5-2023

# Social Dominance Alters Stress-Induced Neural Activity and Generates Individual Differences in Stress Vulnerability

Jenna Lee Laymon
*University of Tennessee, Knoxville*, jlaymon3@vols.utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Jenna Lee Laymon entitled "Social Dominance Alters Stress-Induced Neural Activity and Generates Individual Differences in Stress Vulnerability." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Psychology.

Matthew A. Cooper, Major Professor

We have read this thesis and recommend its acceptance:

Keerthi Krishnan, Phyllis Thompson, Lowell Gaertner, Matthew Cooper

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

# Social Dominance Alters Stress-Induced Neural Activity and Generates Individual Differences in Stress Vulnerability

A Thesis Presented for the

Master of Arts

Degree

The University of Tennessee, Knoxville

Jenna Lee Laymon

May 2023

**Acknowledgments**

Thank you to the RAM dream team including students, animals, and staff of the University of Tennessee for their participation, patience, and cooperation in the collection and quantification of this data. In particular, I would like to thank Akhil Geddati, Anna Radford, Alonnah Brewer, and Yash Deo for their assistance in the collection and quantification of data for this project.

## Abstract

Identifying the physiological and behavioral mechanisms that underlie stress vulnerability is a crucial step toward identifying novel targets for the prevention and treatment of stress-related disorders. Social status is a key environmental factor that contributes to individual variations in stress vulnerability. In particular, achieving a subordinate social status has been shown to produce susceptibility to anxiety-like and depressive-like behavior. In this project, our aim was to identify neural ensembles regulating how dominance status modulated stress-induced changes in avoidant behavior in male and female Syrian hamsters. Using a viral vector that codes for robust activity marker (RAM), we investigated whether stress-induced RAM expression in the infralimbic (IL) region of the prefrontal cortex and posteroventral medial amygdala (MePV) accounts for status-dependent variation in stress vulnerability. We found that dominant male and female hamsters showed differences in stress-induced neural activity in the IL and MePV compared to their subordinate counterparts. We found that latency to approach the light zone of a light/dark transition test predicted the acquisition of social dominance for females and this activity was positively associated with greater IL activation. We also showed that time spent in the dark zone of a light/dark transition test predicted the acquisition of a subordinate social status for males and was negatively associated with IL activity. Overall, we found several various experience-dependent changes in anxiety-like behaviors displayed in social avoidance, light/dark transition, and conditioned defeat tests. These findings suggest that social dominance alters stress-induced neural activity in the IL which underlies status-dependent differences in stress vulnerability.

## Table of Contents

## List of Attachments

Attachments – Figures:  Photos of neural and behavioral data graphs

## Chapter One Introduction

Stress has largely been defined as an event or experience that causes disturbances in the neuroendocrine mechanisms that regulate homeostasis (Selye, 1956). Social stress includes traumatic events such as domestic violence and sexual assault, and these experiences can lead to stress-related mental illnesses such as post-traumatic stress disorder (PTSD). Although social stress is all too common, relatively few individuals develop stress-related psychopathologies (Yehuda, 2007, Sinha, 2008, Hart, 2012). Differences in neurological processes, genetic background, emotional regulation, and cognitive flexibility each contribute to the variability found in the prevalence of PTSD, major depression, bipolar, and social anxiety disorders (Shin, 2006, Soloff, 2017, Fenster, 2016, Rauch, 2006). Resilience to stress is regarded as an individual's capacity to cope with an aversive experience and avoid developing negative physiological and behavioral consequences (Maier, 2010). Importantly, stress resilience is characterized as not only a lack of negative consequences but is also an active process by which cellular and molecular mechanisms interact with the environment to mitigate the effects of stress experience (Faye, 2018). The motivation of this study was to identify the relationship between stress-induced neural activity and behavioral susceptibility with the aim to expand our understanding of the neurobiological mechanisms underlying experience-dependent changes in risk for stress-related mental illnesses.

### *Dominance Status & Stress Vulnerability*

Several factors contribute to the etiology of stress-related disorders such as genetic predispositions, familial and cultural history, and adverse childhood experiences (Seigli, 2022, Huhman, 2006, Mahan and Ressler, 2012) Because the majority of individuals who experience stress do not go on to develop psychopathologies, there has been a steady increase in research

focused on factors that contribute to stress resilience (CDC, 2022). Individual variations in ability to cope with stress also contributes to variation in stress resilience, and factors that alter coping ability include behavioral and cognitive flexibility, experiencing play, social support, humor and emotional regulation, religion, and active engagement with the stressor (Southwick, 2005, Russo, 2012, Rosado, 2023, Faye, 2018). Although several psychosocial factors contributing to risk for psychopathologies have been identified, studies investigating genetic or biological mechanisms of stress resilience are still under development. Utilizing animal models of stress research is necessary for not only valuing the complexity of human beings but also to identify the implications of clinical therapeutic practices. It is, therefore, essential to investigate the neural mechanisms of stress resilience in animal models of anxiety-like and depression-like behavior.

The social environment can alter stress susceptibility by affecting opportunities for stress coping. A wide variety of psychosocial factors that modulate stress vulnerabilities have been identified including social buffering, neglect, social bonding, overcrowding, social play, and dominance status (Snyder-Mackler, 2020, Hofmann, 2014, Dagnino-Subiabre, 2021, Gutzeit, 2020, Zlatkovic, 2014, Overli, 2004, Burleson, 2016, Cooper et al., 2023). A dominant social status, in particular, is a critical environmental factor that alters neuroendocrine, physiological, and behavioral responses to stress (Cooper, 2015, Clinard, 2016, Karamihalev, 2020, Williamson, 2019). Animals obtain a dominance status by competing for resources and either winning or losing these aggressive encounters. In Syrian hamsters both males and females form stable dominance relationships and we have shown that animals that acquire a dominant social status exhibit less stress-induced anxiety compared to their subordinate counterparts (Morrison et al., 2014, Cooper et al., 2021). The effect of dominance status on stress-related behavior is consistent

with literature showing that social dominance is associated with increased emotional regulation, cognitive flexibility, rapid negative feedback of stress hormone, and reduced anxiety-like and depressive-like behavior (Gross, 2018, Snyder-Mackler and Kohn, 2016, Williamson, 2019, Karamihalev, 2020). In contrast, forming a subordinate social status increases susceptibility to stress as indicated by dysregulated hypothalamic-pituitary adrenal axis activity and activation of inflammatory pathways in microglia and other immune cells, which have been associated with depressive-like and anxiety-like behaviors and a shorter expected lifespan (Korzan, 2006, Snyder-Mackler, 2016). In humans, the agonistic encounters that characterize dominance relationships are consistent with coercion theory, which describes a parent-child aggressive interaction that leads to negative reinforcement of aggressive conduct and increases the risk for adolescent antisocial behavior (Patterson, 1984, Smith, 2014).

*Medial Prefrontal Cortex*

The prefrontal cortex and amygdala are both key brain regions that underlie individual differences in stress vulnerability. The prefrontal cortex plays an essential role in behavioral control, including flexible coping, and emotional regulation. A subregion known as the ventromedial prefrontal cortex (vmPFC) integrates sensory and social cues with long-term memory and gathers information representing the 'self' (Roy, 2012). The prefrontal cortex has been a critical region of interest in stress research since separate neural networks within this subregion are necessary for emotional and social behavior, reward and punishment, long-term, short-term and working memory, and autonomic functioning (Roy, 2012, Maier, 2010, Petrides, 2000, Funahashi, 1994, Euston, 2012, Kern, 2008, Sullivan, 2002, 2006). Research has shown that the vmPFC has a bidirectional modulatory influence on limbic structures, which contributes to behavioral inhibition in response to stress and dysregulation of vmPFC neural pathways has

been implicated in stress susceptibility (Vidal-Gonzalez, 2006, Berretta, 2005). Specifically, the infralimbic (IL) region of the vmPFC has been associated with the degree to which an individual controls an aversive event and, in turn, elevated activity in vmPFC neurons is associated with reduced stress-related behavioral responses (Maier and Seligman, 1976, Maier, 2010, Amat, 2005).

There has been a distinction between active forms of coping, often referred to as the flight or fight response, and reactive forms of coping. Active forms of coping have been defined as locomotive responses focused on controlling and resisting a stressor (Koolhaas, 1999). In humans, active coping can include behaviors such as exercise, support seeking, play, and creative expression, but can also include behaviors such as binge alcohol intake and substance abuse, emotional eating, and increased relational arguments (Sinha, 2016, Johnson, 2022, Singleton et al., 2022). On the other hand, reactive coping behaviors have been associated with susceptibility to stress including submission or freezing in rodents and rumination or dissociation in humans (Maxwell and Siu, 2007, Morroquin, 2010, Kecala, 2023, Finklesein, 2012, Lui, 2022, Rosado, 2023). The opportunity to escape or win a stressful social encounter allows for an active coping response that controls the occurrence or duration of stress. Specifically, active escape from a stressor has been associated with increased IL activity within the vmPFC and is essential for stress resilience in a learned helplessness model (Southwick, 2005, Koolhaas, 1999, Seligman, 1976, Amat, 2005, Christianson, 2009). Because winning an aggressive encounter provides similar control over a social interaction, dominant animals may exhibit increased activity of IL pyramidal cells, and in turn, can effectively regulate behavioral output when perceiving threat.

The IL receives bidirectional top-down and bottom-up information that plays a key role in the regulation of conditioned fear through its connectivity with the basolateral amygdala

(BLA) and central amygdala (CEA) (Cho, 2013, Amir, 2011, Berretta, 2005, Kredlow and Fenster, 2022). Electrophysiological approaches have also shown that IL stimulation suppresses spontaneous post-synaptic potentials in the lateral amygdala and disrupts the acquisition of conditioned fear (Rosenkraz, 2003). In addition, optogenetic stimulation of IL cells during extinction training facilitates the storage of extinction memories in target structures such as the BLA (Do-Monte, et al., 2015). Consistent with the fear conditioning literature, we have shown that reduced stress-related behavior in dominant hamsters is associated with greater stress-induced neural activity in IL cells projecting to the BLA (Dulka, et. al., 2018). Using a chemogenetic approach, we found that activation of the IL-BLA pathway during social defeat stress reduced conditioned defeat responses in animals with a subordinate status, suggesting that activation of this pathway increased stress resilience (Dulka 2020). However, studies capturing stress-related behavior in female hamsters have been limited since they do not show robust submissive behavior in a conditioned defeat test. Therefore, it is important to include measures focusing on female anxiety-like behaviors to understand differences in the behavioral expression of stress-related vulnerability. Importantly, Kim Huhman and colleagues have used a social avoidance test to show that female hamsters exhibit increased avoidance of novel animals following an acute social defeat stressor (Rosenhauer et al., 2017) Although data in female rodents are limited, these findings suggest that the vmPFC regulates stress-induced changes in social behavior through its interconnections with the social behavior neural network, including several regions of the amygdala (Newman, 1999, 2006, O'Connell, 2018, Cooper, 2021, Pincus, 2021).

*Medial Amygdala*

The medial amygdala (MeA) is a central node in the social behavior neural network and contains steroid hormone receptors that modulate sex-dependent behaviors such as reproduction, parental behavior, aggression, and defensive behaviors (Petrulis, 2020). The anterior MeA receives direct projections from the vomeronasal organ, which provides social odor cues and gates the expression of sexual behavior (Luiten, 1985). In addition, researchers have investigated the posterior MeA's role in stress-related behavior with a specific focus on defensive behavior, aggression, and proactive coping (Markhum, 2008, Miller 2019, Nordman, 2020). Specifically, the ventral portion of the posterior medial amygdala (MePV) has been linked to defensive aggressive behavior through neuronal projections to regions of the hypothalamus (Choi et. al., 2005). Our lab has shown that a pharmacological blockade of androgen receptors in posterior MeA increased the conditioned defeat response in males with a dominant social status, which suggests that expression of MePV androgen receptors is necessary for stress resilience in dominant males (Cooper, 2021). However, it is unknown whether estrogen alpha receptors in the MePV of female hamsters modulates stress-induced changes in behavior.

The MeA contains several cell types that produce a great deal of heterogeneity in MeA circuits (Keshavarzi et al., 2014). Because MeA projection neurons can be either glutamatergic or GABAergic, non-cell type specific manipulation of MeA activity has produced contradictory findings. Several studies have shown that MeA lesions cause a reduction in aggressive behaviors in the face of threat, while others have shown that MeA lesions increases aggression, and still others have shown no effect (Miller, 2019, Wang, 2013, Vochteloo and Koolhaas, 1987, Busch, 1974, Hong, 2014). Interestingly, Miller et al. (2019) showed that dopamine 1 receptor positive neurons in the MePV increased Ca2+ signaling during a predatory odor task and differentially

regulated approach-avoidant behaviors (Miller, 2019). Additionally, repeated exposure to aggressive encounters are associated with synaptic potentiation in MePV cells projecting to the ventral medial hypothalamus and bed nucleus of the stria terminalis (Nordman et al., 2020). Altogether, these findings suggest that activity of select MePV neurons is associated with experience-dependent changes in aggressive and defensive behavior and may contribute to status-dependent changes in stress susceptibility.

Previous studies in our lab have used c-Fos as a cellular marker of neural activity and have shown that dominant hamsters have greater immunoreactivity in the IL and MePV during social defeat stress compared to subordinates (Morrison, 2014, Dulka, 2020, Grizzell, 2020). In addition, dominant hamsters exhibit less anxiety-like behavior in the days following social defeat stress compared to subordinates (Clinard, 2016, Dulka, 2020, Cooper, 2021). However, linking patterns of neural activity with stress vulnerability has been difficult because previous techniques required that c-Fos and stress-related behavior be measured in separate animals. The aim of this study is to use a novel approach to tag neural activity in the IL and MePV during social defeat stress and predict subsequent changes in anxiety-like behaviors in the same animal. We hypothesize that achieving a dominant social status will lead to a coordinated change in IL and MePV neural activity, proactive coping responses, and stress-related avoidance compared to social subordination. Specifically, we predict that dominant male and female hamsters will show greater neural activity in the MePV, which will be associated with proactive coping during social defeat stress compared to same-sex subordinate counterparts. We also predict that dominant males and females will show greater neural activity in the IL, which will be associated with reduced social avoidance in females and a reduced conditioned defeat response in males.

## Chapter Two Methods

*Subjects*

Subjects consisted of adult female and male Syrian hamsters (*Mesocricetus Auratus*) obtained from our breeding colony of animals that originated from Charles River Laboratories (Wilmington, MA, USA). Subjects were 9 weeks of age and weighed (110g-180g) at the start of this study. Animals were individually housed for one week prior to behavioral testing which allowed them time to establish territories in their home cage. During this one-week interval, female hamsters were estrous cycled, and males were handled for 5 minutes per day. All animals were housed in polycarbonate cages (20 x 40 x 20 cm) with wire mesh tops, corn cob bedding, and cotton nesting materials. All animals had access to standard rodent chow, water *ad libitum,* and received doxycycline (DOX) chow as needed. Cage changes occurred every two weeks to minimize disruption of territorial behavior. To minimize circadian variation in agonistic behavior, all behavioral tests were performed within the first three hours of their 14:10 light/dark cycle and were housed in a temperature-controlled colony room (21 ± 2 °C). The University of Tennessee Institutional Animal Care and Use Committee approved the protocols used in this study. We minimized suffering by treating wounded animals through the application of Betadine to open injuries, which is consistent with the National Institute of Health Guidelines. No animals had surgical complications, and each had 48 hours of post-operative welfare checks. Animals used as resident aggressors during social defeat encounters were >6m old and weighed 150-200g. Resident aggressors (RAs) were screened for reliable territorial aggression and readily attacked subjects during social defeat stress. Non-aggressive Intruders (NAIs) were 7 weeks old and weighed 100g – 130g. We had a total of 64 animals for this study. Of those, we had 23 stable dyadic pairs and excluded 5 unstable dyads for this project. Unfortunately, we had to drop 9

dyadic pairs due to neurological abnormalities. Therefore, we had a sample of 9 male and 9 female pairs.

*Experimental Design*

We used a viral vector approach to identify neural ensembles activated during social defeat stress. The Robust Activity Marker (RAM) virus uses an immediate early gene promoter to tag neural activity and uses a Tet-OFF system in which doxycycline (DOX) chow inhibits virus expression (Sorensen, 2016). We used the Tet-OFF system to isolate neural activity at social defeat by feeding animals DOX chow and only removing DOX chow from the diet during social defeat stress. Our first aim was to validate this method by showing that social defeat stress was necessary to produce robust expression of RAM+ cells in the IL and MePV and that virus expression could be temporally controlled by the removal of DOX chow (Figure 1a, 1b). First, I demonstrated that without the addition of DOX chow animals showed robust RAM expression in both the IL and MePV without experiencing social defeat stress. I also found that with the addition of DOX chow, animals showed no significant expression of RAM in either brain region even if they were exposed to social defeat stress. These findings validate the use of RAM virus in Syrian hamsters by showing that robust RAM expression in hamster brain can be silenced by the addition of DOX chow. I also showed that hamsters expressed significantly more RAM activity in the IL and MePV when DOX chow was removed for 4 days compared to 3 days (Figure 2a, 2b). This finding indicates that 4 days removal of DOX chow is necessary for temporal control of RAM expression within the IL and MePV in our hamster model.

Our second aim was to test whether dominance status altered the stress-induced expression of RAM+ cells in the IL and MeA as well as produce status-dependent differences in anxiety-like behavior (Figure 2a, 2b). Hamsters were switched to DOX chow to silence virus

expression at least 24 hours prior to stereotaxic surgery and allowed a minimum of 48 hours of recovery before beginning behavioral testing (Figure 3). On proestrus day, animals went through their baseline approach/avoidance measures which consisted of a light/dark (LD) transition test and a social avoidance (SA) test with a 30 min inter-trial interval. These measures were taken in sequential order, starting with the least threatening stimuli, to minimize potential carry-over effects. Then, animals began their dyadic dominance encounters on diestrus I and continued with 12 daily encounters while skipping every fourth day to avoid testing females during estrus. Because female hamsters have a consistent 4-day estrous cycle and show reduced aggression during estrus, and we avoided testing females during estrus to reduce the risk of animals flipping their status. To prevent a sex-biased experimental design, males followed the same testing schedule as females. After animals formed a dominant/subordinate relationship, we measured their behavior in both light/dark transition (LD) and social avoidance (SA) tests. After the subjects' post-status behavioral testing, animals were removed from DOX chow and given standard rodent chow. Animals remained off DOX chow for 4 days prior to social defeat since I found that defeat-induced RAM expression in the IL and MeA was greater after 4-day removal compared to 3 days. During social defeat stress, animals were exposed to three novel resident aggressors (RAs). Animals were then placed back in the colony room and 24 hours later were placed back on DOX chow to silence RAM expression. Then, 48 hours after social defeat, animals received conditioned defeat (CD) testing and were exposed to their third and final round of LD and SA testing. Following behavior testing, animals were sacrificed, and brains were collected for the quantification of RAM+ cells.

**Chapter Three Procedures**

Observers were trained for inter-rater reliability and achieved 90% agreement for all behavioral and cell quantification.

*Intracranial Surgery*

Hamsters were anesthetized with isoflurane, fixated on a stereotaxic apparatus, and kept under anesthesia for the remainder of surgery using medical-grade oxygen and an isoflurane nose cone. The animal's body temperature was also maintained at 37°C using a heating pad. A small incision was made, and a craniotomy was performed to allow microinjections. We used a 33-gauge injection needle to deliver an infusion that included (80% AAV8-RAM-mKATE2 and 20% AAV8-hSyn-GFP). Unilateral infusions were made into both the IL (20° angle stereotaxic arm: +1.67 mm anterior to bregma, +3.6 mm lateral to bregma, - 4.7 mm ventral to dura) and the MePV (0° angle stereotaxic arm, -0.4 mm posterior to bregma, +2.78 mm lateral to bregma, -8.12 mm ventral to dura). Animals were given 500nL of the virus with an injection rate of 50nL per min and the needle was left in place for 10 min after injection to allow diffusion. Injections in the left and right hemispheres were counterbalanced and data were collapsed since no hemispheric differences were found in the expression of RAM+ cells of either the IL ($F_{(1,17)}$ = 1.35, P = .80) or MeA ($F_{(1,11)}$ = 1.16, P = .75).

*Light/Dark Transition Test*

Half of the LD chamber was illuminated by white light, while the other half was dark and covered with a solid black lid top. Animals were placed at the back wall of the dark chamber and allowed 5 min to explore the arena (23 x 35 x 20 cm). We measured the animals' latency to enter the light zone, the frequency of transitions between chambers, and the total duration spent in the

dark zone. Anxiety-like behavior was indicated by a longer latency to leave the dark chamber, a longer duration of time spent within the dark chamber, and a low number of transitions. (Figure 4)

*Social Avoidance Test*

Our SA test was modeled after similar procedures used in mice (Duque-Wilckens, 2018) (Krishnan, 2007). Testing was conducted in an open field arena (32 x 32 x 16 cm) under red light conditions. The SA test consisted of three testing phases that were 3 min each. The first phase involved an acclimation period, the second included a target-absent trial, and the third contained the social target-present trial. First, animals were placed in the open field to allow habituation to the arena. The second phase involved a target-absent trial in which we placed a perforated box into the arena and allowed the subject to acclimate to the novel object. In the third phase, we placed a novel adult RA inside a perforated container and quantified the subject's approach/avoidance behaviors to the social target stimulus. An observer that was blinded to treatment conditions quantified latency to approach the target stimulus, duration spent attending to the social target within the interaction zone, and duration of time spent in corners of the arena. Anxiety-like behaviors were indicated as a long latency to approach the social stimulus, a long duration of time spent in the corners, and a short duration of time interacting with the social target. (Figure 5)

*Dyadic Dominance Encounters*

Subjects were exposed to 12 daily social encounters in a resident/intruder procedure to create a dyadic dominant and subordinate relationship. Animals were randomly selected as residents or intruders given that we have previously shown that residency status does not predict social status. In the current study, 9 animals became dominant in the resident role and 9 animals

became dominant in the intruder role. Animals were weight-matched, sex-matched, and estrous cycle-matched with their partner. We used animals from either different litters or different juvenile social groups to ensure animals were unfamiliar with their partner. We utilized Koolhaas (2013) adaptation of a resident intruder paradigm that allows dominance relationships to form (Koolhaas, 2013). Agonistic behavior quantified during dominance encounters included latency to attack, frequency of attacks, latency to submit, and frequency of flees. A dominance relationship was indicated by the unidirectional display of agonistic behavior in which one animal reliability showed submissive behavior (e.g., tail-up posture, avoid, and flee) while the other consistently showed aggression behaviors (e.g., side aggressive posture, chase, attack) throughout the 12-day encounters. Although dyads often show unidirectional agonistic behavior within 5 days, some dyads did not form stable dominance relationships and were not analyzed in this project. Encounters were 10 min in duration until a dominance relationship was formed and they were reduced to 5 min thereafter. In this study, there were two conditions for status which includes a dominant and subordinate animal.

*Social Defeat Stress*

Our social defeat procedure was intended to produce acute traumatic social stress and was adapted from (Huhman et al. 2003). Subjects were placed into the home cage of a larger aggressive animal, which we define as the resident aggressor (RA). Subjects were exposed to three separate RAs for 5 min each and were given 5 min of rest in their home cage between stress encounters. To standardize the number of attacks received by subjects, we used a perforated barrier to separate the subject from RA once they submitted and received 5 attacks. This allowed the subject to still obtain defeat stress and olfactory cues from the RA but also reduced physical wounding. Behaviors measured during social defeat stress include latency to

submit after being aggressed upon by RA (Lat_to_Sub = RA_Lat_to_Aggress – Subject_Lat_to_Submit) and the rate of attacks during mutual aggression behavior. Actively fighting back against the RA and having a long latency to submit is consistent with an active coping response (Koolhaas, 1987, Wood and Bhatnagar, 2015).

*Conditioned Defeat Test*

Following post-defeat LD and SA tests, conditioned defeat (CD) responses were quantified. CD testing involves a 5 min social interaction with a non-aggressive intruder (NAI) as described by Huhman et al. (2003). These NAIs were younger, group-housed animals that did not display aggressive behaviors toward the subjects. We placed a small, same-sex NAI into the home cage of the subject and measured the duration of submissive/defensive, aggressive, social, and non-social behaviors, as well as the number of flees displayed by the subject. A CD response consists of a complete loss of typical territorial aggression and elevated submissive/defensive behavior consistent with learned helplessness literature.

*Histology*

Following the final behavioral tests, animals were anesthetized using isoflurane and perfused with 100mL of 0.1M phosphate buffer saline (PBS) and 100 mL of 4% paraformaldehyde, pH 7.4. Brains were extracted and post-fixed in 10mL of 4% paraformaldehyde for 24h, then moved to PBS 30% sucrose solution at 4°C for 48hrs. Then, 40 $\mu$m coronal sections were cut using a cryostat and collected directly onto slides or stored free-floating in 0.1 M PBS. We collected every third tissue sample to avoid counting the same cells in adjacent sections and to provide backup tissue samples for future analysis. After drying onto slides, tissue sections were cover-slipped using a DAPI mountant and imaged.

*Cell Quantification*

Images were captured at 10x magnification using an Olympus BX51 microscope with a 500x500µm clip region. Injection sites were indicated by the presence of a GFP reporter and only injection sites with GFP+ cells centered within the brain region of interest were quantified. The number of RAM+ cells were quantified in the IL and MePV. We defined RAM+ cells based on the size and intensity of mKate2 fluorescent protein expression. Observers quantified cells using PIPSQUEEK AI software, were blinded to treatment conditions, and manually corrected for type I and type II errors made by the AI. As an indicator of virus transfection, we recorded both the average number of GFP+ cells per clip region and collected a minimum of 6 images for each subject. We used this method to confirm that animals with low RAM+ expression had no significant differences in the spread or number of GFP+ cells compared to animals with high RAM+ expression. We found no significant differences between groups on the average amount of GFP+ cells per clip region in the IL ($P = 0.13$) or MePV ($P = 0.6$).

*Statistical Analysis*

Analyses were conducted using Proc Mixed and Proc Glimmix of SAS statistical software, version 9.4 for Windows. I assessed model fit using Bayesian Information Criterion (BIC) and -2 log likelihood before analyzing behavioral outcomes. Subject data were nested within dyadic pairs during repeated measures; thus, I used a multilevel modeling approach to assess random effects of social status (dominant vs. subordinate) on neural activity (IL and MePV) to predict subsequent avoidant behaviors (CD, LD, and SA tests) using restricted maximum likelihood (REML). The number of RAM+ cells, in the IL and MePV, was calculated as total cell counts and the data showed a negative binomial distribution. As a result, I used Proc Glimmix of SAS to regress the number of RAM+ cells activated at social defeat stress (time 3)

on a factorial crossing of social status (dominant vs. subordinate), and sex (male and female) with a Poisson Distribution and estimated a random intercept for dyadic pairs. When data were repeated measures, in a normal distribution, and categorically defined (submissive, aggressive, avoidant), I used Proc Mixed of SAS to regress behavioral measures (e.g. duration in the dark zone) on a factorial crossing of time (1, 2, 3), status (dominant vs. subordinate), and sex with a random intercept for hamsters (nested in pairs) and a random intercept for pairs. I used Kenward Roger's degrees of freedom approximation for estimating covariance when using Proc Mixed and our set alpha levels at ($p \leq .05$) and presented data as mean ± SE. When correlating predictions of neural ensembles on behavioral outcomes, I used a generalized linear mixed model utilizing Proc Glimmix of SAS.

## Chapter Four Results

*Experience-Dependent Changes in Anxiety-like Behavior*

*Light/Dark Transition Test*

To measure changes in anxiety-like responses before and after the formation of dominance status and after social defeat stress, we repeatedly tested animals in a LD transition test. We used a multiple regression mixed model to regress the number of transitions between light and dark zones on a factorial crossing of time (Pre-Status, Post-Status, Post-Defeat), status (dominant and subordinate), and sex with a random intercept for hamster (nested in pairs) and a random intercept for pairs. There was significant 3-way interactions on the frequency of transitions between zones ($F_{(2,64)} = 4.53$, $P = .0145$). Also, dyadic pairs explained a significant portion of variance in the number of transitions exhibited by our subjects ($R^2 = -.66$). We found a significant interaction between day and sex on the frequency of transitions ($F_{(2,64)} = 3.6$, $P = .033$). We also found a significant interaction between sex and status on the frequency of transitions ($F_{(1,27.6)} = 3.55$, $P = .0162$). For animals tested, pre-status, we observed an effect of sex in which females showed significantly more transitions than males ($F_{(1,27.5)} = 3.6$, $P = .0282$; Figure 6a). During the post-status phase, dominant females transitioned more frequently than all other groups ($F_{(2,64)} = 4.53$, $P < .0001$). There were no significant effects or sex of status on the number of transitions post-defeat. However, we found a status by stress effect in which dominant females significantly decreased their frequency of transitions post-defeat compared to post-status ($F_{(2,64)} = 4.53$, $P = .0003$).

We also performed a similar regression model on the amount of time animals spent in the dark zone of the arena. We found a 3-way interaction between sex, status, and time on the

duration of time spent in the dark zone ($F_{(2,64)} = 3.95$, $P = .0242$). Variance in duration in the dark was significantly explained by subjects ($R^2 = -.66$). Interestingly, I found that duration in the dark zone predicted a subordinate social status in males. Specifically, males who became subordinate spent significantly more time in the dark zone during the pre-status phase compared to all other groups ($F_{(2,64)} = 3.95$ $P = .0338$; Figure 6b). Also, subordinate females spent significantly more time in the dark during the post-status phase compared to their dominant counterparts ($F_{(2,64)} = 3.45$, $P = .0093$), and a similar status effect was shown in subordinate males ($F_{(2,64)} = 3.45$, $P = .0319$). Animals did not significantly differ in the time spent in the dark zone during the post-defeat phase. However, dominant animals showed an effect of social defeat stress such that they significantly increased their time spent in the dark from post-status to post-defeat ($F_{(2,64)} = 3.45$, $P < .0001$).

To further examine changes in anxiety-like behavior, we performed a similar analysis on latency to enter the light zone of the arena. I found a main effect of social status such that dominant animals entered the light chamber more quickly than subordinates ($F_{(1,80)} = 15.38$, $P = .0002$). Also, dyadic pairs explained a significant portion of variance in avoidance behaviors exhibited by our subjects ($R^2 = -.41$). We found no difference in subjects' latency to enter the light zone during the pre-status. However, males who achieved a dominant status entered the light zone more quickly compared to their subordinate counterparts during the post-status phase ($F_{(1,80)} = .09$, $P = .0172$), and a similar trend was found for dominant females ($F_{(1,80)} = .09$, $P = .0682$; Figure 6c). Critically, we found a main effect of stress in which all animals exhibited a significantly greater latency to enter the light zone at post-defeat compared to post-status ($F_{(1,80)} = .09$, $P < .0001$) Also, in the post-defeat phase, dominant males showed a trend to more quickly enter the light zone compared to subordinate males ($F_{(1,80)} = .09$, $P = .0585$). The difference

between dominant and subordinate females in their latency to enter the light zone after social defeat stress did not reach statistical significance ($F_{(1,80)} = .09$, $P = .124$).

*Social Avoidance Test*

We used a SA test to measure social approach/avoidance behavior before and after the formation of dominance relationships and after social defeat stress. We utilized a generalized linear mixed model to analyze the effects of time (pre-status, post-status, post-defeat), status (dominant and subordinate), and sex (male and female) with a random intercept for hamster (nested in pairs) and a random intercept for dyads. We found no significant 3-way interaction between sex, status, and day on the time spent in the corners during the social target trial. Also, Also, dyadic pairs did not explain a significant portion of the variance in avoidance behaviors exhibited by our subjects. However, we found a significant interaction between day and dominance status ($F_{(2,64)} = 4.6$, $P = .0136$). During the pre-status phase, there were no significant differences in the amount of time animals spent in the corners during the social-target trial across groups. However, during the post-status phase, we showed that subordinate males spent significantly more time in the corners compared to their dominant counterparts ($F_{(2,64)} = 4.60$, $P = .0002$), and females showed a similar trend although nonsignificant ($F_{(2,64)} = 4.60P=.521$; Figure 7a). We showed no significant differences in the effect of social defeat stress between groups. Importantly, we observed an effect of stress in which dominant males significantly increased their amount of time spent in the corners in the post-defeat phase compared to the post-status phase ($F_{(2,64)} = 4.60P = .0039$).

We performed a similar analysis on the amount of time animals spent in the interaction zone during the social target trial and found a main effect of social status ($F_{(1,64)} = 7.48$, $P = .0081$). Also, dyadic pairs explained a significant portion of variance in avoidance behaviors

exhibited by our subjects ($R^2$ = -.24). During the pre-status phase, there were no significant differences across groups ($F_{(1,64)}$ = 7.48, P = .785). However, during the post-status phase, dominant females spent more time in the social interaction zone than their subordinate counterparts ($F_{(1,64)}$ = 7.48, P = .0021), and males showed the same effect ($F_{(1,64)}$ = 7.48, P = .006; Figure 7b). Following social defeat, I found a sex effect in which females interacted with the social stimulus significantly longer than males ($F_{(1,64)}$ = 7.48, P = .0048). In addition, dominant males ($F_{(1,64)}$ = 7.48, P < .0001) and females ($F_{(1,64)}$ = 7.48, P = .0027) showed an effect of stress in which they significantly decreased their time in the social interaction zone compared to the post-status phase.

We used a similar analysis for the latency to approach the social target and found no significant 3-way interaction between sex, status, and time. Interestingly, the latency to approach the social target predicted acquisition of a dominant social status for female hamsters ($F_{(2,64)}$ = 4.97, P = .0099; Figure 7c). Specifically, females that became dominant approached the social target more quickly during the pre-status phase compared to all other groups ($F_{(2,64)}$ = 4.97, P = .0245). There were no significant differences in latency to approach the social target after the formation of a social status and after experiencing social defeat stress. Dyadic pairs explained a portion of variance in avoidance behaviors exhibited by our subjects ($R^2$ = -.54).

*Stress-Induced Behavioral Responses and Neural Ensemble Activity*

*Defeat-Induced RAM expression*

I used a generalized linear model to regress the number of RAM+ cells in the IL on a factorial crossing of status (dominant and subordinate) and sex (male and female) and found a significant interaction between sex and social status ($F_{(1,16.14)}$ = 5.4, P = .0335). We found that

subordinate males had significantly more RAM+ cells in the IL compared to their dominant counterparts ($F_{(1,16.14)} = 5.4$, P = .0282). Meanwhile, females showed an opposite pattern of activation, although the effect of dominance status was not statistically significant ($F_{(1,16.14)} = 5.4$, P = .4058; Figure 8). There was no sex-by-dominance status interaction on the number of RAM+ cells in the MePV. However, dominant males showed a trend for having more RAM+ cells in the MePV than subordinate males ($F_{(2,64)} = 7.85$, P = .0846), while the effect of dominance status was not significant in females ($F_{(2,64)} = 7.85$, P = .5878; Figure 9). Interestingly, dyadic pairs did not explain variance of RAM expression exhibited by our subjects in either the IL or MePV.

*Coping with Social Defeat Stress*

Because latency to submit is a measure of coping with social defeat stress, I used a generalized linear mixed model to regress latency to submit during social defeat stress on a factorial crossing of status and sex and found a significant interaction ($F_{(1,32)} = 4.53$, P = .0463). (Wood and Bhatnagar 2015) Importantly, dyadic pairs explained a significant portion of nested variance of submission behaviors exhibited by our subjects ($R^2 = -.43$). We found that subordinate males exhibited a significantly shorter latency to submit during social stress compared to their dominant counterparts ($F_{(1,32)} = 4.3$, P = .0223; Figure 10). We found no significant effect of dominance status on females' latency to submit during social defeat stress, although the pattern of results was the opposite of males. Also, I found a significant interaction between sex and status in the rate of attacks exhibited by subjects before losing the social defeat encounter ($F_{(1,32)} = 4.69$, P = .038). Specifically, we found that dominant males attacked the resident aggressor significantly more often than their subordinate counterparts ($F_{(1,32)} = 4.69$, P = .009). Females showed a similar, albeit non-significant pattern of mutual aggression (Figure 11).

*Conditioned Defeat Response*

We tested animals 48 hours after social defeat stress in a CD test and used a generalized linear model to analyze submissive/defensive and aggressive behaviors on a factorial crossing of status (dominant and subordinate) and sex (male and female). I found a main effect of sex in which males exhibited significantly more submissive/defensive behaviors when confronted with a non-aggressive intruder compared to females ($F_{(1,32)} = 8.66$, P =.006; Figure 12). Dominance status did not significantly alter the amount of submissive/defensive behavior displayed. Importantly, dyadic pairs explained a significant portion of nested variance of submission behaviors exhibited by our subjects ($R^2 = -.45$). Also, we found a main effect of sex in which females showed more aggression toward an intruder during CD testing compared to males ($F_{(1,31)} = 14.16$, P = .0007; Figure 13). There were no significant differences in aggression across status conditions.

*Neural Predictions of Stress-Related Behavior*

We regressed the number of RAM+ cells in the IL and MePV on latency to submit during social defeat stress as well as on anxiety-like behavior during post-defeat LD, SA, and CD tests. In each case we used a generalized linear model to test a factorial crossing of sex and status. There was a trend for a positive correlation between the MePV RAM+ cells and latency to submit during social defeat stress ($F_{(1,29)} = 3.19$, P = .0847), although there were no significant interactions with dominance status or sex. Interestingly, the association between IL RAM expression and latency to submit during defeat stress was significantly different between males and females ($F_{(1,16.3)} = 4.83$, P =.0427). Specifically, RAM+ expression in the IL negatively

predicted latency to submit in males ($F_{(1,27.3)} = 4.65$, P = .0399; Figure 14). In contrast, IL RAM expression in females showed an opposite, albeit nonsignificant, association with latency to submit. I also regressed the rate of attack during social defeat stress on RAM+ cells in the MePV and found a trend for a positive correlation (P = .1095).

I regressed IL RAM expression on latency to enter the light zone during post-defeat LD testing and with a factorial crossing of sex and status. I found that latency to enter the light zone was significantly associated with the number of IL RAM+ cells ($F_{(1,33)} = 6.24$, P = .0176; Figure 15) There was a trend for males and females to differ in this association ($F_{(1,29.1)} = 3.35$, P = .0773). Interestingly, IL RAM+ cells positively predicted males' latency to enter the light ($F_{(1,29.1)} = 29.5$, P = .0092), but was nonsignificant for females (Figure 16). RAM expression in the MePV was not associated with latency to enter the light zone. Also, there was a trend for a positive correlation between IL RAM+ expression and the amount of time spent in the dark zone ($F_{(1,33)} = 2.85$, P = .1006; Figure 17). There was a significant sex difference in correlations of the number of IL RAM+ cells and time spent in the dark zone ($F_{(1,22.6)} = 10.3$, P =.0039). This interaction indicated that IL RAM+ cells positively predicted duration in the dark zone for males ($F_{(1,22.6)} = 10.3$, P = .0012), but not for females (Figure 18). RAM expression in the MePV was not significantly associated with duration in the dark zone.

I regressed the duration of submissive/defensive behavior during CD testing on RAM expression with a factorial crossing of sex and status. I found a significant positive correlation between IL RAM+ expression and the duration of submissive behavior during CD testing ($F_{(1,31.6)} = 5.63$, P = .0239; Figure 19). Also, males and females significantly differed in the correlation between IL RAM+ expression and submissive behavior ($F_{(1,30.9)} = 5.96$, P = .0206). Specifically, the number of RAM+ cells in the IL at defeat positively predicted

submissive/defensive behavior in males during CD testing (P = .0043). Meanwhile, females showed an opposite behavioral pattern, albeit this difference was not significant. I found no significant associations between MePV RAM expression and duration of submissive behavior in CD testing (Figure 20).

I found no other significant associations between defeat-induced RAM expression in the IL and MePV and measures of post-defeat anxiety-like behaviors, including behavioral responses in the SA test. Specifically, RAM+ cells were not significantly associated with latency to approach the social interaction zone, duration of time in the social interaction zone, or time spent in the corner zone during SA test. Similarly, RAM+ cells were not significantly associated with zone transitions in the LD test or aggression in the CD test.

## Chapter Five Discussion

The present study was designed to elucidate the contribution of neural ensembles in the IL and MeA to the development of stress-related vulnerabilities. Here, we demonstrated neural activity in the IL was implicated in stress-induced changes of anxiety-like behavior, while MePV neural activity was more closely associated with resisting social defeat stress. There was a nonsignificant trend for RAM+ cells in the MePV to be associated with a proactive coping response during social defeat stress, which is consistent with the role of the MePV in experience-dependent changes in defensive aggression (Nordman, 2020). Meanwhile, RAM+ cells in the IL predicted various anxiety-like behavioral expressions following social defeat stress, suggesting that activity of IL neural ensembles was linked to stress-related fear and anxiety. These findings are consistent with the critical role IL neurons play in the extinction of fear memories (Ressler and Fenster, 2018). In this study, activity of IL neurons were associated with behaviors such as submissive/defensive behaviors during conditioned defeat testing in male hamsters and avoidant behaviors in a LD transition test in females. Interestingly, male and female hamsters express stress-induced anxiety in separate behavior assays, which suggests that a battery of behavioral assays should be included to account for sex dependent differences in stress vulnerability. In addition, we have shown that responses in CD and SA tests are not correlated with each other, which suggests that different tests measure separate aspects of stress-related anxiety (Grizzell, 2019). Conditioned defeat tests include a smaller non-aggressive animal in the subjects' home cage, while social avoidance tests utilize larger adults as the social stimuli in a novel arena. The environmental context and the specific social stimulus can lead to separate responses because of potential dysregulation of threat perception. Overall, IL neural ensembles contributed to

experience-dependent changes in stress-induced anxiety-like behaviors differentially in males and females.

Intriguingly, we found that male hamsters that spend more time in the dark zone in a LD test prior to daily dominance encounters were more likely to acquire a subordinate social status. Likewise, we found the female hamsters transitioned between zones in the LD test more frequently than males prior to daily dominance encounters. These findings suggest that a timid temperament may contribute to the development of a subordinate social status and a bold temperament may contribute to a dominant social status in females. However, we do not expect that pre-existing temperament differences account for all status-dependent changes in behavior because dominant and subordinate animals show larger differences in anxiety-like behavior after the formation of a dominance relationship. This finding is consistent with previous research showing that 2 weeks of daily dominance encounters are required for stress-induced activation of IL neurons (Morrison et al., 2014). Also, the NIH guidelines for the care and use of laboratory animals describe three principles to ensure that researchers replace, refine, and reduce the number of animals used for a study, which is seemingly well intended, but can create complications in the interpretation of neural and behavioral results. For example, we used non-aggressive intruders as later subjects, which is consistent with the policy of reducing the number of animals needed for the study. However, because non-aggressive intruders may have received aggression during conditioned defeat testing, some of our subjects could have experienced social defeat stress during adolescence. Animals also likely create social hierarchy when they are group housed in late adolescence, suggesting that animals may have had prior experiences winning or losing aggressive social encounters which were not captured in this study. Social stress is known to alter the development of agonistic behavior in male hamsters (Wommack et al., 2003), and

some of our subjects' prior social experiences could have contributed to the individual differences observed during the pre-status phase of the LD and SA tests. Thus, although hamsters clearly show differences in anxiety-like behavior before and after the development of a dominance relationship, future research will be needed to determine how these status-dependent differences in behavior arise.

Interestingly, we found that neural activity in the MePV did not correlate with behavior during stress, though we did find that IL neural activity during social defeat stress correlated with latency to submit. These results suggest that IL activity is a critical region regulating coping behavior during social stress and the development of subsequent anxiety-like behavior. Latency to submit during aggressive encounters has been associated with proactive coping styles in rat models (Wood, 2010, 2015). In the current study, MePV activity was marginally associated with latency to submit and frequency of attacks displayed by the subjects during social defeat stress. Activation of GABAergic neurons in the MePV promotes attack behaviors and is necessary for defensive aggression (Hong et al., Padilla et al., 2016). In addition, the experience of winning aggressive encounters generates synaptic plasticity in MePV glutamatergic cells that sent projections to the bed nucleus of the stria terminalis (Nordman, 2016). These findings suggest that specific cell types in the MePV are related to mutual aggression and long latencies to submit that characterize the response to social defeat stress in dominant hamsters. Our lab found that a blockade of androgen receptors in the MeA during social defeat stress reduces conditioned defeat response without altering latency to submit during social defeat stress itself (Cooper et al., 2021). These findings suggest that non-androgen receptor-positive cells in the MePV contribute to proactive responses during social defeat or that activity of neural ensembles outside of the MePV are also necessary for proactive coping responses. Interestingly, we showed that dominant

animals express more endogenous c-Fos in IL-BLA and IL-DRN pathways compared to subordinate animals and activity in these pathways is correlated with latency to submit, supporting the idea that the IL is implicated in both coping responses during social stressors and resistance to stress-related social avoidance (Dulka, 2018, Grizzell, 2020). Studies have also shown that distinct cell types within the IL and prelimbic (PL) cortex play a critical role in behavioral responses during stress experience. Rosado et al., (2022) found that animals who display flexible coping responses to stress exhibit increased dendritic complexity in pyramidal cells within the IL compared to animals who respond with conditioned behavioral responses. Overall, flexible coping may be critical for increasing the neuroplasticity in the mPFC that underlies resilience to stress-related disorders.

Counterintuitively, we found that social defeat stress produced greater RAM expression in the IL of subordinate males compared to dominants, which is opposite than patterns previously observed for c-Fos expression (Morrison et al., 2012; Morrison et al., 2014) Subordinate males may have activated more GABAergic cells during social defeat stress, which would have inhibited IL pyramidal cells projecting to limbic targets such as the amygdala. The RAM viral vector approach is not without limitations. For instance, the RAM virus uses a promoter from the c-Fos family of immediate early genes, but its expression is not identical to endogenous c-Fos. Variation in RAM expression is likely dependent on the type of cell that is activated during stress experience. Sorensen et al (2016) showed that inescapable stress produces robust RAM expression in GABAergic interneurons in the PL and that RAM expression is far more common in somatostatin neurons compared to parvalbumin neurons. Importantly, the types of cells expressing RAM are critical for interpreting the pattern of neural activity shown by subordinate and dominant males during social defeat stress. Inhibition of IL pyramidal cells during social

defeat stress would be expected to increase the conditioned defeat response and would be consistent with studies showing that pharmacological inactivation of the IL increases the acquisition of a CD response (Morrison et al., 2013, Markham et al., 2012). In contrast, dominant females may have activated more pyramidal cells in the IL compared to same-sex subordinates, although the mechanisms underlying a sex difference in types of IL cells expressing RAM virus are unclear. Future directions should concentrate on delineating cell types within the IL and MePV that show robust defeat-induced RAM expression and its association to stress-related behavior.

The IL and MePV have a critical role in regulating behavior during social stress experiences. The MePV has been implicated in defensive aggression and we found a trend for this region to be associated with proactive coping and fighting back during social stress. However, we found no evidence to indicate that MePV activity predict the development of stress-induced anxiety, which suggests that other brain regions might account for the link between proactive coping and stress resilience. Interestingly, IL activity was strongly associated with passive coping in males, as a portion animals submitted before receiving aggression from the resident aggressor and had the greatest number of RAM+ cells. In addition, RAM expression in the IL strongly predicted the development of anxiety-like behaviors following stress experience, as greater RAM expression was associated with increased time in the dark chamber of the LD test and elevated submissive behavior during CD testing. These findings are consistent with the role of corticotrophin releasing factor in the IL, which has been shown to alter the ratio of excitation to inhibition in IL pyramidal cells (Alizamini et al., 2022). Similarly, dysregulated functional connectivity between the vmPFC and the amygdala is a common characteristic of stress-related psychopathologies (Shin et al., 2004; Rauch et al., 2006). Because we found

opposite results than hypothesized for dominant and subordinate males, future studies should use methods that address the balance of excitation and inhibition within the IL. For example, Jing et al. (2022) showed that stress exposure increased the frequency but not the amplitude of spontaneous inhibitory presynaptic currents in the IL, which supports the possibility that stress increases presynaptic GABA release. Intriguingly, the activity of GABAergic cells may have been captured by our use of the RAM method. Therefore, future studies should include neural markers that delineate excitation/inhibition ratio during stress experience and its effect on the development of stress-related behaviors.

*Conclusions*

Significant advancements have been made in understanding the neurobiological mechanisms that underlie individual differences in stress vulnerability, including critical neural circuitry involved in the acquisition of stress-related disorders. Researchers have been recently investigating the interplay between bottom-up exaggerated responses of the amygdala and top-down regulation from mPFC in the involvement of behavioral habituation to stress experiences. The present study suggests that treatments which address fear memories, such as extinction therapy, could potentially rebalance the ratio of excitation and inhibition in the mPFC. Treatments that balance the activity of glutamatergic and GABAergic cells should also correct dysregulated activity in direct long-range projections from the IL to amygdala as well as indirect local circuits within the IL. Ultimately, behavioral treatments that improve flexible coping skills and strengthen fear inhibition will be essential for promoting the critical neuroplasticity underlying recovery from traumatic stress.

**References**

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 neuroscience*, *8*(3), 365-371.

Amir, A., Amano, T., & Pare, D. (2011). Physiological identification and infralimbic responsiveness of rat intercalated amygdala neurons. *Journal of neurophysiology*, *105*(6), 3054-3066.

Berretta, S., Pantazopoulos, H., Caldera, M., Pantazopoulos, P., & Pare, D. (2005). Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience*, *132*(4), 943-953.

Burleson, C. A., Pedersen, R. W., Seddighi, S., DeBusk, L. E., Burghardt, G. M., & Cooper, M. A. (2016). Social play in juvenile hamsters alters dendritic morphology in the medial prefrontal cortex and attenuates effects of social stress in adulthood. *Behavioral Neuroscience*, *130*(4), 437.

Busch, D. E., & Barfield, R. J. (1974). A failure of amygdaloid lesions to alter agonistic behavior in the laboratory rat. *Physiology & behavior*, *12*(5), 887-892.

Cho, J. H., Deisseroth, K., & Bolshakov, V. Y. (2013). Synaptic encoding of fear extinction in mPFC-amygdala circuits. *Neuron*, *80*(6), 1491-1507.

Clinard, C. T., Barnes, A. K., Adler, S. G., & Cooper, M. A. (2016). Winning agonistic encounters increases testosterone and androgen receptor expression in Syrian hamsters. *Hormones and behavior*, *86*, 27-35.

Cooper, M. A., Clinard, C. T., & Morrison, K. E. (2015). Neurobiological mechanisms supporting experience-dependent resistance to social stress. *Neuroscience*, *291*, 1-14.

Cooper, M. A., Clinard, C. T., Dulka, B. N., Grizzell, J. A., Loewen, A. L., Campbell, A. V., & Adler, S. G. (2021). Gonadal steroid hormone receptors in the medial amygdala contribute to experience-dependent changes in stress vulnerability. *Psychoneuroendocrinology*, *129*, 105249.

Cooper, M. A., Grizzell, J. A., Whitten, C. J., & Burghardt, G. M. (2023). Comparing the Ontogeny, Neurobiology, and Function of Social Play in Hamsters and Rats. *Neuroscience & Biobehavioral Reviews*, 105102.

Christianson, J. P., Thompson, B. M., Watkins, L. R., & Maier, S. F. (2009). Medial prefrontal cortical activation modulates the impact of controllable and uncontrollable stressor exposure on a social exploration test of anxiety in the rat. *Stress*, *12*(5), 445-450.

Dagnino-Subiabre, A. (2021). Stress and Western diets increase vulnerability to neuropsychiatric disorders: A common mechanism. *Nutritional Neuroscience*, *24*(8), 624-634.

Do-Monte, F. H., Manzano-Nieves, G., Quiñones-Laracuente, K., Ramos-Medina, L., & Quirk, G. J. (2015). Revisiting the role of infralimbic cortex in fear extinction with optogenetics. *Journal of Neuroscience*, *35*(8), 3607-3615.

Dulka, B. N., Bress, K. S., Grizzell, J. A., & Cooper, M. A. (2018). Social dominance modulates stress-induced neural activity in medial prefrontal cortex projections to the basolateral amygdala. *Neuroscience*, *388*, 274-283.

Dulka, B. N. (2018). The Neurochemistry and Neural Circuitry of Stress Resilience.

Dulka, B. N., Bagatelas, E. D., Bress, K. S., Grizzell, J. A., Cannon, M. K., Whitten, C. J., & Cooper, M. A. (2020). Chemogenetic activation of an infralimbic cortex to basolateral amygdala projection promotes resistance to acute social defeat stress. *Scientific Reports*, *10*(1), 6884.

Duque-Wilckens, N., Steinman, M. Q., Busnelli, M., Chini, B., Yokoyama, S., Pham, M., ... & Trainor, B. C. (2018). Oxytocin receptors in the anteromedial bed nucleus of the stria terminalis promote stress-induced social avoidance in female California mice. *Biological psychiatry*, *83*(3), 203-213.

Faye, C., McGowan, J. C., Denny, C. A., & David, D. J. (2018). Neurobiological mechanisms of stress resilience and implications for the aged population. *Current neuropharmacology*, *16*(3), 234-270.

Fenster, R. J., Lebois, L. A., Ressler, K. J., & Suh, J. (2018). Brain circuit dysfunction in post-traumatic stress disorder: from mouse to man. *Nature Reviews Neuroscience*, *19*(9), 535-551.

Finklestein, M., Laufer, A., & Solomon, Z. (2012). Coping strategies of Ethiopian immigrants in Israel: Association with PTSD and dissociation. *Scandinavian journal of psychology*, *53*(6), 490-498.

Funahashi, S., & Kubota, K. (1994). Working memory and prefrontal cortex. *Neuroscience research*, *21*(1), 1-11.

Grizzell, J. A., Clarity, T. T., Graham, N. B., Dulka, B. N., & Cooper, M. A. (2020). Activity of a vmPFC-DRN pathway corresponds with resistance to acute social defeat stress. *Frontiers in Neural Circuits*, *14*, 50.

Gross, M., Romi, H., Miller, A., & Pinhasov, A. (2018). Social dominance predicts hippocampal glucocorticoid receptor recruitment and resilience to prenatal adversity. *Scientific Reports*, *8*(1), 1-13.

Gutzeit, V. A., Ahuna, K., Santos, T. L., Cunningham, A. M., Sadsad Rooney, M., Muñoz Zamora, A., ... & Donaldson, Z. R. (2020). Optogenetic reactivation of prefrontal social

neural ensembles mimics social buffering of fear. *Neuropsychopharmacology*, *45*(6), 1068-1077.

Hart, H., & Rubia, K. (2012). Neuroimaging of child abuse: a critical review. *Frontiers in human neuroscience*, *6*, 52.

Hofmann, H. A., Beery, A. K., Blumstein, D. T., Couzin, I. D., Earley, R. L., Hayes, L. D., ... & Rubenstein, D. R. (2014). An evolutionary framework for studying mechanisms of social behavior. *Trends in ecology & evolution*, *29*(10), 581-589.

Hong, W., Kim, D. W., & Anderson, D. J. (2014). Antagonistic control of social versus repetitive self-grooming behaviors by separable amygdala neuronal subsets. *Cell*, *158*(6), 1348-1361.

Huhman, K. L. (2006). Social conflict models: can they inform us about human psychopathology?. *Hormones and behavior*, *50*(4), 640-646.

Huhman, K. L., Solomon, M. B., Janicki, M., Harmon, A. C., Lin, S. M., Israel, J. E., & Jasnow, A. M. (2003). Conditioned defeat in male and female Syrian hamsters. *Hormones and behavior*, *44*(3), 293-299.

Mahan, A. L., & Ressler, K. J. (2012). Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends in neurosciences*, *35*(1), 24-35.

Johnson, K. T., Williams, P. G., Aspinwall, L. G., & Curtis, B. J. (2022). Resilience to stress-related sleep disturbance: Examination of early pandemic coping and affect. *Health Psychology*, *41*(4), 291.

Karamihalev, S., Brivio, E., Flachskamm, C., Stoffel, R., Schmidt, M. V., & Chen, A. (2020). Social dominance mediates behavioral adaptation to chronic stress in a sex-specific manner. *Elife*, *9*, e58723.

Kecala, N. M., Goodman, B. F., & Griffin, M. G. (2023). Dissociation as a Distinct Peritraumatic Coping Response: A Preliminary Analysis. *Journal of Trauma & Dissociation*, *24*(1), 79-94.

Keshavarzi, S., Sullivan, R. K., Ianno, D. J., & Sah, P. (2014). Functional properties and projections of neurons in the medial amygdala. *Journal of Neuroscience*, *34*(26), 8699-8715.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., ... & Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, *23*(7), 925-935.

Koolhaas, J. M., Coppens, C. M., de Boer, S. F., Buwalda, B., Meerlo, P., & Timmermans, P. J. (2013). The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *JoVE (Journal of Visualized Experiments)*, (77), e4367.

Korzan, W. J., Øverli, Ø., & Summers, C. H. (2006). Future social rank: forecasting status in the green anole (Anolis carolinensis). *acta ethologica*, *9*, 48-57.

Kredlow, M., Fenster, R. J., Laurent, E. S., Ressler, K. J., & Phelps, E. A. (2022). Prefrontal cortex, amygdala, and threat processing: implications for PTSD. *Neuropsychopharmacology*, *47*(1), 247-259.

Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., ... & Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*, *131*(2), 391-404.

Lui, F., Espinosa, A., & Anglin, D. M. (2022). Latent class analysis of racial microaggressions and institution-specific racial discrimination at a US minority-serving university: Implications for mental health and coping. *American Journal of Orthopsychiatry*

Luiten, P. G. M., Koolhaas, J. M., de Boer, S., & Koopmans, S. J. (1985). The cortico-medial amygdala in the central nervous system organization of agonistic behavior. *Brain research*, *332*(2), 283-297.

Maier, S. F., & Seligman, M. E. (1976). Learned helplessness: theory and evidence. *Journal of experimental psychology: general*, *105*(1), 3.

Maier, S. F., & Watkins, L. R. (2010). Role of the medial prefrontal cortex in coping and resilience. *Brain research*, *1355*, 52-60.

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*(1), 6-12.

Maxwell, J. P., & Siu, O. L. (2008). The Chinese Coping Strategies Scale: Relationships with aggression, anger, and rumination in a diverse sample of Hong Kong Chinese adults. *Personality and Individual Differences*, *44*(5), 1049-1059.

Miller, S. M., Marcotulli, D., Shen, A., & Zweifel, L. S. (2019). Divergent medial amygdala projections regulate approach–avoidance conflict behavior. *Nature neuroscience*, *22*(4), 565-575.

Morrison, K. E., Bader, L. R., Clinard, C. T., Gerhard, D. M., Gross, S. E., & Cooper, M. A. (2014). Maintenance of dominance status is necessary for resistance to social defeat stress in Syrian hamsters. *Behavioural brain research*, *270*, 277-286.

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior a node in the mammalian social behavior network. *Annals of the New York Academy of Sciences*, *877*(1), 242-257.

Nordman, J. C., Ma, X., Gu, Q., Potegal, M., Li, H., Kravitz, A. V., & Li, Z. (2020). Potentiation of divergent medial amygdala pathways drives experience-dependent aggression escalation. *Journal of Neuroscience, 40*(25), 4858-4880.

O'Connell, G., Hsu, C. T., Christakou, A., & Chakrabarti, B. (2018). Thinking about others and the future: Neural correlates of perspective taking relate to preferences for delayed rewards. *Cognitive, Affective, & Behavioral Neuroscience, 18*, 35-42.

Øverli, Ø., Korzan, W. J., Höglund, E., Winberg, S., Bollig, H., Watt, M., ... & Summers, C. H. (2004). Stress coping style predicts aggression and social dominance in rainbow trout. *Hormones and behavior*, *45*(4), 235-241.

Padilla SL, Qiu J, Soden ME, Sanz E, Nestor CC, Barker FD, Quintana A, Zweifel LS, Ronnekleiv OK, Kelly MJ, Palmiter RD (2016) Agouti-relatedpeptide neural circuits mediate adaptive behaviors in the starved state. Nat Neurosci 19:734–741.

Patterson, G. R., Dishion, T. J., & Bank, L. (1984). Family interaction: A process model of deviancy training. *Aggressive behavior*, *10*(3), 253-267

Petrides, M. (2000). The role of the mid-dorsolateral prefrontal cortex in working memory. *Experimental brain research*, *133*, 44-54.

Petrides, K. V., & Furnham, A. (2000). Gender differences in measured and self-estimated trait emotional intelligence. *Sex roles*, *42*, 449-461.

Petrulis, A. (2020). Structure and function of the medial amygdala. In *Handbook of behavioral neuroscience* (Vol. 26, pp. 39-61). Elsevier.

Pincus, M., Godfrey, J. R., Feczko, E., Earl, E., Miranda-Dominguez, O., Fair, D., ... & Kelly, C. (2021). Chronic psychosocial stress and experimental pubertal delay affect

socioemotional behavior and amygdala functional connectivity in adolescent female rhesus macaques. *Psychoneuroendocrinology*, *127*, 105154.

Rauch, S. L., Shin, L. M., & Phelps, E. A. (2006). Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research—past, present, and future. *Biological psychiatry*, *60*(4), 376-382.

Rosado, A. F., Bevilacqua, L. M., Moreira, E. L. G., & Kaster, M. P. (2023). Behavioral flexibility impacts on coping and emotional responses in male mice submitted to social defeat stress. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *123*, 110696.

Rosenhauer, A. M., McCann, K. E., Norvelle, A., & Huhman, K. L. (2017). An acute social defeat stressor in early puberty increases susceptibility to social defeat in adulthood. *Hormones and behavior*, *93*, 31-38.

Rosenkranz, J. A., Moore, H., & Grace, A. A. (2003). The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *Journal of Neuroscience*, *23*(35), 11054-11064.

Roy, M., Shohamy, D., & Wager, T. D. (2012). Ventromedial prefrontal-subcortical systems and the generation of affective meaning. *Trends in cognitive sciences*, *16*(3), 147-156.

Russo, S. J., Murrough, J. W., Han, M. H., Charney, D. S., & Nestler, E. J. (2012). Neurobiology of resilience. *Nature neuroscience*, *15*(11), 1475-1484.

Seiglie, M. P., Lepeak, L., Velázquez-Sanchez, C., Ferragud, A., Le, T., Cottone, P., & Sabino, V. (2022). The Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) System of the Central Amygdala Mediates the Detrimental Effects of Chronic Social Defeat Stress in Rats. *Eneuro*.

Selye, H. (1956). *The stress of life*.

Singleton, G., Johnson, L., Singleton, N., & Li, H. (2022). COVID-19-related anxiety: How do coping and optimism relate to substance use in African–American young adults?. *Journal of Community Psychology*.

Sinha, R., Lacadie, C. M., Constable, R. T., & Seo, D. (2016). Dynamic neural activity during stress signals resilient coping. *Proceedings of the National Academy of Sciences*, *113*(31), 8837-8842.

Shin, L. M., Rauch, S. L., & Pitman, R. K. (2006). Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Annals of the New York Academy of Sciences*, *1071*(1), 67-79.

Sinha, R. (2008). Chronic stress, drug use, and vulnerability to addiction. *Annals of the New York Academy of Sciences*, *1141*(1), 105-130.

Smith, J. D., Knoble, N. B., Zerr, A. A., Dishion, T. J., & Stormshak, E. A. (2014). Family check-up effects across diverse ethnic groups: Reducing early-adolescence antisocial behavior by reducing family conflict. *Journal of Clinical Child & Adolescent Psychology*, *43*(3), 400-414.

Snyder-Mackler, N., Burger, J. R., Gaydosh, L., Belsky, D. W., Noppert, G. A., Campos, F. A., ... & Tung, J. (2020). Social determinants of health and survival in humans and other animals. *Science*, *368*(6493).

Snyder-Mackler, N., Sanz, J., Kohn, J. N., Brinkworth, J. F., Morrow, S., Shaver, A. O., ... & Tung, J. (2016). Social status alters immune regulation and response to infection in macaques. *Science*, *354*(6315), 1041-1045.

Sørensen, A. T., Cooper, Y. A., Baratta, M. V., Weng, F. J., Zhang, Y., Ramamoorthi, K., ... & Lin, Y. (2016). A robust activity marking system for exploring active neuronal ensembles. *Elife*, *5*, e13918.

Southwick, S. M., Vythilingam, M., & Charney, D. S. (2005). The psychobiology of depression and resilience to stress: implications for prevention and treatment. *Annu. Rev. Clin. Psychol.*, *1*, 255-291.

Soloff, P. H., Abraham, K., Burgess, A., Ramaseshan, K., Chowdury, A., & Diwadkar, V. A. (2017). Impulsivity and aggression mediate regional brain responses in Borderline Personality Disorder: An fMRI study. *Psychiatry Research: Neuroimaging*, *260*, 76-85.

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S. L., & Quirk, G. J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learning & memory*, *13*(6), 728-733.

Vochteloo, J. D., & Koolhaas, J. M. (1987). Medial amygdala lesions in male rats reduce aggressive behavior: interference with experience. *Physiology & behavior*, *41*(2), 99-102.

Wang, Y., He, Z., Zhao, C., & Li, L. (2013). Medial amygdala lesions modify aggressive behavior and immediate early gene expression in oxytocin and vasopressin neurons during intermale exposure. *Behavioural brain research*, *245*, 42-49.

Williamson, C. M., Lee, W., DeCasien, A. R., Lanham, A., Romeo, R. D., & Curley, J. P. (2019). Social hierarchy position in female mice is associated with plasma corticosterone levels and hypothalamic gene expression. *Scientific reports*, *9*(1), 7324.

Wommack, J. C., Taravosh-Lahn, K., David, J. T., & Delville, Y. (2003). Repeated exposure to social stress alters the development of agonistic behavior in male golden hamsters. *Hormones and Behavior*, *43*(1), 229-236.

Wood, S. K., Walker, H. E., Valentino, R. J., & Bhatnagar, S. (2010). Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: role of corticotropin-releasing factor. *Endocrinology, 151*(4), 1795-1805.

Wood, S. K., & Bhatnagar, S. (2015). Resilience to the effects of social stress: Evidence from clinical and preclinical studies on the role of coping strategies. *Neurobiology of stress, 1*, 164-173.

Yehuda, R., & LeDoux, J. (2007). Response variation following trauma: a translational neuroscience approach to understanding PTSD. *Neuron, 56*(1), 19-32.

Zlatković, J., Todorović, N., Bošković, M., Pajović, S. B., Demajo, M., & Filipović, D. (2014). Different susceptibility of prefrontal cortex and hippocampus to oxidative stress following chronic social isolation stress. *Molecular and cellular biochemistry, 393*, 43-57.

## Vita

Jenna Laymon was born in Dowagiac, MI, on July 11, 1995. They attended various elementary schools and graduated from Dowagiac Union High School with honors in 2013. The following August, they entered Great Lakes Christian College and transferred to Lansing Community College thereafter where they received an Associate of Arts degree. Jenna finished her Bachelor of Science degree concentrating in Cognitive Psychology at Michigan State University in 2020. Then, continued her education at the University of Tennessee, Knoxville in July of 2021 and received a Master of Arts Degree in Experimental Psychology focusing on Neuroscience and Behavior in May of 2023.