THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

Exploring the Effects of Adolescent Exposure to Social Instability Stress on Anxiety and Risk-Assessment Behavior in Male C57BL/6J and C57BL/6NJ Mice

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ABSTRACT

Adolescence is a critical period for emotional and cognitive development, during which stress can have lasting effects on behavior and mental health, including the development of anxiety. Social instability stress (SIS), a model of chronic early-life stress, mimics social challenges often faced by adolescents in human populations and induces psychological stress in rodents. Risk-assessment behavior, involving cautious exploration and threat evaluation, is crucial for survival and plays a significant role in human maturation. Risk assessment and anxiety are closely intertwined, as heightened anxiety can lead to risk aversion and/or changes in risk-assessment behavior. Understanding how SIS impacts anxiety-like and risk-assessment behaviors during adolescence is essential, as the behaviors are closely linked to long-term mental health outcomes, including anxiety disorders and how adolescents respond to stress. This honors thesis investigated the effects of SIS on anxiety-like and risk-assessment behaviors in male adolescent C57BL/6J and C57BL/6NJ mice, also exploring the genetic influences on stress susceptibility during adolescence. It was hypothesized that SIS would increase anxiety-like and risk-assessment behavior. Mice were randomly assigned to a SIS or control condition and tested on the elevated plus maze (EPM). Results showed that SIS increased anxiety-like behavior and affected risk-assessment behavior in the EPM, emphasizing the impact of adverse social experiences on health throughout development. A strain-stress interaction for stretch attend posture (SAP) frequency suggests genetic differences in stress regulation and response, which could explain why individuals differ in stress susceptibility. These findings indicate the potential for future research and have important implications for how stress is regulated and how the onset of stress impacts risk-assessment behavior.

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Finally, and most importantly, I want to thank my parents. Thank you for always believing in me and encouraging me to follow my dreams. It is because of you that I have the courage to "risk going too far". I couldn't have survived these four years at Penn State without your love and support, and I am forever grateful for everything you have done – and continue to do - for me. This thesis is dedicated to you both.

Chapter 1

Introduction

Adolescence is a critical developmental period in which social stimuli are more salient as humans mature and is a sensitive time for the effects of stress on mental and behavioral health (Mason et al., 2016). It is associated with the onset of psychiatric disorders and behaviors that affect long-term health, including anxiety (Paus, Keshavan, & Giedd, 2009). In addition, during early to mid-adolescence, heightened risk-taking behavior is typically observed and could be explained by an imbalance that favors behavior driven by emotion and rewards over rational choices (Sawyer et al., 2012). Further, a multitude of health-related risky behaviors are initiated during this time, including smoking, alcohol and drug use, physical activity, and sexual behavior, all of which can maintain themselves into adult life (Viner et al., 2015).

Genetics have been shown to affect stress susceptibility and how an individual can evaluate and respond to stress (Buchanan & Lovallo, 2020). Although research is limited, studies have shown a genetic influence on stress susceptibility (Maul et al., 2019), which could explain why some individuals are more susceptible to stress and some are more resilient. Inbred mouse strains serve as valuable tools to explore genetic influences due to their highly similar genetic makeup. C57BL/6J and C57BL/6NJ mice were used as subjects in this study due to their striking genetic similarity, differing by only 34 single nucleotide polymorphisms, 2 indels in coding regions, and 15 structural gene variants (Sturm et al., 2014). Despite this limited genetic variability, notable differences in stress responses have been observed, highlighting genetics' subtle but significant role in susceptibility to stress. For instance, previous research found that

C57BL/6J mice exhibit less anxious behavior than C57BL/6NJ mice (Bryant, 2016), demonstrating a measurable phenotypic difference. Studying these closely related sub-strains allows for controlled comparisons and models the human condition, where individuals with similar genetic backgrounds may still differ in their responses to stress.

Anxiety and risk assessment are two interrelated behavioral processes that are modulated through adolescence and affected by stress. Anxiety disorders have a high prevalence in adolescence (Mathews et al., 2016) and can lead to several adverse outcomes, such as decreased academic performance, less peer acceptance, increased aggression, and increased depression (Mathews et al., 2016). Moreover, risk behavior is a natural part of adolescent development and peaks in late adolescence to early adulthood (Graham and Kahn, 2020). In mice, risk-taking behavior and risk assessment naturally exist to gather information and evaluate risks in new environments (Marques et al., 2007).

Studies suggest that heightened risk-taking in adolescence is the product of the interaction between two brain networks: a socioemotional network and a cognitive-control network (Steinberg, 2007), leading to the interaction between social and emotional stimuli and executive functioning resulting in risk-taking. Further, studies have found associations between anxiety and risk-taking behavior. Individuals with relatively high levels of trait anxiety showed less willingness to engage in risky behavior (Maner & Schmidt, 2006). Additionally, anxiety and risk-taking in adolescence seem to be somewhat transient, as children manifest many fears and anxieties as part of typical development (Beesdo, Knappe, & Pine, 2011), and risky choices seem to decline with advancing age (Mata et al., 2012). Studying the transient effects of anxiety and risk in adolescence is essential to fully understand how it can affect later-life mental health outcomes and development.

Further, the relationship between stress, anxiety, and risk is important to consider. The diagnostic and statistical manual of mental disorders includes many variations of anxiety, such as generalized anxiety disorder, panic disorder, and social anxiety disorder (American Psychiatric Association, 2022). Early life stressors and sensitivity to stress are strongly linked to many of these syndromes (Faravelli et al., 2012), highlighting the importance of exploring the relationship between adolescent stress and anxiety-like behavior. Further, evidence suggests that risk-taking behavior is influenced by emotional state, especially social stress, resulting in risk avoidance in low-emotion contexts and risk seeking in high-emotion contexts (Reynolds et al., 2013). Understanding the dynamics behind stress, anxiety, and risk is critical to consider as adolescents navigate uncertainty and make decisions in unfamiliar settings.

Social instability stress (SIS) is a well-documented paradigm that can induce anxiety-like behavior in rodents (Yohn et al., 2019). It is characterized by unpredictable changes in social hierarchies and interactions, typically achieved by frequently changing cage mates to create an unstable social environment (Koert et al., 2021). This models human social stress, such as shifting peer dynamics, social hierarchies, or interpersonal conflict. Adolescence is a particularly sensitive period for social stress, as the brain is in a high state of plasticity and vulnerability (Sterlemann et al., 2010). Research in both animal and human models has shown that social exclusion and stress increase anxiety more prominently during adolescence than in adulthood or early childhood (Fuhrmann et al., 2015).

The elevated plus maze (EPM) is one of the most widely used tests for assessing anxiety-like and risk-assessment behaviors in mice (Haller et al., 2013). It relies on rodents' natural aversion to open areas versus their exploratory drive to test for anxiety phenotypes and the presence of risk assessment (Kestering-Ferreira et al., 2021). The EPM is configured like a plus

sign and has two open arms across from each other that are perpendicular to two closed arms with a center platform. Open arms have no walls, and closed arms have a high wall to enclose the arm (Komada, Takao, & Miyakawa, 2008). Measures of anxiety include the number of open arm entries and time spent in the open arms (Rodgers et al., 1997), as mice with greater anxiety levels will spend less time in and make fewer entries into the exposed open arms (Pellow et al., 1985). The EPM can also be used to collect data on risk assessment behaviors such as head dipping, rearing, and stretch attend postures (Walf & Frye, 2007; Loh et al., 2022). Head dipping is the exploratory downward movement toward the floor from the open arms, rearing is the vertical standing of the rodent on two hindlegs and stretch attend posture is when the rodent is motionless but has body stretched around a wall of the EPM (Walf & Frye, 2007). These behaviors are highly responsive to changes in stress and anxiety, making the EPM a valuable tool for studying the impact of stress on adolescent behavior.

The relationship between social instability, stress, anxiety, and risk-assessment behaviors in adolescents prompts further investigation into how these factors interact and shape decision-making processes. Understanding the impact of stress on adolescents' cognitive and emotional responses to risk is crucial in comprehending the broader effects of social instability on behavior and mental health. Questions arise about how stress can influence not only individual choices but also the long-term developmental outcomes of those exposed to chronic stress.

The main goals of this study were (1) To assess the effects of social instability stress on anxiety-like behavior in adolescents and, (2) To assess how social instability stress affected risk-assessment behavior in adolescents and, (3) To distinguish differences in anxiety-like and risk-assessment behavior based on genetic factors that could contribute to individual susceptibility to

stress. We predicted that interactions between strain and stress would contribute to differences in anxiety-like and risk-assessment behavior.

Chapter 2

Methods

2.1 Subjects

16 male C57BL/6J and 16 male C57BL/6NJ mice were ordered from Jackson Laboratory (Bar Harbor, ME, USA). Mice arrived at our facility on postnatal day (PND) 28 and were allowed to acclimate to the animal facilities. Mice were housed in pairs, with ad libitum access to food (Lab Diet 5053, Lab Diet, St. Louis, Mo, USA) and water. Enrichment items in the form of one weekly piece of nesting material (Bed-r'Nest, The Andersons, Maumee, OH, USA) and one red polycarbonate mouse igloo (Bio-Serv, Flemington, NJ, USA) were in each cage. All treatments and testing occurred between 7 AM and 6 PM, which was within the light period of a 12-hour light/dark cycle (lights on at 7:00 AM). The Penn State Institutional Animal Care and Use Committee approved all described procedures, which were conducted per the NIH Guide for the Care and Use of Laboratory Animals. All ages listed are approximations and represent subject ages in postnatal days +/- 3 days.

2.2 Procedures

Mice were given a one-week acclimation period in the animal facility before beginning experimental procedures, placing them at PND 35 for the start of testing. The study followed a 2x2 factorial design, with two factors: Treatment (Control vs. 2 Weeks of Social Instability Stress (SIS)) and Strain (C57BL/6J (B6J) vs C57BL/6NJ (B6NJ)). After the acclimation period, half of the mice from each strain were assigned to the stress condition. From PND 35 to PND 49,

mice in the stress group were exposed to two weeks of SIS, as outlined in section 2.3. On PND 50, the mice were behaviorally tested on the Elevated Plus Maze (EPM) for five minutes, as performed in previous studies (Seemiller et al., 2023). Between PND 51 and PND 59, additional behavioral tests were conducted to assess the effects of adolescent social stress on anxiety-like, depressive-like, and social behaviors. These behaviors are not included in this paper as they are part of an ongoing experiment beyond the scope of this study. On PND 59, following the conclusion of all testing, mice were euthanized in a CO2 chamber, and hair samples were subsequently collected. The backs of the mice were shaved with a hair clipper, and the samples were stored in aluminum foil on ice before being transferred to a -80° C freezer for future sample processing. All procedures are summarized in Figure 1.

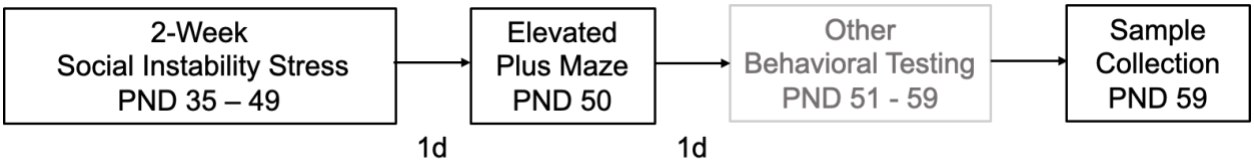

Figure 1. Procedure Timeline.

From PND 51-59, other behavioral testing is colored in light grey to denote that although additional behavioral testing was completed to assess anxiety-like, depressive-like, and social behavior, it will not be discussed in detail in this thesis.

2.3 Social Instability Stress Paradigm

Social instability stress was completed in three-day cycles, repeated twice weekly. On the morning of Day 1, mice in the stress condition were transported to the procedure room and restrained for 30 minutes in perforated 50 mL Falcon tubes secured to the procedure table to

prevent movement. After restraint, mice were returned to their home cages in the vivarium. In the afternoon of Day 1, mice in the stress condition were re-transported to the procedure room, placed in isolation for one hour in standard cages without bedding, nesting material, food, or water, and then subsequently moved to a new home cage with clean bedding, nesting material, a red igloo, regular food and water access, and a new cage mate before being returned to the vivarium.

Mice were left undisturbed overnight, and on Day 2, the same procedures were repeated. On the morning of Day 3, mice were briefly housed individually before being reassigned to a new home cage with their original weaning mate. They remained undisturbed for the remainder of the day. Day 4 was a rest day with no manipulations. From Days 5 to 7, the second cycle of stress was performed identically to Days 1 to 3. These cycles continued for two weeks.

Treatment groups were pseudorandomized, balancing strains to ensure each mouse encountered eight unfamiliar cage mates – four C57BL/6J and four C57BL/6NJ – over the two weeks. All stress procedures occurred outside the colony room or in a different room to avoid potential disruption from vocalizations and/or odor cues emitted by stressed mice. Control mice remained undisturbed in the vivarium. (Novoa, 2022).

2.4 Elevated Plus Maze (EPM)

On PND 50, subjects were placed in the EPM arena (55 cm tall; opposing 30 L x 7 W cm open arms and 30 L x 7 W x 13H cm closed arms; 6 L x 6 W cm center; gray acrylic) in dim lighting and tracked by Noldus Ethovision (Wageningen, Netherlands) for five minutes. Percent time in open arms was calculated as time spent in any open arm/total test time (300 s). Distance traveled was quantified in cm. Behaviors were manually scored to analyze risk assessment

behavior, analyzing the frequency and duration of each type of risk assessment behavior. Risk assessment was defined as head dips (the downward motion of mice's head toward the floor from the open arms), rears (vertical standing of rodent on two hindlegs), and stretch-attend postures (when the rodent is motionless but has body stretched forward/toward a stimulus or around the EPM walls) (Walf & Frye, 2007).

2.5 Hair Corticosterone

Upon completion of behavioral testing on PND 59, mice were sacrificed in a CO2 chamber. Immediately following euthanasia, hair samples were collected. Mice were shaved along the back with hair clippers, and samples were wrapped in aluminum foil and kept on regular ice for transport to the -80 C refrigerator. Samples were kept in the refrigerator for storage purposes until sample processing.

2.6 Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics 28. Thirty-two animals were distributed into four groups: C57BL/6J-control (n=8), C57BL/6J-SIS (n=8), C57BL/6NJ-control (n=8), and C57BL/6NJ-SIS (n=8). The significance level was set up to $\alpha < 0.05$ for all instances. Data are presented as group averages ± SEM. Figures were generated using Microsoft Excel. Risk assessment behavior was manually scored under blind conditions. Upon completion of scoring behaviors, data was analyzed using a two-way ANOVA test (Strain, Stress), followed by post-hoc comparisons when interactions were detected. Correlations were assessed using Spearman's rho correlation matrix.

For anxiety-like behaviors, we evaluated time spent in the open arms (calculated as time spent in any open arm / total test time (300s) and number of entries into the open arms. Risk-assessment behaviors were evaluated as rearing frequency and percentage time exhibiting rearing, head dip frequency and percentage time exhibiting head dipping, and stretch attend posture frequency and percentage time exhibiting stretch attend posture.

Chapter 3

Results

Adolescent social instability stress and genetic background moderated adult anxiety-like behavior (Figure 2). A main effect of strain was observed for percentage time in the open arms ($F_{(1/32)} = 7.75$, $p < 0.05$), overall C57BL/6J mice spent more time in the open arms, suggesting lower anxiety-like behaviors compared to C57BL/6NJ mice. There was also a main effect of stress on percentage time in the open arms ($F_{(1/32)} = 4.27$, $p < 0.05$); overall, mice exposed to adolescent social instability stress spent less time in the open arms, suggesting increased anxiety-like behaviors compared to control mice. There were no significant interaction effects between strain and stress ($F_{(1/32)} = .019$, $p > 0.05$), suggesting that both strains respond similarly to stress even though they have initial differences in anxiety behavior.

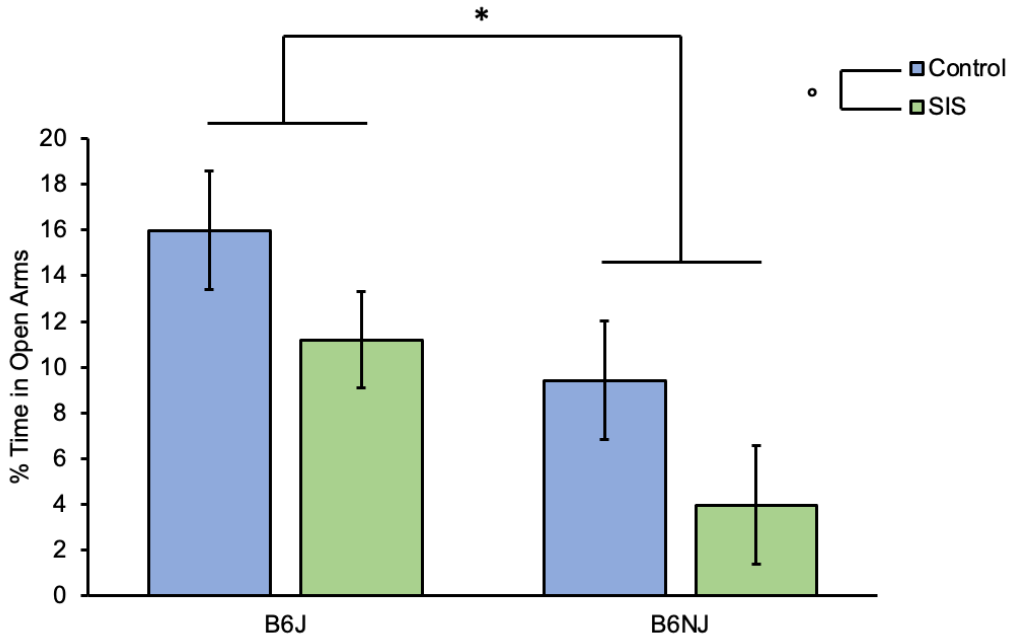

Figure 2. Percentage Time in the Open Arms.

Time spent in the open arms of the EPM as a display of anxiety-like behavior. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

Genetic background moderated spontaneous locomotor activity in the elevated plus maze (Figure 3). A main effect of strain on distance traveled was observed ($F_{(1/32)} = 23.7$, $p < 0.05$); overall, C57BL/6J mice traveled further in the elevated plus maze. No main effect of stress was observed for distance traveled ($F_{(1/32)} = 1.89$, $p > 0.05$), demonstrating that treatment does not significantly affect locomotion. No interaction was observed between strain and stress on distance traveled ($F_{(1/32)} = 3.067$, $p > 0.05$), suggesting that both strains reacted similarly to stress.

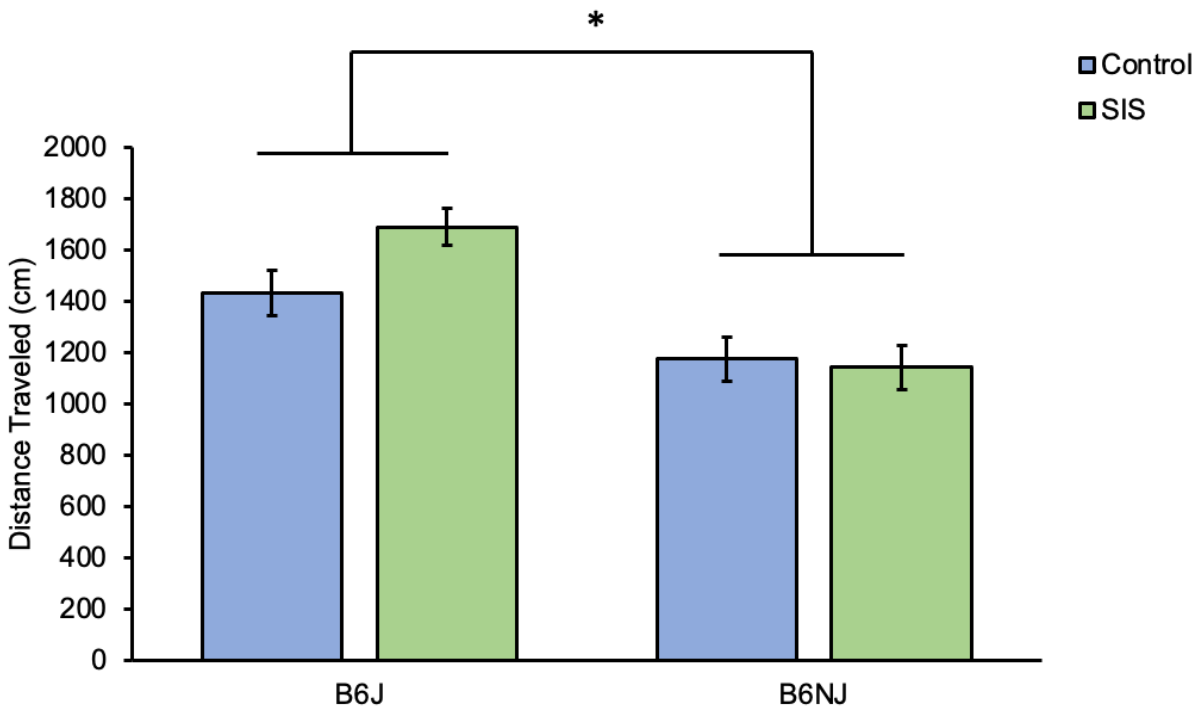

Figure 3. Distance Traveled in the EPM.

Distance traveled in the EPM in cm as a measure of locomotion. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

The frequency of risk-assessment behavior, as seen in rearing, was increased by social instability stress (Figure 4). A main effect of stress on rearing frequency was observed ($F_{(1/32)} = 7.95$, $p < 0.05$); overall, mice exposed to adolescent social instability stress displayed an increased number of rearing behaviors, suggesting that risk assessment increases following stress. No main effect of strain on rearing frequency was observed ($F_{(1/32)} = 1.03$, $p > 0.05$), and no interaction effect between strain and stress was observed ($F_{(1/32)} = .36$, $p > 0.05$). The lack of interaction effects suggests that both strains reacted similarly to stress.

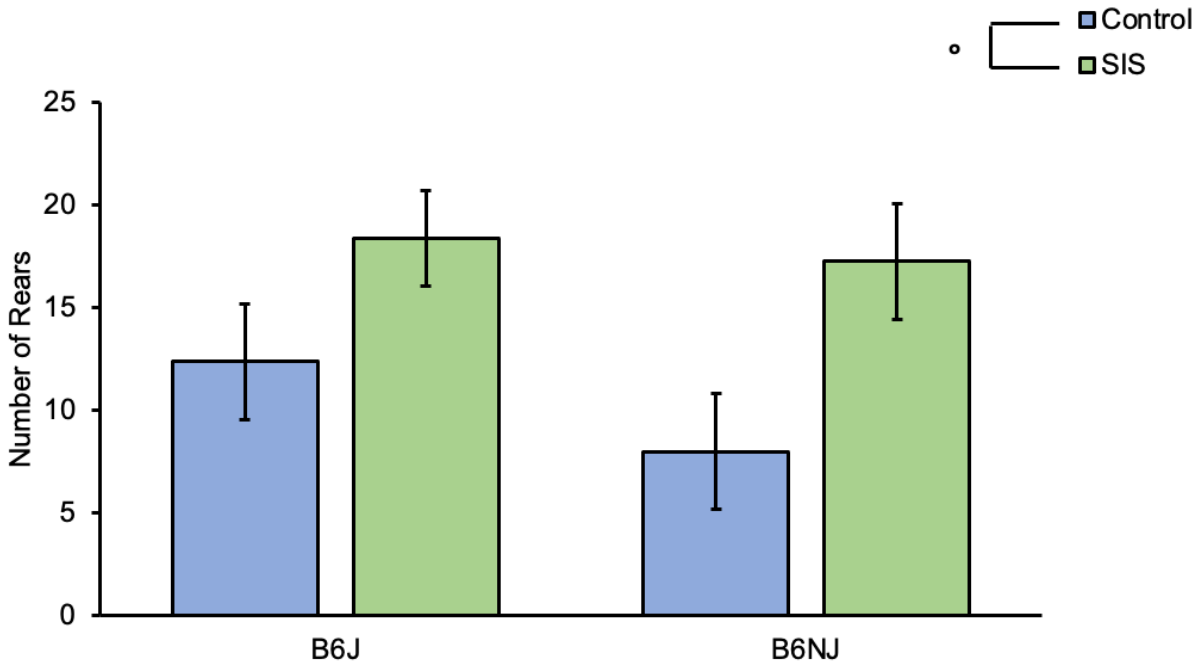

Figure 4. Rearing Frequency in the EPM.

Rearing frequency in the EPM as a measure of risk-assessment behavior. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

The percentage of time spent exhibiting risk-assessment behavior demonstrated by rearing was moderated by social instability stress (Figure 5). A main effect of stress was observed ($F_{(1/32)} = 8.61$, $p < 0.05$); overall, mice exposed to adolescent social instability stress displayed increased time spent exhibiting rearing behavior, suggesting an increase in risk-assessment behavior following stress. No main effect of strain was observed ($F_{(1/32)} = 1.09$, $p > 0.05$), and no significant interactions between strain and stress ($F_{(1/32)} = 1.34$, $p > 0.05$) were observed, suggesting that both strains reacted similarly to stress.

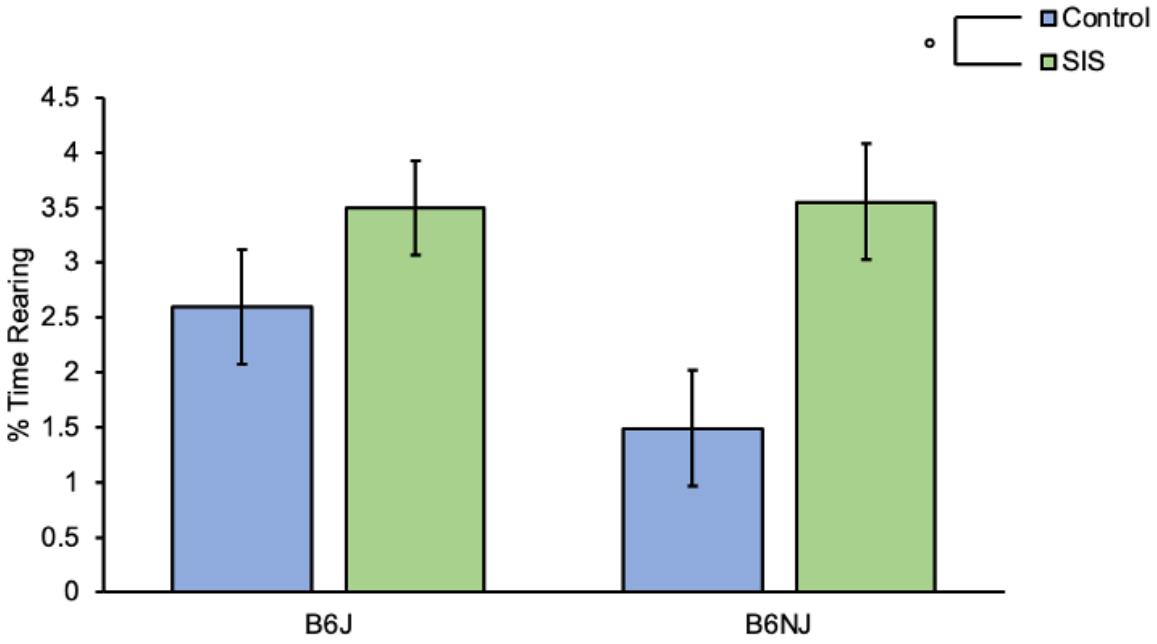

Figure 5. Percentage Time Rearing in the EPM.

Percentage time exhibiting rearing behavior in the EPM as a measure of risk-assessment behavior. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

Social instability stress affected risk-assessment behavior as seen as stretch-attend postures depending on the strain (Figure 6). No main effect of stress or strain was observed (Stress: $F_{(1/32)} = 1.44$, $p > 0.05$; Strain: $F_{(1/32)} = 3.45$, $p > 0.05$). However, there was a significant interaction of strain with stress ($F_{(1/32)} = 4.71$, $p < 0.05$), suggesting that social instability stress affects stretch-attend posture frequency differently for each strain. Post hoc comparisons showed that stressed mice act differently between strains (mean diff = 4.5, 0.95 CI = .484, 8.516, $p < 0.05$) and that only stressed C57BL/6J mice increase their stretch-attend posture frequency compared with their controls (mean diff = 5.375, 0.95 CI = 1.359, 9.391, $p < 0.05$), suggesting that genetic factors play a role in moderating susceptibility to stress on risk-assessment behavior.

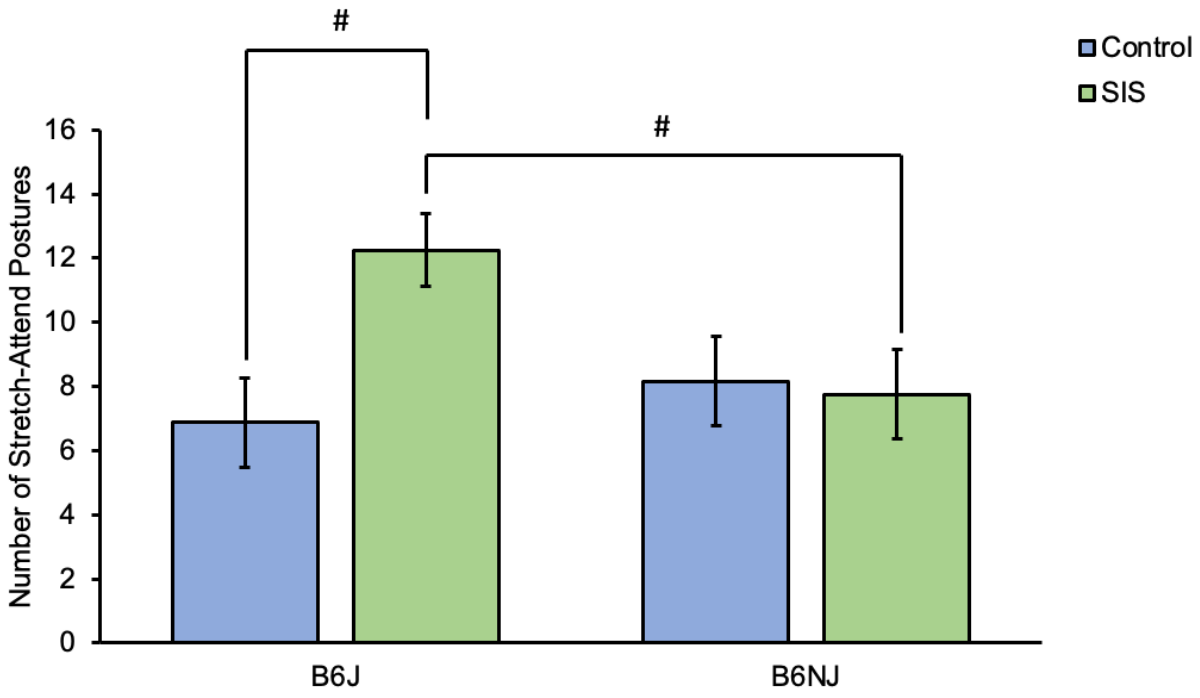

Figure 6. Frequency of Stretch-Attend Postures in the EPM.

Stretch-attend posture frequency in the EPM as a measure of risk-assessment behavior. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. Pound symbols represent significant interaction effects. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

Social instability stress increased risk-assessment behavior as seen in stretch attend posture behavior in mice (Figure 7). A main effect of stress was observed for the percentage of time spent exhibiting stretch attend posture ($F_{(1/32)} = 6.27$, $p < 0.05$). Overall, mice exposed to adolescent social instability stress displayed increased time spent exhibiting SAP, suggesting that social instability stress increases how long mice spend demonstrating stretch attend postures, displaying increased risk-assessment behavior. No main effect of strain on SAP percentage was observed ($F_{(1/32)} = .46$, $p > 0.05$), and no interaction between strain and stress was observed ($F(1/32) = .157$, $p > 0.05$), suggesting that both strains reacted similarly to stress.

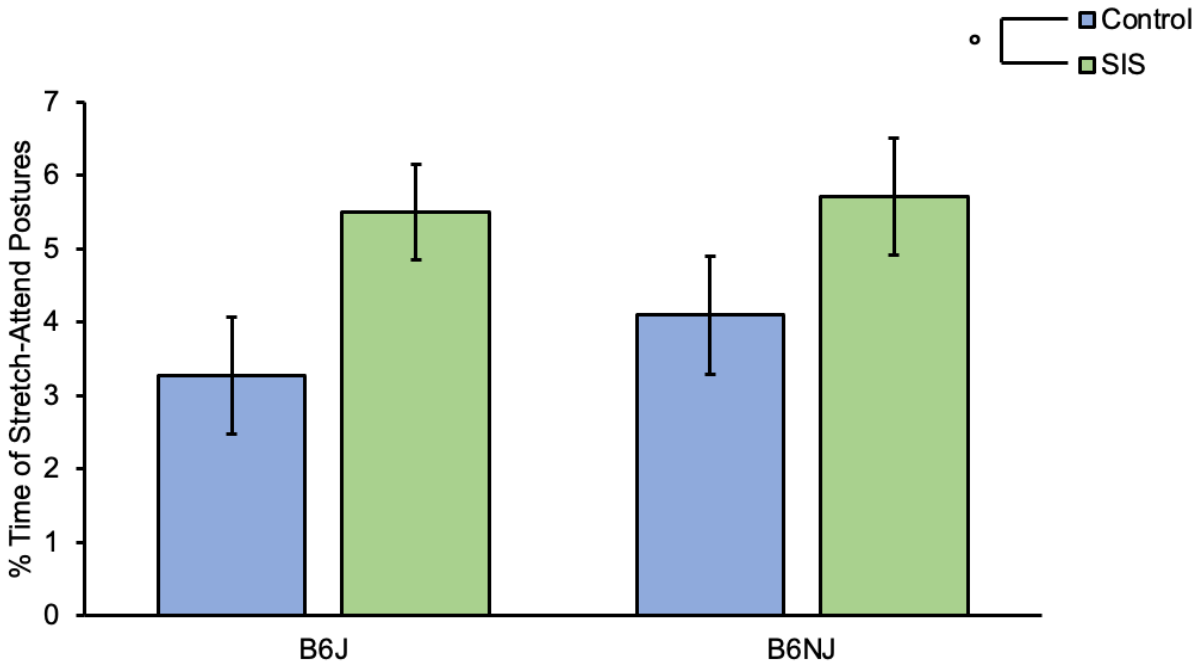

Figure 7. Percentage Time of Stretch-Attend Postures in the EPM.

Percentage time exhibiting stretch-attend posture in the EPM as a measure of risk-assessment behavior. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

Social instability stress decreased head-dipping behavior in mice (Figure 8). A main effect of stress was observed in the number of head dips mice displayed in the EPM ($F_{(1/32)}$ = 6.05, $p < 0.05$); overall, mice exposed to social instability stress showed fewer head dips, suggesting that social instability stress decreased this type of risk-assessment behavior. No main effect of strain on the number of head dips was found ($F_{(1/32)}$ = 2.95, $p > 0.05$), and there was no interaction between strain and stress ($F_{(1/32)}$ = .034, $p > 0.05$), suggesting that both strains reacted similarly to stress.

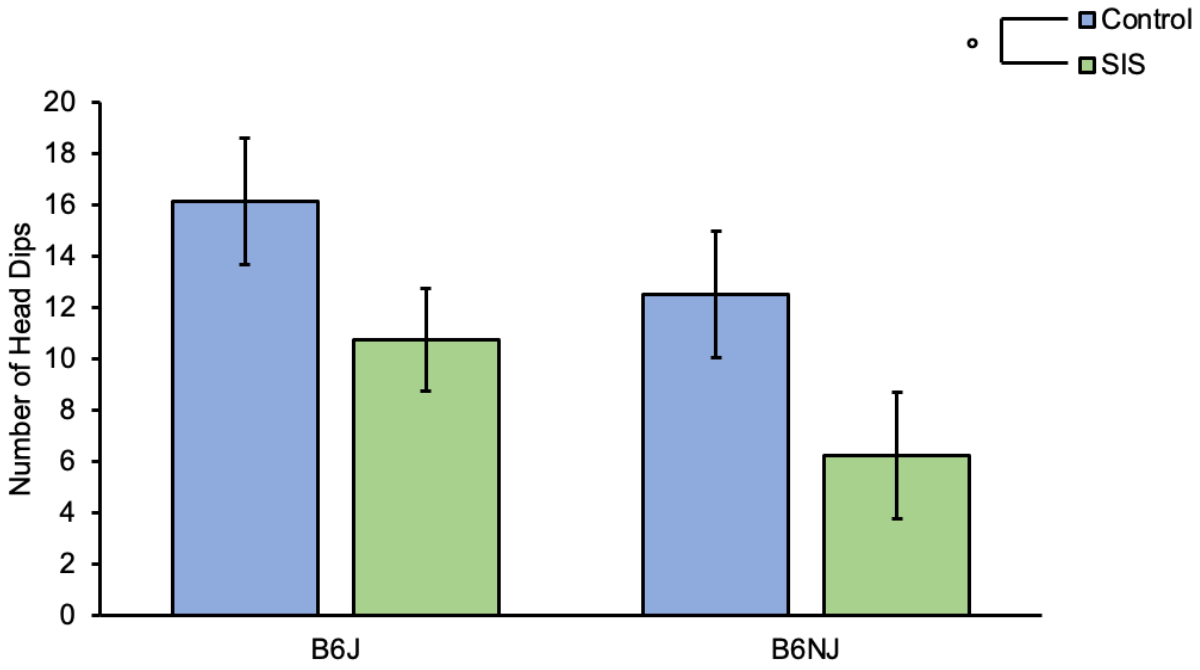

Figure 8. Frequency of Head Dipping in the EPM.

Frequency of head dips in the EPM as a measure of risk-assessment behavior. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

Neither genetic background nor social instability stress influenced the percentage of time spent head dipping in the EPM (Figure 9). No significant main effects were found for strain ($F_{(1/32)} = 1.89$, $p > 0.05$) or stress ($F_{(1/32)} = 3.47$, $p > 0.05$) for the percentage of time spent displaying head dips in the EPM. There were no interaction effects between strain and stress ($F_{(1/32)} = .003$, $p > 0.05$). Although no significant effects were found in the percentage time spent head dipping, the pattern seen in the group means mirrors the patterns seen in the frequency of head dipping behaviors, as shown in Figure 8.

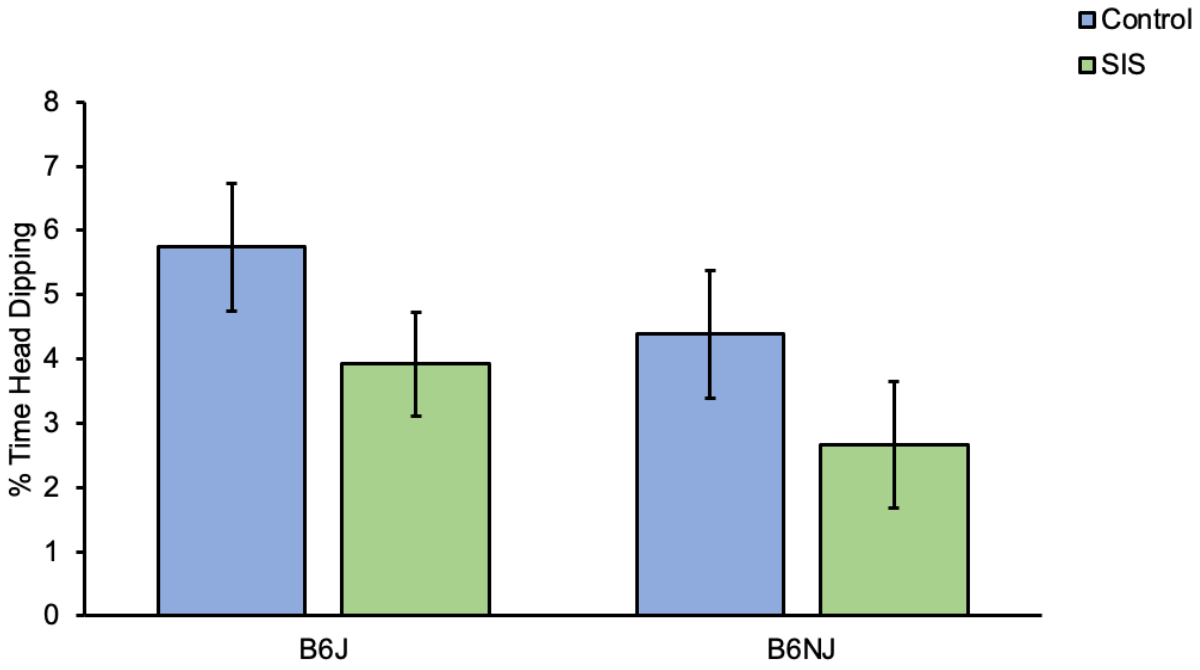

Figure 9. Percentage Time Head Dipping in the EPM.

Percentage time head dipping in the EPM as a measure of risk-assessment behavior. Data are presented as means ± SEM. Asterisks represent significance between strains. Degree symbols represent significance between stress conditions. B6J represents C57BL/6J mice, whereas B6NJ represents C57BL/6NJ mice.

After initial analysis, pairwise correlations were completed to evaluate the association between different behaviors in the EPM (Table 1). A Spearman's rho correlation matrix was utilized to conduct these correlations.

The percentage of time spent exhibiting stretch attend posture was negatively correlated with the percentage time spent in the open arms (rho = -.392, $p < 0.05$), suggesting that this form of risk-assessment behavior is more frequent in mice that exhibit more anxiety-like behavior. The number of head dip behaviors was strongly positively correlated with the percentage of time spent in the open arms (rho = .875, $p < 0.05$), suggesting that this behavior is associated with decreased anxiety-like behavior. Additionally, the percentage time spent in the open arms was positively correlated with distance traveled in the EPM (rho = .649, $p < 0.05$), suggesting that increased locomotion may be associated with less anxiety as measured by more time spent in the open arms. The percentage of time spent exhibiting head dipping behavior was also correlated with the percentage of time spent in the open arms (rho = .680, $p < 0.05$), as well as distance traveled in the EPM (rho = .506, $p < 0.05$), suggesting the same results as the head dipping frequency. Distance traveled in the EPM and percentage time spent in the open arms were also positively correlated (rho = .663, $p < 0.05$), suggesting that locomotion and anxiety-like behaviors are associated in the EPM. Rears were not correlated with any other behaviors.

Table 1. Correlational Matrix of Behaviors Assessed in the EPM

	% Open	Distance	# Rears	% Rearing	# SAP	% SAP	# Dips
Distance	**.663***						
# Rears	0.037	0.257					
% Rearing	0.032	0.252	**0.972**				
# SAP	-0.022	0.161	-0.306	-0.268			
% SAP	**-.392***	-0.115	-0.16	-0.116	**0.697**		
# Dips	**.875***	**0.649**	-0.044	-0.051	0.076	-0.255	
% Dipping	**.680***	**0.506**	0.116	0.104	-0.061	-0.088	**0.85**

% Open represents the percentage time spent in the open arms of the EPM. Distance represents distance traveled in the EPM. # Rears represents the frequency of rearing behaviors. % Rearing represents the percentage of time spent exhibiting rearing behavior. # SAP represents the frequency of stretch-attend postures. % SAP represents the percentage of time spent exhibiting stretch-attend postures. # Dips represents the frequency of head dipping behavior. % Dipping represents the percentage of time spent exhibiting head dipping behavior.

Chapter 4

Discussion

In this honors thesis, we assessed the effects of social instability stress on adolescent male C57BL/6J and C57NL/6NJ mice on anxiety-like and risk-assessment behavior. Notable differences were observed between stress and control conditions. After completing the social instability stress protocol and behavioral testing, we found that social instability stress increased the percentage time spent exhibiting rearing behavior and stretch attend posture but decreased the percentage time exhibiting head dipping behavior, suggesting that certain forms of risk-assessment behavior increase after exposure to stress, but decreases in others dependent on the type of action. Exposure to social instability stress also reduced the percentage time spent in the open arms, validating the social instability stress protocol as a way of inducing anxiety-like behavior in adolescent mice. We also found a strain by stress interaction in the frequency of stretch attend postures, suggesting a genetic influence on risk assessment behavior.

Anxiety-Like Behavior

Our first finding was concurrent with previous studies (Koert et al., 2021), as mice exposed to social instability stress spent less time in the open arms of the EPM, suggesting increased anxiety-like behaviors compared to control mice. This finding supports the conclusion that social instability stress in adolescence can lead to anxiety-like behaviors, highlighting the sensitivity of the adolescent period to stress and the development of behavioral disorders. This relationship is crucial for identifying potential strategies to mitigate anxiety-related outcomes

later in life. Although this study only assesses effects subsequently after adolescent exposure to stress, evidence suggests that adolescent social stress has a lasting impact on anxiety-like behavior (Sterlemann et al., 2008). Further, we assessed locomotion in the EPM by determining distance traveled. Although no significant effects of stress were found, a significant positive correlation was found between distance traveled and percentage time spent in the open arms, suggesting a relationship between the two variables. This data suggests that increased locomotion is associated with greater time in the open arms, indicating that higher locomotion is linked to lower anxiety, as more anxious mice typically avoid the open arms (Pellow et al., 1985 and less anxious mice spend more time exploring the open arms.

Additionally, we observed that C57BL/6J mice spent significantly more time in the open arms of the EPM, suggesting lower anxiety-like behavior compared to the C57BL/6NJ mice. This aligns with findings by Matuso et al. (2010), who reported that C57BL/6J mice exhibited the highest percentage of time in the open arms and demonstrated the least anxiety-like behaviors among C57BL/6 sub-strains. These results highlight the influence of genetic background on behavioral phenotypes, emphasizing the importance of sub-strain selection in anxiety research. The behavioral divergence may be rooted in genetic variations affecting neurobiological pathways, such as differences in HPA axis regulation (Marchette et al., 2018). Understanding these mechanisms could provide insights into the genetic basis of anxiety disorders and create targeted interventions.

Risk-Assessment Behavior

Rearing was assessed as a measure of risk-assessment behavior (Blanchard et al., 1991; Walf & Frye, 2007). Mice exposed to social instability stress during adolescence exhibited a higher frequency and percentage of time spent rearing compared to controls. This increase suggests that stress exposure may heighten risk-assessment behaviors, potentially reflecting an adaptive response to perceived environmental threats. Rearing allows rodents to survey their surroundings better (Shan et al., 2023), which could indicate an attempt to mitigate uncertainty in unknown situations. Alternatively, increased rearing could be seen as elevated anxiety-like behaviors, as seen in some studies (Borta & Schwarting, 2005); however, this finding is not consistent across all studies. These findings highlight the complexity of interpreting rearing behavior and showcase the need for further research.

As another measure of risk-assessment behavior, stretch attend posture (SAP) - characterized when a rodent is motionless but has its body stretched forward/toward a stimulus or around the EPM walls - was assessed (Loh et al., 2022). Interestingly, social instability stress affected SAP frequency in a strain-dependent manner. C57BL/6J mice exposed to SIS demonstrated an increased frequency of SAP, whereas C57BL/6NJ mice exposed to SIS showed no significant change. This interaction suggests a potential strain-specific difference in stress responsiveness or coping strategies, possibly influenced by genetic variations affecting risk-related decision-making (Oron et al., 2019). This finding, taken with the previous finding that C57BL/6J mice typically exhibit the least anxious behavior, is interesting because it suggests that genetic background is critical in shaping how individuals evaluate and respond to stress. Furthermore, SIS-exposed mice demonstrated an overall increase in the percentage of time spent exhibiting SAP behavior compared to controls, reinforcing the idea that adolescent social stress

can increase risk-assessment behavior. This finding is important to consider as exposure to stress can result in more cautionary, exploratory behavior to assess risk in adolescence.

As a final measure of exploratory and risk-assessment behavior, head dipping - defined as the downward motion of the rodent's head toward the floor from the open arms - was assessed (Loh et al., 2022). Interestingly, mice exposed to social instability stress demonstrated a reduction in head dips compared to controls, suggesting a decrease in exploratory behavior. This result contrasts with our other results that indicated heightened risk-assessment behaviors, such as increased rearing or stretch-attend postures, in the same group of stressed mice. The decreased frequency of head dipping could reflect a more cautious or inhibited response to stimuli, but this behavior partially depends on the time spent in the open arms. Anxious mice spent less time in the open arms, which could explain the decrease in head dipping behavior rather than the increase observed in other behaviors that take place behind the EPM walls.

This study presents a few limitations that should be considered when interpreting the results. The sample size of 32 mice may have limited the study's statistical power, making it challenging to detect significant effects. A larger sample size in future studies would enhance the reliability of findings, allowing for more definitive conclusions about the observed behaviors.

Second, the reliance on manual scoring introduces the possibility of human error and subjective bias. Although this study did not include a direct inter-rater reliability measure, the consistency of findings across studies supports the credibility of our observations. This study has replicated previous studies that have found similar observations. Notably, Novoa et al. (2023) demonstrated the same reduction in head dips in a separate study using the same stress exposure paradigm, reinforcing the reliability of this behavioral outcome despite potential scoring variability. This consistency suggests that while human error remains a factor, the observed

effects are reproducible. Further studies could incorporate more inter-rater reliability protocols or try automated behavioral analysis techniques to enhance accuracy and reduce potential biases.

Third, the study's focus on male mice limits the generalizability of the findings. Research suggests that adolescent female mice exhibit more risk-avoidant behavior (Gomes et al., 2022), highlighting potential sex differences. Additionally, studies show that women have consistently higher prevalence rates of anxiety disorders (McLean et al., 2011). Future studies should include female mice to explore sex differences in stress responses, anxiety-like, and risk-assessment behavior.

This study demonstrates that social instability stress during adolescence significantly impacts anxiety-like and risk-assessment behaviors in male C57BL/6J and C57BL/6NJ mice. Mice exposed to stress showed increased time spent exhibiting rearing and stretch-attend postures, alongside reduced head-dipping and open-arm exploration, indicating heightened vigilance and anxiety-like responses. These behavioral patterns suggest that adolescent stress exposure may change how threats are perceived and managed. This study also found an interesting interaction between strain and stress in stretch-attend posture risk-assessment behavior, suggesting the role of genetic factors in susceptibility to stress. Further studies should be done to investigate which specific factors are contributing to the difference in behavior.

Future research should be done to replicate these results and dive more into the mechanism behind risk-assessment behavior. Given the relevance of adolescence as a formative period for emotional development, these findings offer important insights into how early-life stress may contribute to anxiety disorders in humans and affect how adolescents respond to stress in novel environments. The observed behaviors mirror aspects of human risk assessment and anxiety, underscoring the importance of strategies for stress resilience.

Chapter 5

Conclusions

Investigating the relationships between social stress, genetics, anxiety, and risk assessment is crucial for understanding how early-life stress influences anxiety and risk assessment behavior, with long-term implications for mental health. Here, we examined how exposure to adolescent social instability stress affects anxiety-like and risk assessment behavior in male mice. Our results revealed that exposure to social instability stress led to increased anxiety-like behavior, as shown by the decreased time spent in the open arms of the EPM, a well-established indicator of anxiety. Furthermore, stress exposure significantly altered risk assessment behavior, including an increase in the percentage time spent exhibiting rearing and stretch attend postures. However, we also observed a decrease in the percentage of time spent head dipping. This suggests that stress exposure has a complex relationship with risk assessment, increasing certain behaviors that may reflect heightened vigilance while reducing others, particularly those linked to exploration in open space.

Additionally, we found a strain by stress interaction in the frequency of stretch attend postures, highlighting the potential role of genetics in shaping how people react to stressful situations. Ultimately, this study underscores the importance of understanding how adolescent stress exposure can lead to changes in anxiety and risk assessment behaviors, offering valuable insight into how individuals react and respond to stressful situations. Future research should explore the genetic mechanisms behind strain differences and investigate sex differences by including female mice to deepen our understanding of how adolescent stress affects anxiety and risk assessment behavior.

References

American Psychiatric Association. What are anxiety disorders? (n.d.). Retrieved March 17, 2025, from https://www.psychiatry.org:443/patients-families/anxiety-disorders/what-are-anxiety-disorders

Beesdo, K., Knappe, S., & Pine, D. S. (2009). Anxiety and anxiety disorders in children and adolescents: Developmental issues and implications for DSM-v. *Psychiatric Clinics of North America*, *32*(3), 483–524. https://doi.org/10.1016/j.psc.2009.06.002

Borta, A., & Schwarting, R. K. W. (2005). Inhibitory avoidance, pain reactivity, and plus-maze behavior in Wistar rats with high versus low rearing activity. *Physiology & Behavior*, *84*(3), 387–396. https://doi.org/10.1016/j.physbeh.2005.01.009

Bryant, C. D. (2011). The blessings and curses of C57BL/6 substrains in mouse genetic studies. *Annals of the New York Academy of Sciences*, *1245*(1), 31–33. https://doi.org/10.1111/j.1749-6632.2011.06325.x

Buchanan, T. W., & Lovallo, W. R. (2019). The role of genetics in stress effects on health and addiction. *Current Opinion in Psychology*, *27*, 72–76. https://doi.org/10.1016/j.copsyc.2018.09.005

Faravelli, C., Lo Sauro, C., Godini, L., Lelli, L., Benni, L., Pietrini, F., Lazzeretti, L., Talamba, G. A., Fioravanti, G., & Ricca, V. (2012). Childhood stressful events, HPA axis and anxiety disorders. *World Journal of Psychiatry*, *2*(1), 13–25. https://doi.org/10.5498/wjp.v2.i1.13

Fuhrmann, D., Knoll, L. J., & Blakemore, S.-J. (2015). Adolescence as a sensitive period of brain development. *Trends in Cognitive Sciences*, *19*(10), 558–566. https://doi.org/10.1016/j.tics.2015.07.008

Gomes, M. G. S., Tractenberg, S. G., Orso, R., Viola, T. W., & Grassi-Oliveira, R. (2022). Sex differences in risk behavior parameters in adolescent mice: Relationship with brain-derived neurotrophic factor in the medial prefrontal cortex. *Neuroscience Letters*, *766*, 136339. https://doi.org/10.1016/j.neulet.2021.136339

Haller, J., Aliczki, M., & Gyimesine Pelczer, K. (2013). Classical and novel approaches to the preclinical testing of anxiolytics: A critical evaluation. *Neuroscience & Biobehavioral Reviews*, *37*(10), 2318–2330. https://doi.org/10.1016/j.neubiorev.2012.09.001

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016). Regulation of the hypothalamic-pituitary-adrenocortical stress response. In Y. S. Prakash (Ed.), *Comprehensive Physiology* (1st ed., pp. 603–621). Wiley. https://doi.org/10.1002/cphy.c150015

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of dsm-iv disorders in the national comorbidity survey replication. *Archives of General Psychiatry*, *62*(6), 593. https://doi.org/10.1001/archpsyc.62.6.593

Kestering-Ferreira, E., Tractenberg, S. G., Lumertz, F. S., Orso, R., Creutzberg, K. C., Wearick-Silva, L. E., Viola, T. W., & Grassi-Oliveira, R. (2021). Long-term effects of maternal separation on anxiety-like behavior and neuroendocrine parameters in adult balb/c mice. *Chronic Stress*, *5*, 24705470211067181. https://doi.org/10.1177/24705470211067181

Koert, A., Ploeger, A., Bockting, C. L. H., Schmidt, M. V., Lucassen, P. J., Schrantee, A., & Mul, J. D. (2021). The social instability stress paradigm in rat and mouse: A systematic review of protocols, limitations, and recommendations. *Neurobiology of Stress*, *15*, 100410. https://doi.org/10.1016/j.ynstr.2021.100410

Komada, M., Takao, K., & Miyakawa, T. (2008). Elevated plus maze for mice. *Journal of Visualized Experiments*, *22*, 1088. https://doi.org/10.3791/1088

Loh, M. K., Ferrara, N. C., Torres, J. M., & Rosenkranz, J. A. (2022). Medial orbitofrontal cortex and nucleus accumbens mediation in risk assessment behaviors in adolescents and adults. *Neuropsychopharmacology*, *47*(10), 1808–1815. https://doi.org/10.1038/s41386-022-01273-w

Maner, J. K., & Schmidt, N. B. (2006). The role of risk avoidance in anxiety. *Behavior Therapy*, *37*(2), 181–189. https://doi.org/10.1016/j.beth.2005.11.003

Marchette, R. C. N., Bicca, M. A., Santos, E. C. D. S., & De Lima, T. C. M. (2018). Distinctive stress sensitivity and anxiety-like behavior in female mice: Strain differences matter. *Neurobiology of Stress*, *9*, 55–63. https://doi.org/10.1016/j.ynstr.2018.08.002

Marques, J. M., Olsson, I. A. S., Ögren, S. O., & Dahlborn, K. (2008). Evaluation of exploration and risk assessment in pre-weaning mice using the novel cage test. *Physiology & Behavior*, *93*(1–2), 139–147. https://doi.org/10.1016/j.physbeh.2007.08.006

Mason, M. J., Zaharakis, N. M., & Sabo, R. (2016). Reducing social stress in urban adolescents with peer network counseling. *Journal of Child and Family Studies*, *25*(12), 3488–3496. https://doi.org/10.1007/s10826-016-0515-5

Mata, R., Josef, A. K., Samanez-Larkin, G. R., & Hertwig, R. (2011). Age differences in risky choice: A meta-analysis. *Annals of the New York Academy of Sciences*, *1235*(1), 18–29. https://doi.org/10.1111/j.1749-6632.2011.06200.x

Mathews, B. L., Koehn, A. J., Abtahi, M. M., & Kerns, K. A. (2016). Emotional competence and anxiety in childhood and adolescence: A meta-analytic review. Clinical Child and Family Psychology Review, 19(2), 162–184. https://doi.org/10.1007/s10567-016-0204-3

Maul, S., Giegling, I., Fabbri, C., Corponi, F., Serretti, A., & Rujescu, D. (2020). Genetics of resilience: Implications from genome-wide association studies and candidate genes of the stress response system in posttraumatic stress disorder and depression. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *183*(2), 77–94. https://doi.org/10.1002/ajmg.b.32763

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *Journal of Psychiatric Research*, *45*(8), 1027–1035. https://doi.org/10.1016/j.jpsychires.2011.03.006

National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Division of Behavioral and Social Sciences and Education; Board on Children, Youth, and Families; Committee on Applying Lessons of Optimal Adolescent Health to Improve Behavioral Outcomes for Youth; Kahn NF, Graham R, editors. Promoting Positive Adolescent Health Behaviors and Outcomes: Thriving in the 21st Century. Washington (DC): National Academies Press (US); 2019 Dec 12. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554992 / doi:10.17226/2555

Novoa, C. 2022. Social Instability Stress Protocol.

Novoa, C., Garcia-Trevizo, P., Afiouni, E.G., Logue, S., & Gould, T.J. (2023, June 25-30). *Psychobiological sequelae of adolescent social stress in male mice* [Poster presentation]. IBNS 32nd Annual Meeting, Niagara Falls, Ontario, Canada. Poster IBNS 2023.pptx

Oron, O., Getselter, D., Shohat, S., Reuveni, E., Lukic, I., Shifman, S., & Elliott, E. (2019). Gene network analysis reveals a role for striatal glutamatergic receptors in dysregulated risk-assessment behavior of autism mouse models. *Translational Psychiatry*, *9*(1), 257. https://doi.org/10.1038/s41398-019-0584-5

Paus, T., Keshavan, M., & Giedd, J. N. (2008). Why do many psychiatric disorders emerge during adolescence? *Nature Reviews Neuroscience*, *9*(12), 947–957. https://doi.org/10.1038/nrn2513

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*(3), 149–167. https://doi.org/10.1016/0165-0270(85)90031-7

Reynolds, E. K., Schreiber, W. M., Geisel, K., MacPherson, L., Ernst, M., & Lejuez, C. W. (2013). Influence of social stress on risk-taking behavior in adolescents. *Journal of Anxiety Disorders*, *27*(3), 272–277. https://doi.org/10.1016/j.janxdis.2013.02.010

Rodgers, R. J., Cao, B.-J., Dalvi, A., & Holmes, A. (1997). Animal models of anxiety: An ethological perspective. *Brazilian Journal of Medical and Biological Research*, *30*(3), 289–304. https://doi.org/10.1590/S0100-879X1997000300002

Rodgers, R., Haller, J., Holmes, A., Halasz, J., Walton, T., & Brain, P. (1999). Corticosterone response to the plus-mazeHigh correlation with risk assessment in rats and mice. *Physiology & Behavior*, *68*(1–2), 47–53. https://doi.org/10.1016/S0031-9384(99)00140-7

Sawyer, S. M., Afifi, R. A., Bearinger, L. H., Blakemore, S.-J., Dick, B., Ezeh, A. C., & Patton, G. C. (2012). Adolescence: A foundation for future health. *The Lancet*, *379*(9826), 1630–1640. https://doi.org/10.1016/S0140-6736(12)60072-5

Seemiller, L. R., Garcia-Trevizo, P., Novoa, C., Goldberg, L. R., Murray, S., & Gould, T. J. (2023). Adolescent intermittent alcohol exposure produces strain-specific cross-sensitization to nicotine and other behavioral adaptations in adulthood in C57BL/6J and DBA/2J mice. *Pharmacology Biochemistry and Behavior*, *232*, 173655. https://doi.org/10.1016/j.pbb.2023.173655

Shan, X., Contreras, M. P., Sawangjit, A., Dimitrov, S., Born, J., & Inostroza, M. (2023). Rearing is critical for forming spatial representations in pre-weanling rats. *Behavioural Brain Research*, *452*, 114545. https://doi.org/10.1016/j.bbr.2023.114545

Steinberg, L. (2007). Risk taking in adolescence: New perspectives from brain and behavioral science. *Current Directions in Psychological Science*, *16*(2), 55–59. https://doi.org/10.1111/j.1467-8721.2007.00475.x

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., Müller, M. B., & Schmidt, M. V. (2008). Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: Implications for stress-related disorders. *Hormones and Behavior*, *53*(2), 386–394. https://doi.org/10.1016/j.yhbeh.2007.11.001

Sterlemann, V., Rammes, G., Wolf, M., Liebl, C., Ganea, K., Müller, M. B., & Schmidt, M. V. (2010). Chronic social stress during adolescence induces cognitive impairment in aged mice. *Hippocampus*, *20*(4), 540–549. https://doi.org/10.1002/hipo.20655

Sturm, M., Becker, A., Schroeder, A., Bilkei-Gorzo, A., & Zimmer, A. (2015). Effect of chronic corticosterone application on depression-like behavior

in C57BL / 6N and C57BL / 6J mice. *Genes, Brain and Behavior*, *14*(3), 292–300. https://doi.org/10.1111/gbb.12208

Viner, R. M., Ross, D., Hardy, R., Kuh, D., Power, C., Johnson, A., Wellings, K., McCambridge, J., Cole, T. J., Kelly, Y., & Batty, G. D. (2015). Life course epidemiology: Recognising the importance of adolescence. *Journal of Epidemiology and Community Health*, *69*(8), 719–720. https://doi.org/10.1136/jech-2014-205300

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322–328. https://doi.org/10.1038/nprot.2007.44

Yohn, C. N., Ashamalla, S. A., Bokka, L., Gergues, M. M., Garino, A., & Samuels, B. A. (2019). Social instability is an effective chronic stress paradigm for both male and female mice. *Neuropharmacology*, *160*, 107780. https://doi.org/10.1016/j.neuropharm.2019.107780