 cm) where the RA is located during testing. The RA and subject are separated through a perforated Plexiglas wall (0.8 cm thick) to allow for the movement of air throughout the maze. The screen in front of the stimulus box is permanent whereas the screen that separates the start box from the stem is removable to allow the subject to explore the maze. Air is drawn from the stimulus boxes to the start box by a fan mounted on the outside of the start box.

Y-maze testing consisted of two, 3-minute trials. The first trial was an empty trial to determine the animal's preferred arm of the Y-maze. In the second trial, the RA was placed in the stimulus box of the subject's preferred arm to avoid confounding natural tendencies with avoidance of the RA. The Y-maze was cleaned with 70% ethanol after each subject was tested to remove any residual odors. When scoring the location of the subject within the Y-maze, we divided it into six compartments: the start box, the stem of the Y, the basal part of each arm of the Y, and the distal part of each arm of the Y. The location of the subject within the Y-maze was determined by the location of its nose. We

also scored the amount of time spent in olfactory investigation (sniffing), which is defined as time spent with the hamster's nose within 2 cm of the perforated screen of the stimulus box.

Open Field

The open field arena was an 80 × 80 × 40 cm acrylic box with black sides and a white grid on the floor. The grid was divided into 16 cm² squares. Subjects were initially placed underneath a plastic hut in one corner of the open field at the beginning of the 5-minute trial in order to measure latency to withdraw from the hut into the open field. The open field was cleaned between each trial with 70% ethanol to remove any residual odors left by the subject. Tests were recorded and scored by an observer blind to the experimental conditions. We scored the latency to exit the hut, the number of line crosses, and the number of center entries within the grid. A line cross was defined as the point at which a hamster's nose crossed the grid line.

Drug Treatment

We dissolved (±)-8-Hydroxy-2-(dipropylamino)tetralin hydrobromide (8-OH-DPAT; Sigma-Aldrich) in sterile saline (pH=5.5-6.0), which was used as a vehicle control at the same pH (Ricci et al., 2006). 8-OH-DPAT is a nonselective 5-HT_{1A} receptor agonist that has also been shown to act as an agonist at 5-HT₇ receptors (Hedlund et al., 2004). All drugs were administered with a 0.3 mL intraperitoneal injection (i.p.).

Experiment 1: 5-HT1A receptor agonist and behavioral testing

Experiment 1 was designed to investigate whether injection of a 5-HT1A receptor agonist would reduce the acquisition of conditioned defeat, reduce avoidance of former opponents, and modulate defeat-induced behavior in the open field. We injected 8-OH-DPAT (0.25 mg/kg, N=29; or 0.5 mg/kg, N=14) or vehicle (N=31) 20 minutes prior to 3, 5-minute social defeats. For no defeat controls, we injected 8-OH-DPAT 0.25 mg/kg, N=23) or vehicle (N=21) 20 minutes prior to exposure to 3, 5-minute empty resident aggressor cages. Twenty-four hours later, animals were tested for conditioned defeat behavior.

Immediately following conditioned defeat testing, about half of the defeated animals (0.25 mg/kg, N=15; or 0.5 mg/kg, N=14; or vehicle, N=16) and half of the non-defeated animals (0.25 mg/kg, N=11; or vehicle N=10) were tested for avoidance of the RA in a Y-maze. The other half of defeated animals (0.25 mg/kg, N=14; or vehicle N=15) and non-defeated animals (0.25 mg/kg, N=12; or vehicle N=12) were tested for anxiety-like behavior in the open field immediately following CD testing.

Experiment 2: 5-HT1A receptor agonist and Arc immunohistochemistry

In order to investigate whether a 5-HT1A receptor agonist would reduce defeat-induced Arc expression in the BLA, we injected 8-OH-DPAT (0.25 mg/kg; N=10) or vehicle (N=10) 20 minutes prior to 3, 5-minute social defeats. For no defeat controls, we injected 8-OH-DPAT (0.25 mg/kg; N=8) or vehicle (N=10) 20 minutes prior to 3, 5-minute exposures to empty resident aggressor cages. We selected 0.25 mg/kg of 8-OH-DPAT because it was the most effective dose at CD testing. Two hours following the

end of defeat, all animals were anesthetized with 4% isoflurane. Animals were then transcardially perfused with 100 ml of 0.1 M PBS followed by 100 ml of 4% paraformaldehyde. Brains were removed and soaked in 4% paraformaldehyde for 24 hours, followed by 0.1 M PBS/30% sucrose solution for 48 hours, and then were stored in cryoprotectant, all at 4°C. A consecutive series of 30 µm coronal sections were cut on a vibrating microtome and collected into three vials as free floating sections in cryoprotectant at 4°C. The collected sections were processed for Arc protein immunohistochemistry using the following protocol. Sections were washed five times in PBS + 0.2% Triton before each incubation, which were conducted at room temperature unless otherwise stated. Sections were incubated for 25 minutes in 0.3% hydrogen peroxide and methanol solution. Sections were then incubated with 1% bovine serum (BSA) in PBS + 0.2% Triton for 60 minutes before being incubated at room temperature for 24 hours in mouse anti-Arc polyclonal primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a final dilution of 1:500 in 1% BSA in PBS + 0.2% Triton. Sections were then washed five times with PBS + 0.2% Triton, incubated for 60 minutes in biotinylated secondary anti-mouse IgG (Vector Laboratories, Burlingame, CA) at a final dilution of 1:200 with 0.1% BSA in PBS + 0.2% Triton. Sections were then incubated in avidin-biotin-complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) with PBS for 60 minutes, and the peroxidase reaction was visualized using a 15 minute incubation in 3,3'-diaminobenzidine (DAB tablet, Sigma-Aldrich, St. Louis, MO) and nickel dissolved in PBS. The sections were washed five times with PBS and five times with distilled H₂O prior to being mounted onto glass microscope slides. After air-drying, sections were dehydrated using a series of alcohols, cleared with citrisolv and

coverslipped using DPX mountant (Sigma-Aldrich, St. Louis, MO). For each brain region, the tissue from all subjects was processed simultaneously.

Images were captured at 10X magnification using an Olympus BX41 microscope. The number of Arc immunopositive cells was determined in select brain regions using MCID Core image analysis software (InterFocus Imaging, Cambridge, England). We quantified the number of Arc immunopositive cells in the basolateral amygdala (BLA). For the BLA, we recorded background immunoreactivity in unstained regions of each image. We then defined immunopositive cells as those that showed staining 1.5X darker than the specific background immunoreactivity calculated for each image. Cell counts were limited to the area within defined boxes that were tailored to the size of the BLA (Fig. 1). A sample image of Arc immunoreactivity within a 350 x 350 μm box in the BLA is shown in (Fig. 2). For each brain region we quantified three to six sections per individual.

Data analysis

Some animals were not included in statistical analysis because of insufficient aggression during social defeat training (N=2). Two animals also failed to show any conditioned defeat behavior and were excluded from analysis. We also excluded one animal because of an attack by the RA during testing in the Y-maze. Two animals were excluded because of damage to brain tissue when sliced on the vibrating microtome. For cell counting analysis, we excluded animals (N=4) with an average of less than ten positively stained cells within the BLA.

We performed two-way ANOVAs to investigate an interaction between defeat (2 levels) and drug treatment (2 levels) on CD, Y-maze, and open field tests. For each

behavioral test, we performed planned comparisons to investigate dose-response relationships and non-selective drug effects in non-defeated controls. One-way ANOVAs with LSD post-hoc tests were run for dose-response relationships and t-tests were used for non-defeated controls. For Arc immunohistochemical data, we performed a two-way ANOVA with defeat experience and drug treatment as independent variables. All statistical tests were two-tailed, the alpha level was $p < 0.05$, and data are presented as mean $\pm$ S.E.

CHAPTER II RESULTS

Experiment 1: 8-OH-DPAT and acquisition of conditioned defeat

Injection of 8-OH-DPAT prior to social defeat decreased the acquisition of conditioned defeat (Fig 3). We found a significant drug by defeat interaction for the duration of submissive and defensive behavior ($F_{(1,100)} = 6.029, p = 0.016$). Also, defeated subjects showed a significant decrease in submissive and defensive behavior at both 0.25 mg/kg ($F_{(2,71)} = 4.517, p = 0.014$, LSD, $p = 0.006$) and 0.5 mg/kg of 8-OH-DPAT ($p = 0.044$). Drug treatment did not significantly change the frequency of flight ($F_{(1,100)} = 2.790, p = 0.068$) or stretch-attend postures ($F_{(1,100)} = 1.854, p = 0.164$) compared to vehicle controls (Table 1). Also, injection of 8-OH-DPAT prior to social defeat did not alter the durations of non-agonistic social ($F_{(1,100)} = 2.348, p = 0.129$), nonsocial ($F_{(1,100)} = 1.336, p = 0.251$), or aggressive ($F_{(1,100)} = 1.766, p = 0.187$) behaviors.

No defeat controls showed less submissive and defensive behavior at testing compared to defeated subjects (Fig 3; $F_{(1,102)} = 37.362, p = 0.000$). However, increases in duration of aggression ($F_{(1,100)} = 1.766, p = 0.187$) and number of attacks ($F_{(1,102)} = 2.926, p = 0.060$) were non-significant compared to defeated subjects. We also investigated whether 8-OH-DPAT altered agonistic behavior in the absence of social defeat stress. Injections of 8-OH-DPAT in no defeat controls did not alter the duration of submissive and defensive ($t_{(1,42)} = -0.509, p = 0.396$), aggressive ($t_{(1,42)} = 0.771, p = 0.171$), non-agonistic social ($t_{(1,42)} = 0.848, p = 0.751$), or nonsocial behavior ($t_{(1,42)} = -0.1305, p = 0.591$).

8-OH-DPAT and behavior in the Y-maze

Injection of 8-OH-DPAT prior to social defeat increased the amount of time spent in the distal compartment of the Y-maze near the RA (Figure 4). We found a significant drug by defeat interaction for the amount of time spent in the distal compartment of the Y-maze near the RA (Figure 4a; $F_{(1,48)} = 5.444, p = 0.024$). However, when we tested for a dose-response relationship in defeated animals we did not find a significant effect of drug dose on the amount of time spent in the distal compartment of the Y-maze near the RA (Figure 4a; $F_{(2,42)} = 2.002, p = 0.148$). We also found a significant drug by defeat interaction for the amount of time spent sniffing the RA combined with the amount of time spent in the distal compartment of the Y-maze near the RA (Figure 4c; $F_{(1,48)} = 5.007, p = 0.030$). However, we did not find a significant dose-response relationship for sniffing combined with proximity (Figure 4c; $F_{(2,42)} = 2.595, p = 0.087$). We did not find a significant drug by defeat interaction for the amount of time spent sniffing the RA (Figure 4b; $F_{(1,48)} = 1.677, p = 0.202$) as well as the latency to sniff the RA (Figure 4d; $F_{(1,48)} = 0.699, p = 0.407$). We did find a main effect of defeat on the amount of time spent sniffing the RA (Figure 4b; $F_{(1,48)} = 16.307, p = 0.000$), the amount of time spent sniffing combined with proximity (Figure 4c; $F_{(2,42)} = 11.406, p = 0.001$), and the latency to sniff the RA (Figure 4d; $F_{(1,48)} = 7.969, p = 0.007$).

We investigated whether drug treatment had non-selective effects in non-defeated controls. Injection of 8-OH-DPAT in no defeat controls did not alter the amount of time spent sniffing ($t_{(1,42)} = 0.736, p = 0.396$), the time spent in the target distal compartment ($t_{(1,42)} = 1.258, p = 0.974$), the amount of time spent sniffing combined with the amount

of time spent in the distal compartment of the Y-maze near the RA ($t_{(1,42)} = 0.953$, $p = 0.977$) or latency to sniff the RA ($t_{(1,42)} = 0.265$, $p = 0.126$).

8-OH-DPAT and behavior in the open field

Injection of 8-OH-DPAT prior to defeat increased locomotion in the open field (Fig 5). We found a significant drug by defeat interaction for the number of line crosses within the open field ($F_{(1,50)} = 4.812$, $p = 0.033$). An interaction for the number of entries into the center of the open field approached significance ($F_{(1,50)} = 3.314$, $p = 0.075$), but we did not find a significant interaction for latency to exit the hut into the open field ($F_{(1,50)} = 0.432$, $p = 0.514$). These results indicate that 8-OH-DPAT treatment increased locomotion in the open field for defeated animals although it decreased locomotion for non-defeated controls.

Experiment 2: 8-OH-DPAT and Arc expression in the BLA

Injection of 8-OH-DPAT prior to defeat resulted in a nearly significant drug by defeat interaction for the number of Arc immunopositive cells within the BLA (Fig. 6) ($F_{(1,34)} = 3.519$, $p = 0.069$). These results indicate a tendency for 8-OH-DPAT treatment to reduce the number of Arc immunopositive cells in defeated subjects but not in non-defeated controls.

CONCLUSION

We found that administration of 8-OH-DPAT, a nonselective 5-HT_{1A} receptor agonist, prior to social defeat reduced the acquisition of submissive and defensive behaviors present at conditioned defeat testing. Because 8-OH-DPAT did not alter agonistic behavior in non-defeated animals, it suggests that 5-HT_{1A} receptor activation disrupts defeat-induced changes in behavior but not aggressive behavior in general. These results support earlier findings that the activation of 5-HT_{1A} receptors in the BLA prior to social defeat reduces the development of conditioned defeat (Morrison and Cooper, 2012). We also found that administration of 8-OH-DPAT prior to social defeat reduces avoidance of former opponents in a Y-maze. These results suggest that activation of 5-HT_{1A} receptors impairs avoidance of familiar opponents. In this study, we found that activation of 5-HT_{1A} receptors prior to social defeat resulted in increased line crossings in the open field but not an increase in center grid entries. This suggests that activation of 5-HT_{1A} receptors increased defeat-induced locomotion but not defeat-induced anxiety. Finally, we found that injection of 8-OH-DPAT prior to social defeat produced a trend for fewer Arc immunopositive cells in the BLA compared to vehicle-treated controls. This suggests that the activation of 5-HT_{1A} receptors disrupts defeat-induced neural plasticity within the BLA. Together, these results suggest that activation of 5-HT_{1A} receptors reduces the acquisition of conditioned defeat by disrupting neurochemicals critical for the formation of fear memories associated with specific opponents.

Administration of 8-OH-DPAT has been shown to increase the expression of social behavior when given prior to a social interaction test. Although 8-OH-DPAT has

often been used to target 5-HT_{1A} receptors in an attempt to reduce anxiety-like behavior and fear memory, some researchers have failed to find effects of 8-OH-DPAT treatment. Davis et al. (1988) found that administration of 8-OH-DPAT had no effect on the expression of fear-potentiated startle. Similarly, Conti (2012) found no reduction in prepulse inhibition (PPI) of the acoustic startle response in rats administered 8-OH-DPAT prior to PPI testing. The effects of 8-OH-DPAT on acquisition of fear and anxiety have also been mixed. Dos Santos et al. (2005) found that 8-OH-DPAT treatment reduced the acquisition of inhibitory avoidance but not escape behavior in the elevated T-maze. Also, Misane et al. (1998) found that administration of 8-OH-DPAT prior to training reduced passive avoidance retention (latency to escape) in a light/dark transition box test.

Although 8-OH-DPAT has mostly been used as a 5-HT_{1A} receptor agonist, it can also bind to 5-HT₇ receptors at higher doses. This nonselective binding of 8-OH-DPAT could explain the non-linear dose response curve in our results. We found that 0.25 mg/kg of 8-OH-DPAT reduced the acquisition of conditioned defeat, whereas 0.5 mg/kg was less effective. It is possible that at higher doses 8-OH-DPAT binds to 5-HT₇ receptors, which counteracts the reduction in conditioned defeat mediated by 5-HT_{1A} receptors. This interpretation is consistent with other research showing the effects of 8-OH-DPAT on 5-HT_{1A} and 5-HT₇ receptors in the brain. Eriksson et al. (2008) found that administration of 8-OH-DPAT prior to training leads to impairments in a passive avoidance task and when combined with SB-269970, a 5-HT₇ receptor antagonist, the impairments were even greater. However, SB-269970 alone had no effect on passive avoidance retention. These results suggest that when serotonin binds to the 5-HT₇

receptor, it produces an opposite effect compared to the inhibitory effects of serotonin at the 5-HT_{1A} receptor.

The open field test has been used as a validated measure of anxiety-like behavior in which anxiety is characterized by a decrease in the amount of time spent in the center of the open field or the number of entries into the center compared to time spent the perimeter (Kinsey et al., 2007). Kinsey et al. (2007) found that social defeat reduced the amount of time spent in the center of the open field, reduced the number of center entries, and had no effect on locomotion, defined as line crosses, 24 hours after the defeat experience. Similarly, Prut and Belzung (2003) describe anxiety-like behavior in the open field as a reduction in the number of center entries and anxiolytic drugs increase the number of center entries whereas anxiogenic drugs do the opposite. We found that administration of 8-OH-DPAT prior to social defeat resulted in an increase in the number of line crosses but did not significantly alter the number of center entries compared to vehicle animals. In sum, although 8-OH-DPAT treatment increased locomotion, our data suggest that 8-OH-DPAT did not alter classic measures of anxiety in the open field test.

Stress has been found to desensitize 5-HT_{1A} autoreceptors receptors and induce anxiety-like behavior. Short et al. (2000) found that uncontrollable stress resulted in 5-HT_{1A} receptor desensitization and downregulation which in turn increased 5-HT activity in the dorsal raphe nucleus. Similarly, Greenwood et al. (2003) found that freewheel running increased 5-HT_{1A} receptor mRNA in the dorsal raphe nucleus which prevented 5-HT hyperactivity following uncontrollable stress. Chronic uncontrollable stress also leads to the desensitization of 5-HT_{1A} autoreceptors in the dorsal raphe and enhanced anhedonia-like behavior in rats (Bambico et al., 2009).

The desensitization of 5-HT_{1A} autoreceptors following stress is related to increased 5-HT levels in the dorsal raphe nucleus. 8-OH-DPAT treatment may mimic stress-induced activity at 5-HT_{1A} receptors and lead to their desensitization. We found that 8-OH-DPAT treatment reduced locomotion in control animals and this effect could be related to 5-HT_{1A} receptor desensitization. Activation of 5-HT_{1A} receptors by a low dose of 8-OH-DPAT prior to testing has been shown to reduce locomotor activity in an open field (Carey et al., 2004). Carli et al. (1989), also found that administration of 8-OH-DPAT prior to open field testing prevented the reduction in locomotion induced by restraint stress. While these studies explain the effects of 8-OH-DPAT on the expression of open field locomotion, they could help to understand why our non-defeated animals displayed reduced locomotion in the open field after 8-OH-DPAT treatment prior to social defeat. Similar to Carli et al. (1989), 8-OH-DPAT may also have the ability to prevent the reduction in locomotion displayed in our stressed hamsters.

Several studies indicate that the BLA is a critical neural substrate for the development of CD. Injection of a NMDA receptor antagonist into the BLA impairs the acquisition of CD, and CREB overexpression induced by a viral vector increases the acquisition of CD (Jasnow et al., 2005, Day et al., 2011). Administration of muscimol, a GABA(A) receptor agonist, into the BLA also reduces the acquisition of CD (Jasnow and Huhman, 2001). BDNF, a protein important for memory formation, increases after social defeat in hamsters, and blockade of the TrkB receptors in the BLA where BDNF binds impairs the acquisition of CD (Taylor et al., 2011). In sum, the conditioned defeat response is an amygdala-dependent behavioral change that requires many of the neurochemicals critical for conditioned fear.

The immediate early gene Arc has been shown to be necessary for the expression of long-term potentiation and is associated with experience-dependent plasticity and the acquisition of auditory fear conditioning (Messaoudi et al., 2007, Maddox and Schafe, 2011). Infusion of a viral vector into the lateral amygdala that knocks down Arc protein has been shown to impair memory formation in Pavlovian fear conditioning (Ploski et al., 2008). Also, social defeat enhances Arc expression in the prefrontal cortex, hippocampus, and nucleus accumbens (Coppens et al., 2011). We found that administration of 8-OH-DPAT prior to social defeat tended to reduce Arc expression in the basolateral amygdala. This suggests that Arc may be involved in the formation of defeat-related memories and that activation of 5-HT_{1A} receptors may disrupt defeat-induced Arc expression. We also found that 8-OH-DPAT treatment disrupted avoidance of familiar opponents suggesting that 5-HT_{1A} activation may impair defeat-related memories.

In sum, our findings suggest the hypothesis that 5-HT_{1A} receptor activation disrupts Arc expression in the BLA and thereby impairs the development of a fear memory that is essential for the conditioned defeat response. In the future, we will investigate the role of 5-HT_{2A} receptors in the acquisition of conditioned and if blockade of these receptors impairs the anxiety-like behavior associated with the conditioned defeat response.

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APPENDICES

Table 1.

Behavior	D 0.0 mg/kg	D 0.25 mg/kg	D 0.5 mg/kg	ND 0.0 mg/kg	ND 0.25 mg/kg	p value
Flee	1.032 ± .487	.034 ± .035	.071 ± .071	.000 ± .000	.000 ± .000	0.095
Stretch Attend	.452 ± .166	.517 ± .236	.071 ± .071	.048 ± .048	.130 ± .072	0.960
Attack	.000 ± .000	.172 ± .141	.071 ± .071	2.190 ± .575	1.261 ± .346	0.062

The frequencies of flee, stretch attend, and attack (mean ± SE) during conditioned defeat testing are shown. Defeated (D) animals were treated with 0.0 mg/kg, 0.25 mg/kg, or 0.5 mg/kg of 8-OH-DPAT prior to social defeat. No defeat (ND) animals were treated with 0.0 mg/kg or 0.25 mg/kg of 8-OH-DPAT before exposure to an aggressor's empty cage. Subjects did not significantly differ in any category of behavior.

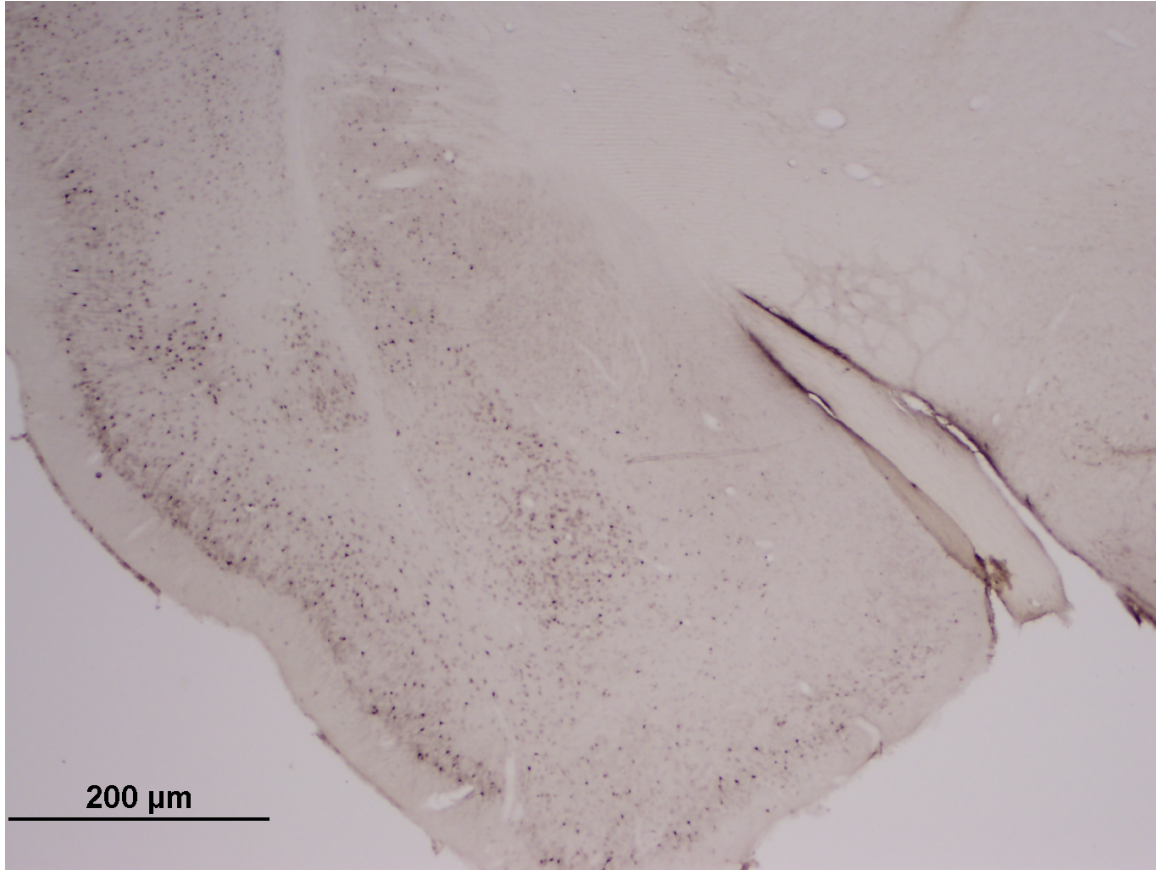


Figure 1. Photograph (2X magnification) of the BLA from a defeated hamster used in Arc quantification. Black dots represent Arc immunopositive nuclei. The box size used for quantification (width x height) was 350 μm x 350 μm.

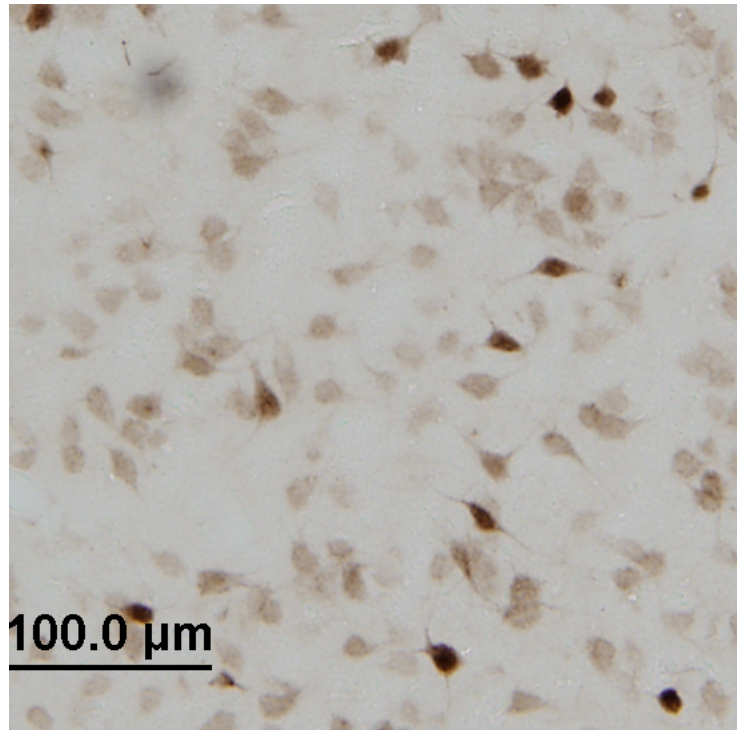


Figure 2. Representative photomicrograph (10X magnification) of the BLA from a defeated hamster used in Arc quantification. Black dots represent Arc immunopositive nuclei.

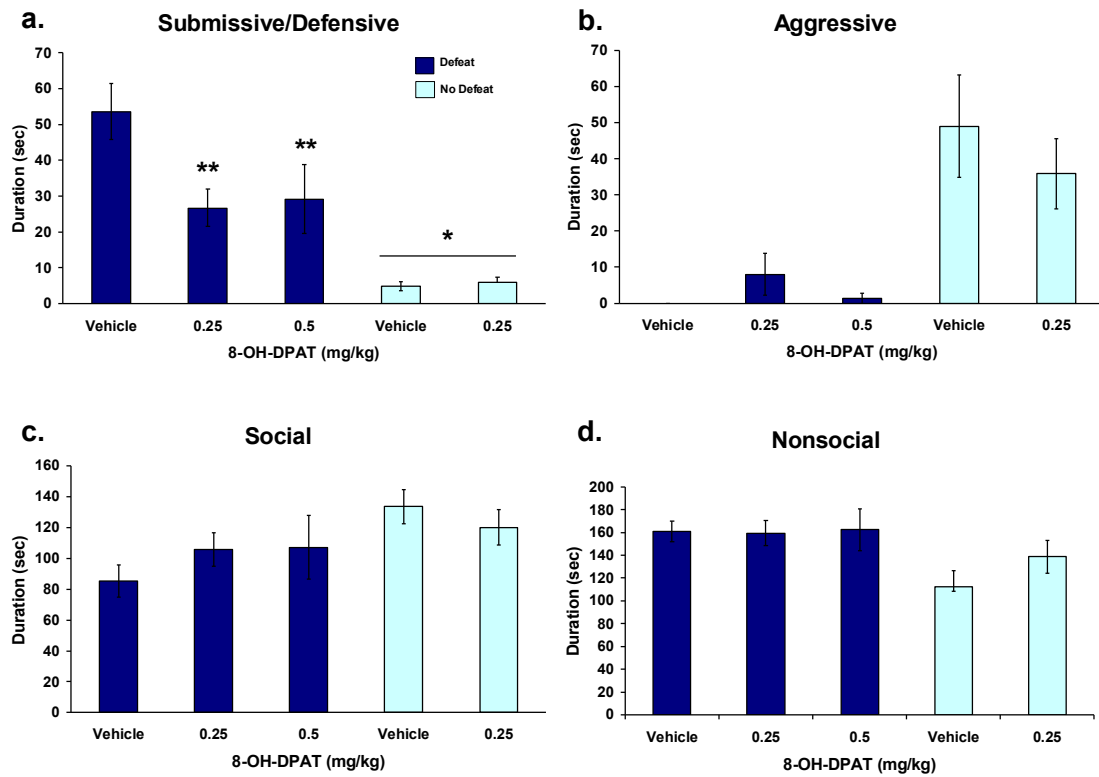


Figure 3. Durations (mean $\pm$) of submissive, aggressive, non-agonistic social, and nonsocial behavior are shown for a 5-minute test with a novel, non-aggressive opponent. Defeated animals received an injection of 8-OH-DPAT (0.25 mg/kg, N=29 or 0.5 mg/kg, N=14) or vehicle (N=31) 20 minutes before social defeat training. Likewise, controls received an injection of 8-OH-DPAT (0.25 mg/kg, N=23) or vehicle (N=21) 20 minutes before exposure to a resident aggressor's empty cage. ** Indicates significantly different than defeated, vehicle controls. * Indicates significantly different than defeated subjects.

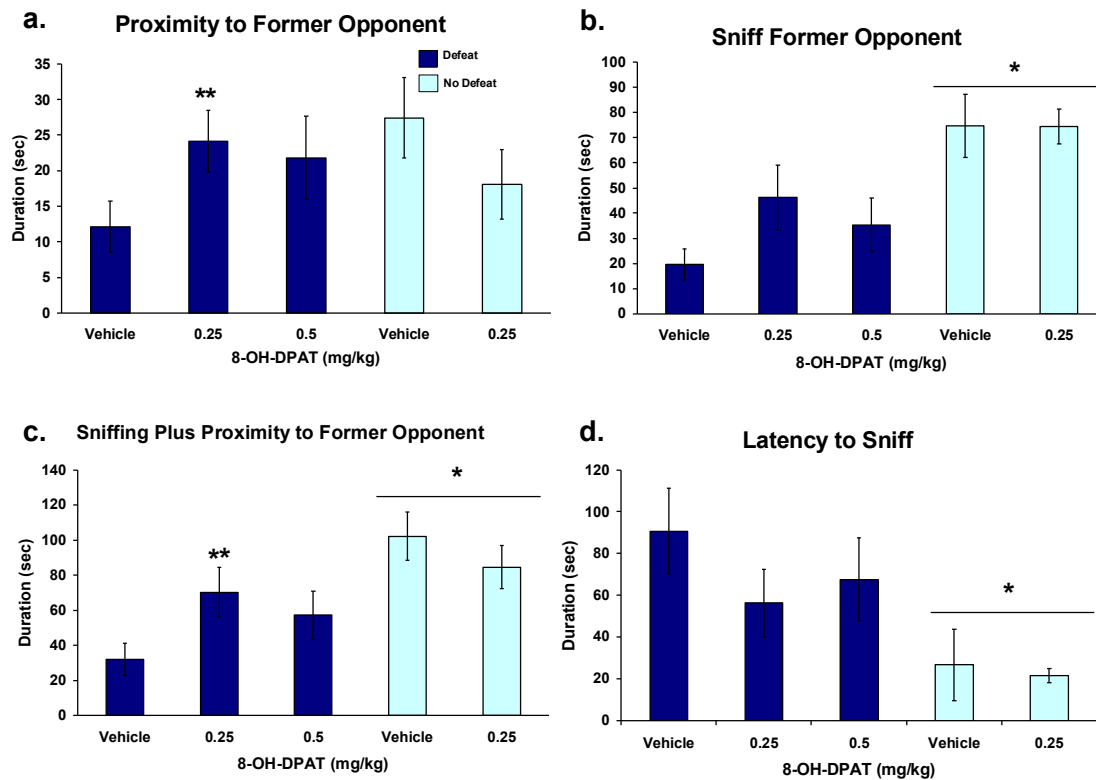


Figure 4. Durations (mean $\pm$) of time spent in proximity to former opponent, sniffing the former opponent, and sniffing plus proximity to the former opponent, and latency to sniff the former opponent are shown for a 3-minute test in the Y-maze. Defeated animals received an injection of 8-OH-DPAT (0.25 mg/kg, N=15 or 0.5 mg/kg, N=14) or vehicle (N=16) 20 minutes before social defeat training. Likewise, controls received an injection of 8-OH-DPAT (0.25 mg/kg, N=11) or vehicle (N=10) 20 minutes before exposure to a resident aggressor's empty cage. ** Indicates significantly different than defeated, vehicle controls. * Indicates significantly different than defeated subjects.

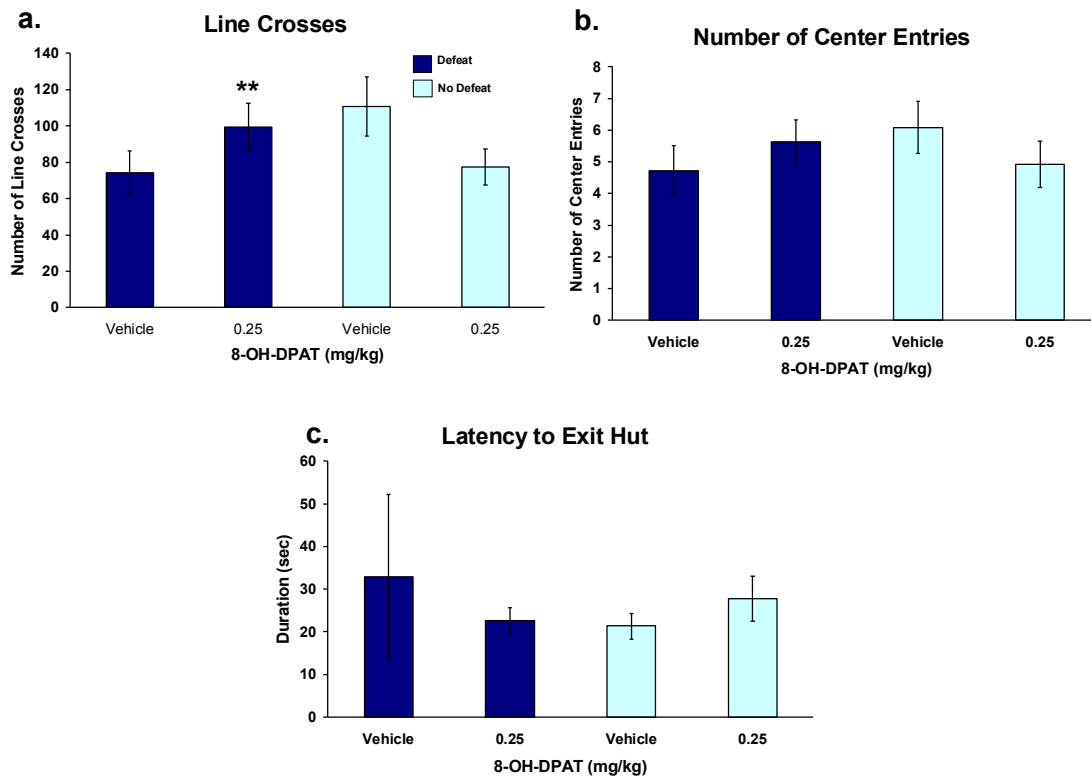


Figure 5. Number (mean $\pm$) of line crosses, center entries, and the latency to exit the hut are shown for a 5-minute test in an open field. Defeated animals received an injection of 8-OH-DPAT (0.25 mg/kg, N=14) or vehicle (N=15) 20 minutes before social defeat training. Likewise, controls received an injection of 8-OH-DPAT (0.25 mg/kg, N=12) or vehicle (N=12) 20 minutes before exposure to a resident aggressor's empty cage. ** Indicates significantly different than defeated, vehicle controls. * Indicates significantly different than defeated subjects.

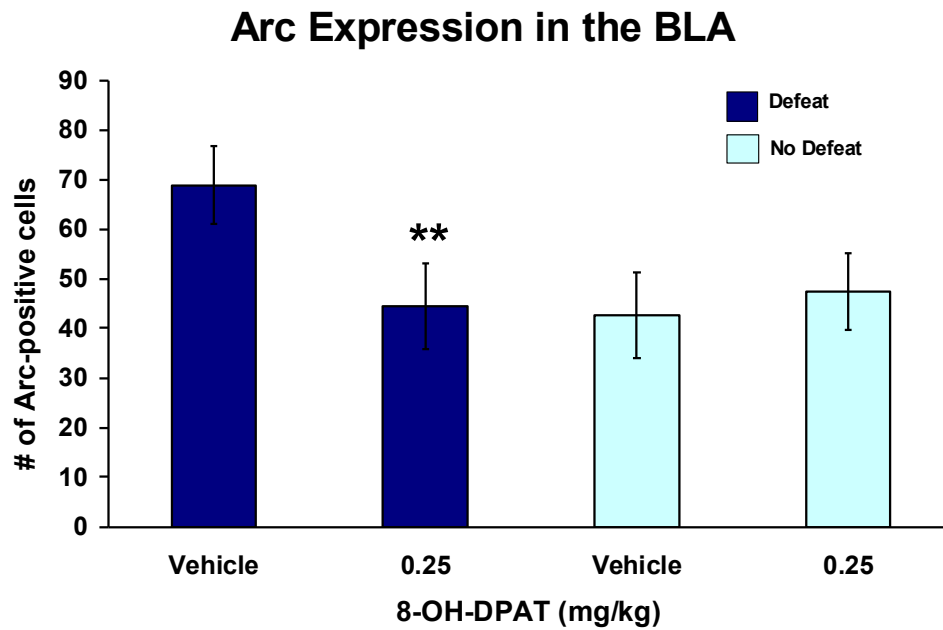


Figure 6. Number (mean $\pm$ SE) of Arc immunopositive cells in the BLA measured following social defeat training. Defeated animals received an injection of 8-OH-DPAT (0.25 mg/kg, N=10) or vehicle (N=10) 20 minutes before social defeat training. Likewise, controls received an injection of 8-OH-DPAT (0.25 mg/kg, N=8) or vehicle (N=10) 20 minutes before exposure to a resident aggressor's empty cage. ** Indicates significantly different than defeated, vehicle controls.

Vita

Lauren Bader was born to parents Bradley and Debra Bader in Cape Girardeau, Missouri on December 4, 1987. She obtained a B.A. degree in Biology at Drury University in Springfield, Missouri in 2010. In the fall of 2010, she entered the graduate program in Psychology at the University of Tennessee, Knoxville and studied in the behavioral neuroscience lab of Dr. Matthew Cooper. After obtaining a M.A. degree in Psychology in May 2013, she accepted at doctoral position in the Department of Child and Family Studies at the University of Tennessee, Knoxville. She plans to pursue a career in cross-cultural child development research as well as undergraduate teaching.