Neural and Behavioral Predictors of Vulnerability to Chronic Social Stress in Mice

by

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Dedication

This dissertation is dedicated to my grandparents, whose resilience is defined by their compassion and selflessness. Thank you for being my source of inspiration, in science and in life.

وأهدي هذا البحث لجدي وجدتي اللذين تميز صمودهما بالتعاطف ومحبة الغير على أمل أن أسير على دربهما

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Abstract

Evaluating and coping with stressful social events as they unfold is a critical strategy in overcoming them without long-lasting detrimental effects. Individuals display a wide range of responses to stress, which can manifest in a variety of outcomes for the brain as well as subsequent behavior. Importantly, how an individual responds during the initial stress exposure has the potential to shape future behaviors and susceptibility to stress-related disorders. Chronic Social Defeat Stress (CSDS) in mice has been widely used to model individual variation following a social stressor. Following a course of repeated intermittent psychological and physical stress, mice diverge into separate populations of social reactivity: Resilient (socially interactive) and Susceptible (socially avoidant) animals. A rich body of work reveals distinct neurobiological and behavioral consequences of this experience that map onto the resilient and susceptible phenotypes. By contrast, we have less insight as to when and how the individual differences in social stress reactivity arise. In this thesis, we therefore sought to answer two questions: 1) when and how do individual differences to reactivity to social stress arise and 2) are there distinct patterns of neural network activation during the initial stress exposure that predict resilient and susceptible outcomes?

To address these questions, we focused on behaviorally characterizing resilient and susceptible mice before, during, and following CSDS. We found that behavioral coping strategies used by the mice early on during their initial social stress encounter can distinguish animals that will eventually be classified as resilient or susceptible. In particular, mice that will emerge as susceptible display greater escape behavior on Day 1 of social defeat than those that

will emerge as resilient, indicating early differences in coping mechanisms used between the two groups. We further show that the final social avoidance phenotype in susceptible mice is specific to the aggressor strain and does not generalize to conspecifics or other strains, indicating that threat discrimination is heightened in susceptible mice.

The dilemma in the field has been that the resilience/susceptible classification requires several days to emerge and defining neural circuit activity on Day 1 that was predictive of future classification was a major challenge. We used novel mouse technology (termed FosTRAP) to capture brain-wide neural activation patterns elicited during the initial stress exposure, and to examine whether they predict the social outcome. We were able to identify selective brain areas and networks that showed a distinctive social defeat signature, as well as brain regions that were predictive of coping behavior during the first encounter, and subsequently, the propensity to develop susceptibility.

Our findings point to the initial experience with a social stressor as a major variable that impacts both brain and behavior to set the stage for the final social phenotype. The findings outlined in this thesis provide an important framework for characterizing temporal behavioral and neural dynamics of the stress response that elicit individual differences in the development of adaptive or maladaptive social behaviors.

Chapter 1 Introduction

1.1 Chronic Social Stress and the Development of Affective Disorders

Many social animals, including humans, rely on social interactions as a key source of survival. How an individual navigates social interactions is highly complex, as one needs to pay attention to the environmental context, rely on feedback from other social partners, and respond with appropriate emotional control. While positive social relationships increase overall health and well-being, negative social relationships can induce highly stressful and vulnerable states, leading to psychiatric disorders. Therefore, social interactions provide a framework for uncovering how neural, environmental, and behavioral factors converge to impact reactivity to social stress.

When negative social encounters are a constant source of stress in the environment, the propensity to develop vulnerability increases. However, the perception of social stress is often subjective, and the outcomes can be variable. Prior social experiences play a crucial role in an individual's perception of and subsequent response to future social events. Notably, the initial response often serves as the anchor upon which subsequent similar experiences are built. Acute stressors are known to facilitate memory of context and habit formation (Schwabe et al., 2022). For example, in threatening scenarios such as humiliation or bullying, an individual may first react by either actively confronting the aggressor or taking on a more avoidant state to remain silent or run-away (Pontillo et al., 2019). This initial reaction has the potential to set the course for how an individual assesses and reacts to threats in similar future scenarios.

Following the initial exposure, later repeated exposures to social stress can lead to various outcomes. On the one hand, repeated exposures are perceived as familiar and predictable, which can lead to adaptive health outcomes, such as the ability to exercise cognitive and behavioral control under distress. On the other hand, repeated social stress exposure, at the extremes, can develop into social anxiety disorder or hyper-aggression. In some cases, these disorders are specific to the social context, but in others, may reflect more generalized affective and aggressive states.

While the cumulative effects of chronic stress on the brain and body have been well-studied, less is known about how the initial exposure can shape the final affective state. Early patterns of responsiveness, such as coping strategies and activation of brain-wide circuits, likely set the stage for future social reactivity (Palamarchuk and Vaillancourt, 2021; Sinha et al., 2016; Snyder et al., 2015). Here we ask whether there are predictive elements present early on during the stress response that act as risk factors in the outcome of stress reactivity.

1.2 Affective Disorders Associated with Sociability are Moderately Heritable

Genetics may provide a basis for predicting how an individual will react under social stress. Maladaptive social patterns can present either as a specific social anxiety disorder (SAD) or as a feature in other psychiatric disorders, such as major depressive disorder (MDD), generalized anxiety disorder (GAD), and panic disorder (PD) (Lydiard, 2001). Studies in multi-generational families and twins have been useful in estimating the contribution of genetic versus environmental factors. In these studies, psychiatric disorders that have reduced sociability as a common feature are known to be moderately heritable (27% for SAD, 37% for MDD, 30% GAD, 30% for PD) (Hettema et al., 2005, 2001; Sullivan et al., 2000). While these epidemiological studies indicate that disorders with low and anxious sociability have a genetic

component and biological underpinning, there remains a large proportion of the variance (~60%) that is attributable to the environment. Therefore, the development of psychiatric disorders and the biological basis of sociability can be seen as an interaction between genetic and environmental factors, with environmental factors playing the more dominant role. However, the contribution of early experience, as opposed to other environmental factors, remains unclear.

In order to uncover the genetic underpinnings of social behaviors, researchers have focused their efforts on conducting candidate gene and genome-wide association studies (GWAS). Candidate gene studies are hypothesis-driven and probe whether a single gene or a small number of genes are implicated in the causes or presentation of a disorder. By contrast, GWAS are agnostic and use a discovery approach to identify genetic variants across the entire genome that are associated with a disorder (Duncan et al., 2019). In an example of the candidate approach, the oxytocin (OTXR) gene and system have been implicated in regulating complex social behaviors. A meta-analysis of 2 promising OTXR single nucleotide polymorphisms (SNPs) candidates revealed no meaningful effect on human social behavior (Bakermans-Kranenburg and van Ijzendoorn, 2014). More broadly, candidate gene approaches for many psychiatric disorders have not borne fruit, even in highly heritable diseases such as schizophrenia (Scull, 2022). This approach likely underestimates the heterogeneity and gene x environment interactions that contribute to complex behaviors associated with psychiatric disease.

Given the heterogeneity of social behavior and associated disorders, it may not be surprising that GWAS analyses of social anxiety disorders uncovered few if any, SNPs that significantly associate with these disorders (Bralten et al., 2021; Day et al., 2018; Stein et al., 2017). Even when analyzing for specific types of social behaviors (social interaction, social avoidance, social isolation), the heritability estimates were <5% (Bralten et al., 2021). The

limited results are likely due to the fact that GWAS and candidate gene studies are often

hampered by variable sample sizes, low replicability between studies, and differences that arise

from age, sex, and race (Tam et al., 2019). For example, in MDD, a meta-analysis of over a

million individuals was necessary to uncover ~87 significant genes that explain only a small

percentage of the variance (Howard et al., 2019). These studies suggest that while heritable

genetic factors may underlie affective disorders that have a socially avoidant component, they

are likely highly heterogeneous across individuals and families, with multiple paths leading to a

similar clinical outcome. Moreover, genetics may bias social interactions, but they clearly do not

determine them. Consequently, behavioral and environmental factors may be more reliable

predictors of social stress-related disorders.

1.3 Temperament is an Indicator of Future Affective State

Untangling how the environment influences both genetics and behavior may provide a

clearer lens by which to risk factors that contribute to stress reactivity. An individual's

temperament, or their style of response to challenges in the environment, may play a significant

role in their response to social stress and, therefore, the propensity to develop vulnerability

following stress. Introversion and extraversion are two personality traits that encompass the

larger construct of temperament and form the basis of internalizing and externalizing mental

health disorders. In general, introverted individuals may display neuroticism and negative affect

(fear, shyness, anger, deficits in social skills), while extraverted individuals display positive

emotionality and are reward-sensitive to novel people and situations. (Sanson et al., 2004).

Moreover, these traits map onto internalizing disorders, such as SAD, GAD, MDD, and

externalizing disorders, such as antisocial personality disorder, substance abuse disorders, and

aggression-related disorders. These traits are thought to have a substantial level of heritability,

as they emerge during the early stages of development and have shown to be highly stable over the lifetime (Heath et al., 1994; Thompson and Lamb, 1982). Indeed, even when controlling for age, neuroticism and extraversion were significant predictors in whether or not an individual presented with SAD (Costache et al., 2020). These temperamental tendencies can also be seen in animals. For example, a rodent model of temperament that uses selective breeding based on novelty seeking has led to two lines of rats that exhibit several highly contrasting characteristics that map onto human internalizing and externalizing behavioral profiles(Birt et al., 2021; Clinton et al., 2011; Flagel et al., 2014; Prater et al., 2017; Stedenfeld et al., 2011; Turner et al., 2017). Therefore, the individual variation in temperamental traits may provide a key insight into how sociability is acquired.

In particular, these temperament systems map onto differences in coping behaviors, as those with an introverted temperament tend to engage in avoidance, while those who exhibit extraversion tend to engage in active strategies to change or think more positively about the stress at hand (Rueda and Rothbart, 2009) (See **Figure 1.1** for more details). Based on these findings, temperament and the type of coping response that arises during stress may be features of heritability that allow for better prediction of social stress reactivity. However, it is important to note that there is an ongoing interaction between these intrinsic characteristics of temperament and the surrounding environment; while temperament has an inherent biological basis, its expression and impact on individual reactivity may be moderated by external societal and cultural pressures (Sanson et al., 2004). It remains to be seen how these underlying biological predispositions towards a particular temperament, the environmental context, and experience-dependent learning all come together to influence stress reactivity.

Figure 1.1 contents (text within the figure):

Temperament Systems and **Associated Response Patterns**   **Coping Strategies**

Negative Affectivity →
Avoidance
Escape
Behavioral inhibition
Anxiety
Aggression

Effortful Control →
Control of attention
Inhibition of inappropriate (dominant) responses
Activation of appropriate (nondominant) responses
Error detection
Planning

Extraversion/Surgency →
Approach of novelty
Risk taking
Social proximity (*)
Optimism

Active coping
Approach response to either change the situation or think about it more positively

Avoidant coping
Avoid the stressful situation or avoid thinking about the problem (including wishful thinking)

Distraction
Involvement in some other activity to keep oneself from thinking about the problem

Support seeking
Use of social support both to solve the problem and to reduce negative emotions

Note: Gray lines represent hypothesized relationships based on conceptual connections. Black lines represent evidence-based relations. Solid lines represent positive relationships, and dotted lines represent negative relationships.

Figure 1.1 Temperament Systems, Associated Response Patterns, and Coping Strategies.
Adapted by permission from John Wiley and Sons: New Directions for Child & Adolescent Development.
The Influence of temperament on the development of coping: The role of maturation and experience.
Rothbart and Rueda (2009). DOI: 10.1002/cd.240, PMID: 19536792 © 2009.

1.4 Coping Behaviors May Provide Better Prediction for Stress Reactivity

How an individual evaluates and copes with negative social encounters as they unfold plays a significant role in overcoming stress without long-lasting detrimental effects. Here we define coping as the "cognitive or behavioral responses to stressors, intended to remedy a stressful situation or dampen the emotional response to a stressful situation" (Lazarus & Folkman, 1984). In humans, Endler and Parker (1990) defined three major types of coping styles in response to stress: task-oriented, emotional-oriented, and avoidant. Task-oriented focuses on active problem-solving approach by reframing and reasoning through stress, a feature that has also been associated with low levels of depression and anxiety. In contrast, anxiety has been seen to manifest in both emotional-oriented (rumination and negative association of stress) and avoidance (avoiding the problem) coping styles (Kendler et al., 1991; Parker and Endler, 1990; Wonderlich-Tierney and Vander Wal, 2010).

For example, individuals with SAD engage in avoidance and safety behaviors when faced with a social encounter (Hofmann, 2007). During the interaction, safety behaviors are used to mask anxiety-related behaviors, such as stereotypical movements ('playing with hair, shaking, inappropriate emotional reactions). Following the encounter, the individual will ruminate about the event and exacerbate the negative associations (emotional-oriented coping). This then leads to avoidance, as the individual will avoid similar social situations in the future (Hofmann, 2007). These anxious feelings have been shown to be strongly encoded in neural circuits involving stress and memory (Boehme et al., 2014; Nakao et al., 2011; Savage et al., 2020).

As for the heritability of coping styles, monozygotic twin studies have shown that all three types of coping styles had varying degrees of heritability, ranging from 30-34% for task-oriented, 19-35% for emotional-oriented styles, and 0-19% for avoidance (Busjahn et al., 1999; Kendler et al., 1991; Kozak et al., 2005). Candidate gene studies and genome-wide approach studies have been used to assess the genetic basis for different types of coping styles but were often underpowered, leading to mixed results (Dunn and Conley, 2015; Shimanoe et al., 2019). This suggests that environmental influences account for the majority of the variability in coping style, primarily influenced by experience unique to each individual (Jang et al., 2007). These experiences can range from early life stress or previous experiences with stress that influence threat and fear assessment, which are encoded in the brain (Britton et al., 2013; Pechtel and Pizzagalli, 2011). How an individual from the same genetic background (such as the monozygotic twins) engages in different coping responses to influence stress reactivity outcome may therefore be due to differences in neural circuitry.

1.5 Brain Activity Patterns During Stress Reflect Type of Coping Style

Using functional magnetic resonance imaging (fMRI), researchers have shown that individual differences in coping styles manifest at the level of neural activity patterns. fMRI studies allow for investigating neural activity patterns via changes in blood oxygen level detection (BOLD) signal. In one study, Sinha et al. assessed dynamic neural activity patterns in an fMRI task in which participants were shown highly aversive images and non-aversive neutral images in an unpredictable pattern (Sinha et al., 2016). When first shown the aversive images, increases in brain activity were seen in cortico-limbic regions associated with the traditional stress response. When images were flashed from stressful to neutral, another distinct temporal pattern emerged, in which early increases of activation were then followed by reduction, suggesting a pattern of stress adaptation. This dynamic response pattern was particularly observed in the vmPFC, indicating that it was a key hub region in this overall emotional and behavioral control network. Greater flexibility of activation exhibited in the vmPFC strongly correlated with active coping behaviors in real life. In contrast, lower dynamic activation of the vmPFC was correlated with maladaptive coping behaviors, such as emotional eating, frequency of arguments, and alcohol intake. This suggests that early patterns of responsiveness and adaptation to stress, as seen in the vmPFC, may set the course for the type of coping behavior an individual uses and, therefore, the propensity to develop vulnerability to social stress disorders.

In sum, some features of coping style are influenced by temperament, which is heritable. However, coping behavior is also strongly influenced by environmental context and experience. Even within the same genetic background, such as monozygotic twins, there is significant variability in coping responses (Busjahn et al., 1999). These differences likely result from environmental challenges in particular contexts that shape experience and lead to the

establishment of certain habits (Mellins et al., 1996). The initial feedback from stress and the environment on behavioral and neural activity patterns can color subsequent responses to stress (Evans and Kim, 2013; Folkman and Moskowitz, 2004; McEwen, 1998a). A single stress experience may then set the stage for a distinctive neural and behavioral activity pattern that predisposes the development of either susceptibility or resilience to future stress

1.6 Significant Questions Remain Unanswered to Predict an Individual's Reactivity to Chronic Stress

The sections above have reviewed a significant body of work in humans that have identified differences in genetic, behavioral, and neural mechanisms that contribute to the manifestation of stress-related disorders. Despite this, we have limited insight into the risk factors that predict whether an individual is vulnerable and likely to develop a stress-related disorder such as major depression or anxiety disorders. Here, we summarize the conclusions of the previous sections and outline the outstanding questions.

- While there is genetic and epidemiological evidence for the heritability of affective disorders, much of the variance can be attributed to the environment or environment-by-gene interaction that is brought about by environmental stressors.
  - What environmental conditions are necessary to bring about the emergence of stress-related disorders? Are there characteristics of acute, intermittent, or chronic stress that are especially powerful in triggering these disorders, and how do they interact with genetic and developmental factors.
  - How do individuals with the same genetic background and family environment manifest different levels of susceptibility to stress-related disorders?

- The way that an individual reacts to a stressor is highly dependent on their temperament and the type of behavioral coping strategy that is deployed during the stress to elicit future responses.
  - Are there coping behaviors that eventually confer higher susceptibility to stress disorders?
  - Can we use behavioral outputs as measurable risk factors for predicting the development of affective disorders?
- While there are neural correlates with specific brain regions that correspond to distinct types of coping styles, these markers are often studied after an individual has significant experience with that class of stress in their daily life.
  - Are there patterns of neural activity that predispose to certain early responses to stress and shape coping strategies?
  - Can the neural response during the initial stress experience prime a pattern of behavior that shapes subsequent responses to the same stressor?
  - Does this combination of neural activity and associated behavior shape vulnerability or resilience to chronic stress?

In brief, what is lacking is a scientific understanding of the role of an early experience in shaping vulnerability or resilience—i.e., how individual variation in coping styles and neural circuit activity that arise during the initial stress response set the stage for future reactivity. Developing a better understanding of the role of the initial response to an experience may lead to identification of specific behavioral, neurobiological, genetic, or environmental interactions that are triggered by the stress response to mediate vulnerability outcomes.

1.7 Importance of Animal Models of Social Stress

Capturing the responses to initial experiences in humans and their subsequent role in shaping long-term reactivity can be challenging. Animal models of chronic stress allow researchers to address the interplay between initial experience and long-term reactivity in a tightly controlled setting to investigate the associated neural mechanisms in greater depth. Importantly, animal models of individual differences in coping responses to social stress may reflect elements in the development of human psychiatric disorders.

In vertebrate and invertebrate animals alike, the establishment of hierarchy and territorial control is associated with physical and psychosocial stress (Gottier, 1968; Lord et al., 2021; Mason and Mendoza, 1993; Meese and Ewbank, 1973; Williamson et al., 2016). Characterized by a series of aggressive conflicts, the establishment of dominant and subordinate relationships arise. However, it is the individual variation in the response and reaction to the environmental challenge during these encounters that mediate the final state. Below we will describe a series of studies that explore how differences in behavioral response patterns, neuroendocrine expression, and neural circuits contribute to social coping and the further development of vulnerability.

1.8 Coping Responses in Rodents

Coping during stress can be seen in a number of social species in addition to humans, such as fish, primates, pigs, and rodents (de Boer et al., 2017; de Polavieja and Orger, 2018; Kanitz et al., 2019; Lyons et al., 2010; Wong et al., 2019). This interaction between stress and coping results from adaptive mechanisms that have been positively selected for through evolution. Therefore, the success of a coping style is based on its ability to reduce physiological features of stress and its effectiveness in eliminating the aversive situation (Wechsler, 1995).

In the laboratory setting, rodent models provide the optimal framework for assessing how adaptive and maladaptive states arise from different types of coping styles. Koolhaas defines two overall main coping styles that rodents engage in: active or passive. Active coping (or proactive) is characterized by behavioral control and a fight or flight response, while passive coping (reactive) is characterized by low levels of aggression and greater immobility (Koolhaas et al., 1999). When faced with a threat, an animal with an active response will either engage in the aggression and fight, or engage in escape behavior, characterized by the rodent fleeing to shelter. In contrast, animals with a passive response will conserve their energy and freeze, a feature of self-preservation (Wechsler, 1995).

While an animal may engage in active or passive coping strategies, these strategies themselves are not inherently adaptive or maladaptive. The environmental context and energy expenditure required by the stress response are factors to be considered in assessing the adaptability of the behavior. This can be seen through the lens of allostasis, which refers to the "active process of maintaining stability" (McEwen, 1998b). If a stress is unpredictable and long-lasting, it can lead to inefficient termination of the stress response, resulting in a higher allostatic load. This increases the risk of detrimental health effects at the level of metabolic, immune, and neural systems (Dhabhar, 2014; McEwen and Akil, 2020; Rabasa and Dickson, 2016). For example, heightened levels of aggression can be seen as adaptive during an agonistic encounter, but if that aggression continues after the threat has passed, then hyper-aggressive behavior can ensue and be associated with biological, social, and health costs (Haller et al., 2005, 2001; König et al., 1996; Tóth et al., 2008). In contrast, heightened threat detection during stress is adaptive and necessary to ensure the survival of the organism, but if that threat detection is persistent

and/or it becomes generalized to other contexts, then maladaptive states such as PTSD can result (Norrholm et al., 2011; Pamplona et al., 2011; Thome et al., 2018).

Thus, each coping style has some advantages but is associated with potential costs. It is necessary to consider the physiological effects these different strategies have on the dysregulation of homeostasis in the organism.

1.8.1 Neuroendocrine Correlates of Differences in Coping Styles

The interplay between environmental stressors and the animal's behavioral response begins at the level of the nervous system. A major component of the stress response is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. When activated, neural stress circuitry triggers a cascade of neural and endocrine events that result in the synthesis and release of glucocorticoid hormones (corticosterone (CORT) in rodents) from the adrenal gland, which in turn impact the entire body, including the brain. The rapid rise in glucocorticoids activates a negative feedback loop that allows the body to return to homeostasis. (Akil, 2005; Cullinan et al., 1993; Herman et al., 1995).

More broadly, stress is a brain-wide process that involves the integration of inputs and outputs that converge onto the paraventricular nucleus of the hypothalamus (PVN) and control the HPA axis. The elements of the neural circuitry that are activated encode various characteristics of the stress input and shape the ensuing stress response (Cullinan et al., 1993; Herman et al., 1995). One feature of these complex responses can be seen in **Figure 1.2**, which outlines how a stressor activates distinct but overlapping neural circuits depending on whether it is a physical (in pink) or psychological stressor (in purple). The brain regions involved in these responses reflect highly complex integrations of sensory and emotional modalities (Godoy et al., 2018). Notably, differences in inhibition or activation at any point in this system may reflect

different coping styles and adaptations to stress (McEwen, 1998b). Therefore, coping behavior

and brain-wide neural circuitry may represent different facets of risk factors that are apparent

during the initial stress response.
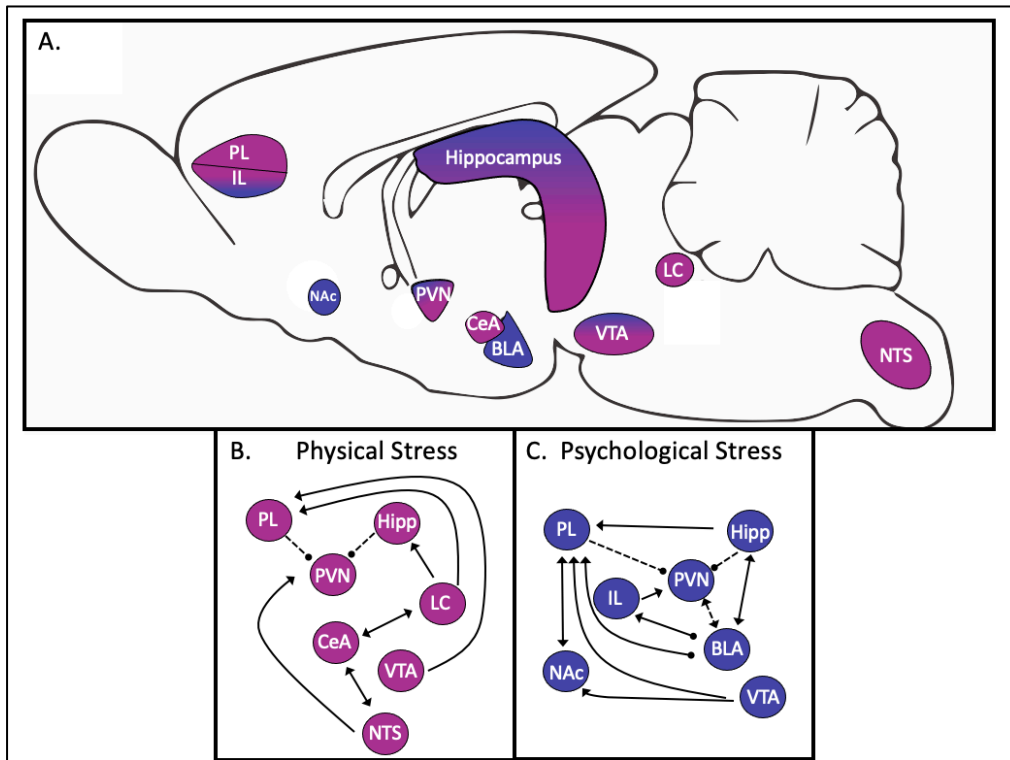
Figure 1.2 Neuroanatomy of the Stress Response
"Schematic representation of primarily neuroanatomical substrates responsible for physical (pink) and psychological (blue) stressors processing. Upper panel shows that neural processing for different types of stressors s(A). Bottom panels represent how physical and psychogenic stressors require engagement of different networks (B, C, respectively). Physical stressors mainly activate structures related to vital functions control located on brainstem and hypothalamus. Structures such as the nucleus of the solitary tract (NTS) and locus coeruleus (LC) have an important role in the physical stress pathways. However, prosencephalic regions also participate in physical stress processing, such as prelimbic area (PL) in pre-frontal cortex (PFC). The central nucleus of the amygdala (CeA) participates in autonomic response integration. For instance, psychological stressors are perceived in an anticipatory condition, which may heavily rely on limbic structures and can be modulated by the reward system. The PFC is critical to develop appropriate responses to environment changes, and it is densely innervated by dopaminergic projections from the Ventral Tegmental Area (VTA) and Nucleus Accumbens (NAc). Although PFC involvement is complex and integrates different stress responses in general, PL and Infralimbic (IL) regions coordinate a top-down control. The amygdaloid complex also participates on psychological stress circuitry and with PFC disruption its involvement becomes more prevalent, and the circuitry switches to a bottom-up control. The Hippocampus (Hipp) CA1 region has important connections with the above-mentioned limbic structures and Hipp is an important structure of the HPA axis negative feedback. The paraventricular nucleus of hypothalamus (PVN) the main relay of the stress response triggering the HPA axis. Adapted by permission from Frontiers: Frontiers in Behavioral Neuroscience. A Comprehensive Overview on Stress Neurobiology: Basic Concepts and Clinical Implications. Godoy et al. (2018). DOI: 10.3389/fnbeh.2018.00127, PMID: 30034327© 2018

Agonistic encounters present a naturalistic paradigm to assess differences in coping strategies. Depending on the duration of the encounter and the genetic background of the model, differences in how neuroendocrine responses map onto behavioral coping styles emerge. Wild house mice that were selectively bred for either short attack latencies (SAL) or long attack latencies (LAL) showed differential CORT expression after being placed across from a male of opposite genotype for five days. Following five days of this purely sensory contact stress, mice with LAL, and therefore more passive response patterns, showed higher CORT levels and adrenal hypertrophy (Veenema et al., 2005). In a different study where the resident-intruder paradigm occurred with the sensory and physical component over a series of 12 days, outbred OF-1 mice that engaged in a passive strategy had higher serum CORT and lower serum adrenaline when placed in an additional social defeat challenge in comparison to active coping mice (De Miguel et al., 2011). In contrast, in another study where 21 days of defeat occurred in outbred OF-1 mice that were either classified as active or passive based on coping strategies established by day 18, an increase of plasma CORT following an additional social stress was seen in comparison to controls but showed no difference between each other (Pérez-Tejada et al. 2013). From these studies, it is apparent that either a genetic component, introduced by selective breeding and/or the use of an outbred strain led to differences in CORT between passive and active coping mice. However, these differences tend to disappear with an environmental manipulation of increasing the duration of social defeat. Therefore, the intersection of the stress response and active and passive coping strategies may represent a highly dynamic process influenced by environmental manipulations. In addition, these observed differences in CORT expression are measured following the stress and, therefore, may or may not reflect individual differences that arose due to adaptation to stress over time. The stress response system varies

over many temporal windows (acute vs. during vs. chronic stress). Thus, the dynamic change of these responses and how they map onto the coping style used during stress is unclear.

1.9 Neural Circuits Involved in Social Behaviors and Coping

While the above provides some insight into the neural encoding of a stress signal, an important complement is the encoding of the behavioral coping style that the animal mounts in response to the stressor. Examining the dynamic interplay between these two facets of stress—perception and coping, may be essential for understanding how various elements of an initial stress experience shape subsequent adaptation to stress. Agonistic encounters provide a naturalistic model to assess how social stress and coping responses manifest. From rodent work, it is apparent that fight, escape, freeze, and social circuits all converge within the same brain regions involving the limbic-hypothalamic-midbrain pathways (Ko, 2017). Here, we will review the current literature on neural circuits that mediate specific behavioral elements of active and passive coping. These studies have focused on using optogenetic and electrophysiological tools to induce the presence of a single specific behavior (i.e., aggression vs. no aggression) to uncover the circuitry that mediates its execution. Most of them have not carried out the work in the context of individual differences in coping strategies. A visual summary of these circuitry findings is in Figure 1.3.
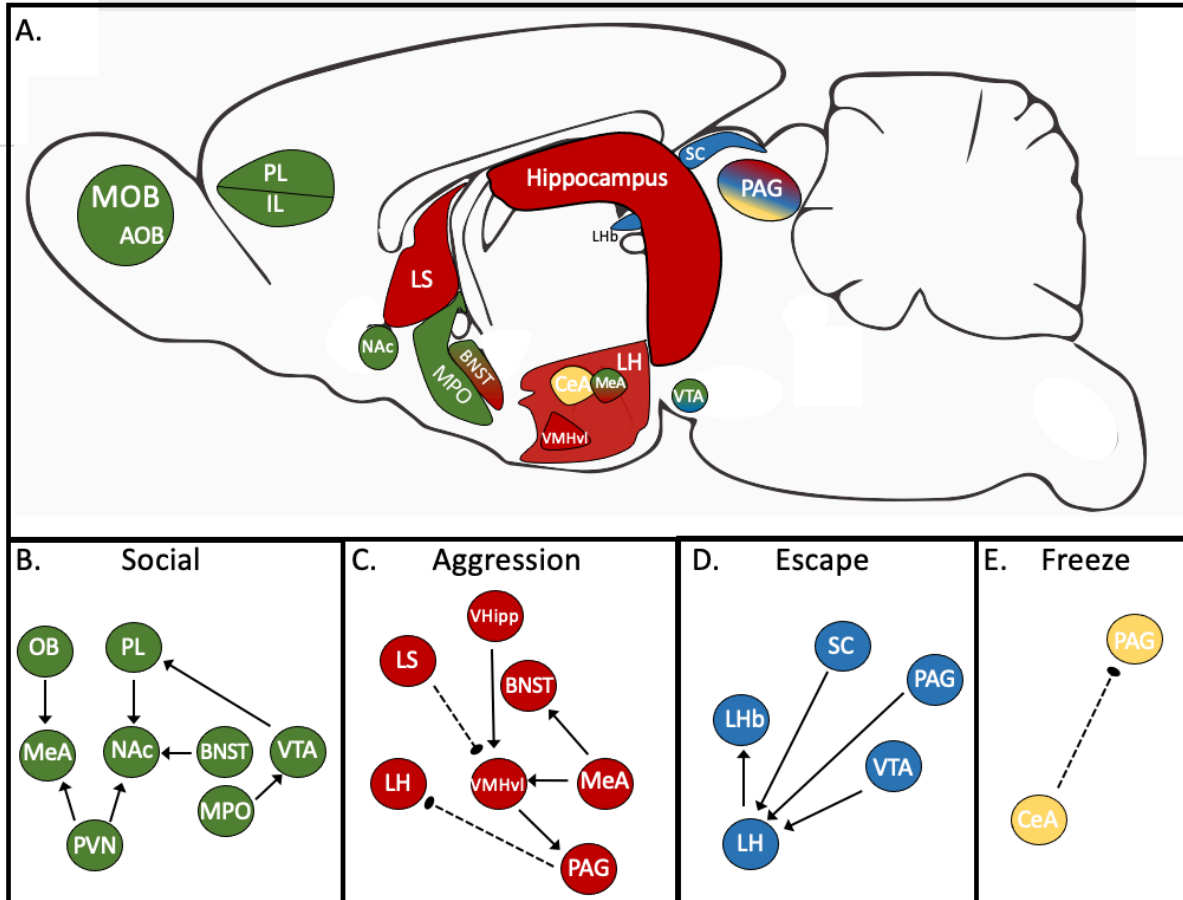
Figure 1.3 An Illustration of Social, Aggression, Escape, and Freezing Response Circuits
Top Panel (A) shows that neural processing for social and coping behaviors involve multiple selective and similar brain regions. Green(B)- Social, Red(C)- Aggression, Blue(D)- Escape, and Yellow(E)- Freeze. Solid arrows represent activation, while dashed lines represent inhibition. OB: Olfactory Bulb, PL: Prelimbic, IL: Infralimbic, NAc: Nucleus Accumbens, MPO: Medial Preoptic Area, LS: Lateral Septum, BNST: Bed Nucleus of the Stria Terminalis, VMHvl: Ventromedial Hypothalamus, LH: Lateral Hypothalamus, CeA: Central Amygdala, MeA: Medial Amygdala, vHipp: Ventral Hippocampus, LHb: Lateral Habenula, VTA: Ventral Tegmental Area, SC: Superior Colliculus, and PAG: Periaqueductal Gray

1.9.1 Neural Circuits Involved in Social Behaviors (Fig. 1.3B, Green)

During the initiation of social behaviors in rodents, the olfactory sensory neurons in the main olfactory bulb initiate a cascade of activation depending on the valence attached to the social interaction. From there, regions associated with fear (medial amygdala- MeA), stress (PVN), reward (nucleus accumbens -NAc), bed nucleus of the stria terminalis -BNST), ventral tegmental area -VTA), and social behaviors (medial preoptic area -MPO) are activated.

Information and social context clues arise from cortical regions, such as the prelimbic cortex (PL), which also relays this information to midbrain regions (Miura et al., 2020). Depending on the type of social interaction (attack, copulation, parental care), specific neural circuits are activated within these regions to engage in the social response (Ko, 2017; Wei et al., 2021).

1.9.2 Neural Circuits Elicited by Aggression During Agonistic Encounters (Fig. 1.3C, Red)

Optogenetic and electrophysiology studies have elucidated the pathways in which initiation, duration, and intensity of fight behavior are mediated. The ventral part of the ventromedial hypothalamus (VMHvl) has been a well-studied hub in generating aggressive, attack behavior. The VMHvl receives inputs from various brain regions, including the MeA, BNST, lateral septum (LS), periaqueductal gray (PAG), and medial preoptic nucleus (MPO) (Falkner et al., 2020; Kim et al., 2019; Lo et al., 2019; Wang et al., 2019). It was found that high-frequency optogenetic stimulation of MeA synapses to the BNST regulated the initiation of attack, while stimulation of MeA synapses to the VMHvl maintained the attack behavior (Nordman et al., 2020). The act of winning or losing an agonistic encounter influenced the plasticity between the MeA projections. Specifically, when Nordman et al. measured field excitatory postsynaptic potentials (fEPSP), higher initial winning attack experience enhanced the synaptic transmission between the MeA to the BNST and VMHvl prior to future encounters (Nordman et al., 2020). Moreover, the VMHvl also undergoes learning-dependent changes, as repetitive optogenetic stimulation of its neurons accelerates aggression latency and increases the intensity of attack during future agonistic encounters (Falkner et al., 2016). The VMHvl also receives input from the ventral hippocampus (vHipp), a region that has been implicated in many neuropsychiatric disorders. Optogenetic stimulation of vHipp neurons projecting to the VMHvl induced attack behavior (Chang and Gean, 2019).

Motor initiation and termination of attack have been shown to be mediated through the LS and PAG. Optogenetic stimulation of excitatory glutamatergic projections from the VMHvl to the PAG and inhibitory GABAergic projections from the PAG to the lateral hypothalamus (LH) were necessary for the downstream coordination and execution of motor behavior during attack (Falkner et al., 2020). And finally, optogenetic stimulation of GABAergic cells projecting from the LS, a region known for evoking aggression, to the VMHvl, terminated ongoing attack (Wong et al., 2016). Overall, these aggression circuit studies demonstrate that the experience of the agonistic encounter and the active fight coping style is regulated by the strength and potential plasticity of this circuit.

1.9.3 Neural circuits Involved in Escaping an Approaching Threat (Fig. 1.3D, Blue)

Another active coping style that rodents may engage in during stress is escape (or flight) behavior (Evans et al., 2019). This behavior is characterized by the animal 'jumping' over an approaching aggressor or away from a looming threat, allowing them to move safely towards a space of shelter (De Franceschi et al., 2016). Similar to aggression behavior, escape behavior is a dynamic response that rapidly updates through experience to adapt to threatening environments (Vale et al., 2017). Studies of escape behavior have primarily focused on the role of the LH. Excitatory glutamatergic afferents from the LH to the lateral habenula (LHb) are necessary to promote aversion-driven escape behavior (Lecca et al., 2017; Li et al., 2018). In addition, upstream LH afferents from the VTA were shown to be necessary for the initiation of the escape response, as the inhibition or ablation of glutamatergic VTA neurons disrupted escape responses (Barbano et al., 2020). Moreover, optogenetic and in-vivo calcium imaging studies of freely behaving mice have shown that activity in the medial superior colliculus (mSC), a region where integration of sensory modalities occurs, was predictive of perceiving threat prior to escape

behavior, and that glutamatergic neurons in the PAG encode escape choice and vigor (Evans et al., 2018). While not assessed, the mSC is also known to project to the LH (Fallon and Moore, 1979). In contrast to aggression behavior, these PAG-projecting LH glutamatergic neurons control escape and evasion (Li et al., 2018), suggesting that these two active responses (fight and escape) may be mediated through opposing circuits.

1.9.4 Neural Circuit of the Initiation of the Freeze Response

When the stress is uncontrollable and appears as though there is no escape, the rodent will display fear-induced freezing (Eilam, 2005). Similar to active responses, passive freezing behaviors are also modulated by experience. Previous exposure to unfamiliar and unpredictable stress, such as random foot shocks, increases the propensity for the rodent to freeze in future paradigms (Atsak et al., 2011; Rau et al., 2005). This is indeed the basis of numerous studies in fear conditioning (De Oca et al., 1998; Lavond et al., 1993; Ledoux, 1998; LeDoux et al., 1988; Maren et al., 1996).

The central amygdala (CeA) is a critical node in controlling freezing behavior. Specifically, GABAergic CeA neurons have been shown to project onto and inhibit the PAG to induce freezing behavior (Tovote et al., 2016). As is the case with aggression and escape, the PAG appears to be a central node that mediates these different types of active and passive coping responses.

1.9.5 Conclusions on the Neural Circuitry of Social and Coping Responses

Overall, it is apparent that the interplay between the neural circuitry involved in social encounters and active or passive coping strategies overlap, but also have distinct features that mediate the expression of the behavior. Notably, these several strands of work indicate that

coping response may be a learned behavior depending on how the animal perceives the encounter (winning or losing), resulting in the strengthening or weakening of certain circuits. This highly intertwined and regulated system allows for feedback from the environment to elicit coping responses and, most likely, strengthens the type of coping style based on subsequent experiences of stress.

However, these studies were all conducted on mice that were explicitly manipulated (via optogenetics) to express the presence of one specific type of coping style. In a naturalistic rodent environment where social stress is elicited via multiple agonistic encounters that have both a physical and psychosocial element, as in the resident-intruder paradigm, mice express a variety of coping responses during the defeat. Importantly, these coping responses are expressed to different degrees, reflecting the element of individual variation in social coping with stress. Therefore, while these studies provide the basis for which brain regions and circuits become activated under this controlled environment, the feature of individual variation is lost. A key question that remains is if individual variation in coping style used during defeat is reflected in neural circuitry that appears early on during the initial encounter to mediate the degree of final social stress reactivity.

1.10 Resident-Intruder Paradigm as a Model of Individual Variation in Stress Reactivity

In rodents, the resident-intruder paradigm has been used as a naturalistic social stress model to evaluate individual differences in the development of vulnerability (Hollis and Kabbaj, 2014). In this paradigm, aggressive, agonistic encounters are conducted until social hierarchy is established, with rodents that arise as dominant 'winners' and those that are subordinate as 'losers'(Hammels et al., 2015; Kabbaj et al., 2001; Kollack-Walker et al., 1999). Importantly, individual variation in the response and reaction to threat in the environment (i.e., coping)

emerges during these encounters. While the previous section outlined specific circuits that mediate coping responses, how individual differences in coping strategies map onto the expression of resilience or vulnerability to chronic social stress is not as well known. This animal model provides the basis to explore this question.

In our lab, different models have been used to assess the effects of the resident-intruder paradigm (also known as social defeat) in establishing these hierarchies. In one model based on temperament, outbred male rats are selectively bred into high responders (bHRs) and low responders (bLRs) based on their locomotor activity when exposed to a novel environment (Flagel et al., 2014; Turner et al., 2017). At baseline, bHRs are highly exploratory and present increased aggression, while bLRs are highly inhibited and avoidant and present anxiety- and depression-like behavior. When placed through social defeat, HRs engaged in less passive and submissive behaviors and, when recovering from defeat, had higher CORT levels in comparison to LRs. This was further reflected in differences in mineralocorticoid/glucocorticoid ratio in the hippocampus (Hipp) in bHR but not bLR rats (Calvo et al., 2011). In one of the earliest studies of brain correlates of agonistic behavior, male Syrian hamsters were separated into dominant and subordinate groups based on behavior shown during the initial aggression encounter. These dominant males exhibited chase and attack responses, while the subordinate males took on more vigilant, defensive, and escape reactions. To assess differences in neural circuitry involved between these two groups, Kollack-Walker et al. used c-fos mRNA as a proxy for neural activity patterns. Interestingly, brain regions were selectively activated depending on whether defensive (subordinate) or offensive (dominant) behavior was used during the defeat (Kollack-Walker et al., 1997). Moreover, repeated exposure to social defeat resulted in differential adaptation in different brain regions (Kollack-Walker et al., 1999). Overall, these findings provide evidence

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that 'winning' or 'losing' agonistic encounters arises from specific coping styles and are associated with distinct patterns of neural circuit activation that are altered with repeated exposure. Thus, they lay the foundation for linking initial coping style, experience, and subsequent adaptation to specific neural circuits.

1.10.1 Chronic Social Defeat Stress in Mice

In mice, the resident-intruder paradigm is referred to as the chronic social defeat stress (CSDS) model. Traditionally the CSDS paradigm is run on genetically inbred mice; thus, unlike the above models, the differences in stress reactivity observed are mainly due to environmental influences (Golden et al., 2011; Hammels et al., 2015). Thus, this model provides the optimal framework to control for genetics in assessing how specific characteristics and features of behavioral and neural responses come together to impact individual reactivity to stress.

The CSDS paradigm consists of an intruder C57BL6/J mouse repeatedly subjected to daily 5-minute bouts of defeat by a larger, aggressive CD-1 mouse. Following the physical defeat, the C57BL6/J mouse is placed across a clear perforated divider in a shared home cage from the CD1 mouse for 24 hrs. to induce continuous psychosocial stress. Traditionally, this is repeated for ten days, with the C57BL6/J mouse rotated to a new CD1 each day to prevent habituation. Following CSDS, C57BL6/J mice are placed in a social interaction measure, where they diverge into two separate populations of socially interactive (resilient) and socially avoidant (susceptible) mice (Golden et al., 2011) (**Figure 1.4**, below). In contrast, C57BL6/J control mice are rotated across a clear perforated divider in a shared home cage from each other but never physically interact. While defeated mice are termed 'resilient' and 'susceptible' based on their social behavior following stress, it is essential to consider what neural and behavioral factors contribute to the affective makeup of these states. The following sections describe what is known

in the literature about resilient and susceptible behavioral and neural signatures in this CSDS

paradigm.
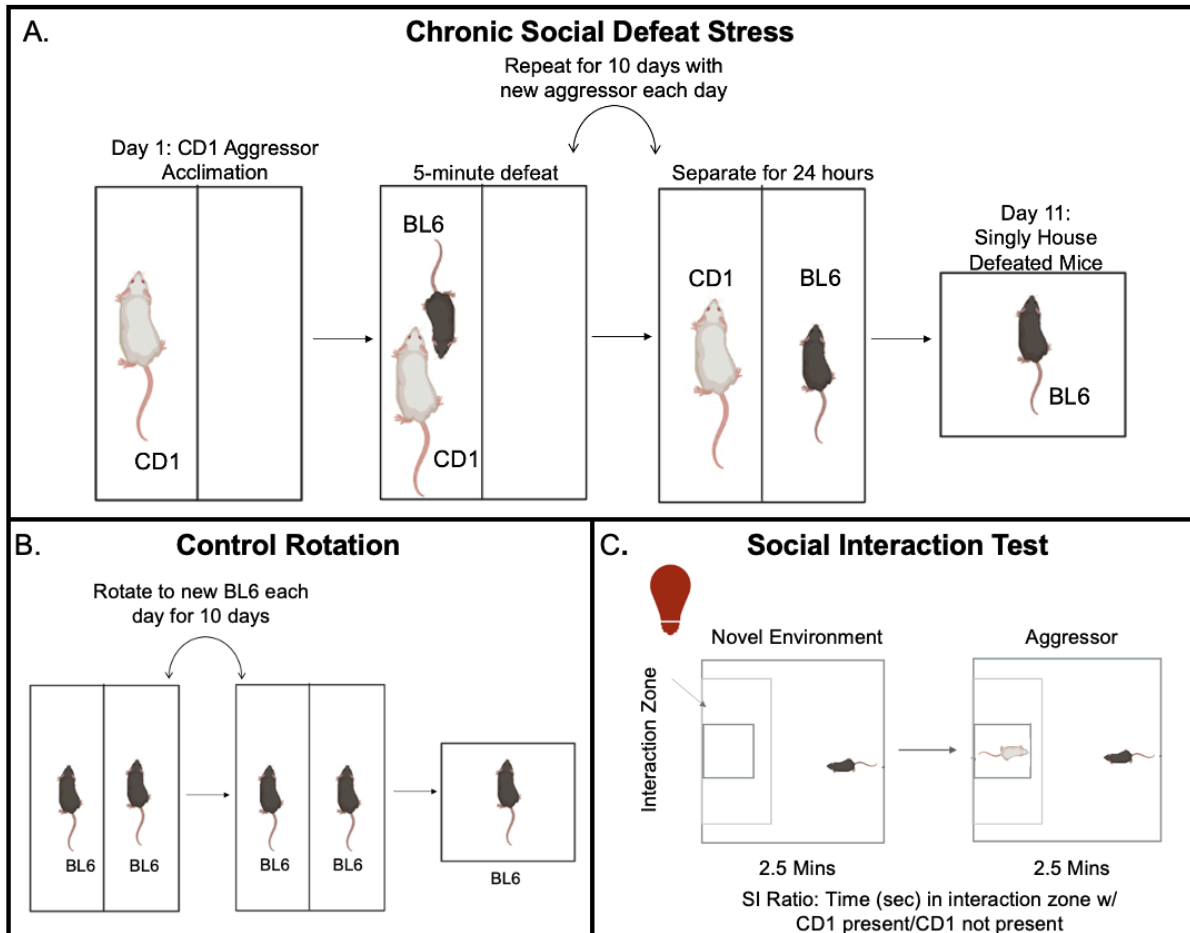
Figure 1.4 Chronic Social Defeat Stress (CSDS) Paradigm in Mice
A. The CSDS paradigm occurs in a home cage separated by a perforated divider. Before CSDS, aggressive CD1 mice are acclimated to the cage to establish home cage dominance. Next, C57BL6/J(BL6) mice are placed in the CD1 'section' for 5-minutes to induce physical defeat. Following the defeat, mice are separated across the perforated divider for 24 hours to induce sensory, but not physical, stress. This is repeated over the course of 10 days, with the BL6 experiencing a novel CD1 each day. Following CSDS, BL6 mice are singly housed for downstream behavioral testing. B. Control Rotation: BL6 mice are placed across the perforated divider from each other and are rotated to a new partner each day for ten days. No physical contact occurs. C. Following CSDS, mice are placed in the social interaction test under red light, which measures the amount of time the BL6 mouse spends in the interaction zone when the CD1 is not present in the wire mesh cage and when the CD1 is present. The SI ratio is calculated as CD1 present/CD1 not present. Figure Created with BioRender.com.

1.10.2 Behavioral Summary of CSDS

In addition to the individual variation seen in the social interaction measure, resilient and

susceptible populations have also been shown to map onto other downstream behavioral tests. In

a comprehensive study, Krishnan et al. characterized resilient and susceptible mice in several

behavioral and physiological domains following social defeat. While defeat alone was sufficient

to induce overall depression-like behavior (increase of immobility in both the tail suspension and

forced swim test) and anxiety-like behavior (decrease of time spent in the open field), the split

between resilient and susceptible was only apparent in measures of anhedonia (decreased sucrose

preference in susceptible mice) and addiction-like behavior (increase of cocaine placed

preference in susceptible mice). Physiologically, serum CORT between the two groups remained

high in comparison to controls, while susceptible animals had a decrease of circadian amplitude

and an increase of social hyperthermia in comparison to resilient and control mice (Krishnan et

al., 2007). However, it is important to note that these behavioral and physiological findings have

not always been consistent across studies. Other groups have found either no differences in

anhedonia measures or have found differences in anxiety-like and depression-like behavioral

tests between resilient and susceptible animals (Alves-Dos-Santos et al., 2020; He et al., 2018;

Lee et al., 2021; K. Zhang et al., 2021). Moreover, varying the length of social defeat has been

shown to alter the proportion of resilient and susceptible mice, as well as the strength of

displaying increased vulnerability in other behavioral tasks. For example, increasing the length

of defeat from 10 to 21 days increases the number of susceptible mice and provokes a more

generalized anxiety phenotype in other measures of affective-like behavior (Fang et al., 2020). In

contrast, a shorter period of repeated social defeat (5 days) is not sufficient to induce the resilient

or susceptible outcome, suggesting that the evolution of the stress response overtime is necessary

to establish the final classification (Bagot et al., 2015). Taken together, the variability in

behavioral outputs in resilient and susceptible mice, beyond the robust social interaction

measure, suggests that the socially avoidant phenotype may more accurately map onto measures from the environment and onto the context of the defeat.

As evidenced by the different outcomes in varying lengths of defeat, the experience of the stress itself may be more indicative of how resilient and susceptible outcomes manifest. For example, Yoshida et al. trained mice on a food-seeking lever-press operant task before social defeat, then during all ten days of defeat, mice were evaluated for their goal-directed behavior. Day 1 of defeat alone increased latency to lever press for food, and each consecutive day steadily increased latency and number of incomplete trials in all defeated mice (Yoshida et al., 2021). This longitudinal study revealed that as the stress continues, the reduction in goal-directed behavior can be seen as an aggregating effect of repeated stress exposure. In order to assess differences that arise at the level of the brain, Yoshida et al. used fiber photometry to assess differences in neural activity patterns within the ventral CA1 (vCA1) during the goal-directed task following Day 1 of stress and Day 10 of stress exposure. A successful completion of the task revealed that activity within the vCA1 becomes suppressed. Control and defeated mice appeared to have the same suppressed activity pattern on Day 1 during the task. However, after ten days of social defeat, only susceptible mice diminished this suppression activity and exhibited hyperactivity in the vCA1. Overall, at the level of brain and behavior, it appears that chronic stress dynamically alters the type of strategy that an animal engages in and results in changes of specific circuitry that is reflected in the social outcome.

1.10.3 Baseline Predictors of Resilient and Susceptible Populations

Recently there has been a greater emphasis on uncovering whether it is possible to predict resilient and susceptible outcomes before the stress occurs. Using an integrated model that combined baseline anxiety-like behavior and an immune biomarker, IL-6, Nasca et al. found that susceptibility could be predicted by 80% when mice exhibited a low anxiety-like score and low expression of the IL-6 biomarker (Nasca et al., 2019). In another measure of anxiety-like behavior, Milic et al. found that low levels of exploratory drive in novel environments was predictive of future susceptibility (Milic et al., 2021). In a voluntary wheel-running task, Zhang et al. found that susceptibility could be predicted by 92% when mice exhibited low baseline physical activity in the wheel (J. Zhang et al., 2021). And finally, Larrieu et al. found that the prior establishment of social hierarchy in the home cage was predictive of future sociability; mice that were initially ranked higher in dominance in their home cage were predicted by 89% to develop susceptibility following stress (Larrieu et al., 2017). Taken together, differences in baseline anxiety-like behavior, immune responses, locomotor activity, and aggression appear to represent facets of risk factors that can confer increased susceptibility in this animal model.

1.11 Neural Circuits of CSDS

A series of gene expression, optogenetic, and anatomical studies have dissected the neural circuits that mediate sociability following CSDS. The following studies emphasize that brain-wide activation networks are necessary to maintain and induce the resilient and susceptible outcomes. See **Figure 1.5** for a summary of known circuits/brain regions of CSDS.
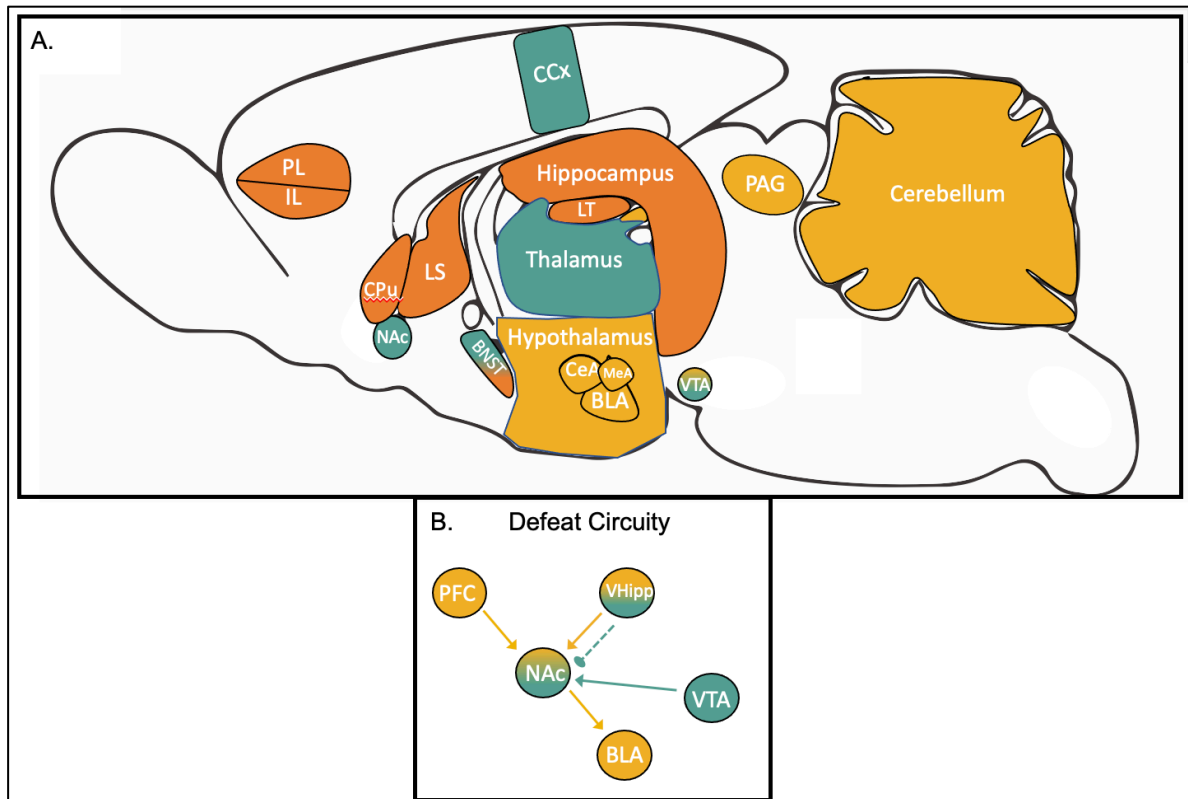
Figure 1.5 Neural Circuits Underlying CSDS

A: Summary of Circuitry Involved in Defeat, Resilience, and Susceptibility. Orange= Activated by defeat, Yellow= specific to susceptible, Green= Specific to Resilient. B: Panel B: Known circuits in mediating resilient/susceptible outcomes. Arrows= activation, Dashed lines= inhibition, yellow= susceptible, green= resilient. Coordinated oscillatory signals from the PFC to the NAc to the BLA is present in susceptible animals (Hultman et al., 2018). Optogenetic activation of glutamatergic vHipp afferents to the NAc induces susceptibility, while inhibition of those same afferents induces resilience (Bagot et al., 2015; Muir et al., 2020). Inhibition of the PFC by the VTA induces susceptibility, while activation of the NAc by the VTA induces resilience (Chaudhury et al., 2013). PFC: Prefrontal Cortex (PL, IL), PL: Prelimbic, IL: Infralimbic, CPu: Caudate Putamen, NAc: Nucleus Accumbens, LS: Lateral Septum, BNST: Bed Nuclei of the Stria Terminalis, LT:Lateral Thalamus, CeA: Central Amygdala, BLA: Basolateral Amygdala, MeA: Medial Amygdala, CCx: Cingulate Cortex, VTA: Ventral Tegmental Area, PAG: Periaqueductal Gray.

1.11.1 Network Gene Expression Studies Indicate Brain-Wide Differences in Response to CSDS

Recently, weighted gene co-expression network analysis (WGCNA) has been used to identify changes in networks of transcriptomic signatures across multiple brain regions and animal models (Bagot et al., 2016; Caradonna et al., 2021; Huang et al., 2016; Kwon et al., 2019; Zhao et al., 2010). Two WGCNA studies have been conducted in CSDS. To assess circuit-wide

pattern differences in gene expression, Bagot et al. first selected four brain regions associated with stress: NAc, vHipp, prefrontal cortex (PFC), and AMY. Then, the researchers conducted RNA-seq and WGCNA analysis at early (48 hours) and late (27 days) timepoints post-CSDS in resilient, susceptible, and control mice. Forty-eight hours following stress, differential gene expression patterns revealed that resilient mice had increased synchrony of transcriptional regulation (overlap of gene expression profiles) between the PFC and NAc, while susceptible mice had increased synchrony between the PFC and vHipp. This suggested that these differentially circuit-based co-regulated gene expression networks are indicative of driving early differences in social reactivity. Analysis between the early and late time points revealed that susceptible mice had an increase in highly coordinated gene expression profiles between all four brain regions compared to control and resilient mice, indicating that larger coordinated changes in gene expression networks are a feature of susceptibility to stress (Bagot et al., 2016). In another study using WGCNA analysis, Caradonna et al. found that in two models of stress (CSDS and chronic oral corticosterone), there was a similar gene expression profile in the vHipp of both CSDS and CORT-induced susceptibility (Caradonna et al., 2021). As these circuit-wide gene co-expression studies demonstrate, there appear to be distinct transcriptional networks following stress that regulate the development of resilience or susceptibility.

1.11.2 Fos Studies Indicate Differences in Neural Activation Patterns Arise as a Result of CSDS

In order to gain anatomical specificity as to which brain regions become activated as a result of stress, immediate early genes, such as Fos, have been used as a marker of neural activity. Fos is induced specifically to bouts of activity and is transient in nature, allowing for the capture of distinct neural patterns that appear during behavior (Bullitt, 1990). Both Delta FosB

(ΔFosB) and Fos protein have been used to probe differences in brain-wide circuits of social reactivity following CSDS. ΔFosB, a transcription factor within the Fos protein family, is unique in that it accumulates over time in neurons and has a half-life of eight days. It has been used as a proxy for identifying neurons that have been repeatedly activated through long courses of stress. In comparison to controls, defeated mice had an overall increase in ΔFosB in the BNST, LS, LH, vHipp, hypothalamus (HT), lateral thalamus (LT), and caudate putamen (CPu). (Laine et al., 2017). Across resilient, susceptible, and control mice, resilient mice had an increase in ΔFosB in the NAc compared to susceptible and controls (Vialou et al., 2010). This ΔFosB increase In resilient mice was seen specifically in D1 MSNs of the NAc (Lobo et al., 2013). In combining information from ΔFosB studies, Muir et al. used in-vivo fiber photometry to confirm that an increase of baseline D1-MSN activity within the NAc was a predictive marker of future resilience but not susceptibility (Muir et al., 2018). Collectively, these experiments show that brain regions are selectively being repeatedly activated by defeat alone throughout the paradigm, and that resilient and susceptible populations also exhibit selective activation within the NAc. This selective activation between stress reactivity groups was apparent at the level of in vivo recordings before stress, suggesting that certain brain regions may be primed before defeat to induce future vulnerability. This initial priming may then set the course for any experience-dependent changes and plasticity that arise between brain regions and subsequently alter neuronal circuits that eventually confer stress reactivity.

1.11.3 Electrophysiology And Optogenetics Studies Provide a Mechanistic Role of Neural Circuits Mediating Individual Reactivity to Social Stress

In addition to genetic and neuronal markers of activation, researchers have also focused on identifying the temporal and spatial dynamics of brain regions in mediating the outcome to

CSDS. In order to assess brain-wide differences in activity patterns, Hultman et al. used multi-circuit in vivo recordings of local field potentials (LFPs) across the PL, IL, NAc, CeA, basolateral amygdala (BLA), VTA, and vHipp (Hultman et al., 2018). Differences in the coordination of LFPs were present depending on whether mice were grouped as "defeat" or whether the specific acquisition of susceptibility arose. Defeat in general increased coordinated oscillatory signals from the NAc to the vHipp and VTA, but this pathway was also seen to be increased more in susceptible mice compared to resilient. In addition, susceptibility was associated with increased coordinated oscillatory signals from the PFC and NAc to the BLA. Similar circuits have also been found in optogenetics studies in mediating resilient and susceptible phenotypes. Bagot et al. found that low-frequency optogenetic stimulation (LTD) of glutamatergic afferents from the vHipp to NAc induced resilience, while high-frequency optogenetic stimulation (LTP) induced susceptibility (Bagot et al., 2015). Moreover, Chaudhury et al. found that optogenetic phasic firing of VTA dopaminergic neurons projecting to the NAc induced resilience (Chaudhury et al., 2013). From these findings, it is apparent that the stress reactivity phenotype is a result of coordinated and specific brain-wide circuitry.

1.11.4 Anatomical Studies Indicate Structural Differences in Individual Variation to CSDS

While the above studies focused on similar brain regions associated with reward-related circuits, repeated exposure to stress additionally profoundly impacts cognitive functions involving memory, attention, and decision-making. Thus, the impacts of CSDS must be assessed brain-wide. To assess structural differences in the brain following CSDS, Anacker et al. conducted magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) on resilient, susceptible, and control mice (Anacker et al., 2016). MRI allows for assessing structural differences in brain regions, while DTI images the white matter of the brain to infer the

directional preference of diffusion along the axon (i.e., fractional anisotropy (FA)). Susceptible mice selectively showed increases in area in the AMY, Hipp, PAG, HT, VTA, habenular commissure, and cerebellum (Cb) compared to resilient. In contrast, the cingulate cortex (CCx), NAc, BNST, Raphe nuclei, and thalamus were all larger in resilient mice compared to susceptible. Moreover, DTI revealed that the Hipp and HT both had correlations between social avoidance (susceptibility) and FA, suggesting that more diffusion across the axons in this brain region is associated with increased vulnerability (Anacker et al., 2016). While these brain regions show structural differences between resilient and susceptible mice following stress, it is unknown whether these differences existed before or early in the stress experience. Despite this, it is apparent that defeat affects the resilient and susceptible brain in a selective manner.

1.11.5 Conclusions From Circuit CSDS Studies

Overall, these studies indicate that the individual variation in the stress response manifests not only behaviorally in social avoidance, but likely as a coordinated neural response that engages multiple brain regions and networks of activation. While collectively these brain-wide and circuit-specific studies indicate that brain regions known to be involved in reward and stress are implicated in defeat and social reactivity, these anatomical studies have all been conducted following defeat. Moreover, these brain regions have all been selected a priori, therefore, the majority of findings have been limited to the same set of regions. However, it is apparent from circuitry involved in social, coping, and stress, as well as the anatomical differences seen following defeat, that the impact of CSDS on neural circuitry may extend beyond the traditional regions that have already been assessed (See **Figure 1.6**). Gaining resolution on which neural circuits are established before or early on during the stress experience is a critical next step in assessing how brain-wide networks of activation confer vulnerability.
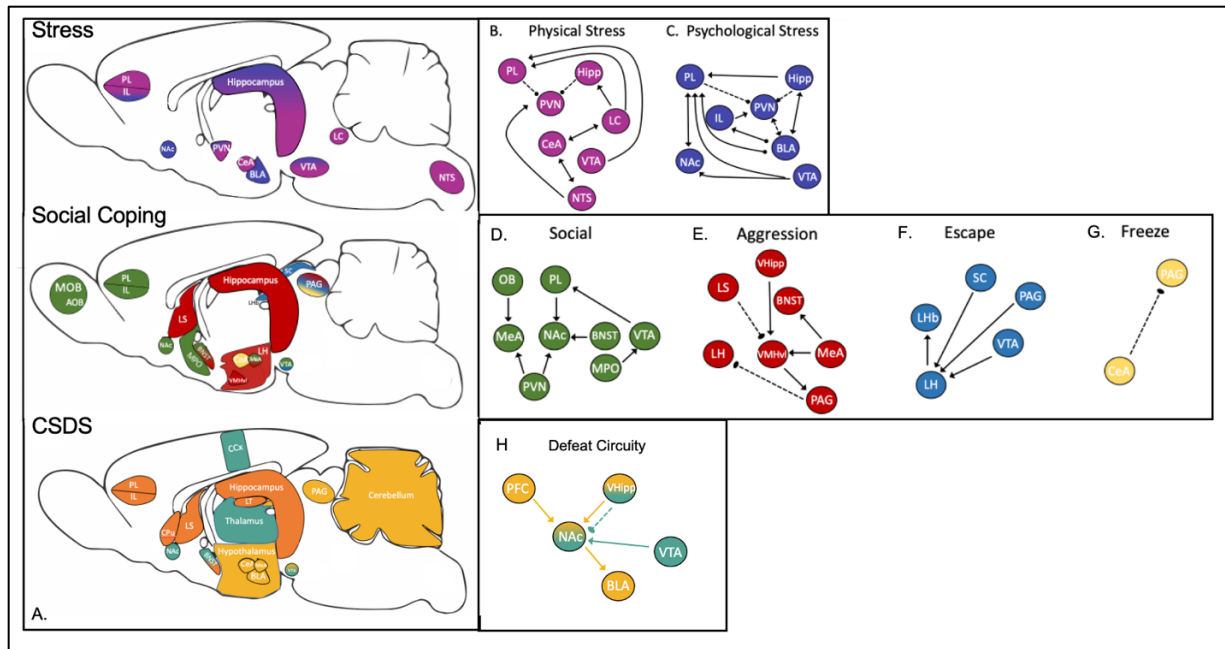
Figure 1.6 Summary Figure of Stress, Social Coping, and CSDS Circuits
A: represents the schematics of stress, social coping, and CSDS circuits. B&C represent Stress: B. Physical Stress, C. Psychological Stress, D-E Represent social and coping behaviors: D. Circuitry involved in social behaviors, E. Aggression, F. Escape, G. Freezing. H is known defeat circuitry. Overall, it is apparent that these circuits converge on similar, but slightly distinct brain regions and circuits to elicit specific responses. These responses are reflected in brain-wide circuits. Acronyms: PL: prelimbic cortex, IL: infralimbic cortex, PVN: paraventricular nucleus, CeA: central amygdala, VTA: ventral tegmental area, LC: locus coeruleus, NTS: nucleus tractus solitarius, MOB: main olfactory bulb, AOB: accessory olfactory bulb, NAc: nucleus accumbens, LS: lateral septum, VMHvl: ventral hypothalamus, MeA: medial amygdala, LH: lateral hypothalamus, SC: superior colliculus, LHb: lateral habenula, PAG: periaqueductal gray, BLA: basolateral amygdala, CCx: cingulate cortex, LT: lateral thalamus. Arrows represent activation and dashed lines represent inhibition. Colors: Pink & Purple: Physical and Psychological Stress, Green: Social Circuitry, Red: Aggression, Blue: Escape, Light Yellow: Freeze, Orange: Defeat, Yellow: Susceptible Only, Blue-Green= Resilient Only.

1.12 Hypothesis and Specific Aims of the Dissertation

Uncovering the risk factors that underlie individual differences in emotional reactivity to stress is critical in understanding how vulnerability is developed. Importantly, individual differences in response to stress significantly modify the risk of developing psychiatric disorders, such as major depressive, generalized anxiety, and social anxiety disorders. These individual differences may arise from a sequence of events due to the environmental context or a variety of gene-by-environment interactions, but without understanding the initial trigger, resolution is lost to understand the dynamic changes that emerge to elicit the final state. Rodent models provide a foundation to characterize differences in vulnerability that arise due to environmental stressors.

The CSDS paradigm is a benchmark rodent model for measuring facets of individual differences to stress-related disorders, and has been put forward as offering high etiological, discriminative, and face validity. However, the majority of studies have focused on behavioral and neural characteristics that arise after resilience and susceptibility have been established. While there has been some headway in understanding behavioral and neural factors before stress that contribute to the social outcome, the role of how the initial stress encounter shapes this final reactivity is unclear.

Hypothesis: Our overarching hypothesis is that brain-wide activation patterns and behavioral coping styles that emerge during the initial defeat encounter map onto future resilience or susceptibility.

Major Questions:

This dissertation, therefore, endeavors to answer the following questions:

1. *When and how do individual differences in reactivity to social stress arise?*

2. *Are there distinct patterns of neural network activation during the initial social stress experience that predict eventual susceptibility or resilience to CSDS?*

In **Chapter 2**, we assess Question 1 from above. When and how do individual differences in reactivity to social stress arise? We characterized behavioral, physiological, and neuroendocrine profiles of mice in three separate phases: Before, during, and following CSDS. We hypothesized that coping strategies that mice exhibit during the first stress experience would predict whether resilient or susceptible reactivities arose following repeated social stress. Moreover, we characterized resilient and susceptible groups along additional dimensions throughout the process to provide a more comprehensive understanding of this animal model of stress. Our

findings enhance our current understanding of the temporal dynamics of behavioral and neuroendocrine features of stress reactivity in the resilient and susceptible phenotypes.

In **Chapter 3,** we assess Question 2 from above. Are there distinct patterns of neural network activation during the initial social stress experience that predict eventual susceptibility or resilience to chronic social defeat? In this chapter, we use a new technique (FosTRAP) to capture neural patterns of activation during the first encounter with the social defeat experience, while allowing the animals to remain alive, undergo chronic social stress, and emerge from it as either susceptible or resilient. We were able to capture specific regional differences associated with the defeat experience broadly (vs. control), as well as specific regional differences associated with coping style that are apparent as early as the first episode of defeat. The challenge was then to a) assess whether there were differences in brain-wide activity patterns present on Day 1 and b) relate these activated networks with specific features of behavior to create a predictive model of vulnerability following subsequent chronic stress. Using several analytical strategies, we are able to depict the unique and highly distinctive signature of social defeat across numerous brain regions. We were also able to identify brain areas and networks associated with defensive behavior during the first encounter that are predictive of future susceptibility or resilience. In **Chapter 4,** we integrate the behavioral and neural findings and discuss their implications.

The body of work described in this dissertation sheds light on how an initial experience with a social stressor can differentially impact both brain and behavior and set the stage for subsequent differences in coping with social stress. This is a key first step in elucidating the temporal dynamics that can shape eventual resilience or susceptibility to repeated social stress. This work has translational implications as it may uncover critical points of possible intervention that could minimize the risk of developing stress-related disorders in humans.

1.13 References

Akil, H., 2005. Stressed and depressed. Nat. Med. 11, 116–118. doi:10.1038/nm0205-116

Alves-Dos-Santos, L., Resende, L. de S., Chiavegatto, S., 2020. Susceptibility and resilience to chronic social defeat stress in adolescent male mice: No correlation between social avoidance and sucrose preference. Neurobiol. Stress 12, 100221. doi:10.1016/j.ynstr.2020.100221

Anacker, C., Scholz, J., O'Donnell, K.J., Allemang-Grand, R., Diorio, J., Bagot, R.C., Nestler, E.J., Hen, R., Lerch, J.P., Meaney, M.J., 2016. Neuroanatomic differences associated with stress susceptibility and resilience. Biol. Psychiatry 79, 840–849. doi:10.1016/j.biopsych.2015.08.009

Atsak, P., Orre, M., Bakker, P., Cerliani, L., Roozendaal, B., Gazzola, V., Moita, M., Keysers, C., 2011. Experience modulates vicarious freezing in rats: a model for empathy. PLoS One 6, e21855. doi:10.1371/journal.pone.0021855

Bagot, R.C., Cates, H.M., Purushothaman, I., Lorsch, Z.S., Walker, D.M., Wang, J., Huang, X., Schlüter, O.M., Maze, I., Peña, C.J., Heller, E.A., Issler, O., Wang, M., Song, W.-M., Stein, J.L., Liu, X., Doyle, M.A., Scobie, K.N., Sun, H.S., Neve, R.L., Geschwind, D., Dong, Y., Shen, L., Zhang, B., Nestler, E.J., 2016. Circuit-wide Transcriptional Profiling Reveals Brain Region-Specific Gene Networks Regulating Depression Susceptibility. Neuron 90, 969–983. doi:10.1016/j.neuron.2016.04.015

Bagot, R.C., Parise, E.M., Peña, C.J., Zhang, H.-X., Maze, I., Chaudhury, D., Persaud, B., Cachope, R., Bolaños-Guzmán, C.A., Cheer, J.F., Deisseroth, K., Han, M.-H., Nestler, E.J., 2015. Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. Nat. Commun. 6, 7062. doi:10.1038/ncomms8062

Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2014. A sociability gene? Meta-analysis of oxytocin receptor genotype effects in humans. Psychiatr Genet 24, 45–51. doi:10.1097/YPG.0b013e3283643684

Barbano, M.F., Wang, H.-L., Zhang, S., Miranda-Barrientos, J., Estrin, D.J., Figueroa-González, A., Liu, B., Barker, D.J., Morales, M., 2020. VTA glutamatergic neurons mediate innate defensive behaviors. Neuron 107, 368–382.e8. doi:10.1016/j.neuron.2020.04.024

Birt, I.A., Hagenauer, M.H., Clinton, S.M., Aydin, C., Blandino, P., Stead, J.D.H., Hilde, K.L., Meng, F., Thompson, R.C., Khalil, H., Stefanov, A., Maras, P., Zhou, Z., Hebda-Bauer, E.K., Goldman, D., Watson, S.J., Akil, H., 2021. Genetic Liability for Internalizing Versus Externalizing Behavior Manifests in the Developing and Adult Hippocampus: Insight From a Meta-analysis of Transcriptional Profiling Studies in a Selectively Bred Rat Model. Biol. Psychiatry 89, 339–355. doi:10.1016/j.biopsych.2020.05.024

Boehme, S., Ritter, V., Tefikow, S., Stangier, U., Strauss, B., Miltner, W.H.R., Straube, T., 2014. Brain activation during anticipatory anxiety in social anxiety disorder. Soc. Cogn. Affect. Neurosci. 9, 1413–1418. doi:10.1093/scan/nst129

Bralten, J., Mota, N.R., Klemann, C.J.H.M., De Witte, W., Laing, E., Collier, D.A., de Kluiver, H., Bauduin, S.E.E.C., Arango, C., Ayuso-Mateos, J.L., Fabbri, C., Kas, M.J., van der Wee, N., Penninx, B.W.J.H., Serretti, A., Franke, B., Poelmans, G., 2021. Genetic underpinnings of sociability in the general population. Neuropsychopharmacology 46, 1627–1634. doi:10.1038/s41386-021-01044-z

Britton, J.C., Grillon, C., Lissek, S., Norcross, M.A., Szuhany, K.L., Chen, G., Ernst, M., Nelson, E.E., Leibenluft, E., Shechner, T., Pine, D.S., 2013. Response to learned threat: An FMRI study in adolescent and adult anxiety. Am. J. Psychiatry 170, 1195–1204.

doi:10.1176/appi.ajp.2013.12050651

Bullitt, E., 1990. Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. J. Comp. Neurol. 296, 517–530. doi:10.1002/cne.902960402

Busjahn, A., Faulhaber, H.D., Freier, K., Luft, F.C., 1999. Genetic and environmental influences on coping styles: a twin study. Psychosom. Med. 61, 469–475. doi:10.1097/00006842-199907000-00011

Calvo, N., Cecchi, M., Kabbaj, M., Watson, S.J., Akil, H., 2011. Differential effects of social defeat in rats with high and low locomotor response to novelty. Neuroscience 183, 81–89. doi:10.1016/j.neuroscience.2011.03.046

Caradonna, S.G., Zhang, T.-Y., O'Toole, N., Shen, M.-J., Khalil, H., Einhorn, N.R., Wen, X., Parent, C., Lee, F.S., Akil, H., Meaney, M.J., McEwen, B.S., Marrocco, J., 2021. Genomic modules and intramodular network concordance in susceptible and resilient male mice across models of stress. Neuropsychopharmacology. doi:10.1038/s41386-021-01219-8

Chang, C.-H., Gean, P.-W., 2019. The Ventral Hippocampus Controls Stress-Provoked Impulsive Aggression through the Ventromedial Hypothalamus in Post-Weaning Social Isolation Mice. Cell Rep. 28, 1195–1205.e3. doi:10.1016/j.celrep.2019.07.005

Chaudhury, D., Walsh, J.J., Friedman, A.K., Juarez, B., Ku, S.M., Koo, J.W., Ferguson, D., Tsai, H.-C., Pomeranz, L., Christoffel, D.J., Nectow, A.R., Ekstrand, M., Domingos, A., Mazei-Robison, M.S., Mouzon, E., Lobo, M.K., Neve, R.L., Friedman, J.M., Russo, S.J., Deisseroth, K., Nestler, E.J., Han, M.-H., 2013. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature 493, 532–536. doi:10.1038/nature11713

Clinton, S.M., Stead, J.D.H., Miller, S., Watson, S.J., Akil, H., 2011. Developmental underpinnings of differences in rodent novelty-seeking and emotional reactivity. Eur. J. Neurosci. 34, 994–1005. doi:10.1111/j.1460-9568.2011.07811.x

Costache, M.E., Frick, A., Månsson, K., Engman, J., Faria, V., Hjorth, O., Hoppe, J.M., Gingnell, M., Frans, Ö., Björkstrand, J., Rosén, J., Alaie, I., Åhs, F., Linnman, C., Wahlstedt, K., Tillfors, M., Marteinsdottir, I., Fredrikson, M., Furmark, T., 2020. Higher- and lower-order personality traits and cluster subtypes in social anxiety disorder. PLoS One 15, e0232187. doi:10.1371/journal.pone.0232187

Cullinan, W.E., Herman, J.P., Watson, S.J., 1993. Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. J. Comp. Neurol. 332, 1–20. doi:10.1002/cne.903320102

Day, F.R., Ong, K.K., Perry, J.R.B., 2018. Elucidating the genetic basis of social interaction and isolation. Nat. Commun. 9, 2457. doi:10.1038/s41467-018-04930-1

de Boer, S.F., Buwalda, B., Koolhaas, J.M., 2017. Untangling the neurobiology of coping styles in rodents: Towards neural mechanisms underlying individual differences in disease susceptibility. Neurosci. Biobehav. Rev. 74, 401–422. doi:10.1016/j.neubiorev.2016.07.008

De Franceschi, G., Vivattanasarn, T., Saleem, A.B., Solomon, S.G., 2016. Vision guides selection of freeze or flight defense strategies in mice. Curr. Biol. 26, 2150–2154. doi:10.1016/j.cub.2016.06.006

De Miguel, Z., Vegas, O., Garmendia, L., Arregi, A., Beitia, G., Azpiroz, A., 2011. Behavioral coping strategies in response to social stress are associated with distinct neuroendocrine,

monoaminergic and immune response profiles in mice. Behav. Brain Res. 225, 554–561. doi:10.1016/j.bbr.2011.08.011

De Oca, B.M., DeCola, J.P., Maren, S., Fanselow, M.S., 1998. Distinct regions of the periaqueductal gray are involved in the acquisition and expression of defensive responses. J. Neurosci. 18, 3426–3432. doi:10.1523/JNEUROSCI.18-09-03426.1998

de Polavieja, G.G., Orger, M.B., 2018. Social behavior: A neural circuit for social behavior in zebrafish. Curr. Biol. 28, R828–R830. doi:10.1016/j.cub.2018.06.065

Dhabhar, F.S., 2014. Effects of stress on immune function: the good, the bad, and the beautiful. Immunol. Res. 58, 193–210. doi:10.1007/s12026-014-8517-0

Duncan, L.E., Ostacher, M., Ballon, J., 2019. How genome-wide association studies (GWAS) made traditional candidate gene studies obsolete. Neuropsychopharmacology 44, 1518–1523. doi:10.1038/s41386-019-0389-5

Dunn, S.H., Conley, Y.P., 2015. A systematic review of genetic influences on coping. Biol Res Nurs 17, 87–93. doi:10.1177/1099800414527340

Eilam, D., 2005. Die hard: a blend of freezing and fleeing as a dynamic defense--implications for the control of defensive behavior. Neurosci. Biobehav. Rev. 29, 1181–1191. doi:10.1016/j.neubiorev.2005.03.027

Evans, D.A., Stempel, A.V., Vale, R., Branco, T., 2019. Cognitive control of escape behaviour. Trends Cogn. Sci. (Regul. Ed.) 23, 334–348. doi:10.1016/j.tics.2019.01.012

Evans, D.A., Stempel, A.V., Vale, R., Ruehle, S., Lefler, Y., Branco, T., 2018. A synaptic threshold mechanism for computing escape decisions. Nature 558, 590–594. doi:10.1038/s41586-018-0244-6

Evans, G.W., Kim, P., 2013. Childhood poverty, chronic stress, self-regulation, and coping. Child Dev Perspect 7, 43–48. doi:10.1111/cdep.12013

Falkner, A.L., Grosenick, L., Davidson, T.J., Deisseroth, K., Lin, D., 2016. Hypothalamic control of male aggression-seeking behavior. Nat. Neurosci. 19, 596–604. doi:10.1038/nn.4264

Falkner, A.L., Wei, D., Song, A., Watsek, L.W., Chen, I., Chen, P., Feng, J.E., Lin, D., 2020. Hierarchical Representations of Aggression in a Hypothalamic-Midbrain Circuit. Neuron 106, 637–648.e6. doi:10.1016/j.neuron.2020.02.014

Fallon, J.H., Moore, R.Y., 1979. Superior colliculus efferents to the hypothalamus. Neurosci. Lett. 14, 265–270. doi:10.1016/0304-3940(79)96159-7

Fang, W., Zhang, J., Hong, L., Huang, W., Dai, X., Ye, Q., Chen, X., 2020. Metformin ameliorates stress-induced depression-like behaviors via enhancing the expression of BDNF by activating AMPK/CREB-mediated histone acetylation. J. Affect. Disord. 260, 302–313. doi:10.1016/j.jad.2019.09.013

Flagel, S.B., Waselus, M., Clinton, S.M., Watson, S.J., Akil, H., 2014. Antecedents and consequences of drug abuse in rats selectively bred for high and low response to novelty. Neuropharmacology 76 Pt B, 425–436. doi:10.1016/j.neuropharm.2013.04.033

Folkman, S., Moskowitz, J.T., 2004. Stress, appraisal, and coping, in: Encyclopedia of Health and Behavior. SAGE Publications, Inc., 2455 Teller Road, Thousand Oaks California 91320 United States . doi:10.4135/9781412952576.n198

Godoy, L.D., Rossignoli, M.T., Delfino-Pereira, P., Garcia-Cairasco, N., de Lima Umeoka, E.H., 2018. A comprehensive overview on stress neurobiology: basic concepts and clinical implications. Front. Behav. Neurosci. 12, 127. doi:10.3389/fnbeh.2018.00127

Golden, S.A., Covington, H.E., Berton, O., Russo, S.J., 2011. A standardized protocol for

repeated social defeat stress in mice. Nat. Protoc. 6, 1183–1191. doi:10.1038/nprot.2011.361

Gottier, R.F., 1968. The dominance-submission hierarchy in the social behavior of the domestic chicken. J. Genet. Psychol. 112, 205–226. doi:10.1080/00221325.1968.10533796

Haller, J., Tóth, M., Halász, J., 2005. The activation of raphe serotonergic neurons in normal and hypoarousal-driven aggression: a double labeling study in rats. Behav. Brain Res. 161, 88–94. doi:10.1016/j.bbr.2005.01.006

Haller, J., van de Schraaf, J., Kruk, M.R., 2001. Deviant forms of aggression in glucocorticoid hyporeactive rats: a model for "pathological" aggression? J. Neuroendocrinol. 13, 102–107. doi:10.1046/j.1365-2826.2001.00600.x

Hammels, C., Pishva, E., De Vry, J., van den Hove, D.L.A., Prickaerts, J., van Winkel, R., Selten, J.-P., Lesch, K.-P., Daskalakis, N.P., Steinbusch, H.W.M., van Os, J., Kenis, G., Rutten, B.P.F., 2015. Defeat stress in rodents: From behavior to molecules. Neurosci. Biobehav. Rev. 59, 111–140. doi:10.1016/j.neubiorev.2015.10.006

He, Y., Li, W., Tian, Y., Chen, X., Cheng, K., Xu, K., Li, C., Wang, H., Qu, C., Wang, C., Li, P., Chen, H., Xie, P., 2018. iTRAQ-based proteomics suggests LRP6, NPY and NPY2R perturbation in the hippocampus involved in CSDS may induce resilience and susceptibility. Life Sci. 211, 102–117. doi:10.1016/j.lfs.2018.09.016

Heath, A.C., Cloninger, C.R., Martin, N.G., 1994. Testing a model for the genetic structure of personality: a comparison of the personality systems of Cloninger and Eysenck. J. Pers. Soc. Psychol. 66, 762–775. doi:10.1037//0022-3514.66.4.762

Herman, J.P., Cullinan, W.E., Morano, M.I., Akil, H., Watson, S.J., 1995. Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis. J. Neuroendocrinol. 7, 475–482. doi:10.1111/j.1365-2826.1995.tb00784.x

Hettema, J.M., Neale, M.C., Kendler, K.S., 2001. A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am. J. Psychiatry 158, 1568–1578. doi:10.1176/appi.ajp.158.10.1568

Hettema, J.M., Prescott, C.A., Myers, J.M., Neale, M.C., Kendler, K.S., 2005. The structure of genetic and environmental risk factors for anxiety disorders in men and women. Arch. Gen. Psychiatry 62, 182–189. doi:10.1001/archpsyc.62.2.182

Hofmann, S.G., 2007. Cognitive factors that maintain social anxiety disorder: a comprehensive model and its treatment implications. Cogn Behav Ther 36, 193–209. doi:10.1080/16506070701421313

Hollis, F., Kabbaj, M., 2014. Social defeat as an animal model for depression. ILAR J. 55, 221–232. doi:10.1093/ilar/ilu002

Howard, D.M., Adams, M.J., Clarke, T.-K., Hafferty, J.D., Gibson, J., Shirali, M., Coleman, J.R.I., Hagenaars, S.P., Ward, J., Wigmore, E.M., Alloza, C., Shen, X., Barbu, M.C., Xu, E.Y., Whalley, H.C., Marioni, R.E., Porteous, D.J., Davies, G., Deary, I.J., Hemani, G., Berger, K., Teismann, H., Rawal, R., Arolt, V., Baune, B.T., Dannlowski, U., Domschke, K., Tian, C., Hinds, D.A., 23andMe Research Team, Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium, Trzaskowski, M., Byrne, E.M., Ripke, S., Smith, D.J., Sullivan, P.F., Wray, N.R., Breen, G., Lewis, C.M., McIntosh, A.M., 2019. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. Nat. Neurosci. 22, 343–352. doi:10.1038/s41593-018-0326-7

Huang, J.-Y., Tian, Y., Wang, H.-J., Shen, H., Wang, H., Long, S., Liao, M.-H., Liu, Z.-R.,

Wang, Z.-M., Li, D., Tao, R.-R., Cui, T.-T., Moriguchi, S., Fukunaga, K., Han, F., Lu, Y.-M., 2016. Functional genomic analyses identify pathways dysregulated in animal model of autism. CNS Neurosci Ther 22, 845–853. doi:10.1111/cns.12582

Hultman, R., Ulrich, K., Sachs, B.D., Blount, C., Carlson, D.E., Ndubuizu, N., Bagot, R.C., Parise, E.M., Vu, M.-A.T., Gallagher, N.M., Wang, J., Silva, A.J., Deisseroth, K., Mague, S.D., Caron, M.G., Nestler, E.J., Carin, L., Dzirasa, K., 2018. Brain-wide Electrical Spatiotemporal Dynamics Encode Depression Vulnerability. Cell 173, 166–180.e14. doi:10.1016/j.cell.2018.02.012

Jang, K.L., Thordarson, D.S., Stein, M.B., Cohan, S.L., Taylor, S., 2007. Coping styles and personality: a biometric analysis. Anxiety. Stress. Coping 20, 17–24. doi:10.1080/10615800601170516

Kabbaj, M., Norton, C.S., Kollack-Walker, S., Watson, S.J., Robinson, T.E., Akil, H., 2001. Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. Psychopharmacology 158, 382–387. doi:10.1007/s002130100918

Kanitz, E., Tuchscherer, M., Otten, W., Tuchscherer, A., Zebunke, M., Puppe, B., 2019. Coping style of pigs is associated with different behavioral, neurobiological and immune responses to stressful challenges. Front. Behav. Neurosci. 13, 173. doi:10.3389/fnbeh.2019.00173

Kendler, K.S., Kessler, R.C., Heath, A.C., Neale, M.C., Eaves, L.J., 1991. Coping: a genetic epidemiological investigation. Psychol. Med. 21, 337–346. doi:10.1017/s0033291700020444

Kim, D.-W., Yao, Z., Graybuck, L.T., Kim, T.K., Nguyen, T.N., Smith, K.A., Fong, O., Yi, L., Koulena, N., Pierson, N., Shah, S., Lo, L., Pool, A.-H., Oka, Y., Pachter, L., Cai, L., Tasic, B., Zeng, H., Anderson, D.J., 2019. Multimodal analysis of cell types in a hypothalamic node controlling social behavior. Cell 179, 713–728.e17. doi:10.1016/j.cell.2019.09.020

Ko, J., 2017. Neuroanatomical substrates of rodent social behavior: the medial prefrontal cortex and its projection patterns. Front. Neural Circuits 11, 41. doi:10.3389/fncir.2017.00041

Kollack-Walker, S., Don, C., Watson, S.J., Akil, H., 1999. Differential expression of c-fos mRNA within neurocircuits of male hamsters exposed to acute or chronic defeat. J. Neuroendocrinol. 11, 547–559. doi:10.1046/j.1365-2826.1999.00354.x

Kollack-Walker, S., Watson, S.J., Akil, H., 1997. Social stress in hamsters: defeat activates specific neurocircuits within the brain. J. Neurosci. 17, 8842–8855.

König, M., Zimmer, A.M., Steiner, H., Holmes, P.V., Crawley, J.N., Brownstein, M.J., Zimmer, A., 1996. Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 383, 535–538. doi:10.1038/383535a0

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935. doi:10.1016/s0149-7634(99)00026-3

Kozak, B., Strelau, J., Miles, J.N.V., 2005. Genetic determinants of individual differences in coping styles. Anxiety, Stress & Coping 18, 1–15. doi:10.1080/10615800500040844

Krishnan, V., Han, M.-H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., Laplant, Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga,

C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391–404. doi:10.1016/j.cell.2007.09.018

Kwon, J., Kim, Y.J., Choi, K., Seol, S., Kang, H.J., 2019. Identification of stress resilience module by weighted gene co-expression network analysis in Fkbp5-deficient mice. Mol. Brain 12, 99. doi:10.1186/s13041-019-0521-9

Laine, M.A., Sokolowska, E., Dudek, M., Callan, S.-A., Hyytiä, P., Hovatta, I., 2017. Brain activation induced by chronic psychosocial stress in mice. Sci. Rep. 7, 15061. doi:10.1038/s41598-017-15422-5

Larrieu, T., Cherix, A., Duque, A., Rodrigues, J., Lei, H., Gruetter, R., Sandi, C., 2017. Hierarchical status predicts behavioral vulnerability and nucleus accumbens metabolic profile following chronic social defeat stress. Curr. Biol. 27, 2202–2210.e4. doi:10.1016/j.cub.2017.06.027

Lavond, D.G., Kim, J.J., Thompson, R.F., 1993. Mammalian brain substrates of aversive classical conditioning. Annu. Rev. Psychol. 44, 317–342. doi:10.1146/annurev.ps.44.020193.001533

Lecca, S., Meye, F.J., Trusel, M., Tchenio, A., Harris, J., Schwarz, M.K., Burdakov, D., Georges, F., Mameli, M., 2017. Aversive stimuli drive hypothalamus-to-habenula excitation to promote escape behavior. Elife 6. doi:10.7554/eLife.30697

Ledoux, J., 1998. The Emotional Brain: The Mysterious Underpinnings of Emotional Life, illustrated, reprint. ed. Simon and Schuster.

LeDoux, J.E., Iwata, J., Cicchetti, P., Reis, D.J., 1988. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J. Neurosci. 8, 2517–2529. doi:10.1523/JNEUROSCI.08-07-02517.1988

Lee, C.-W., Fang, Y.-P., Chu, M.-C., Chung, Y.-J., Chi, H., Tang, C.-W., So, E.C., Lin, Hsin-Chuan, Lin, Hui-Ching, 2021. Differential mechanisms of synaptic plasticity for susceptibility and resilience to chronic social defeat stress in male mice. Biochem. Biophys. Res. Commun. 562, 112–118. doi:10.1016/j.bbrc.2021.05.064

Li, Y., Zeng, J., Zhang, J., Yue, C., Zhong, W., Liu, Z., Feng, Q., Luo, M., 2018. Hypothalamic circuits for predation and evasion. Neuron 97, 911–924.e5. doi:10.1016/j.neuron.2018.01.005

Lo, L., Yao, S., Kim, D.-W., Cetin, A., Harris, J., Zeng, H., Anderson, D.J., Weissbourd, B., 2019. Connectional architecture of a mouse hypothalamic circuit node controlling social behavior. Proc. Natl. Acad. Sci. USA 116, 7503–7512. doi:10.1073/pnas.1817503116

Lobo, M.K., Zaman, S., Damez-Werno, D.M., Koo, J.W., Bagot, R.C., DiNieri, J.A., Nugent, A., Finkel, E., Chaudhury, D., Chandra, R., Riberio, E., Rabkin, J., Mouzon, E., Cachope, R., Cheer, J.F., Han, M.-H., Dietz, D.M., Self, D.W., Hurd, Y.L., Vialou, V., Nestler, E.J., 2013. ΔFosB induction in striatal medium spiny neuron subtypes in response to chronic pharmacological, emotional, and optogenetic stimuli. J. Neurosci. 33, 18381–18395. doi:10.1523/JNEUROSCI.1875-13.2013

Lord, J.P., Moser, R.M., Buonocore, E.M., Sylvester, E.E., Morales, M.J., Granitz, A.P., Disipio, A., Blakely, E., O'Sullivan-Evangelista, S.L., Mateo, T.F., Chlebove, G.J., Carey, C.M., Lucas, O., 2021. Dominance hierarchies in marine invertebrates. Biol Bull 240, 2–15. doi:10.1086/712973

Lydiard, R.B., 2001. Social anxiety disorder: comorbidity and its implications. J. Clin. Psychiatry 62 Suppl 1, 17–23; discussion 24.

Lyons, D.M., Buckmaster, P.S., Lee, A.G., Wu, C., Mitra, R., Duffey, L.M., Buckmaster, C.L., Her, S., Patel, P.D., Schatzberg, A.F., 2010. Stress coping stimulates hippocampal neurogenesis in adult monkeys. Proc. Natl. Acad. Sci. USA 107, 14823–14827. doi:10.1073/pnas.0914568107

Maren, S., Aharonov, G., Fanselow, M.S., 1996. Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behav. Neurosci. 110, 718–726. doi:10.1037/0735-7044.110.4.718

Mason, W.A., Mendoza, S.P. (Eds.), 1993. Primate Social Conflict, illustrated. ed. SUNY Press.

McEwen, B.S., 1998a. Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171–179. doi:10.1056/NEJM199801153380307

McEwen, B.S., 1998b. Stress, adaptation, and disease: Allostasis and allostatic load. Ann. N. Y. Acad. Sci. 840, 33–44. doi:10.1111/j.1749-6632.1998.tb09546.x

McEwen, B.S., Akil, H., 2020. Revisiting the stress concept: implications for affective disorders. J. Neurosci. 40, 12–21. doi:10.1523/JNEUROSCI.0733-19.2019

Meese, G.B., Ewbank, R., 1973. The establishment and nature of the dominance hierarchy in the domesticated pig. Anim. Behav. 21, 326–334. doi:10.1016/S0003-3472(73)80074-0

Mellins, C.A., Gatz, M., Baker, L., 1996. Children's methods of coping with stress: a twin study of genetic and environmental influences. J. Child Psychol. Psychiatry 37, 721–730. doi:10.1111/j.1469-7610.1996.tb01464.x

Milic, M., Schmitt, U., Lutz, B., Müller, M.B., 2021. Individual baseline behavioral traits predict the resilience phenotype after chronic social defeat. Neurobiol. Stress 14, 100290. doi:10.1016/j.ynstr.2020.100290

Miura, I., Overton, E.T.N., Nakai, N., Kawamata, T., Sato, M., Takumi, T., 2020. Imaging the Neural Circuit Basis of Social Behavior: Insights from Mouse and Human Studies. Neurol Med Chir (Tokyo) 60, 429–438. doi:10.2176/nmc.ra.2020-0088

Muir, J., Lorsch, Z.S., Ramakrishnan, C., Deisseroth, K., Nestler, E.J., Calipari, E.S., Bagot, R.C., 2018. In vivo fiber photometry reveals signature of future stress susceptibility in nucleus accumbens. Neuropsychopharmacology 43, 255–263. doi:10.1038/npp.2017.122

Muir, J., Tse, Y.C., Iyer, E.S., Biris, J., Cvetkovska, V., Lopez, J., Bagot, R.C., 2020. Ventral Hippocampal Afferents to Nucleus Accumbens Encode Both Latent Vulnerability and Stress-Induced Susceptibility. Biol. Psychiatry. doi:10.1016/j.biopsych.2020.05.021

Nakao, T., Sanematsu, H., Yoshiura, T., Togao, O., Murayama, K., Tomita, M., Masuda, Y., Kanba, S., 2011. fMRI of patients with social anxiety disorder during a social situation task. Neurosci. Res. 69, 67–72. doi:10.1016/j.neures.2010.09.008

Nasca, C., Menard, C., Hodes, G., Bigio, B., Pena, C., Lorsch, Z., Zelli, D., Ferris, A., Kana, V., Purushothaman, I., Dobbin, J., Nassim, M., DeAngelis, P., Merad, M., Rasgon, N., Meaney, M., Nestler, E.J., McEwen, B.S., Russo, S.J., 2019. Multidimensional predictors of susceptibility and resilience to social defeat stress. Biol. Psychiatry 86, 483–491. doi:10.1016/j.biopsych.2019.06.030

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. J. Neurosci. 40, 4858–4880. doi:10.1523/JNEUROSCI.0370-20.2020

Norrholm, S.D., Jovanovic, T., Olin, I.W., Sands, L.A., Karapanou, I., Bradley, B., Ressler, K.J., 2011. Fear extinction in traumatized civilians with posttraumatic stress disorder: relation to symptom severity. Biol. Psychiatry 69, 556–563. doi:10.1016/j.biopsych.2010.09.013

Palamarchuk, I.S., Vaillancourt, T., 2021. Mental Resilience and Coping With Stress: A

Comprehensive, Multi-level Model of Cognitive Processing, Decision Making, and Behavior. Front. Behav. Neurosci. 15, 719674. doi:10.3389/fnbeh.2021.719674

Pamplona, F.A., Henes, K., Micale, V., Mauch, C.P., Takahashi, R.N., Wotjak, C.T., 2011. Prolonged fear incubation leads to generalized avoidance behavior in mice. J. Psychiatr. Res. 45, 354–360. doi:10.1016/j.jpsychires.2010.06.015

Parker, J., Endler, N., 1990. Coping Inventory for Stressful Situations (CISS). North Tonawanda, NY: Multi-Health Systems.

Pechtel, P., Pizzagalli, D.A., 2011. Effects of early life stress on cognitive and affective function: an integrated review of human literature. Psychopharmacology 214, 55–70. doi:10.1007/s00213-010-2009-2

Pérez-Tejada, J., Arregi, A., Gómez-Lázaro, E., Vegas, O., Azpiroz, A., Garmendia, L., 2013. Coping with chronic social stress in mice: hypothalamic-pituitary-adrenal/ sympathetic-adrenal-medullary axis activity, behavioral changes and effects of antalarmin treatment: implications for the study of stress-related psychopathologies. Neuroendocrinology 98, 73–88. doi:10.1159/000353620

Pontillo, M., Tata, M.C., Averna, R., Demaria, F., Gargiullo, P., Guerrera, S., Pucciarini, M.L., Santonastaso, O., Vicari, S., 2019. Peer victimization and onset of social anxiety disorder in children and adolescents. Brain Sci. 9. doi:10.3390/brainsci9060132

Prater, K.E., Aurbach, E.L., Larcinese, H.K., Smith, T.N., Turner, C.A., Blandino, P., Watson, S.J., Maren, S., Akil, H., 2017. Selectively Bred Rats Provide a Unique Model of Vulnerability to PTSD-Like Behavior and Respond Differentially to FGF2 Augmentation Early in Life. Neuropsychopharmacology 42, 1706–1714. doi:10.1038/npp.2017.37

Rabasa, C., Dickson, S.L., 2016. Impact of stress on metabolism and energy balance. Curr. Opin. Behav. Sci. 9, 71–77. doi:10.1016/j.cobeha.2016.01.011

Rau, V., DeCola, J.P., Fanselow, M.S., 2005. Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder. Neurosci. Biobehav. Rev. 29, 1207–1223. doi:10.1016/j.neubiorev.2005.04.010

Rueda, M.R., Rothbart, M.K., 2009. The influence of temperament on the development of coping: The role of maturation and experience. New Dir. Child Adolesc. Dev. 2009, 19–31. doi:10.1002/cd.240

Sanson, A., Hemphill, S.A., Smart, D., 2004. Connections between temperament and social development: A review. Social Development 13, 142–170. doi:10.1046/j.1467-9507.2004.00261.x

Savage, H.S., Davey, C.G., Fullana, M.A., Harrison, B.J., 2020. Threat and safety reversal learning in social anxiety disorder - an fMRI study. J. Anxiety Disord. 76, 102321. doi:10.1016/j.janxdis.2020.102321

Schwabe, L., Hermans, E.J., Joëls, M., Roozendaal, B., 2022. Mechanisms of memory under stress. Neuron. doi:10.1016/j.neuron.2022.02.020

Scull, A., 2022. Desperate Remedies: Psychiatry's Turbulent Quest to Cure Mental Illness, unabridged. ed. Harvard University Press.

Shimanoe, C., Hachiya, T., Hara, M., Nishida, Y., Tanaka, K., Sutoh, Y., Shimizu, A., Hishida, A., Kawai, S., Okada, R., Tamura, T., Matsuo, K., Ito, H., Ozaki, E., Matsui, D., Ibusuki, R., Shimoshikiryo, I., Takashima, N., Kadota, A., Arisawa, K., Uemura, H., Suzuki, S., Watanabe, M., Kuriki, K., Endoh, K., Mikami, H., Nakamura, Y., Momozawa, Y., Kubo, M., Nakatochi, M., Naito, M., Wakai, K., 2019. A genome-wide association study of coping behaviors suggests FBXO45 is associated with emotional expression. Genes Brain

Behav. 18, e12481. doi:10.1111/gbb.12481

Sinha, R., Lacadie, C.M., Constable, R.T., Seo, D., 2016. Dynamic neural activity during stress signals resilient coping. Proc. Natl. Acad. Sci. USA 113, 8837–8842. doi:10.1073/pnas.1600965113

Snyder, K.P., Barry, M., Valentino, R.J., 2015. Cognitive impact of social stress and coping strategy throughout development. Psychopharmacology 232, 185–195. doi:10.1007/s00213-014-3654-7

Stedenfeld, K.A., Clinton, S.M., Kerman, I.A., Akil, H., Watson, S.J., Sved, A.F., 2011. Novelty-seeking behavior predicts vulnerability in a rodent model of depression. Physiol. Behav. 103, 210–216. doi:10.1016/j.physbeh.2011.02.001

Stein, M.B., Chen, C.-Y., Jain, S., Jensen, K.P., He, F., Heeringa, S.G., Kessler, R.C., Maihofer, A., Nock, M.K., Ripke, S., Sun, X., Thomas, M.L., Ursano, R.J., Smoller, J.W., Gelernter, J., Army STARRS Collaborators, 2017. Genetic risk variants for social anxiety. Am. J. Med. Genet. B, Neuropsychiatr. Genet. 174, 120–131. doi:10.1002/ajmg.b.32520

Sullivan, P.F., Neale, M.C., Kendler, K.S., 2000. Genetic epidemiology of major depression: review and meta-analysis. Am. J. Psychiatry 157, 1552–1562. doi:10.1176/appi.ajp.157.10.1552

Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G., Meyre, D., 2019. Benefits and limitations of genome-wide association studies. Nat. Rev. Genet. 20, 467–484. doi:10.1038/s41576-019-0127-1

Thome, J., Hauschild, S., Koppe, G., Liebke, L., Rausch, S., Herzog, J.I., Müller-Engelmann, M., Steil, R., Priebe, K., Hermans, D., Schmahl, C., Bohus, M., Lis, S., 2018. Generalisation of fear in PTSD related to prolonged childhood maltreatment: an experimental study. Psychol. Med. 48, 2223–2234. doi:10.1017/S0033291717003713

Thompson, R.A., Lamb, M.E., 1982. Stranger sociability and its relationships to temperament and social experience during the second year. Infant Behavior and Development 5, 277–287. doi:10.1016/S0163-6383(82)80037-4

Tóth, M., Halász, J., Mikics, E., Barsy, B., Haller, J., 2008. Early social deprivation induces disturbed social communication and violent aggression in adulthood. Behav. Neurosci. 122, 849–854. doi:10.1037/0735-7044.122.4.849

Tovote, P., Esposito, M.S., Botta, P., Chaudun, F., Fadok, J.P., Markovic, M., Wolff, S.B.E., Ramakrishnan, C., Fenno, L., Deisseroth, K., Herry, C., Arber, S., Lüthi, A., 2016. Midbrain circuits for defensive behaviour. Nature 534, 206–212. doi:10.1038/nature17996

Turner, C.A., Flagel, S.B., Blandino, P., Watson, S.J., Akil, H., 2017. Utilizing a unique animal model to better understand human temperament. Curr. Opin. Behav. Sci. 14, 108–114. doi:10.1016/j.cobeha.2017.02.002

Vale, R., Evans, D.A., Branco, T., 2017. Rapid spatial learning controls instinctive defensive behavior in mice. Curr. Biol. 27, 1342–1349. doi:10.1016/j.cub.2017.03.031

Veenema, A.H., Sijtsma, B., Koolhaas, J.M., de Kloet, E.R., 2005. The stress response to sensory contact in mice: genotype effect of the stimulus animal. Psychoneuroendocrinology 30, 550–557. doi:10.1016/j.psyneuen.2005.01.003

Vialou, V., Robison, A.J., Laplant, Q.C., Covington, H.E., Dietz, D.M., Ohnishi, Y.N., Mouzon, E., Rush, A.J., Watts, E.L., Wallace, D.L., Iñiguez, S.D., Ohnishi, Y.H., Steiner, M.A., Warren, B.L., Krishnan, V., Bolaños, C.A., Neve, R.L., Ghose, S., Berton, O.,

Tamminga, C.A., Nestler, E.J., 2010. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. Nat. Neurosci. 13, 745–752. doi:10.1038/nn.2551

Wang, L., Talwar, V., Osakada, T., Kuang, A., Guo, Z., Yamaguchi, T., Lin, D., 2019. Hypothalamic Control of Conspecific Self-Defense. Cell Rep. 26, 1747–1758.e5. doi:10.1016/j.celrep.2019.01.078

Wechsler, B., 1995. Coping and coping strategies: a behavioural view. Appl. Anim. Behav. Sci. 43, 123–134. doi:10.1016/0168-1591(95)00557-9

Wei, D., Talwar, V., Lin, D., 2021. Neural circuits of social behaviors: Innate yet flexible. Neuron 109, 1600–1620. doi:10.1016/j.neuron.2021.02.012

Williamson, C.M., Lee, W., Curley, J.P., 2016. Temporal dynamics of social hierarchy formation and maintenance in male mice. Anim. Behav. 115, 259–272. doi:10.1016/j.anbehav.2016.03.004

Wonderlich-Tierney, A.L., Vander Wal, J.S., 2010. The effects of social support and coping on the relationship between social anxiety and eating disorders. Eat Behav 11, 85–91. doi:10.1016/j.eatbeh.2009.10.002

Wong, L.C., Wang, L., D'Amour, J.A., Yumita, T., Chen, G., Yamaguchi, T., Chang, B.C., Bernstein, H., You, X., Feng, J.E., Froemke, R.C., Lin, D., 2016. Effective Modulation of Male Aggression through Lateral Septum to Medial Hypothalamus Projection. Curr. Biol. 26, 593–604. doi:10.1016/j.cub.2015.12.065

Wong, R.Y., French, J., Russ, J.B., 2019. Differences in stress reactivity between zebrafish with alternative stress coping styles. R. Soc. Open Sci. 6, 181797. doi:10.1098/rsos.181797

Yoshida, K., Drew, M.R., Kono, A., Mimura, M., Takata, N., Tanaka, K.F., 2021. Chronic social defeat stress impairs goal-directed behavior through dysregulation of ventral hippocampal activity in male mice. Neuropsychopharmacology 46, 1606–1616. doi:10.1038/s41386-021-00990-y

Zhang, J., He, Z.-X., Qu, Y.-S., Li, L.-F., Wang, L.-M., Yuan, W., Hou, W.-J., Zhu, Y.-Q., Cai, W.-Q., Zhang, X.-N., Guo, Q.-Q., An, S.-C., Jia, R., Tai, F.-D., 2021. Different baseline physical activity predicts susceptibility and resilience to chronic social defeat stress in mice: Involvement of dopamine neurons. Eur. Neuropsychopharmacol. 45, 15–28. doi:10.1016/j.euroneuro.2021.02.011

Zhang, K., Sakamoto, A., Chang, L., Qu, Y., Wang, S., Pu, Y., Tan, Y., Wang, X., Fujita, Y., Ishima, T., Hatano, M., Hashimoto, K., 2021. Splenic NKG2D confers resilience versus susceptibility in mice after chronic social defeat stress: beneficial effects of (R)-ketamine. Eur Arch Psychiatry Clin Neurosci 271, 447–456. doi:10.1007/s00406-019-01092-z

Zhao, W., Langfelder, P., Fuller, T., Dong, J., Li, A., Hovarth, S., 2010. Weighted gene coexpression network analysis: state of the art. J Biopharm Stat 20, 281–300. doi:10.1080/10543400903572753

Chapter 2 Characterizing the Behavioral and Neuroendocrine Features of Susceptibility and Resilience to Social Stress

2.1 Introduction

For humans, social interactions can be a source of support or stress. The nature of interpersonal relationships and interactions play a crucial role in the development of an affective state. Whereas positive social relationships can strengthen mental and physical well-being, negative social relationships have the propensity to induce highly stressful and harmful environments. During social stress, different individuals rely on various coping styles or strategies that may prove either adaptive or maladaptive in the long term (Billings and Moos, 1984; Connor-Smith and Compas, 2002; Wood and Bhatnagar, 2015).

In rodents, social defeat studies provide a naturalistic dominance model that uses agonistic encounters as a trigger, and social coping as a dependent measure (Hollis and Kabbaj, 2014). An agonistic encounter is often characterized by intense, aggressive interactions among animals of the same species that ultimately leads to individuals emerging as either 'winners' or 'losers' following the stress (Kollack-Walker et al., 1999; Kabbaj et al., 2001; Hammels et al., 2015). In mice, the Chronic Social Defeat Stress (CSDS) model relies on a paradigm where an experimental mouse is exposed physically and psychosocially to a more aggressive mouse from a different strain. Following the end of the chronic stress, the defeated animals diverge into two separate populations of social reactivity: a "resilient" group that is socially interactive and a "susceptible" group that is socially avoidant (Golden et al., 2011).

Defeated mice are termed 'resilient' and 'susceptible' based on their social behavior after stress. Understanding when and how these reactivities arise is critical in identifying predictive factors that set the course for vulnerability. For example, baseline differences in wheel running (J. Zhang et al., 2021), exploratory drive (Milic et al., 2021), anxiety-like behavior, hippocampal volume, and systemic immune factors (Nasca et al., 2019), have all been shown to predict the social avoidance outcome to some degree. Additionally, social hierarchies established in the homecage, well before the stress, predict the resilient and susceptible outcome (Larrieu et al., 2017). This is particularly interesting, as it suggests that there may be features of the defeat (e.g., dominance) that reflect intrinsic sociability. During defeat encounters, mice engage in a variety of active and passive coping behaviors, such as fight, escape, and freezing, as they react to the social stressor (McLaughlin et al., 2006). Also involved in the interaction is the ability to distinguish between threatening and non-threatening social cues, such as the environmental context and aggressor (Ayash et al., 2020). Observing the features of how these adaptive and reactive strategies unfold throughout the chronic stress experience may allow us to identify additional predictors of social outcome and paint a more cohesive picture of what constitutes vulnerability.

The behavioral and physiological components of the stress response are deeply intertwined; therefore, it is important to understand how interactions between these traits map onto different social outcomes following social defeat. The primary driver of the stress response is the hypothalamic-pituitary-adrenal (HPA) axis. When activated, a cascade of events leads to the adrenal synthesis and release of corticosteroid hormones—specifically, corticosterone (CORT) in rodents and cortisol in humans—targeting numerous organs, including the brain (Akil, 2005). Through this neuroendocrine axis, stressful experiences can trigger a wave of

physiological consequences, including alterations in the HPA axis itself, as well as changes in body weight regulation and pain sensitivity (Abdallah and Geha, 2017; Butler and Finn, 2009; Iio et al., 2014; Jeong et al., 2013). In humans, affective disorders, including major depressive disorder, have been associated with shifts in circadian cortisol rhythms (Adam et al., 2017) and an initial increase in cortisol in response to a social stress (Adam et al., 2017; Young et al., 2000).

To understand the specific factors that may lead an animal to display features of resilience or susceptibility to social stress, we conducted a series of careful studies examining behavioral and endocrine variables across the CSDS paradigm. We examined mice before (pre-), during, and after (post-) CSDS (**Fig. 2.1A**), generating a detailed temporal characterization of resilient and susceptible mice and allowing identification of key variables that predict phenotype divergence. Importantly, our results suggest that social reactivity to CSDS can be visualized during the defeat itself via inherent differences in coping mechanisms and that social reactivity is specific to the social context.

2.2 Materials and Methods

Animals: All mice were purchased from Charles River Laboratories (Wilmington, MA) and allowed at least one week for habituation to the vivarium prior to use in experiments. Subjects included 80 C57BL/6J male mice aged 8-11 weeks, housed 2-4 per cage upon arrival and until the start of the experiment. A total of 86 Male CD1 retired breeders (3-6 months) were used as aggressors and social interaction stimulus mice. CD1 mice were singly housed one week prior to aggressor screening and the start of the experiment. Black Swiss mice (10-14 weeks) were used as stimulus animals in a subset of social interaction tests. Enrichment in the form of enviro-paks was provided throughout the study. The rooms were kept under a 12 h light:12 h dark cycle

starting at 7 AM. All behavioral tests were conducted under standard overhead lighting conditions unless otherwise noted. Mice were provided with mouse chow and tap water ab libitum and maintained in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. The University of Michigan Institute of Animal Use Care and Use Committee (IACUC) approved all animal protocols utilized.

Chronic Social Defeat Stress (CSDS) and Control Rotation (CR): CSDS was performed according to the previously described protocol (Golden et al., 2011). Briefly, this paradigm consisted of a C57BL/6J intruder male mouse subjected daily to 5-minute bouts of physical defeat from a resident CD1 mouse, for a total of ten consecutive days. Each day of defeat, the intruder mouse was rotated to a new CD1 resident to prevent habituation. All CD1 mice were screened for aggression (defined as <1-minute interval to aggressive behavior) prior to use in CSDS experiments, and CD1 mice were not re-used across cohorts. Following the defeat, the intruder mouse was placed across the resident CD1 in a (26.7x48.3x15.2 cm) cage with a perforated plexiglass divider between them for 24 hours to elicit the psychosocial aspect of the stress. Given the aggressive nature of defeat in mice, minor physical injuries were possible. Animals were closely monitored during each defeat session, and where visible wounding occurred (<1cm), mice were immediately separated, and that defeat trial was concluded. In cases where injuries resulted in open wounds of >1cm, both intruder and CD1 mice were removed from the study (Golden et al., 2011). Control mice were housed 2/cage, separated from one another by a plexiglass divider and rotated to a new conspecific each day for ten days. All CSDS and CR occurred between 9 AM-11 AM each day. Following CSDS or CR, experimental mice were singly housed in the vivarium with enviro-pak enrichment. Defeat sessions were recorded with a video camera and manually scored by a blinded observer. The behaviors measured were

total duration of engagement in the fight and total number of fights, escapes, and upright,

forward, and crouch-back freezing during physical defeat bouts (Table 1). Duration

measurements are reported in seconds, and all other behaviors are reported as a ratio of behavior

measured/total behaviors. Three cohorts were recorded for this analysis, resulting in a total of

n=22 resilient mice and n=26 susceptible mice.

Table 2.1 Summary Table of Behaviors Scored During Physical Defeat Bouts

Behavior Scored	Description
Fight	Intruder mouse actively engages in fight
Escape	When approached, the intruder mouse jumps over the resident mouse
Upright Freeze	Intruder mouse freezes while on hind legs
Forward Freeze	Intruder mouse freezes while on all four legs, facing towards the resident
Crouch Back Freeze	Intruder mouse freezes with all four legs pulled in, facing away from resident

Social Interaction Test (SI): SI was performed according to a previously described protocol

(Golden et al., 2011). SI tests were conducted under red light in a testing arena (41x41x40 cm),

with a removable stimulus cage (10x10x10 cm) used to hold the social stimulus animal. There

were two, 2.5-minute trials. During the first trial, the experimental mouse was allowed to explore

the arena with an empty stimulus cage. The mouse was then removed from the arena, and a

social partner (CD1, unless otherwise noted) was placed inside the stimulus cage. The

experimental mouse was returned to the arena for another 2.5 minutes. Each session was

recorded and exploration of the whole arena, as well as the exploration of the cage/interaction

zone was scored using automatic tracking software (Noldus Ethovision XT software;Noldus

Information Technology, Leesburg, VA). The SI Ratio was calculated as time spent in the

interaction zone with CD1 present/not present. A social interaction score of ≤ 1 indicates susceptible, while > 1 indicates resilient (Assessed in Cohorts 1-4).

Body Weight (BW): Mice were weighed on Day 1 and again on Day 10 of the CR or CSDS. Change in body weight was measured as Day 10 (g) – Day 1 (g). (Assessed in Cohort 1).

Corticosterone (CORT) Measurements: AM/PM Measurements: To assess the circadian rhythm of circulating CORT, AM and PM CORT were assayed three days before and following CSDS or CR. Blood was drawn two hours before lights on and one hour following lights off in the mouse vivarium via tail vein under red light using Sarstedt Inc. MICROVETTE CB300 EDTA tubes (Ref#16.444.100). Blood was centrifuged, and plasma was stored at -80oC until used for CORT analysis with the Arbor Assays CORT ELISA kit (Cat#K014-H1). (Assessed in Cohort 1).

Forced Interaction Test (FIT): After CSDS or CR and one day following AM/PM blood draws, mice were placed across from a novel mouse in a clean CSDS cage, separated by a perforated plexiglass wall for 40 minutes to simulate both SI test and CR/CSDS conditions. No physical interaction was allowed, but sensory interaction was allowed through the perforated divider. Immediately upon the conclusion of this 40-minute test, mice were sacrificed, trunk blood was collected in 1mL tubes with 25 μL of 0.05M EDTA and centrifuged. Plasma was then stored at -80oC until used for CORT analysis with the Arbor Assays CORT ELISA kit (Cat#K014-H1). (Assessed in Cohort 1)

Open Field (OF): After CSDS or CR and one day following SI testing, mice were placed in an arena of 41x41x40 cm in size made of white plexiglass. Mice were allowed to explore the open field for 10 minutes. Time spent in the center was scored by Noldus Ethovision XT software. (Assessed in Cohort 2).

Forced Swim Test (FST): After CSDS or CR and two days following SI testing, mice were recorded from above and placed in a tall container (30x10 cm) filled within 2 inches from the top with 25 +/- 2oC water for 6 minutes. Total time spent immobile was hand-scored using a timer by an observer blind to the groups. Time spent immobile was defined as the least amount of movement to stay afloat (one hind-paw peddling or total immobility). (Assessed in Cohort 2).

Von Frey (VF): After CSDS or CR and one day after SI testing, each mouse was placed in a Plexiglas box atop a mesh platform and allowed to habituate for 30 minutes each day for three days. On the third day, mice were tested. Von Frey filaments of various forces starting from lowest to highest (0.008(g) - 2.0(g) Aesthesio Precise Tactile Sensory Evaluator Item#415000-20C) were placed on the plantar surface of the hind paw (of each foot) until the fiber bends or the mouse withdrew its paw from the stimulus. Each hind paw was tested twice for force necessary to cause a withdrawal response and then all 4 values were averaged for the final score. (Assessed in Cohort 3).

Cohort Breakdown: Four cohorts of mice were used to explore the above behaviors and physiological differences. Breakdown of group sizes and testing conditions are as follows:

Table 2.2 Testing Conditions Per Experimental Cohort

Cohort	Group Size	Test Conditions	Testing Timelines
1	n=20 control, n=16 defeat	SI, Baseline CORT, FIT CORT, Body Weight	Figs. 2A & 5A
2	n=20 control, n=15 defeat	SI, OF, FST	Fig. 4A
3	n=19 control, n=17 defeat	SI, VF	Fig. 4A
4a	n=10 control, n=17 defeat	SI w/ CD1, SI w/ Novel C57BL/6J	Fig. 6A
4b	n=8 control, n=15 defeat	SI w/ CD1, SI w/ Black Swiss	Fig. 6A

Statistics: Graphpad Prism and R (version 4.0.2) were used for figure design and statistical

analyses. In R, pastecs, car, afex, nlme, and emmeans packages were used for statistical analyses

(Fox and Weisberg, 2019; Grosjean and Ibanez, 2018; Lenth, 2020; Pinhero et al., 2020;

Singmann et al., 2016). To determine main effects, one-way ANOVAs were evaluated with the

car package, (aov function). To determine main and interaction effects with multiple time points,

2x2 ANOVAs were evaluated using the afex package (aov_ez function), with between factor =

Group (control, resilient, susceptible) and within factor= time or tester strain. To control for

cohort effects for the during stress behaviors, we added 'cohort' as a covariate to the 2x2

ANOVA. Post-hoc tests were conducted with the emmeans package (emmeans and pairs

function), using a Tukey-HSD test. Mann-Whitney T-test was used for single comparisons

among control and defeat groups. Pearson correlations were conducted via the cor.test function

to assess linear relationships between outcome variables. Multi-level model (MLM) for baseline

CORT data was evaluated using the nlme package (lme function) with estimates optimized using

Restricted Maximum Likelihood (REML) and summarized to produce individual coefficients,

approximate standard errors, and respective p-values for each level of the fixed effects variables.

MLM was used to examine datasets that contain both repeated measures and missing data. There

were missing data points for baseline AM/PM CORT samples due to not collecting enough blood

from the tail vein (data point= blood collection time point--9 data points missing in controls, 7

data points missing in defeated mice). All significance thresholds were set at $p \leq 0.05$. Receiver

operating characteristic curve analysis was done in Prism to test for the sensitivity and specificity

of using Day 1 escape behavior as a predictive variable for social outcome. The Fisher's exact

test was run in prism to analyze the contingency table of resilient and susceptible mice Day 1

escape behavior. Detailed statistics are included in **Appendix A: Chapter 2 Supplement.**

2.3 Results

2.3.1 Characterizing the Resilient-Susceptible Outcome
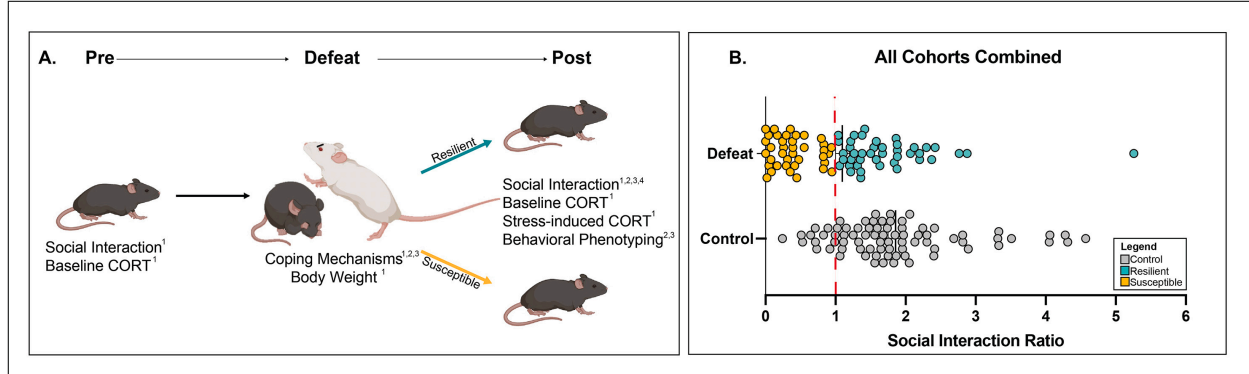
Figure 2.1 Experimental Schematic: Behavioral and Endocrine Profiling Following Chronic Social Defeat Stress (CSDS)
A. Schematic timeline of the 3 timepoints (pre-, during, and post-defeat) used for behavioral phenotyping and endocrine profiling. Each timepoint includes the overall category of experiments conducted. Superscripts indicate the cohorts of mice used for each assay. B. Social Interaction (SI) ratios of all 4 cohorts of mice combined. Defeat (n = 80; mean = 1.096, sd = ±0.877) and Control (n = 77; mean = 1.856, sd = ±0.934). Colors: red dash = SI ratio of 1, yellow = susceptible, blue = resilient, grey = control.

We first examined social interaction behavior following ten days of chronic social defeat stress (CSDS) or control rotation (CR). Across four cohorts, control mice demonstrated a preference for social interaction, with a mean SI Ratio of 1.856 (sd=+/-0.934). In contrast, animals that experienced social defeat displayed a significant reduction in SI Ratios, with a mean of 1.096 (sd=+/-0.877) (**Fig. 2.1B**; p<0.001, Mann-Whitney U=1600). Defeat increased the proportion of socially avoidant mice (SI Ratio ≤1) from 16% (controls) to 48% (defeat). Notably, defeated mice could now be grouped according to two distinct response patterns: socially avoidant (susceptible) and socially interactive (resilient).

To understand this shift in population distribution due to defeat, we asked how the final susceptible and resilient features evolve longitudinally. We characterized defeated mice by observing features of behavioral, physiological, and neuroendocrine read-outs before (pre-), during, and after (post-) CSDS (**Fig. 2.1A**). The information gathered was used to identify which

time(s) and which behavior(s) can predict the divergence of resilient and susceptible mice. This allowed us to ask whether the social avoidance phenotype is apparent early on, or whether it emerges due to the full extent of the repeated stress.

Pre-Defeat: SI Ratios and CORT Levels were not Predictive of Future Reactivity to CSDS
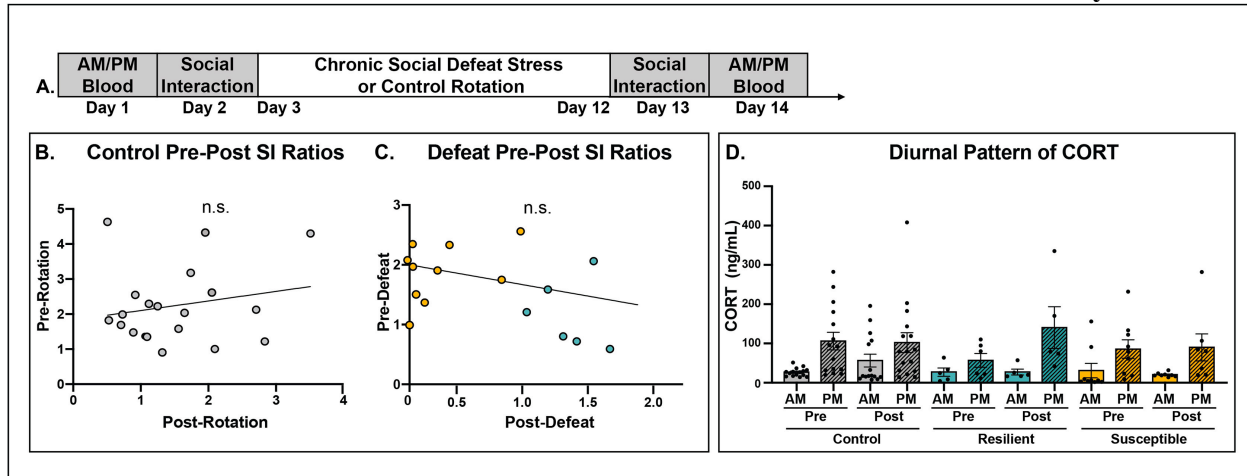
Figure 2.2 Pre-stress: Basal Social Interaction and Corticosterone Levels Did Not Predict Resilience or Susceptibility Post Social Defeat
A. Schematic timeline of pre-post SI test and AM/PM corticosterone (CORT) sampling. B. In control mice, SI Ratios displayed before the rotation did not correlate with SI ratios measured after the rotation. C. In defeated mice, SI ratios measured before defeat did not correlate with SI ratios displayed after defeat. D. CORT ELISA showing diurnal patterns of CORT before and after CSDS/CR. For all groups, the diurnal CORT patterns remained stable throughout the experiment, with higher PM CORT. For either pre- or post-CSDS/CR timepoints, there were no differences in CORT levels between groups. Significance Codes: n.s. = not significant. Colors: grey = control, blue = resilient, yellow = susceptible.

Pre-CSDS SI ratios did not correlate with post-CSDS SI ratios

Given that the most robust finding following CSDS is the split of resilient and susceptible behaviors in the SI test, we asked whether this social behavior is a stable trait that 1) precedes stress and 2) could be used to predict resilient and susceptible groups. To that end, we conducted SI testing one day before and one day after CSDS and CR in the same animals (**Fig. 2.2A**). There was no correlation between SI ratios pre-or post-CR in control mice, nor pre- or post-CSDS in defeated mice (Control: **Fig. 2.2B, Appendix Table A2;** *p*>0.05, Defeat: **Fig 2.2C, Appendix Table A2;** *p*>0.05). These data demonstrate that the development of vulnerability to CSDS cannot be predicted by social behavior before experiencing defeat.

Social reactivity outcome was not predicted by basal diurnal CORT rhythms, either before or

after CSDS

We then wanted to assess if baseline neuroendocrine differences predicted resiliency or

susceptibility. There is a distinct diurnal pattern of CORT in rodents, with relatively low levels in

the morning and high levels in the evening (Moore and Eichler, 1972). We assayed both AM

and PM plasma CORT levels in the same mice taken two days before and two days after the

CSDS/CR (**Fig. 2.2A).** We fit a linear mixed-effects model with CORT (ng/mL) levels as the

outcome variable and pre- vs. post- CSDS/CR, time of day, and group and their interactions as

the fixed effects. Across control, resilient, and susceptible mice, time of day was a significant

predictor of CORT levels (**Appendix Table A1;** *est*=1.552, *sd*=0.503, *p*=0.003), indicating that

the diurnal rhythm of CORT remained robust in all groups. Importantly, this diurnal pattern was

not predictive of social outcome (*p*>0.05), denoting that the resilient/susceptible phenotype did

not shift the circadian cycle of stress hormones. Moreover, pre- and post- CSDS/CR levels were

not predictive (*p*>0.05), suggesting that the process of going through the CSDS/CR itself did not

alter baseline CORT levels (**Fig. 2.2D)**.

2.3.2 During the Initial Defeat Encounter, Susceptible Mice Engaged in More Escape Behavior
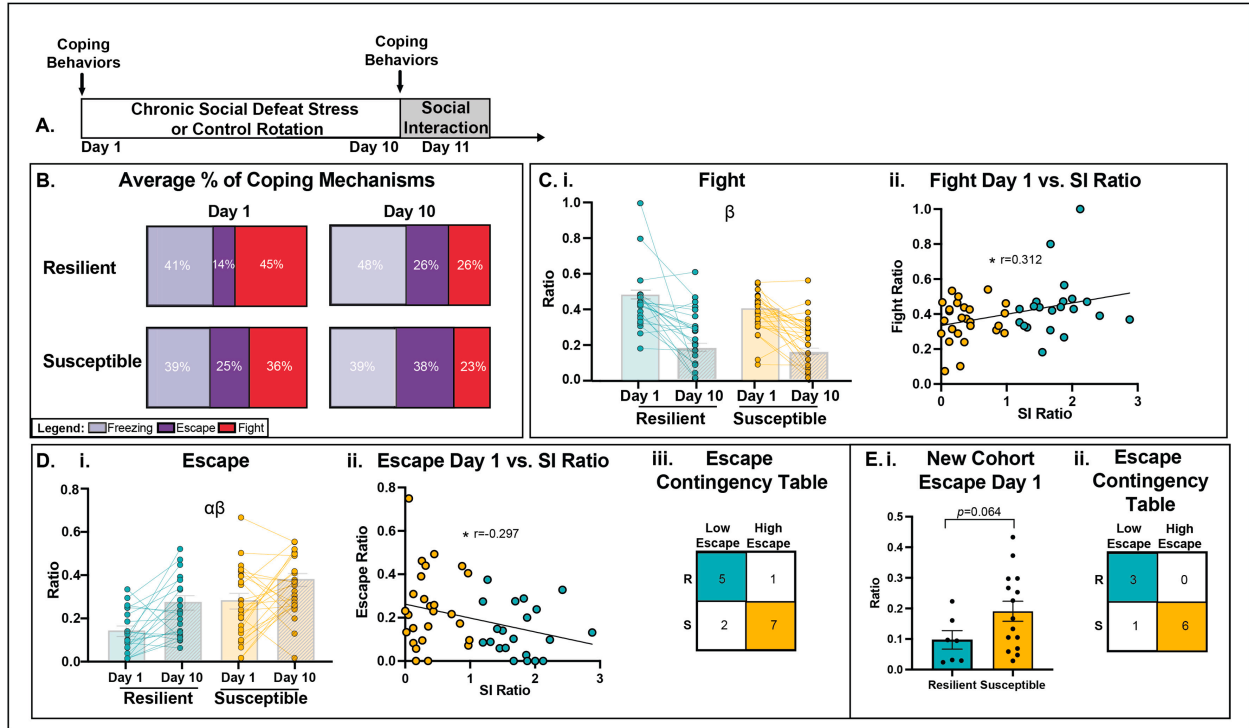
Figure 2.3 During Stress: Mice that End Up Susceptible Used Escape as a Coping Behavior
A. Schematic timeline indicating observation of coping behaviors during Day 1 and Day 10 of defeat. B. Average %
of freezing, escape, and fight behaviors displayed on Day 1 and Day 10, according to resilient and susceptible
phenotype. Ci. Ratio of # of fights/total behaviors observed on Day 1 and Day 10 of defeat. Overall, fight behaviors
decreased from Day 1 to Day 10, but levels of fighting were similar between resilient and susceptible groups. C.ii.
Fight ratios measured on Day 1 of defeat were positively correlated with SI ratios measured post-defeat. D.i Ratio
of # of escapes/total behaviors observed on Day 1 and Day 10 of defeat. Escape behaviors were higher overall in
susceptible compared to resilient mice and also higher overall on Day 10 compared to Day 1. D. ii. Escape ratios
measured on Day 1 of defeat were negatively correlated with SI ratios measured post-defeat. D. iii. Escape
contingency table illustrating a higher proportion of susceptible mice escape compared to resilient on Day 1. E.i.
Replication of Escape analysis in an additional cohort presented a trend for susceptible mice to escape more
compared to resilient. E.ii. Escape contingency table in replicate cohort illustrating a higher proportion of
susceptible mice escape compared to resilient on Day 1. Significance Codes: Group Main Effect α, Day Main Effect
β, *p < 0.05. Colors: blue = resilient, yellow = susceptible.

During agonistic encounters, mice engage in a variety of coping behaviors, including

freeze, fight, and escape (McLaughlin et al., 2006; Scott, 1966). Therefore, we asked whether the

type of coping behavior utilized during the defeat encounter has any predictive value for the

resilient or susceptible outcome. To do this, we quantified these active and passive coping

behaviors (Table 1) on Day 1 (a stress-naïve first encounter) and on Day 10 (experienced final

stress encounter) (**Fig. 2.3A**). As shown in **Fig. 2.3B**, resilient and susceptible mice displayed different patterns of freeze, fight, and escape. We then compared the expression of these behaviors on Day 1 and Day 10 between resilient and susceptible groups.

Freezing behaviors were not related to resilient or susceptible outcomes

For all three freezing behaviors (Upright, Crouch, or Forward), there were no changes across Day or between Groups (Supp. Fig. 1C-D, Supp. Table 5; Day p>0.05, Group p>p0.05). Freezing behaviors were also not correlated with post-defeat SI ratios (*data not shown*). Thus, these data suggest that the freezing response is not related to the sociability outcome. Therefore, we assessed whether differences in the active coping behaviors (fight versus escape) mapped onto future expression of resilience and susceptibility.

Greater initial fighting was correlated with subsequent resilience

Fighting behavior over the course of the defeat protocol is dynamic. Over the course of the ten days of the defeat protocol, there was an overall significant decrease in fight duration (**Supp. Fig. 2.1B**; Test Day $F_{1,44}=16.20$, p<0.001). Across all defeat sessions, neither fight duration nor ratio of total fight engagements differentiated the resilient and susceptible groups, as these groups displayed similar durations of active fighting across the defeat sessions (**Appendix A Fig. 1B**; Group $F_{1,44}=0.030$, p=0.860). Similarly, the fight ratios over the course of defeat were similar between resilient and susceptible mice (**Fig. 2.3C.i**; Group $F_{1,44}=3.45$, p=0.070). However, the amount of fighting behavior displayed early in the course of defeat (in the initial encounter) was related to the eventual sociability phenotype, as indicated by a significant positive correlation between SI ratio and fight behavior on Day 1 (**Fig. 2.3C.ii**; r=0.312, p=0.031). Thus, although overall fighting levels were similar between groups, higher SI ratios correlated with higher levels of fighting on the first aggressive encounter.

Day 1 escape behavior predicted which mice become susceptible

Escape behaviors are also dynamic, but in contrast to fight, escape ratios increased over the course of defeat (**Fig 2.3D. i**.; Test Day $F2,44=29.92$, $p<0.001$). There was also a significant group difference for this coping behavior, as mice that emerged as susceptible exhibited significantly greater escape behaviors on Day 1 than resilient mice (Fig. **2.3D.i**.; Group $F1,44=7.070$, $p=0.011$). Notably, we observed a significant negative correlation between SI Ratio and escape behavior on Day 1 (**Fig. 2.3D.ii**.; $r=-0.297$, $p=0.040$).

We next asked whether escape behavior could predict susceptibility/resilience prospectively. To do this, we separated mice on Day 1 into two groups: high escape vs. low escape, based on whether mice escaped one standard deviation above or below the mean (mean=0.195, sd=+/-0.160). A Fisher Exact Test revealed a significant correlation between SI Ratio and high/low escape behavior ($p=0.041$). A 2x2 contingency table analysis shows that on Day 1, the proportion of high escape mice that become susceptible to CSDS (7 out of 9) was larger than resilient individuals (1 out of 6) (**Fig. 2.3D. iii.**). This was further confirmed by using a receiver operating characteristic curve analysis, which found that escape behavior on Day 1 significantly predicted the outcome of resilient and susceptible groups following CSDS (**Appendix A Fig. A1(2)**; area=0.720, $p=0.009$).

We used an additional cohort to verify that Day 1 high escape behaviors predict the emergence of susceptibility. In this cohort, mice overall escaped at an average ratio of 0.161 (sd= +/- 0.117) with a trend for future susceptible mice to have a higher escape ratio (**Fig. 2.3E.i**.; $p=0.064$). After separating this cohort into high vs. low escape, a Fisher Exact Test revealed again that there was a significant correlation between SI Ratio and high/low escape behavior ($p=0.033$). A 2x2 contingency table analysis shows that on Day 1, the proportion of high escape

mice that become susceptible to CSDS (6 out of 7) was larger than resilient individuals (0 out of 3) (**Fig. 3E. ii**). Thus, examining escape behavior as either a continuous measure or categorical variable shows a predictive relationship between high escape and eventual susceptibility.

2.3.3 Post-Stress: Comprehensive Behavioral Phenotyping Associated with Social Outcome
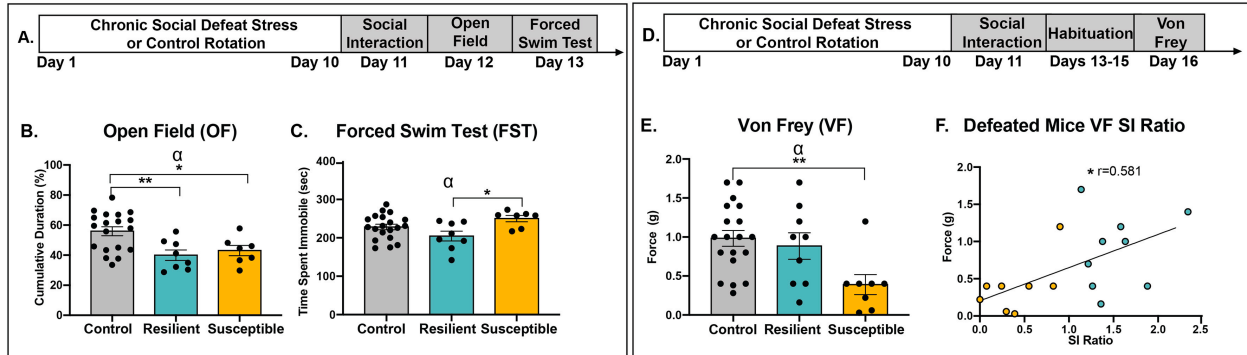
Figure 2.4 Characterization of Behavioral Outcomes.
A. Schematic timeline of assessing traditional read-outs of anxiety-like (Open field, OF) and depression-like (Forced swim test (FST) behavior tests. 4 B. Cumulative duration (%) of time spent in the open area of the OF was overall decreased by defeat. 4C. Resilient mice spent less time immobile compared to susceptible mice. 4D. Schematic timeline of Von Frey (VF) testing. 4 E. In the VF test, susceptible mice had increased pain sensitivity compared to controls. 4 F. Lower SI ratios were correlated with lower force (g) filaments used to elicit a response in the VF test (increased pain sensitivity). Significance Codes: Group Main Effect α, $*p < 0.05$, $**p < 0.01$. Colors: grey = control, blue = resilient, yellow = susceptible.

Traditional Affective Readouts: The Open Field (OF) and Forced Swim Test (FST)

To address whether resilient and susceptible phenotypes (according to the SI test) are generalizable to traditional measures of anxiety- and depressive-like behavior, we conducted the Open Field (OF, anxiety-like measure) and Forced Swim Test (FST, depression-like measure) following CSDS or CR (**Fig. 2.4A**).

In the OF test, social defeat reduced the percent time spent in center compared to controls (**Fig. 2.4B**; $F_{2,31}=6.689$, $p=0.004$), indicating a general increase in anxiety-like behavior following CSDS. However, there was no difference between resilient and susceptible mice ($p>0.05$), and there was no correlation between SI Ratios and percent time spent in the center in defeated mice

($p>0.05$) (**Appendix Table A10**). Therefore, although defeat itself increased anxiety-like behavior in the OF, there was no difference between resilient and susceptible groups.

In the FST, there was a significant difference across groups in time spent immobile (**Fig. 2.4C**; $F_{2,31}=3.93$, $p=0.030$). Compared to controls, there was no significant difference between resilient ($p>0.05$) or susceptible ($p>0.05$) mice. However, susceptible mice spent significantly more time immobile compared to resilient mice ($p=0.023$). In this test, SI Ratios did not correlate with immobility time in defeated mice (**Appendix Table A10**; $p>0.05$).

*Mice with lower SI Ratios displayed greater sensitivity to mechanical nociception **(Fig. 2.4D)***

Due to the physical nature of social defeat, we conducted the Von Frey (VF) test to understand whether pain sensitivity differed between resilient and susceptible mice. There was a significant effect of group on filament force (g) used in the VF test (**Fig. 2.4E**; $F_{2,33}=5.163$, $p=0.011$). Susceptible mice needed a significantly lower filament force to elicit a mechanical response than controls, indicating an increased mechanical nociception sensitivity ($p=0.009$). In contrast, resilient mice had a similar filament force threshold to control mice ($p>0.05$). There was a trend for lower pain threshold in susceptible mice compared to resilient ($p=0.069$). And moreover, within defeated mice, there was a positive correlation between SI Ratio and force (g) of filament threshold (**Fig. 2.4F**; $p=0.015$, $r=0.581$), indicating a relationship between social avoidance and increased pain sensitivity. These data highlight a relationship between social interaction and pain sensitivity that goes beyond the traditional measures of affective-like behaviors.

2.3.4 Post-Stress: Sociability Outcome was Related to Physiological Measures of Weight and CORT
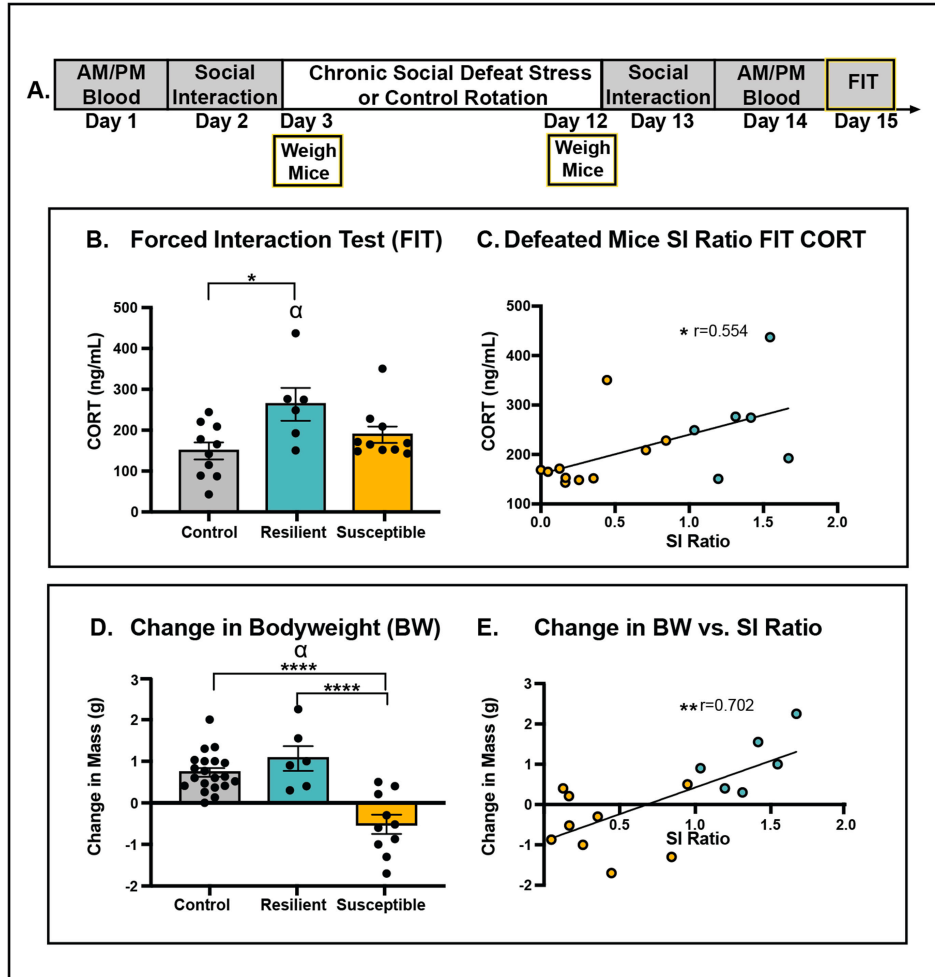
Figure 2.5 Susceptible Mice Gained Less Weight and Mounted a Blunted CORT Response Compared to Resilient Mice

5 A. Schematic timeline of body weight and Forced Interaction Test (FIT) CORT measurements. 5 B. FIT-induced CORT (ng/mL) levels increased in resilient mice compared to controls. 5C. In defeated mice, lower FIT CORT (ng/mL) levels correlated with lower SI Ratios. 5D. Change in BW (Day 10 – Day 1) indicates that susceptible mice had reduced weight gain compared to resilient and control mice. 5 E. In defeated mice, higher SI ratios were significantly correlated with larger weight gains over the course of the experiment. Significance Codes: Group Main Effect α, *p < 0.05, **p < 0.01. Colors: grey = control, blue = resilient, yellow = susceptible.

To determine whether there were neuroendocrine and physiological differences in resilient and susceptible mice following stress, we conducted a social stress challenge and observed changes in body weight and CORT (**Fig. 2.5A**).

During a social stress challenge, lower SI ratios correlated with lower CORT expression

Although pre-defeat experiments showed no differences in basal CORT rhythms, we wanted to determine whether resilient and susceptible mice would display different CORT responses when given a social stress challenge. Following 40-minute exposure to a CD1 mouse, there was an overall group difference between control, resilient, and susceptible mice (**Fig. 2.5B**; $F_{2,33}=4.560$, $p=0.0214$). This group effect was driven by increased CORT expression in resilient mice compared to controls ($p=0.016$). Interestingly, within defeated mice, there was a significant positive correlation between SI ratios and CORT levels ($r=0.055$, $p=0.026$), indicating that the more socially interactive mice mounted higher CORT responses to the social stress challenge (**Fig. 2.5C**).

Susceptible mice had reduced weight gain by the end of CSDS

As an additional physiological measure of the effects of stress, we compared changes in body weight across the experimental period in mice exposed to CSDS or CR (**Fig. 2.5A**). Weight change across social defeat was calculated (Day 10 weight – Day 1 weight) and showed an overall group difference between control, resilient, and susceptible mice (**Fig. 2.5D**; $F_{2,33}=18.60$, $p<0.001$). This difference was driven by a significant reduction in weight in susceptible mice compared to both control ($p<0.001$) and resilient ($p<0.001$) mice. Within the defeated group, there was a significant positive correlation between SI Ratio and body weight (**Fig. 2.5E**; $r=0.492$, $p<0.001$), further indicating that social avoidance was related to reduced weight gain.

We ran a linear regression to test whether there was a relationship between body weight and SI Ratio on FIT CORT expression. We found that while there was an effect of body weight change on SI Ratio ($p=0.017$), there was no effect of weight change on FIT CORT expression

(**Appendix Table A9**; p>0.05). This analysis indicates that although body weight and stress-induced CORT were independently related to social reactivity outcome of defeat, the two physiological variables were not directly related to each other.

2.4 Discussion

The CSDS model has proven valuable in investigating the underlying neurobiological mechanisms of social reactivity after resilient and susceptible groups are established. Following CSDS, neuroanatomical differences (Anacker et al., 2016), transcriptional profiles (Bagot et al., 2016), and neural activity patterns (Hultman et al., 2018; Muir et al., 2020, 2018) have all been linked to susceptibility or resilience. In our study we performed a characterization of the behavioral differences either before or early in the stress experience that may set the course for the social outcome. We assessed behavioral, neuroendocrine, and physiological differences pre-stress, during the early social stress experience, and post-stress. The current work outlines several key findings that shed light on the affective makeup of resilient and susceptible mice.

Key Findings:

a) We did not identify a priori behavioral readouts that predicted resilient or susceptible outcomes. The degree of social interaction before stress is not predictive of social response following stress, suggesting that these animals do not simply differ in "sociability." Moreover, physiologically, basal CORT has no predictive value for social reactivity to CSDS.

b) By contrast, coping style during the first encounter is a key variable in the emergence of the eventual phenotype: higher rates of escape behavior on Day 1 indicate a greater likelihood of a subsequent classification of susceptible. In addition, the propensity for more fighting on Day 1 correlates with greater resilience scores.

c) Following stress, social avoidance is not generalizable to traditional measures of affective behavior. However, it is associated with greater sensitivity to physical pain and significant weight loss. Moreover, lower social interaction ratios correlate with lower CORT expression when mice are placed back in the social stress context.

d) Social avoidance is social context-specific, as susceptible mice do not avoid non-aggressive strains.

Taken together, our findings demonstrate that the susceptible, socially avoidant animals exhibit high reactivity to a specific social stressor, characterized by a greater propensity to escape during the early encounters. This initial difference evolves into a relatively lower endocrine stress response to social threat, along with greater sensitivity to pain and weight loss. However, susceptible mice appear clearly attuned to the nature of the aggressor and do not generalize the threat response to either con-specifics or other novel strains of mice. Through this work, we have expanded our understanding of susceptibility in this animal model, adding context to the socially avoidant phenotype.

2.4.1 Evaluating How Social Outcome Tracks with Traditional Measures of Emotional Vulnerability

Across multiple labs, the variation in behavioral responses to social defeat reflects the complexity of defining vulnerability and the nuanced relationship between stress and affective behaviors. When assessed after defeat, traditional measures of anxiety-like behavior, such as open field and elevated plus maze, generally do not track between resilient and susceptible outcomes (Alves-Dos-Santos et al., 2020; Krishnan et al., 2007). In measures of depression-like behavior, such as the forced swim test, there have been contradictory findings related to the link to resilience or susceptibility (He et al., 2018; Krishnan et al., 2007; Lee et al., 2021; K. Zhang et

al., 2021). Although we detected a difference in FST immobility between resilient and susceptible mice, both groups were comparable to controls. Additionally, within defeated animals, there was no relationship between social reactivity and this particular depressive-like measure. It is important to highlight that CSDS is both a physical and psychosocial stressor—in our work, delineation of resilient and susceptible animals involved measures that were physical (pain sensitivity and body weight) or psychosocial (coping behavior, FIT CORT, and susceptible threat-discrimination). Thus, although anxiety- or depressive-like assays can be useful in characterizing general affective states following CSDS, these tests do not appear to reflect the relevant stimuli encoded by the social stress itself and may be less tied to the social outcome at the time points downstream behavior is observed. Another important variable to consider when parsing susceptible or resilient outcomes of social defeat is the nature of the CSDS protocol itself. For example, increasing the number of defeat sessions from 10 to 21 consecutive days increases susceptibility and provokes stronger and more reliable differences in depression-like and anxiety-like measures (Fang et al., 2020; Lu et al., 2021), suggesting that social interaction outcomes following a more prolonged stress track well with traditional affective assays. In contrast, acute social defeat stress (3 sessions in 1 day) does not reveal any differences between resilient and susceptible outcomes in depression-like or anxiety-like tests (Grossman et al., 2022). Finally, an added layer of complexity for these studies relates to the potential for sex differences in how resilient or vulnerable phenotypes emerge. Examining sex differences in the CSDS model has been complicated by the fact that territorial aggression is provoked by different experimental conditions in male vs. female mice, and the majority of previous CSDS studies to date have focused on male subjects (Carnevali et al., 2020; Hammels et al., 2015; Martinez et al., 1998). Nevertheless, researchers have begun to explore inter-female aggression using modified

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CSDS protocols and have identified unique consequences of this type of social stress in females (Harris et al., 2018; Logan, 2019; Takahashi et al., 2017; van Doeselaar et al., 2021). Although we did not include female mice in our current work, defining the neurobiology of vulnerability and resilience to social stress in both sexes remains a critical gap in this model and will be essential for our understanding of underlying risk factors for stress-related disorders in humans (Lyons et al., 2018; Senst et al., 2016; Varholick et al., 2019).

2.4.2 Predicting the Resilient-Susceptible Outcome

Recently there has been greater emphasis on uncovering what traits or combinations of traits are needed to predict resilient and susceptible outcomes. For example, Nasca et al. found that predicting susceptibility is optimized when multiple behavioral, neuroanatomical, and physiological measures are combined (Nasca et al., 2019). When looking specifically at social behavior features, Larrieu et al. identified that hierarchal status before defeat predicts the social phenotype; mice that are higher-ranked in their home cage are more likely to exhibit susceptible and socially avoidant behaviors following social defeat (Larrieu et al., 2017). Moreover, it has been shown that features of behaviors that can be classified as 'active actions' are predictive of social outcome. Indeed, mice displaying low baseline physical activity in a voluntary wheel running assessment prior to CSDS are more likely to become susceptible compared to more active mice (J. Zhang et al., 2021). Similarly, low levels of exploration in a novel environment (exploratory drive) predict susceptibility following CSDS (Milic et al., 2021). Consistent with a role for active behavioral traits in predicting social outcome, the current results provide novel evidence that less "active" coping behaviors displayed during the initial defeat (e.g., escape) predicted susceptibility. Taken together, this pattern suggests that the resilient or susceptible responses to social stress may reflect broad temperamental differences in active vs. passive traits.

2.4.3 Evaluating the Cost-Benefit Balance in Susceptible Mice

The two main coping behaviors we observed during the defeat, fight and escape, can be understood in the context of evolutionarily conserved predator-prey interactions (Koolhaas et al., 1999). For example, mice can either engage in the fight to assert active territorial control (Anderson and Hill, 1965) or deploy an escape strategy to avoid the looming threat of the approaching predator (De Franceschi et al., 2016; Eilam, 2005; Shang et al., 2018). Mice that go on to become resilient engage in more fights on day 1 of defeat, suggesting that they may be attempting to assert dominance over the social threat through heightened aggression and fight engagements. This interpretation is further supported by our finding that CORT responses to social stress challenge were elevated in resilient mice, as increased plasma glucocorticoids have been linked to increased levels of aggression and fight engagements (Haller et al., 1998). Similarly, Milic et al., found that when assessing CORT expression following an SI test that occurred 1-week post-CSDS, elevated CORT levels were positively correlated with exploration time of a novel CD1 (Milic et al., 2021). A heightened level of CORT in resilient mice may come at a cost, as sustained CORT expression can have detrimental health effects on both body and brain (McEwen and Akil, 2020). Although we did not observe CSDS-induced changes in baseline CORT, other CSDS protocols have cited baseline shifts, perhaps reflecting differences in the exact protocol or blood collection methods (Gururajan et al., 2019). The potential for CSDS to significantly shift HPA function, altering the consequences of subsequent stressors, highlights the need for more studies examining CORT trajectories in resilient and susceptible mice.

Although susceptibility has often been thought to reflect a maladaptive reduction in social motivation, our results suggest possible advantages to this response. In susceptible mice, the high

propensity to escape indicates that these animals tend to employ an evasive strategy to avoid harm and are perhaps highly sensitive to threat. This underlying difference in threat assessment can also be seen in Milic et al., where they observed that susceptible mice show increased avoidance to harm in a passive avoidance task prior to stress (Milic et al., 2021). Interestingly, we showed that susceptible mice do not appear to have reduced social motivation in general, only reduced exploration specific to the strain associated with aggression. Similarly, when using a modified social interaction test in which defeated mice were placed in a three-chambered social approach task, Ayash et al. found that susceptible mice interacted more with the non-aggressive C57BL6/J or 129/Sv than a CD1(Ayash et al., 2020). Both studies show that when tested either sequentially or forced to make a choice, the social avoidance behavior in susceptible mice is specific to the aggressor context. The potential benefits of susceptibility, in terms of increased threat avoidance, appear to come at some cost, as these animals also had reduced weight gain and increased pain sensitivity following CSDS. Other studies have observed overall increased pain sensitivity following defeat (Marco Pagliusi et al., 2020; M Pagliusi et al., 2020), but it is unclear whether individual variation in pain sensitivity precedes the defeat; additional studies are needed to uncover whether this is a predictive factor. Taken together, our results suggest that susceptibility should be viewed in a more nuanced way, as the conditions in which the observed behavior is expressed dictates whether there it is a cost or benefit to the animal.

2.4.4 Concluding Remarks

It is worth recalling that resilient and susceptible mice in these and other studies derive from a single inbred strain and therefore share a common genetic makeup. Despite this, inbred strains exhibit a high degree of individual variability in behavioral outputs (Tuttle et al., 2018; Wahlsten et al., 2006), highlighting the key role of developmental and contextual variables in

shaping emotional reactivity. Some of these variables may arise from differences in environmental enrichment (Caradonna et al., 2021), maternal behavior (Pedersen et al., 2011), or from social dominance hierarchies (Horii et al., 2017). Here, our longitudinal study suggests the existence of an ongoing interplay between the animal's initial propensity for adopting different coping strategies, the specific characteristics of the stress condition, and the cumulative effects of daily stress experience. Over the course of the stress, these factors work together to determine resilient or susceptible outcomes, each with their own set of costs and benefits. This perspective provides a context for future studies investigating the neurobiology underlying social defeat stress, as well as the unique and dynamic changes associated with distinct behavioral phenotypes.

Going forward, detailed quantification of coping behaviors displayed during defeat, and the incorporation of coping strategy itself as a variable in predicting social outcome, will serve useful in the CSDS model. Intervention studies, employing pharmacological or environmental manipulations to shift coping behaviors early in the course of defeat, can be used to directly test the role of coping in shaping final behavioral outcome. In this way, understanding the factors that determine coping traits may provide a valuable clinical framework for defining affective risk, as well as identifying potential behavioral therapeutic interventions that are protective against human stress-related disorders.

2.5 References

Abdallah, C.G., Geha, P., 2017. Chronic pain and chronic stress: two sides of the same coin? Chronic Stress (Thousand Oaks) 1. doi:10.1177/2470547017704763

Adam, E.K., Quinn, M.E., Tavernier, R., McQuillan, M.T., Dahlke, K.A., Gilbert, K.E., 2017. Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. Psychoneuroendocrinology 83, 25–41. doi:10.1016/j.psyneuen.2017.05.018

Akil, H., 2005. Stressed and depressed. Nat. Med. 11, 116–118. doi:10.1038/nm0205-116

Alves-Dos-Santos, L., Resende, L. de S., Chiavegatto, S., 2020. Susceptibility and resilience to chronic social defeat stress in adolescent male mice: No correlation between social avoidance and sucrose preference. Neurobiol. Stress 12, 100221. doi:10.1016/j.ynstr.2020.100221

Anacker, C., Scholz, J., O'Donnell, K.J., Allemang-Grand, R., Diorio, J., Bagot, R.C., Nestler, E.J., Hen, R., Lerch, J.P., Meaney, M.J., 2016. Neuroanatomic differences associated with stress susceptibility and resilience. Biol. Psychiatry 79, 840–849. doi:10.1016/j.biopsych.2015.08.009

Anderson, P.K., Hill, J.L., 1965. Mus musculus: Experimental Induction of Territory Formation. Science 148, 1753–1755. doi:10.1126/science.148.3678.1753

Ayash, S., Schmitt, U., Müller, M.B., 2020. Chronic social defeat-induced social avoidance as a proxy of stress resilience in mice involves conditioned learning. J. Psychiatr. Res. 120, 64–71. doi:10.1016/j.jpsychires.2019.10.001

Bagot, R.C., Cates, H.M., Purushothaman, I., Lorsch, Z.S., Walker, D.M., Wang, J., Huang, X., Schlüter, O.M., Maze, I., Peña, C.J., Heller, E.A., Issler, O., Wang, M., Song, W.-M., Stein, J.L., Liu, X., Doyle, M.A., Scobie, K.N., Sun, H.S., Neve, R.L., Geschwind, D., Dong, Y., Shen, L., Zhang, B., Nestler, E.J., 2016. Circuit-wide Transcriptional Profiling Reveals Brain Region-Specific Gene Networks Regulating Depression Susceptibility. Neuron 90, 969–983. doi:10.1016/j.neuron.2016.04.015

Billings, A.G., Moos, R.H., 1984. Coping, stress, and social resources among adults with unipolar depression. J. Pers. Soc. Psychol. 46, 877–891. doi:10.1037//0022-3514.46.4.877

Butler, R.K., Finn, D.P., 2009. Stress-induced analgesia. Prog. Neurobiol. 88, 184–202. doi:10.1016/j.pneurobio.2009.04.003

Caradonna, S.G., Zhang, T.-Y., O'Toole, N., Shen, M.-J., Khalil, H., Einhorn, N.R., Wen, X., Parent, C., Lee, F.S., Akil, H., Meaney, M.J., McEwen, B.S., Marrocco, J., 2021. Genomic modules and intramodular network concordance in susceptible and resilient male mice across models of stress. Neuropsychopharmacology. doi:10.1038/s41386-021-01219-8

Carnevali, L., Montano, N., Tobaldini, E., Thayer, J.F., Sgoifo, A., 2020. The contagion of social defeat stress: Insights from rodent studies. Neurosci. Biobehav. Rev. 111, 12–18. doi:10.1016/j.neubiorev.2020.01.011

Connor-Smith, J.K., Compas, B.E., 2002. Vulnerability to Social Stress: Coping as a Mediator or Moderator of Sociotropy and Symptoms of Anxiety and Depression. Springer Science and Business Media LLC. doi:10.1023/a:1013889504101

De Franceschi, G., Vivattanasarn, T., Saleem, A.B., Solomon, S.G., 2016. Vision guides selection of freeze or flight defense strategies in mice. Curr. Biol. 26, 2150–2154. doi:10.1016/j.cub.2016.06.006

Eilam, D., 2005. Die hard: a blend of freezing and fleeing as a dynamic defense--implications for the control of defensive behavior. Neurosci. Biobehav. Rev. 29, 1181–1191. doi:10.1016/j.neubiorev.2005.03.027

Fang, W., Zhang, J., Hong, L., Huang, W., Dai, X., Ye, Q., Chen, X., 2020. Metformin ameliorates stress-induced depression-like behaviors via enhancing the expression of BDNF by activating AMPK/CREB-mediated histone acetylation. J. Affect. Disord. 260, 302–313. doi:10.1016/j.jad.2019.09.013

Fox, J., Weisberg, S., 2019. An {R} Companion to Applied Regression, Third Edition [WWW Document]. URL https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Golden, S.A., Covington, H.E., Berton, O., Russo, S.J., 2011. A standardized protocol for repeated social defeat stress in mice. Nat. Protoc. 6, 1183–1191. doi:10.1038/nprot.2011.361

Grosjean, P., Ibanez, F., 2018. pastecs: Package for Analysis of Space-Time Ecological Series [R package version 1.3.21] [WWW Document]. URL https://cran.r-project.org/web/packages/pastecs/index.html

Grossman, Y.S., Fillinger, C., Manganaro, A., Voren, G., Waldman, R., Zou, T., Janssen, W.G., Kenny, P.J., Dumitriu, D., 2022. Structure and function differences in the prelimbic cortex to basolateral amygdala circuit mediate trait vulnerability in a novel model of acute social defeat stress in male mice. Neuropsychopharmacology 47, 788–799. doi:10.1038/s41386-021-01229-6

Gururajan, A., van de Wouw, M., Boehme, M., Becker, T., O'Connor, R., Bastiaanssen, T.F.S., Moloney, G.M., Lyte, J.M., Ventura Silva, A.P., Merckx, B., Dinan, T.G., Cryan, J.F., 2019. Resilience to chronic stress is associated with specific neurobiological, neuroendocrine and immune responses. Brain Behav. Immun. 80, 583–594. doi:10.1016/j.bbi.2019.05.004

Haller, J., Halasz, J., Makara, G.B., Kruk, M.R., 1998. Acute effects of glucocorticoids: behavioral and pharmacological perspectives. Neurosci. Biobehav. Rev. 23, 337–344. doi:10.1016/s0149-7634(98)00035-9

Hammels, C., Pishva, E., De Vry, J., van den Hove, D.L.A., Prickaerts, J., van Winkel, R., Selten, J.-P., Lesch, K.-P., Daskalakis, N.P., Steinbusch, H.W.M., van Os, J., Kenis, G., Rutten, B.P.F., 2015. Defeat stress in rodents: From behavior to molecules. Neurosci. Biobehav. Rev. 59, 111–140. doi:10.1016/j.neubiorev.2015.10.006

Harris, A.Z., Atsak, P., Bretton, Z.H., Holt, E.S., Alam, R., Morton, M.P., Abbas, A.I., Leonardo, E.D., Bolkan, S.S., Hen, R., Gordon, J.A., 2018. A novel method for chronic social defeat stress in female mice. Neuropsychopharmacology 43, 1276–1283. doi:10.1038/npp.2017.259

He, Y., Li, W., Tian, Y., Chen, X., Cheng, K., Xu, K., Li, C., Wang, H., Qu, C., Wang, C., Li, P., Chen, H., Xie, P., 2018. iTRAQ-based proteomics suggests LRP6, NPY and NPY2R perturbation in the hippocampus involved in CSDS may induce resilience and susceptibility. Life Sci. 211, 102–117. doi:10.1016/j.lfs.2018.09.016

Hollis, F., Kabbaj, M., 2014. Social defeat as an animal model for depression. ILAR J. 55, 221–232. doi:10.1093/ilar/ilu002

Horii, Y., Nagasawa, T., Sakakibara, H., Takahashi, A., Tanave, A., Matsumoto, Y., Nagayama, H., Yoshimi, K., Yasuda, M.T., Shimoi, K., Koide, T., 2017. Hierarchy in the home cage affects behaviour and gene expression in group-housed C57BL/6 male mice. Sci. Rep. 7, 6991. doi:10.1038/s41598-017-07233-5

Hultman, R., Ulrich, K., Sachs, B.D., Blount, C., Carlson, D.E., Ndubuizu, N., Bagot, R.C., Parise, E.M., Vu, M.-A.T., Gallagher, N.M., Wang, J., Silva, A.J., Deisseroth, K., Mague, S.D., Caron, M.G., Nestler, E.J., Carin, L., Dzirasa, K., 2018. Brain-wide Electrical Spatiotemporal Dynamics Encode Depression Vulnerability. Cell 173, 166–180.e14. doi:10.1016/j.cell.2018.02.012

Iio, W., Takagi, H., Ogawa, Y., Tsukahara, T., Chohnan, S., Toyoda, A., 2014. Effects of chronic social defeat stress on peripheral leptin and its hypothalamic actions. BMC Neurosci. 15, 72. doi:10.1186/1471-2202-15-72

Jeong, J.Y., Lee, D.H., Kang, S.S., 2013. Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice. Endocrinol Metab (Seoul) 28, 288–296. doi:10.3803/EnM.2013.28.4.288

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935. doi:10.1016/s0149-7634(99)00026-3

Krishnan, V., Han, M.-H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., Laplant, Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga, C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391–404. doi:10.1016/j.cell.2007.09.018

Larrieu, T., Cherix, A., Duque, A., Rodrigues, J., Lei, H., Gruetter, R., Sandi, C., 2017. Hierarchical status predicts behavioral vulnerability and nucleus accumbens metabolic profile following chronic social defeat stress. Curr. Biol. 27, 2202–2210.e4. doi:10.1016/j.cub.2017.06.027

Lee, C.-W., Fang, Y.-P., Chu, M.-C., Chung, Y.-J., Chi, H., Tang, C.-W., So, E.C., Lin, Hsin-Chuan, Lin, Hui-Ching, 2021. Differential mechanisms of synaptic plasticity for susceptibility and resilience to chronic social defeat stress in male mice. Biochem. Biophys. Res. Commun. 562, 112–118. doi:10.1016/j.bbrc.2021.05.064

Lenth, R., 2020. Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.0 [WWW Document]. URL https://CRAN.R-project.org/package=emmeans

Logan, R.W., 2019. Adapting Social Defeat Stress for Female Mice Using Species-Typical Interfemale Aggression. Biol. Psychiatry 86, e31–e32. doi:10.1016/j.biopsych.2019.08.007

Lu, J., Gong, X., Yao, X., Guang, Y., Yang, H., Ji, R., He, Y., Zhou, W., Wang, H., Wang, W., Bai, S., Guo, H., Guo, Z.V., Xie, P., 2021. Prolonged chronic social defeat stress promotes less resilience and higher uniformity in depression-like behaviors in adult male mice. Biochem. Biophys. Res. Commun. 553, 107–113. doi:10.1016/j.bbrc.2021.03.058

Martinez, M., Calvo-Torrent, A., Pico-Alfonso, M.A., 1998. Social defeat and subordination as models of social stress in laboratory rodents: A review. Aggress Behav 24, 241–256. doi:10.1002/(SICI)1098-2337(1998)24:4<241::AID-AB1>3.0.CO;2-M

McEwen, B.S., Akil, H., 2020. Revisiting the stress concept: implications for affective disorders. J. Neurosci. 40, 12–21. doi:10.1523/JNEUROSCI.0733-19.2019

McLaughlin, J.P., Li, S., Valdez, J., Chavkin, T.A., Chavkin, C., 2006. Social defeat stress-induced behavioral responses are mediated by the endogenous kappa opioid system. Neuropsychopharmacology 31, 1241–1248. doi:10.1038/sj.npp.1300872

Milic, M., Schmitt, U., Lutz, B., Müller, M.B., 2021. Individual baseline behavioral traits predict the resilience phenotype after chronic social defeat. Neurobiol. Stress 14, 100290. doi:10.1016/j.ynstr.2020.100290

Moore, R.Y., Eichler, V.B., 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. 42, 201–206. doi:10.1016/0006-8993(72)90054-6

Muir, J., Lorsch, Z.S., Ramakrishnan, C., Deisseroth, K., Nestler, E.J., Calipari, E.S., Bagot, R.C., 2018. In vivo fiber photometry reveals signature of future stress susceptibility in nucleus accumbens. Neuropsychopharmacology 43, 255–263. doi:10.1038/npp.2017.122

Muir, J., Tse, Y.C., Iyer, E.S., Biris, J., Cvetkovska, V., Lopez, J., Bagot, R.C., 2020. Ventral Hippocampal Afferents to Nucleus Accumbens Encode Both Latent Vulnerability and Stress-Induced Susceptibility. Biol. Psychiatry. doi:10.1016/j.biopsych.2020.05.021

Nasca, C., Menard, C., Hodes, G., Bigio, B., Pena, C., Lorsch, Z., Zelli, D., Ferris, A., Kana, V., Purushothaman, I., Dobbin, J., Nassim, M., DeAngelis, P., Merad, M., Rasgon, N., Meaney, M., Nestler, E.J., McEwen, B.S., Russo, S.J., 2019. Multidimensional predictors of susceptibility and resilience to social defeat stress. Biol. Psychiatry 86, 483–491. doi:10.1016/j.biopsych.2019.06.030

Pagliusi, M, Bonet, I.J.M., Brandão, A.F., Magalhães, S.F., Tambeli, C.H., Parada, C.A., Sartori, C.R., 2020. Therapeutic and Preventive Effect of Voluntary Running Wheel Exercise on Social Defeat Stress (SDS)-induced Depressive-like Behavior and Chronic Pain in Mice. Neuroscience 428, 165–177. doi:10.1016/j.neuroscience.2019.12.037

Pagliusi, Marco, Bonet, I.J.M., Lemes, J.B.P., Oliveira, A.L.L., Carvalho, N.S., Tambeli, C.H., Parada, C.A., Sartori, C.R., 2020. Social defeat stress-induced hyperalgesia is mediated by nav 1.8+ nociceptive fibers. Neurosci. Lett. 729, 135006. doi:10.1016/j.neulet.2020.135006

Pedersen, C.A., Vadlamudi, S., Boccia, M.L., Moy, S.S., 2011. Variations in Maternal Behavior in C57BL/6J Mice: Behavioral Comparisons between Adult Offspring of High and Low Pup-Licking Mothers. Front. Psychiatry 2, 42. doi:10.3389/fpsyt.2011.00042

Pinhero, J., Bates, D., S, D., D, S., R Core Team, 2020. nlme: Linear and Nonlinear Mixed Effects Models [R package version 3.1-153] [WWW Document]. URL https://cran.r-project.org/web/packages/nlme/index.html

Scott, J.P., 1966. Agonistic behavior of mice and rats: a review. Am Zool 6, 683–701. doi:10.1093/icb/6.4.683

Shang, C., Chen, Z., Liu, A., Li, Y., Zhang, J., Qu, B., Yan, F., Zhang, Y., Liu, W., Liu, Z., Guo, X., Li, D., Wang, Y., Cao, P., 2018. Divergent midbrain circuits orchestrate escape and freezing responses to looming stimuli in mice. Nat. Commun. 9, 1232. doi:10.1038/s41467-018-03580-7

Singmann, H., Bolker, B., Westfall, J., Aust, F., 2016. afex: Analysis of Factorial Experiments [R package version 1.0-1] [WWW Document]. afex: Analysis of Factorial Experiments. R package version 0.16-1. URL https://cran.r-project.org/web/packages/afex/index.html

Takahashi, A., Chung, J.-R., Zhang, S., Zhang, H., Grossman, Y., Aleyasin, H., Flanigan, M.E., Pfau, M.L., Menard, C., Dumitriu, D., Hodes, G.E., McEwen, B.S., Nestler, E.J., Han, M.-H., Russo, S.J., 2017. Establishment of a repeated social defeat stress model in female mice. Sci. Rep. 7, 12838. doi:10.1038/s41598-017-12811-8

Tuttle, A.H., Philip, V.M., Chesler, E.J., Mogil, J.S., 2018. Comparing phenotypic variation between inbred and outbred mice. Nat. Methods 15, 994–996. doi:10.1038/s41592-018-

0224-7

van Doeselaar, L., Yang, H., Bordes, J., Brix, L., Engelhardt, C., Tang, F., Schmidt, M.V., 2021. Chronic social defeat stress in female mice leads to sex-specific behavioral and neuroendocrine effects. Stress 24, 168–180. doi:10.1080/10253890.2020.1864319

Wahlsten, D., Bachmanov, A., Finn, D.A., Crabbe, J.C., 2006. Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades. Proc. Natl. Acad. Sci. USA 103, 16364–16369. doi:10.1073/pnas.0605342103

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. Neurobiol. Stress 1, 164–173. doi:10.1016/j.ynstr.2014.11.002

Young, E.A., Lopez, J.F., Murphy-Weinberg, V., Watson, S.J., Akil, H., 2000. Hormonal evidence for altered responsiveness to social stress in major depression. Neuropsychopharmacology 23, 411–418. doi:10.1016/S0893-133X(00)00129-9

Zhang, J., He, Z.-X., Qu, Y.-S., Li, L.-F., Wang, L.-M., Yuan, W., Hou, W.-J., Zhu, Y.-Q., Cai, W.-Q., Zhang, X.-N., Guo, Q.-Q., An, S.-C., Jia, R., Tai, F.-D., 2021. Different baseline physical activity predicts susceptibility and resilience to chronic social defeat stress in mice: Involvement of dopamine neurons. Eur. Neuropsychopharmacol. 45, 15–28. doi:10.1016/j.euroneuro.2021.02.011

Zhang, K., Sakamoto, A., Chang, L., Qu, Y., Wang, S., Pu, Y., Tan, Y., Wang, X., Fujita, Y., Ishima, T., Hatano, M., Hashimoto, K., 2021. Splenic NKG2D confers resilience versus susceptibility in mice after chronic social defeat stress: beneficial effects of (R)-ketamine. Eur Arch Psychiatry Clin Neurosci 271, 447–456. doi:10.1007/s00406-019-01092-z

Chapter 3 Individual Differences in Neural Activity Patterns Predict Vulnerability to Chronic Social Defeat Stress

3.1 Introduction

Social stress is experienced by all social animals, including humans. Some individuals are especially sensitive to social stress, and that vulnerability can trigger the development or expression of affective disorders such as clinical anxiety, major depressive disorder, and others (Fox & Kalin, 2014; Lydiard, 2001; Pontillo et al., 2019). This vulnerability to social stress depends on a multitude of factors, including genetic, developmental, environmental, and behavioral. Notably, being able to evaluate and cope with stressful social events as they unfold plays a large role in the capacity to overcome the consequences of a stressor without long-lasting detrimental effects (Bienvenu, Hettema, Neale, Prescott, & Kendler, 2007; Billings & Moos, 1984; Derryberry, Reed, & Pilkenton–Taylor, 2003; K. M. Lee, Shellman, Osmer, Day, & Dempsey, 2016; Wright, Banerjee, Hoek, Rieffe, & Novin, 2010; Yi, Smith, & Vitaliano, 2005). However, the neurobiological mechanisms underlying the role of coping strategies on the long-term impact of social stress remain to be elucidated.

The rodent resident-intruder paradigm, which relies on a series of agonistic encounters between an aggressive resident animal and an intruder conspecific, has been used as a naturalistic social stress model (Hollis & Kabbaj, 2014; Koolhaas et al., 2013). Following these stressful encounters, rodents diverge into groups of 'winners' and 'losers,' establishing dominant and subordinate hierarchies between each pair. Early work from our lab first mapped the neural circuitry associated with social dominance hierarchies. Syrian hamsters that use defensive

(subordinate) versus offensive (dominant) behaviors during the fight showed clear differences in Fos activity patterns that map onto their behavioral profiles (Kollack-Walker, Watson, & Akil, 1997). There is now clear evidence in several species that active versus passive coping strategies are mediated through the activation of distinctive neural circuitry throughout the brain (Barbano et al., 2020; Chang & Gean, 2019; de Boer, Buwalda, & Koolhaas, 2017; De Franceschi, Vivattanasarn, Saleem, & Solomon, 2016; Falkner, Grosenick, Davidson, Deisseroth, & Lin, 2016; Kim et al., 2019; Nordman et al., 2020; Shang et al., 2018; Vale, Evans, & Branco, 2017; Wang et al., 2019; Wei, Talwar, & Lin, 2021; Wong et al., 2016). It is also clear that patterns of neural activation are altered by repeated exposure to the social stressor (Kollack-Walker, Don, Watson, & Akil, 1999). However, what remains unclear is how early behavioral and neural responses to a social stressor shape the eventual long-term response to chronic stress and result in enduring social consequences associated with vulnerability or resilience to social interactions.

In mice, the chronic social defeat stress paradigm (CSDS) is a widely validated model of individual variation in response to social stress (Hammels et al., 2015; Krishnan et al., 2007). In this paradigm, inbred C57BL6/J intruder mice are repeatedly subjected to bouts of physical and psychosocial stress by a larger, aggressive resident CD1 mouse. Following CSDS, the intruder mice diverge into two distinct populations of social reactivity: a socially interactive "Resilient" group and a socially avoidant "Susceptible" group (Golden, Covington, Berton, & Russo, 2011). While it has been established that several days of social defeat are necessary before the Resilient and Susceptible classification emerge (Bagot et al., 2015; Kudryavtseva, 1994; Wells et al., 2017), work from our lab (see Chapter 2) found that behavioral coping strategies used during the initial stress encounter were a predictive factor in whether resilience or susceptibility arose in the final social interaction test. Thus, mice that emerge as Susceptible display greater "escape"

behavior on Day 1 of Defeat compared to those that emerge as Resilient (Murra et al., 2022).

These individual variations in the behavioral response to stress are likely associated with distinct

neural response patterns that likely shape the responses to subsequent social stressors and the

ultimate susceptibility or resilience in a social context.

Several brain regions known to play a role in major depressive disorder (MDD) have also

been implicated in mediating reactivity to social stress, including the prelimbic cortex (PrL),

nucleus accumbens (NAc), amygdala (AMY), ventral hippocampus (vHipp), and ventral

tegmental area (VTA) (Mayberg et al., 2005; Nestler et al., 2002). However, whether this neural

circuitry differs before the chronic social defeat, or whether it emerges as a result of the repeated

stress remains unclear. To our knowledge, only two studies have attempted to address this

question in the context of the CSDS model. One study relied on in vivo fiber photometry and

found that a baseline increase in D1-MSN activity in the NAc was a predictive marker of future

resilience but not susceptibility (Muir et al., 2018). Another study relied on in vivo local field

potential recordings in the MDD-network brain regions and found that before stress, subtle

differences in oscillatory signatures mapped onto future resilience versus susceptibility (Hultman

et al., 2018). Other circuit-based studies have focused on assessing neural circuits after the stress

has occurred (Anacker et al., 2016; Bagot et al., 2015; Chaudhury et al., 2013; Heshmati et al.,

2020; Hultman et al., 2018; Muir et al., 2020; Nam et al., 2019; Vialou et al., 2010). However, to

understand the variables that set the stage for the emergence of susceptibility versus resilience, it

is important to capture differential responses early in the process that can shape the course of

subsequent behavior. The practical challenge resides in the fact that the classification as

Susceptible vs. resilience requires a multi-day course of daily social stress. Recent advances in

mouse technology have allowed us to address this challenge. We can now capture brain-wide

neural activity patterns in living animals during the first episode of social defeat and allow them to live on to undergo the chronic stress experience and be further classified into Resilient or Susceptible groups.

Neuronal activity-related genes, such as the immediate early gene Fos, have been a powerful tool in labeling neuronal ensembles based on behavior, as these genes are rapidly induced, but their activation is transient (Bullitt, 1990). While classic Fos studies can only take place post-mortem, the FosTRAP technique enables in vivo capture of neuronal activity. The FosTRAP mouse line links a promotor-driven Fos Cre-ERT2 system in a tamoxifen-dependent manner to an LSL-tdTomato reporter to allow for permanent brain-wide labeling of activated neurons restricted to a specific time window (DeNardo et al., 2019; Guenthner, Miyamichi, Yang, Heller, & Luo, 2013) (**Fig. 3.1A, B**). Using this system, we labeled and quantified brain-wide ensembles of neurons that are activated on Day 1 of stress to permanently 'TRAP' the stress-naïve circuit to then test the hypothesis that brain-wide activation patterns that emerge during the initial defeat encounter predict eventual social reactivity. We found evidence for selective brain regions and networks that are activated on Day 1 that are specific to Defeat alone, and others that distinguish between groups that will eventually emerge as Resilient or Susceptible.
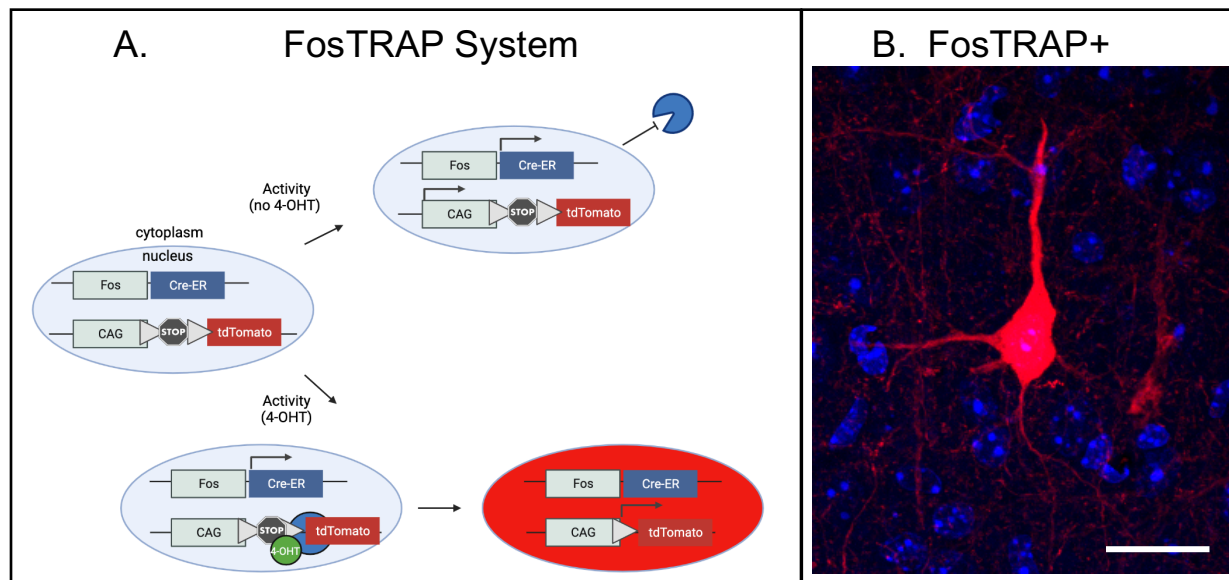
Figure 3.1 FosTRAP System Approach
A. TRAP requires two transgenes: one that expresses CreERT2from an activity-dependent Fos promoter and one that allows expression of an effector gene, such as tdTomato, in a Cre-dependent manner. Without tamoxifen (TM), CreERT2is retained in the cytoplasm of active cellsin which it is expressed, so no recombination can occur (top). In the presence of TM, CreERT2 recombination can occur in active cells (bottom), whereas nonactive cells do not undergo recombination, because they do not express CreERT2 Adapted by permission from Elseiver: Neuron. Permanent Genetic Access to Transiently Active Neurons via TRAP: Targeted Recombination in Active Populations. Guenther (2013). DOI: 10.1016/j.neuron.2013.03.025.PMID: 23764283 B. Visualization of a FosTRAP+ neuron (imaged at 40X). Red= FosTRAP+, blue=DAPI. Scale bar = 20μm.

3.2 Methods

Animals: Transgenic FosTRAP2 (Jackson Laboratory; Fos2mt.1(iCreERT2)Luo/J; strain #(030323)), and Ai14D(Jackson Laboratory; B6l;129S6-Gt(ROSA)26sortm14(CAG-tdtomato)Hze/J; Strain #007908) mouse lines were crossed in-house to generate FosTRAP2-Ai14 mice. In total, 32 FosTRAP2-Ai14 male mice (10-12 weeks of age) were used for the social Defeat and were housed 2-4 per cage until start of experiment. 36 male CD1 retired breeders (3-6 months) from Charles River Laboratories (Wilmington, MA) were used as aggressors and social interaction stimulus mice. Enrichment in the form of enviro-paks was provided before and after social Defeat. Housing rooms were kept under a 12 h light: 12 h dark cycle starting at 7 AM.

Mice were provided with mouse chow and tap water *ab libitum*, in accordance with the *NIH Guidelines for the Care and Use of Laboratory Animals. All animal protocols utilized were approved by the University of Michigan Institute of Animal Use Care and Use Committee (IAUCAC).*

Chroinc social defeat stress (CSDS) and Control Rotation (CR): CSDS and CR was conducted according to a previously described protocol (Golden et al., 2011). CD1 mice were screened for aggression (defined as <1-minute interval latency to attack for two consecutive days) before use in CSDS. During CSDS, FosTRAP2-Ai14 mice were subjected to 5-minute bouts of agonistic encounters from a novel, aggressive CD1 mouse for ten consecutive days. After each session, the FosTRAP2-Ai14 mouse was rotated to a new CD1 aggressor. Following the Defeat, the FosTRAP2-Ai14 mouse was placed in the CD1 resident cage (27x48x15cm) across a plexiglass divider from the CD1. In the same cage set-up, Control mice had no physical interaction but were placed across from each other and rotated to a new partner for ten consecutive days. All sessions occurred between 9 am-11 am each day. Following CSDS or CR, FosTRAP2-Ai14 mice were singly housed in static cages with enviro-pak enrichment.

Coping Behavior: Defeat sessions were recorded with a video camera and were manually scored by a blinded observer. During the physical bouts of Defeat, coping strategies were scored as follows: total duration of engagement in fight action (sec), the total number of fights, escapes, and upright, forward, and crouch-back freezing (see Chapter 2 & Murra et al., 2022 for behavior descriptions). Coping behavior is reported as a ratio of coping strategy measured/total coping strategies.

Social Interaction Test (SI): Following ten days of Defeat, the SI test was conducted in the AM on Day 11. The SI test consists of two 2.5-minute trials that occur in an open field arena

(41x41x40cm) containing a rectangular interaction zone (14x24cm) with a removable wire mesh stimulus cage (10x10x10cm) against a sidewall. During the first trial, the experimental mouse was allowed to explore the arena with the empty cage. Then, the experimental mouse is removed, and a novel, aggressive CD1 mouse, is placed in the cage. During this second trial, the experimental mouse is placed back in the open field to explore again. Each session was recorded, and the mouse's exploration of the interaction zone was scored using automatic tracking software (Noldus Ethovision XT software; Noldus Technology, Leesburg, VA). The SI ratio is calculated as time spent in the interaction zone with CD1 present/CD1 not present. An SI ratio of < 1 indicates Susceptible, while ≥ 1 indicates Resilient.

Tamoxifen: 1 hour following the 5-minute Defeat encounter on Day 1, FosTRAP2-Ai14 were injected with 4-hydroxy-tamoxifen (4-OHT) intraperitoneal at a dose of 50mg/kg. 4TM (Sigma H6278) was dissolved in an aqueous solution containing 10% DMSO and 10% Tween-80 in saline. All cages were changed the following day to avoid the re-uptake of Tamoxifen.

Histology: FosTRAP2-Ai14 mice were perfused transcardially with cold 1X PBS solution followed by 4% PFA. Brains were post-fixed in 4% PFA overnight and dehydrated with 20% sucrose, then flash-frozen in -20°C isopentane. Coronal sections were collected in a series of 10 at 20µM using a freezing cryostat at -24°C and immediately mounted to the glass slide. Slides were stored at -80°C until processing. The sampling rate was every 200µM starting anterior at bregma 1.98 and ending posterior at bregma -3.08. Slides were washed 3x5-min with 1X PBS and then incubated with DAPI 1:10,000 for 10 min. Slides were then washed 3x5min, and coverslipped using Invitrogen™ Prolong™ Gold Antifade Mountant (Cat#P10144).

Imaging Parameters: All images were taken on an Olympus IX83 Inverted Microscope at a 10x objective and imaged at 647nm (FosTRAP+tdTomato cells) and 405nm (DAPI).

Cell Counting and Registration: Individual cell bodies for both FosTRAP+ was identified using the automated cell detection feature in NeuroInfo (MBF Bioscience, Williston, VT). For FosTRAP+ cells, the largest cell diameter detected was 40μM, and the smallest was 6μM at an intensity of 16-26 nearest to the background (depending on the section). The NeuroInfo 'Registration' feature was used on the DAPI channel to register individual sections to the Allen Mouse Brain Atlas.

Statistical Analysis: Graphpad Prism and R (version 4.0.2) were used for figure design and statistical analyses. In R, *pastecs, psych, nlme, pheatmap, and emmeans* packages were used for visual and statistical analyses. *Cor* function was used to assess correlations between all brain regions within Control, Defeat, Resilient, and Susceptible groups. *Pheatmap* was used to visualize clusters of correlations between brain regions and groups. The *nlme* package with the *lm* function was used to run linear regressions on all brain regions between groups and post-hoc Tukey multiple comparisons was done using the *emmeans* package. The *lm* function was used to run a linear regression between brain region and behavioral interactions. For analyzing differences in coping strategies between groups on Day 1, Prism Mann-Whitney T-test function was used. All significance thresholds were set at $p{\leq}0.05$ and FDR at <0.1.

3.3 Results
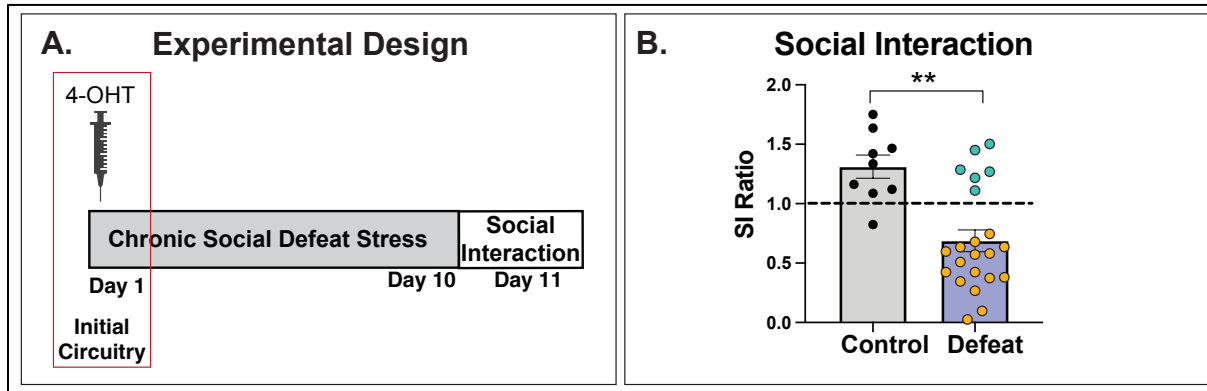
3.3.1 Characterizing the Behavioral Response

Figure 3.2 Chronic Social Defeat Stress in FosTRAP2 Mice
A. Experimental Design: 4-hydroxytamoxifen (4-OHT) is injected 1 hour following 5 minutes of CSDS to capture the neural circuitry on Day 1. B. Social Interaction (SI): Defeated mice show a significant reduction in SI compared to controls (p=0.001, Mann-Whitney U=27).

In order to assess neural circuitry associated with the initial Defeat encounter, we injected FosTRAP2 mice with aqueous 4-hydroxytamoxifen (4-OHT) on Day 1 of social Defeat. FosTRAP2 mice then experienced the traditional trajectory of all 10 days of social Defeat and were then placed in the social interaction test for final classification **(Fig. 3.2A).** Control mice demonstrated a preference for social interaction, with a mean SI Ratio of 1.311 (*sd=+/-0.292*). In contrast, mice that experienced social Defeat displayed a significant reduction in SI Ratio, with a mean of 0.688 (*sd=+/-0.430*) **(Fig. 3.2B**; *p*=0.001, Mann-Whitney U=27). As a result, 6 animals were defined as Resilient (socially interactive) and 16 were defined as Susceptible (socially avoidant).