5-2012

Effect of social status on social defeat-induced neural activation in the dorsal raphe nucleus

Danielle M. Gerhard
University of Tennessee - Knoxville, dgerhard@utk.edu

Kathleen E. Morrison
University of Tennessee - Knoxville, kmcinty1@utk.edu

Matthew A. Cooper
University of Tennessee - Knoxville, mcooper@utk.edu

Effect of social status on social defeat-induced neural activation in the dorsal raphe nucleus

Danielle M. Gerhard, Kathleen E. Morrison, Matthew A. Cooper

Danielle Gerhard
Chancellor's Honors Program Senior Thesis
University of Tennessee
Spring 2012

Abstract

The dorsal raphe nucleus (DRN) has been implicated in the regulation of behavior following stressful events. Previous research indicates that social defeat activates serotonin (5-HT) neurons in select subregions of the DRN and that increased 5-HT activity in the DRN enhances the conditioned defeat response in Syrian hamsters (*Mesocricetus auratus*). Furthermore, research has shown that dominant hamsters exhibit less defensive and submissive behavior at conditioned defeat testing compared to subordinates and controls. The purpose of this study was to determine if social status alters neural activation the in the DRN during social defeat. We tested the hypotheses that following social defeat, dominant hamsters would show decreased c-Fos immunoreactivity compared to subordinates and controls and that neural activation in subordinates would be restricted to the rostral DRN. We found that 3, 5-minute aggressive encounters increased c-Fos expression in subordinates and social experience controls compared to dominants and handled, non-defeated controls. Furthermore, the increased c-Fos expression in subordinates was restricted to the rostral and caudal regions of the ventral DRN. These results suggest that defeat-induced c-Fos immunoreactivity in select subregions of the DRN is status-dependent. Our results support the hypothesis that dominants exhibit a blunted conditioned defeat response and a distinct pattern of neural activation in the DRN, which may contribute to resistance to conditioned defeat.

1. Introduction

A considerable variance in behavioral response exists among individuals that experience traumatic events. Only a portion of those who experience a traumatic event will go on to develop post-traumatic stress disorder (PTSD) and other stress-related mental illnesses (Yehuda, et al., 2006). Resilience to stress in humans can be attributable to complex behavioral adaptation and

personality traits associated with extraversion, high self-esteem, assertiveness, internal locus of control (Agaibi & Wilson, 2005), cognitive flexibility, and positive coping styles (Yehuda et al., 2006). In rodent models of stress, social status (Morrison et al., 2011), coping styles (Koolhass et al., 2010), and stressor controllability (Amat et al., 2005) can alter an individual's stress response.

Syrian hamsters are a solitary species that display territorial aggression. Reliable aggressiveness and the long-lasting effects of acute social defeat make this species ideal for studying the behavioral and neural correlates of social stress. Conditioned defeat is a model in Syrian hamsters in which an acute social defeat results in a loss of territorial aggression and an increase in submissive and defensive behavior in later non-aggressive social encounters. The conditioned defeat model has been used to examine the behavioral and neurobiological consequences of social stress (Huhman, 2006). This model has the benefit of utilizing social defeat, a type of social stressor more commonly experienced by humans than the physical stressors often used in other animal models. Research in our lab has shown that social status alters how individuals respond to social defeat such that dominants show a reduced conditioned defeat response compared to subordinates and controls (Morrison et al., 2011).

Little is known about the neural substrates that underlie variability in how individuals respond to stressful stimuli. Serotonin (5-HT) is a key neurochemical that modulates aggressive behavior, coping style and behavioral responses to social stress. A majority of the 5-HT neurons innervating forebrain structures stems from the dorsal raphe nucleus (DRN). The 5-HT system is implicated in the etiology of stress-related mental illnesses and effectual pharmacological treatments target 5-HT neurons. The selective serotonin reuptake inhibitors (SSRIs) are successful in treating affective and anxiety spectrum disorders (Vaswani et al., 2003) as well as reducing anxiety-like behavior on the elevated plus-maze (Drapier et al, 2007) and depressive-

like behavior in the forced swimming test (Detke et al., 1995). SSRIs prevent the reuptake, and thus metabolism, of serotonin, and therefore produce a therapeutic mechanism of action by altering the 5-HT system. SSRI treatment also alters the expression of 5-HT receptors, including 5-HT1A receptors (Roth, 1994). The 5-HT1A autoreceptor mediates inhibitory neurotransmission and receptor agonists have proven effective in relieving anxiety and depression (Schreiber & De Vry, 1993). Research using knockout mice shows that mice lacking the 5-HT1A receptor display enhanced anxiety and an increased response to stress (Parks et al., 1998). Pharmacological activation of 5-HT1A receptors in the DRN has been found to reduce the behavioral effects of learned helplessness (Greenwood et al., 2003), fear-conditioning (Maier et al., 1995) and conditioned defeat (Cooper et al., 2008). Our lab has previously shown that flesinoxan, a selective 5-HT1A agonist, reduces the amount of submissive/defensive behavior and that WAY 100635, a 5-HT1A antagonist, enhanced conditioned defeat when injected in the DRN prior to social defeat training (Cooper et al., 2008). These results support an overarching hypothesis that the activity of 5-HT cells in the DRN, regulated by 5-HT1A autoreceptors, is instrumental is the formation of conditioned defeat.

The DRN has topographically organized projections to the forebrain that are compartmentalized into the rostral and caudal subdivisions with ventral, dorsal and lateral subregions. In the rat, projections from the DRN to the amygdala originate in the mid-rostrocaudal regions while those that project to the hippocampus stem from the caudal regions, with both areas of the brain heavily implicated in anxiety-related behavior (Imai et al., 1986). Previous research in our lab has shown that subordinate individuals display increased c-Fos expression and decreased 5-HT1A mRNA levels in the rostral portions of the ventral DRN, suggesting that 5-HT neurons are hyperactive following social defeat (Cooper et al., 2009).

The purpose of this study was to determine if social status alters neural activation in the DRN during social defeat in the male Syrian hamster. We hypothesized that dominant individuals would show decreased c-Fos immunoreactivity in the DRN compared to subordinates and controls. Also, we hypothesized that defeat-induced c-Fos expression in subordinates and controls would be restricted to the rostral DRN.

2. Materials and Methods

2.1 Subjects

Subjects were male Syrian hamsters (*Mesocricetus auratus*) obtained from our breeding colony that was originally derived from Charles River Laboratories (Wilmington, MA). Subjects were 8 weeks old and weighed 120-180 g at the start of the study. All subjects were individually housed one week prior to experimental manipulation. Older hamsters (> 6 months, > 190 g) that have been singly housed for a longer period of time and display reliable aggression when faced with intruders were used as resident aggressors for social defeat training. All animals were housed in polycarbonate cages (12 cm x 27 cm x 16 cm) with corncob bedding, cotton nesting materials, and wire mesh tops. Animals were housed in a temperature controlled colony room (21 ± 2° C) and kept on a 14:10 hr light:dark cycle with food and water available *ad libitum*. Cages were not changed for one week prior to dominant-subordinate encounters to allow individuals to scent mark their territory. Subjects were handled daily for one week prior to dominant-subordinate encounters to habituate them to the stress of human handling. All behavioral protocols were performed during the first three hours of the dark phase of their cycle. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Behavioral protocols

2.2.1 Dominant-subordinate daily pairs

Subjects within each cohort were weight-matched and paired in daily social encounters for 14 days. Subjects were randomly assigned as a resident or intruder, and all social encounters occurred in the resident's home cage. The encounter on day 1 was 10 minutes in duration while all subsequent encounters were 5 minutes. Our lab has previously shown that a 10-minute encounter on day 1 facilitates the formation of a dominance relationship and that 5-minute encounters on subsequent days are sufficient to maintain the dominance relationship while reducing the chance of wounding (Morrison et al., 2011). Dominant and subordinate subjects were distinguished by the direction of agonistic behavior within each dyad. The subjects used in the present study came from another project in our lab and therefore follow a dominant-subordinate paradigm that allows time for surgery and recovery partway through the 14 days of daily pairs. After nine days of dominant-subordinate encounters, all subjects underwent stereotaxic surgery to inject the retrograde tracer Cholera toxin B into the basolateral amygdala (BLA). After one day of surgical recovery, dominant-subordinate encounters resumed and continued for five days (Fig. 1). Daily agonistic encounters were digitally recorded for later behavioral analysis.

2.2.1 Social Defeat Training

Twenty-four hours after the last social encounter, all dominants (N=11), subordinates (N=11), and half of the controls (N=12; social experience controls) experienced social defeat training (Fig. 1.1). Social defeat consisted of subjects being placed into the home cages of three different resident aggressors for three separate 5-minute aggressive encounters, and subjects

received a 5-minute resting period in their home cage between each aggressive encounter. To correct for potential variation in the amount of aggression subjects received, we defined social defeat as starting at the resident's first attack that was accompanied by submissive behavior in a subject. Half of the controls (N=10) experienced the no defeat control procedure (no defeat controls), which consisted of subjects being placed into the empty home cages of three different resident aggressors for three separate 5-minute exposures. We placed no defeat controls in dirty resident aggressor cages to control for handling and olfactory cues that may alter neural activation.

We digitally recorded all social defeat training sessions and quantified the behavior of resident aggressors and subjects using Noldus Observer software (Noldus Information Technology, Wageningen, Netherlands). We quantified the total duration of the following behaviors: submissive/defensive (flee, avoid, upright and side defensive postures, tail-up, stretch-attend), aggressive (chase, attack including bite, upright and side offensive postures), nonagonistic social (sniff, approach), and nonsocial (locomotion, grooming, nesting, feeding). We also recorded the frequency of attacks, flees, and stretch-attend postures. A researcher blind to the experimental conditions of the subject and at least 90% reliable performed all behavioral scoring.

2.3 c-Fos immunohistochemistry

Ninety minutes following the start of social defeat, animals were anesthetized with 4% isoflurane. Animals were then transcardially perfused with 100ml of 0.1 M PBS followed by 100 ml of 4% paraformaldehyde solution. 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 submerged

in 0.1 M PBS on a vibrating microtome and collected in glass scintillation vials then stored as free floating sections in cryoprotectant at 4°C.

Tissue sections collected for the present study contained the DRN. The tissue was processed for immunohistochemisty using a primary antiserum directed against the c-Fos protein. All washes, rinses and incubations were performed at room temperature in plastic well plates gently shaken on an orbital shaker throughout immunostaining. Sections were rinsed five times with PBS + 0.2% Triton and then incubated for 20 minutes with 0.3% hydrogen peroxide followed by another five rinses in PBS + 2% Triton. Sections were then incubated in goat serum with PBS + 0.2% Triton for 25 minutes followed by 24 hours in rabbit anti-c-Fos polyclonal primary antibody (Santa Cruz Biotechnologies) at a final dilution of 1:10000 in PBS + 2% Triton. Sections were rinsed five times in PBS + 0.2% Triton, followed by 60 minutes in PBS + 0.2% Triton containing anti-rabbit IgG at a final dilution of 1:200. After staining with the biotylinated secondary antibody, sections were rinsed five times in PBS + 0.2% Triton followed by a 60 minute incubation with an avidin-biotin complex reagent (Vectastain Elite ABC kit, Vector Laboratories). After rinsing with PBS + 0.2% Triton, sections were placed in a solution containing 3,3'-diaminobenzidene (DAB), hydrogen peroxide, and nickel dissolved in PBS for 15 minutes. The peroxide reaction was stopped with a series of five rinses in PBS followed by five rinses in distilled water. The sections were mounted onto microscope slides, air-dried, dehydrated using a series of alcohols, cleared with citrosolv and coverslipped using DPX mountant (Sigma-Aldrich, St. Louis, MO). The tissue from all subjects was processed simultaneously to control for any extraneous variables.

Images of the DRN were captured at 10X magnification using an Olympus BX41 microscope. The number of c-Fos immunopositive cells was determined using MCID Core

image analysis software (InterFocus Imaging, Cambridge, England). We quantified the number of c-Fos immunopositive cells in the ventral, dorsal and lateral regions of the rostral and caudal DRN. For each brain region, we recorded background immunoreactivity in unstained regions of each image. We then defined immunopositive cells as those that showed staining 1.3X darker than the specific background immunoreactivity calculated for each image. We used 1.3X background because it produced cell counts similar to manual quantification. Cell counts were limited to the area within defined boxes that were tailored to the size of each brain region (Fig. 2). A sample image of the ventral DRN shows the result of c-Fos immunostaining (Fig. 3).

2.4 Data Analysis

Data from social defeat training were analyzed with a 1-way analysis of variance (ANOVA) with social status as a between-subjects factor and were performed by doctoral student Kathleen Morrison. For immunohistochemical data, we performed two separate analyses. Immunohistochemical data from defeated subjects were analyzed using a 1-way ANOVA with social status as a between-subjects factor. To investigate the effect of defeat on c-Fos immunoreactivity, the immunohistochemical data from control subjects were analyzed using an independent samples t-test (social experience controls vs. no defeat controls). Significant main effects found in the 1-way ANOVAs were followed by Tukey's HSD post hoc analysis. All results were considered significant when the α level was $p \leq 0.05$.

3. Results

3.1 Dominant-subordinate daily pairs

Dominance relationships were quickly formed and remained stable throughout the experiment. On average, a dominance relationship formed between a pair on day 1.4 (± 0.3). Subordinates showed an increased and consistent level of submissive/defensive behavior during

dominant-subordinate encounters compared to dominants, which never showed submissive/defensive behavior (Fig. 5a). Additionally, dominants reliably displayed aggressive behaviors while subordinates were never aggressive (Fig. 5b).

3.2 Social defeat training

All subjects received equal treatment from the resident aggressors during social defeat training as there was no effect of social status on the total amount of aggression received from the resident aggressors; however, there was an effect of social status on the total amount of submissive and defensive behavior displayed by subjects during social defeat training (p = 0.013) (Table 1). Subordinates displayed significantly more submissive and defensive behavior during social defeat training than did dominants (p = 0.009). Social experience controls displayed an intermediate amount of submissive and defensive behavior that was not significantly different from the amount displayed by either dominants or subordinates ($p > 0.05$).

Social status also had an effect on whether subjects counter attacked the resident aggressor during the first defeat. Dominant individuals were more likely to counter attack the resident aggressor (6 out of 11 dominants) than were subordinates (1 out of 11) ($p < 0.025$). Subordinates and social experience controls (2 out of 12) were equally as likely to counter attack the resident aggressor ($p > 0.05$). Although the difference was nearly significant, social experience controls and dominants did not differ in the number that counter attacked the resident aggressor ($p = 0.056$).

3.3 c-Fos immunohistochemistry

We did not find a main effect of social defeat in any of the DRN subregions. Although social experience controls had more c-Fos immunoreactive cells in the rostral ventral DRN than

did non-defeated, handled controls, the results did not reach statistical significance ($p = 0.0114$). Nevertheless, we found a significant effect of social status in key subregions of the DRN. Subordinate hamsters showed increased c-Fos immunoreactivity in the rostral ventral DRN compared to dominants ($p = 0.002$) and handled controls ($p = 0.002$) (Fig. 4a). Additionally, subordinates showed increased c-Fos immunoreactivity in the caudal ventral DRN compared to dominants ($p = 0.024$) and handled controls ($p = 0.018$). We did not find an effect of social status in rostral or caudal subregions of the dorsal DRN (Fig. 4b), although the differences were nearly significant in the rostral dorsal DRN ($p = 0.055$). Also, no significant differences were found in either the rostral or caudal regions of the lateral DRN ($p > 0.05$) (Fig. 4c).

4. Discussion

These results are consistent with previous research in our lab showing that dominant and subordinate hamsters differ in their susceptibility to conditioned defeat as well as in their pattern of defeat-induced neural activation. We found an effect of social status on the amount of submission produced during social defeat training in that subordinates displayed significantly more submissive and defensive behavior than did dominants. Additionally, social status did affect whether subjects counter attacked the resident aggressor during the first defeat. Dominant were more likely to counter attack the resident aggressor than were subordinates. Subordinates also showed significantly more c-Fos immunoreactivity than did dominants or no defeat controls in the rostral and caudal subdivisions of the ventral DRN. These results suggest that social status has an effect on social defeat-induced neural activation in select subregions of the DRN. The significant difference between subordinates and no defeat controls further supports the overarching hypothesis that subordinates are more sensitive to the effects of social defeat than dominants.

Some of our findings differ from previous research in our lab. We expected that in addition to an effect of social status, there would be an effect of defeat, as seen previously (Cooper et al., 2009). Although the caudal and rostral subdivisions of the ventral DRN appeared to follow a trend for increased activation following social defeat, the differences between social experience controls and no defeat controls was not statistically significant. Furthermore, we have shown that defeat-induced neural activation was restricted to the rostral portions of the DRN (Cooper et al., 2009), whereas we found activation in both the rostral and caudal regions of the DRN in the present study.

The topographic organization of the DRN implies functional differences among the subdivisions. Serotonergic neurons projecting to brain regions that regulate anxiety are concentrated in the mid-rostral, mid-caudal and caudal regions of the DRN (Abrams et al. 2005; Commons et al., 2003). Many of the efferents from the DRN innervate key brain regions implicated in the formation and display of conditioned defeat. The rostral DRN has projections to the amygdala and periaqueductal gray, which modulate emotional reactions and defensive behavior, respectively, while the caudal DRN has connections with the hippocampus, amygdala and locus coeruleus (Rizvi et al., 1991; Imai et al., 1986). Also, the ventral region of the DRN has the highest expression of 5-HT1A receptors (Hale & Lowry, 2010). Taking into account all of the research on the topographic organization of the DRN, research suggests that changes in the neuronal activity of small numbers of serotonergic neurons may have important implications for specific physiologic or behavioral responses. A projection region of particular interest is the basolateral amygdala (BLA), a critical neural structure underlying the formation of conditioned defeat.

Intra-BLA infusion of anisomycin blocks protein synthesis and impairs the acquisition of

conditioned defeat (Markham et al., 2010; Markham & Huhman, 2008) and conditioned freezing (Schafe & LeDoux, 2000). Injections of urocortin 1, a neuropeptide closely related to corticotropin-releasing factor (CRF), into the BLA induced anxiety-like behavior as well as increased c-Fos expression within serotonergic neurons in the rostral and mid-rostrocaudal parts of the ventral DRN in rats exposed to social interaction test but not home-cage controls (Spiga et al., 2006). Activation of serotonin-immunopositive cells in the caudal DRN has been shown in rats following uncontrollable stressors (Grahn et al., 1999). These results suggest that the basolateral amygdala and serotonergic neurons within the dorsal raphe nucleus could be implicated in anxiety- and depression-related behaviors. It may be increased release of 5-HT in the BLA from specific subregions of the DRN that drives conditioned defeat behavior and the resistance associated with dominance may be due to less 5-HT release into the BLA. This possibility is supported by existing literature which shows that approximately 10% of 5-HT neurons in the DRN that give rise to axons in the amygdala are predominantly found in the ventromedial parts (Ma et al., 1991) and a majority of 5-HT1A receptors are located in the ventral DRN (Hale & Lowry, 2010). This study suggests that many of the c-Fos positive cells in the ventral DRN of our study could have been serotonergic. Furthermore, retrograde tracer injections of cholera toxin b into the ventral and dorsal regions of the DRN resulted in labeled cells within the central nucleus of the amygdala (Peyron et al., 1997). Further research is necessary to verify that defeat-induced neural activation in the ventral DRN is in serotonergic cells that project to the BLA.

The study of the neural basis of resilience to stress is an important research topic in the field of behavioral neuroscience. Individuals differentially respond to social stress and social status has been shown to moderate stress-induced changes in behavior following social defeat.

Our results show that subordinate individuals have a heightened response to social defeat, which might lead to an elevated conditioned defeat response. These findings indicate that social status influences individual responses to stress. Understanding the neural circuitry of conditioned defeat will provide clues to potential pharmacological and therapeutic interventions for individuals suffering from stress-related psychopathologies.

Acknowledgements

I would like to thank Dr. Matthew Cooper for the guidance and educational opportunities he has given me through working in his lab. Additionally, I thank our team of graduate and undergraduate students, notably Kathleen Morrison. The present study stemmed from her final dissertation project and she performed all behavioral analyses for this study. I am thankful for her unrelenting willingness to teach from the classroom to the lab over the past few years. This work was supported by an NIH R21 grant (MH085230) to Matthew A. Cooper and a Chancellor's Honors Program Research Grant to Danielle M. Gerhard.

References

Abrams, J.K., Johnson, P.L., Hay-Schmidt, A., Mikkelsen, JD, Shekar, A, & Lowry, C.A. (2005). Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs. *Neuroscience, 133*: 983-97.

Agaibi, C.E. & Wilson, J.P. (2005). Trauma, PTSD, and resilience: A review of the literature. *Trauma, Violence, & Abuse, 6*: 195-216.

Amat, J., Baratta, M.V., Paul E., Bland S.T., Watkins, L.R., & Maier, S.F. (2005). Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nature, 8*: 365-71.

Charney, D.S. (2004). Psychobiological mechanisms of resilience and vulnerability: Implications for successful adaptation to extreme stress. *American Journal of Psychiatry, 161*: 195-216. distinct dorsal raphe-limbic circuit with a potential role in affective disorders. *Neuropsychopharmacology, 28*: 206-15.

Commons, K.G., Connolley, K.R., & Valentino, R.J. (2003). A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. *Neuropsychopharmacology, 28*: 206-15.

Cooper, M.A., McIntyre, K.E., & Huhman, K.L. (2008). Activation of 5-HT1A autoreceptors in the dorsal raphe nucleus reduces the behavioral consequences of social defeat. *Psychneuroendocrinology, 33*: 1236-47.

Cooper, M.A., Grober, M.S., Nicholas, C.R., & Huhman, K.L. (2009). Aggressive encounters alter the activation of serotonergic neurons and the expression of 5-HT1A mRNA in the hamster dorsal raphe nucleus. *Neuroscience, 161*: 680-90.

Detke, M.J., Rickels, M., & Lucki, I. (1995). Active behaviors in the rat forced swimming test differentially produced by serotonergic and nonadrenergic antidepressants. *Psychopharmacology, 121*: 66-72.

Drapier, D., Bentué-Ferrer, D., Laviolle, B., Millet, B., Allain, H., Bourin, M., & Reymann, J. (2007). Effects of acute fluoxetine, paroxetine and desipramine on rats tested on the elevated plus-maze. *Behavioural Brain Research, 176*: 202-209.

Grahn, R.E., Will, M.J., Hammack, S.E., Maswood, S., McQueen, M.B., Watkins, L.R., & Maier, S.F. (1999). Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. *Brain Research, 826*: 35-43.

Greenwood, B.N., Foley, T.E., Day, H., Campisi, J., Hammack, S.H., Campeau, S., Maier, S.F., & Fleshner, M. (2003). Freewheel running prevents learned helplessness/behavioral depression: Role of dorsal raphe serotonergic neurons. *The Journal of Neuroscience, 23*: 2889-98.

Hale, M.W. & Lowry, C.A. (2010). Functional topography of midbrain and pontine serotonergic systems: Implications for synaptic regulation of serotonergic circuits. *Psychopharmacology, 213*: 243-64.

Huhman, K.L. (2006). Social conflict models: Can they inform us about human psychopathology? *Hormones & Behavior, 50*: 640-6.

Imai, H., Steindler da, Kitai, S.T. (1986). The organization of divergent axonal projections from the midbrain raphe nuclei in the rat. *J Comp. Neruol., 243*: 363-80.

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Neuroendocrinology, 31*: 307-21.

Ma, Q.P., Yin, G.F., Ai, M.K., & Han, J.S. (1991). Serotonergic projections form the nucleus raphe dorsalis to the amygdala in the rat. *Neuroscience Letters, 134*: 21-24.

Maier, S.F., Grahn, R.E., & Watkins, L.R. (1995). 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear conditioning and interference with escape produced by exposure to inescapable shock. *Behavioral Neuroscience, 109*: 404-12.

Markham, C.A., Taylor, S.L., Huhman, K.L. (2010). Role of amygdala and hippocampus in the neural circuit subserving conditioned defeat in Syrian hamsters. *Learning & Memory*, *17*: 109-16.

Markham, C.M. & Huhman, K.L. (2008). Is the medial amygdala part of the neural circuit modulating conditioned defeat in Syrian hamsters? *Learning & Memory, 15*: 6-12.

Morin, LP, Wood RI. (2001). A stereotaxic atlas of the golden hamster brain. San Diego: Academic Press.

Morrison, K.E., Swallows, C.L., & Cooper, M.A. (2011). Effects of dominance status on conditioned defeat and expression of 5-HT1A and 5-HT2A receptors. *Physiology & Behavior, 104*: 283-90.

Morrison, K.E. (2012). Neural mechanisms underlying resistance to conditioned defeat. *University of Tennessee Doctoral Dissertations.*

Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T. & Toth, M. (1998). Increased anxiety of mice lacking the 5HT1A receptor. *Proc. Natl. Acad. Sci., 95*: 10734-9.

Paul, E.D., Hale, M.W., Lukkes, J.L., Valentine, M.J., Sarchet, D.M., & Lowry, C.A. (2011). Repeated social defeat increases reactive emotional coping behavior and alters functional

responses in serotonergic neurons in the rat dorsal raphe nucleus. *Physiology & Behavioral, 104*: 272-82.

Peyron, C., Petit, J.-M., Rampon, C., Jouvet, M., & Luppi, P.-H. (1997). Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience, 82*: 443-68.

Rizvi, T.A., Ennis, M., Behbehani, M.M., & Shipley, M.T. (1991). Connections between the central nucleus of the amygdala and the midbrain periaqueductal gray: topography and reciprocity. *J Comp Neurol, 303*: 121-31.

Roth, B.L. (1994). Multiple serotonin receptors: Clinical and experimental aspects. *Ann. Clin. Psychiatry, 6*: 67-78.

Schafe, G., Ledoux, J.E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *Journal of Neuroscience, 20*: RC96.

Schreiber, R. & De Vry, J. (1993). 5-HT1A receptor ligands in animal models of anxiety, impulsivity and depression: Multiple mechanisms of action? *Prog. Neuro-Psychopharmacol. & Biol. Psychiat., 17*: 87-104.

Spiga, F., Lightman, S.L., Shekhar, A., & Lowry, C.A. (2006). Injections of urocortin 1 into the basolateral amygdala induce anxiety-like behavior and c-Fos expression in brainstem serotonergic neurons. *Neuroscience, 138*: 1265-76.

Yehuda, R., Flory, J.D., Southwick, S., & Charney, D.S. (2006). Developing an agenda for t translational studies of resilience and vulnerability following trauma exposure. *Annals of the New York Academy of Sciences, 1071*: 379-96.

Appendix

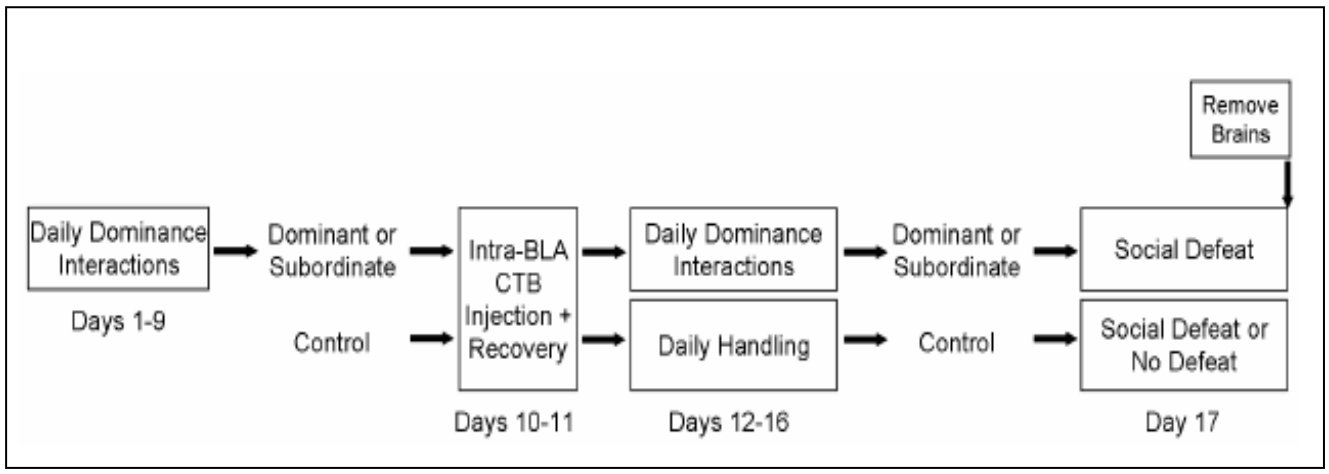


Figure 1 A schematic representation of the experimental design. Dominants, subordinates, and controls received intra-BLA injection of the retrograde tracer CTB seven days prior to social defeat or no defeat. Control subjects were singly housed following CTB injection. Brains were removed 90 minutes following social defeat or no defeat (Morrison, 2012).

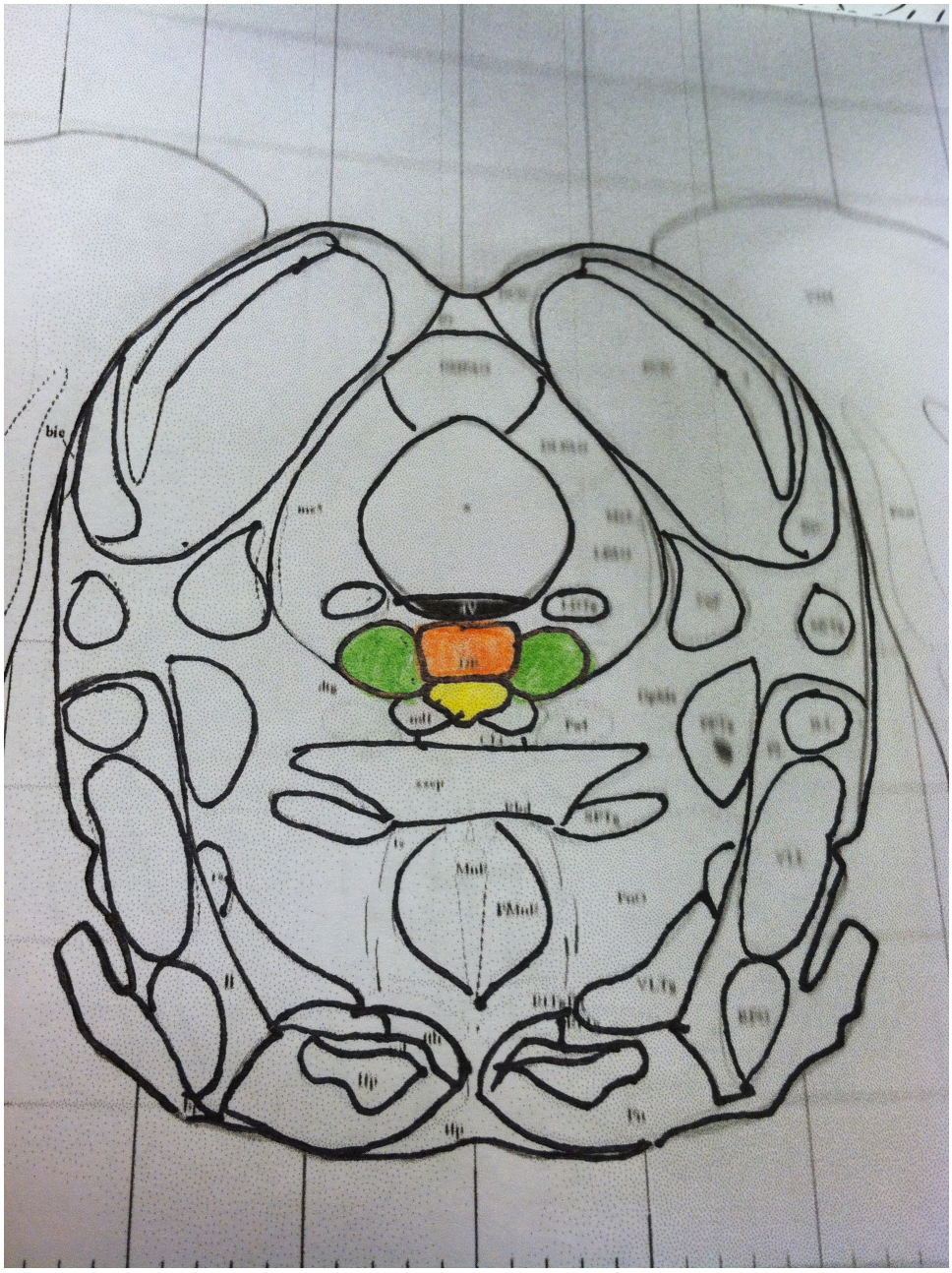


Figure 2 The diagram indicates the location of brain regions selected for c-Fos quantification. The diagram of the midrostral DRN was modified from the hamster atlas of Morin & Wood (Morin & Wood, 2001). The following regions are color-coated: ventral (yellow), dorsal (orange), and lateral (green). The box size used for quantification in the ventral caudal and rostral DRN was 250 μm x 300 μm. The box size used for quantification in the dorsal caudal, midcaudal, midrostral and rostral DRN as well as the ventral and lateral regions of the midrostral and midcaudal DRN was 500 μm x 300 μm.

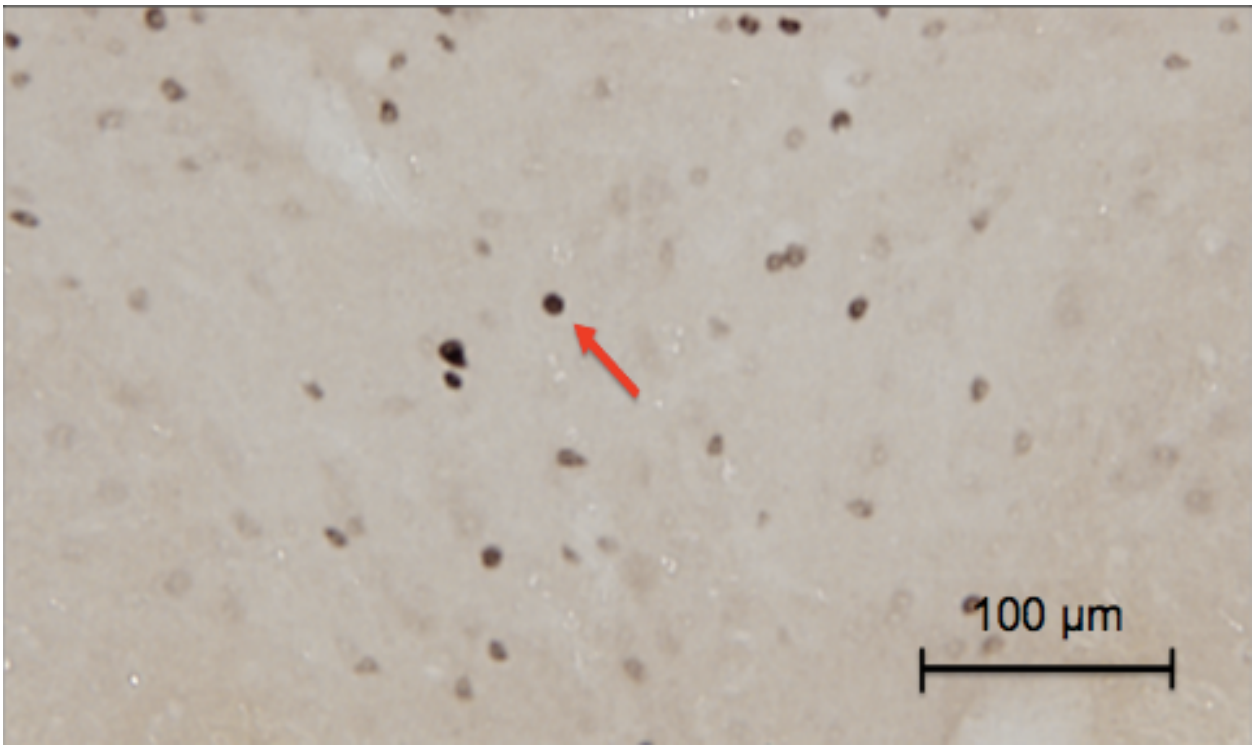
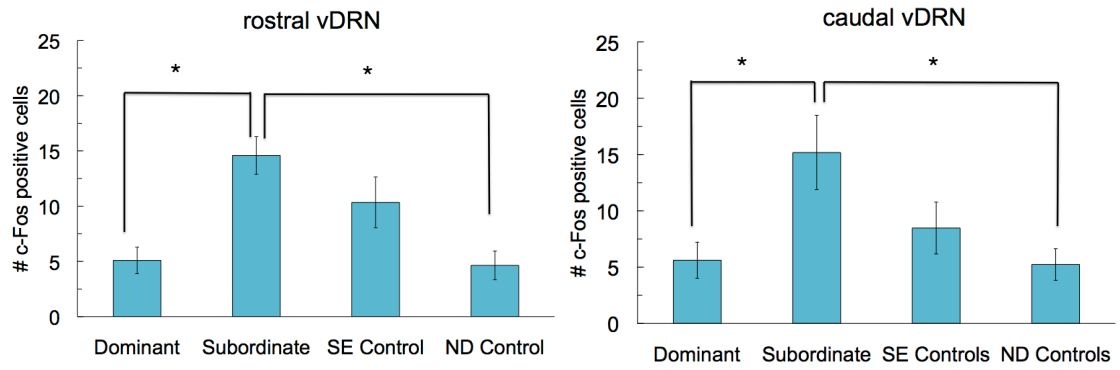
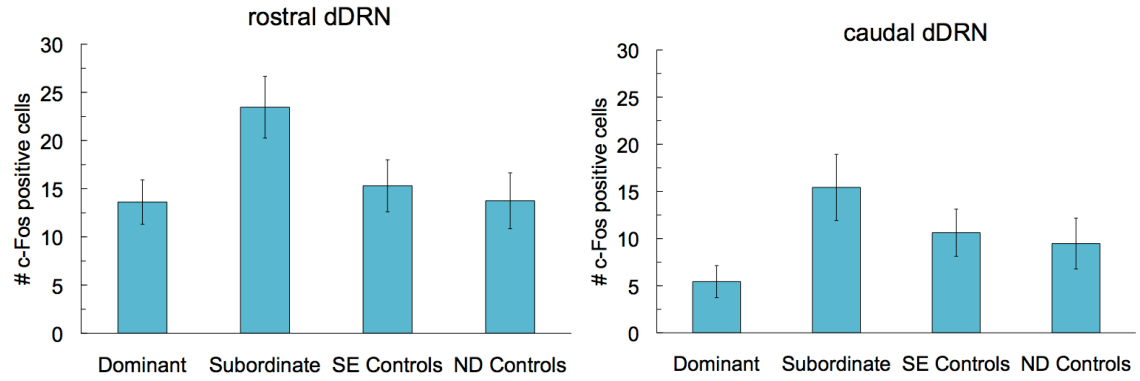


Figure 3 A representative photomicrograph (10x magnification) of a coronal slice through the ventral DRN showing c-Fos in a subordinate individual. A c-Fos immunoreactive cell is indicated with an arrow.

a.



b.



c.

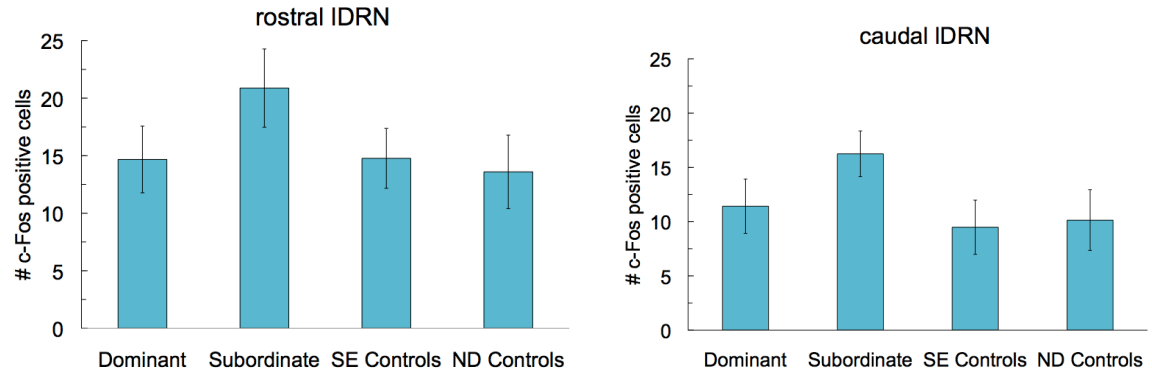


Fig. 4 Number (mean ± SE) of c-Fos immunopositive cells in the rostral and caudal subdivisions of the a) ventral, b) dorsal and c) lateral DRN measured following social defeat training. SE indicates social experience controls and ND indicates no defeat controls. We found an effect of social status in the ventral DRN, and an asterisk (*) indicates a significant difference between bracketed bars ($P < 0.05$).

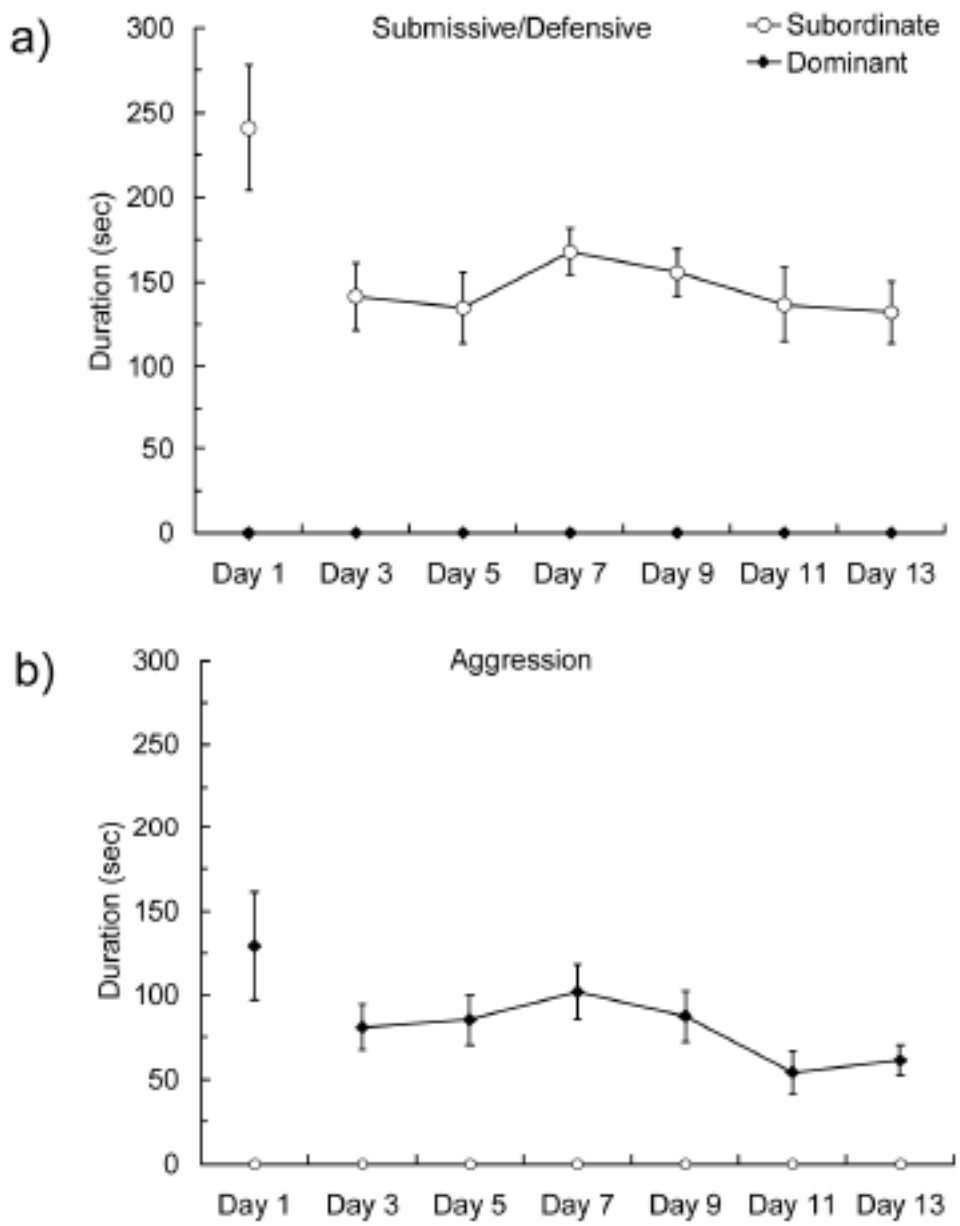


Figure 5 Agonistic behavior during daily encounters. Encounters were 10 min on day 1 and 5 min on days 2-14. a) Duration (mean ± SEM) of submissive/defensive behavior displayed by subordinates (N = 11) and dominants (N = 11) during fourteen days of encounters. Dominants never displayed submissive/defensive behavior. b) Duration (mean ± SEM) of aggressive behavior displayed by dominants (N= 11) and subordinates (N = 11) during fourteen days of encounters, subordinates never displayed aggressive behavior. (Morrison, 2012).

	Dominant (N = 11)	Subordinate (N = 11)	Control (N = 12)	*p*
Aggression Received (sec)	353 ± 26	369 ± 28	426 ± 19	ns
Submission Produced (sec)	654 ± 21[a]	761 ± 26[b]	703 ± 23[a,b]	0.01

Note: Superscript letters indicate the results of the Tukey post-hoc test. Unshared letters indicate significant difference while shared letters indicate there is no difference between the groups. ns – not significant

Table 1 Total durations (mean ± SEM) of behavior during social defeat training. (Morrison 2012).