THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF BIOBEHAVIORAL HEALTH

THE EFFECTS OF CHRONIC VARIABLE SOCIAL STRESS DURING ADOLESCENCE ON LATE-ADOLESCENT ANXIETY-LIKE BEHAVIOR AND ALCOHOL CONSUMPTION IN BALB/CJ INBRED MICE

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ABSTRACT

Adolescence is a critical period of major neurophysiological changes as well as the time when people first experience alcohol. The physiological system that regulates the stress response is especially vulnerable, as repeated hypothalamic-pituitary-adrenal (HPA) axis activation results in a persistent release of the hormone corticosterone in response to stress that lasts well into adulthood. In preclinical studies, alcohol consumption and affect-related disorders are modulated by hypo- and hyper-active HPA-axis activity. We investigated the effect of chronic variable social stress (CVSS) during adolescence on affect-related behavior and alcohol binge-drinking in late adolescent with an inbred mouse model. Mice were separated into either a stress (CVSS) or control (CON) group. Adolescent CVSS mice, from postnatal day (PND) 26-50, were exposed to a chronic variable social stress paradigm, which alternates between 3 days of individual housing followed by 4 days of social reorganization. Affect-related behavior was measured during late adolescence by the elevated plus maze (EPM), a measure of risk-taking behavior on open arms of an elevated platform and physical coat status scoring, assigning higher scores to poorly groomed fur. Binge-like alcohol consumption was measured through the Drinking in the Dark (DID) model during late adolescence. In adulthood (PND 70), anxiety-like behavior was measured by the Social Interaction test, an open field comparison of interaction or avoidance of an unfamiliar social target. A deteriorated physical coat state suggests anxiety-like behavior in male CVSS mice. However, differences between CVSS and CON groups were not observed in alcohol consumption, anxiety-like behavior measured on EPM, or social avoidance/interaction. Gender differences in alcohol consumption and social interaction were observed. These results indicate that the chronic variable social stress model does not induce anxiety-like behavior or alcohol binge-like drinking in late-adolescence. CVSS has been shown to alter adult behavior. This work is an important component of the overall study utilizing CVSS during adolescence because it suggests the effects of CVSS do not appear until adulthood.

TABLE OF CONTENTS

LIST OF FIGURES

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Chapter 1

Background

Alcoholism is a serious problem that has significant costs on our society. Specifically, excessive alcohol consumption on average results in 88,000 deaths per year, and is the third leading preventable cause of death in the country (Gonzales et al., 2014). The financial costs of alcohol abuse to the U.S. economy amounted to $249 billion in 2010, a rise from the $223.5 billion in 2006. The majority of this cost comes from a loss of workplace productivity, however, large portions are also due to health care expenses, law enforcement costs, and motor vehicle crashes (Sacks et al., 2015). Addiction to alcohol is characterized as a cycle of anticipation, binge-like drinking to intoxication, and withdrawal (Koob, 2003). Obsessive preoccupation, failure to control, and continuation of alcohol consumption despite negative consequences are the essential features of alcohol addiction (Goodman, 1990). In addition to societal costs, alcoholism also has detrimental effects on the body, which can have a lasting impact on an individual's financial security and livelihood. Alcohol abuse over time can increase the risk of stroke, high blood pressure, arrhythmias, and cardiomyopathy. It can also affect the liver, leading to alcoholic hepatitis and steatosis, or fatty liver disease. Further, alcohol use can increase the risk for certain cancers, such as breast, liver, throat, esophagus and mouth (NIAAA, 2010).

Adolescence is typically the time when people first experience alcohol. Most adults who meet the criteria for alcohol dependence started drinking during adolescence (Schulenberg, 2002). An analysis of substance use data from the National Comorbidity Survey (NCS) of

individuals in the United States 15-54 years old found the peak of initial use of alcohol was 18 years old and 17-18 years old for individuals meeting the criteria of alcohol dependence syndrome (Wagner and Anthony, 2002). Heavy drinking at 18 years old is a risk factor for alcohol abuse and developing alcohol use disorders in adolescence that last well into late adulthood, further supporting the importance of adolescence in development of alcoholism (Merline et al., 2008). Risk factors for the development of alcohol use disorders include poor relationships among family members, family and life disruptions, and deviant peer interactions during adolescence (Nash et al., 2005; Thompson and Meyer, 2007; Marshall, 2014). Some factors that increase adolescent resilience to developing alcohol addiction include good peer relationships, low family stress and involvement in social activities (Fergusson, Boden, and Horwood, 1999; Jaffee et al., 2007; Marshall, 2014).

Alcohol addiction relies on a defining characteristic of out-of-control use, or lack of impulse control despite the serious consequences to the life of an addicted person (Hyman and Malenka, 2001). Frontal cortical remodeling is very important in the maturation and stabilization in adulthood of risk-taking behavior, motivation, and sensitivity to rewards (Crews, He, and Hodge, 2007). These same regions of the brain that undergo major remodeling and neurogenesis from early-puberty throughout adolescence are involved in the development mental health disorders, such as depression and anxiety-disorders, as well as drug addiction (Young and Dietrich, 2015; Romeo, 2017; Enoch et al., 2011). In fact, adolescence is the period when mental health disorders such as depression and anxiety disorders begin to be observed (Young and Dietrich, 2015). One in four youth will experience a significant mental health disorder by 18 years of age, with 40% of those individuals meeting criteria for having comorbid mental health

disorders (Merikangas et al., 2010). Adolescence is defined as the period between the pubertal period of sexual and physical maturation and adulthood, a time of psychological maturation. The period between 12 and 19 years is typically considered adolescence in humans, whereas, adolescence in mice typically encompasses postnatal days (PND) 28-42 (Spear, 2000). However, the end of adolescence is not entirely concrete and is simply considered to be the time in which a person becomes adult-like in behavior (Lerner and Boyd, 2010). Changes in psychological and emotional growth, as well as increased novel peer interactions and stressful life events associated with social exploration make the adolescent increasingly exposed to chronic stress throughout a vulnerable developmental period (Young and Dietrich, 2015).

Adolescent Vulnerability

Adolescence is a particularly vulnerable time in an individual's lifetime, due to these major developmental, physiological, and neurobiological changes (Ernst et al., 2006; Anderson, 2003). Importantly, this developmental period is critical in both humans and in model organisms. During adolescence, the brain begins to undergo important cortical remodeling including changes to synapses, neurotransmitter receptors, and hormones (Giedd et al., 1999). In late-adolescence, development of the frontal cortex takes place, which is an area crucial for reasoning, impulse control, rewards, and priority setting (Toga, Thompson, and Sowell, 2006). There are also changes in the limbic system that are important for emotions and formation of new memories from past experiences, including the hippocampus, amygdala, nucleus accumbens, and the hypothalamus. There is an overproduction of axons and synapses during that are remodeled through adolescence. Due to these changes, there is a window of vulnerability

to environmental influences on gray matter maturity in these important regions (Crews, He, and Hodge, 2007). This overproduction of synapses and innervation followed by remodeling, pruning, and apoptosis is hypothesized to have the goal of increasing the transmission efficiency at synapses (Changeux and Danchin, 1976; Giedd et al., 1999). This remodeling event in adolescence parallels the regional development of function, influencing transitions in cognition, reasoning, and impulsivity (Spear, 2000; Casey et al., 2008).

The risk of development of affective and alcohol use disorders in adulthood may be increased by the physiological response to stress and perceived stress during adolescence. Excessive drinking has been linked to times of major stress, as well as the anticipation of major stress, as reported by interviewed alcoholics. Further, chronic life stressors are reported as a main reason for relapse in alcoholics (Laurent et al., 1997). The motivation for drinking alcohol may be guided by the anxiolytic, or stress/tension reducing effects of alcohol, which is the basis for the Tension Reduction Theory (Powers and Kutash, 1985). Therefore, prolonged heightened perceived stress in adulthood may be a risk factor for excessive alcohol use in addition to affective disorders, such as anxiety and depression.

Stress & Neurophysiological Development

When exposed to stress, the primary physiological response is to increase glucocorticoid hormone levels. The glucocorticoid response differs between adolescents and adults when exposed to physical and psychological stressors. The adolescent glucocorticoid hormone release is prolonged throughout the stress exposure compared to an adult. This is due to a lack of maturation of the system that otherwise regulates this response (McCormick, 2010). For

example, adolescent rats have been shown to display a sensitivity to the main rodent glucocorticoid, corticosterone. Slow release of corticosterone in adolescent rats, followed by a high-dose injection of corticosterone resulted in elevated NMDA receptor subunit mRNA expression in the hippocampus, indicating proliferation of hippocampal neurons. However, this "acute-stress" dosage of corticosterone did not produce the same effect in adult rats, indicating a lack of capacity for proliferation, or plasticity, in response to chronic low dose corticosterone release (Lee et al., 2003). The areas of the brain that are undergoing development throughout adolescence, such as the prefrontal cortex and hippocampus, have high levels of glucocorticoid receptors (Dziedzic et al., 2014). Importantly, repeated stress exposure during adolescence has been shown to increase the glucocorticoid stress response that lasts well into adulthood (McCormick, 2010). This persisting, prolonged hormone release indicates a vulnerability of the hypothalamic-pituitary-adrenal (HPA) axis, which mediates the physiological response to stress (Lupien et al., 2009). The continued maturation of the adolescent brain, in combination with increased hormonal reactivity to stress and sensitivity to corticosterone, leaves the adolescent particularly vulnerable to lasting impacts on behavior, impulse control, and development of psychological disorders and substance use (Romeo et al., 2017).

The development of social skills highlights the importance of social interaction during adolescence. Social interactions with peers peak in early to mid-adolescence, which has a strong impact on the development of mature social behaviors (Spear, 2000). Social cognition, the way in which we receive and process information about social situations and the people around us, is also drastically changing from adolescence to adulthood (Blakemore, 2012). Stress that is social in nature is a widespread stressor for humans, while animal studies have shown that exposure to social stress increases HPA axis activity and is associated with increased mortality rate (Albeck

et al., 1997). Effects of the stressor are evident after the procedure is finished, that is important for relating to human affect-related disorders and alcohol use disorders, which typically present later in life (Morley, 1983). Studies have found behavioral effects and neuroendocrine changes that persisted well after discontinuation of the social stress procedure (Tsankova et al., 2006). Social stress is often the cause of psychiatric disorders in humans, social stress as a pre-clinical model takes into account the etiology of human disorders that are stress-associated (Brown and Prudo, 1981).

Preclinical Animal Studies

Preclinical studies using rodent models have become invaluable due to parallels between adolescents and adults in both rodents and humans. Behavioral changes that we observe in human adolescents are also seen in rodents (Crews, He, and Hodge, 2007). In order to study the effects of social stress during adolescence on the development of psychosocial disorders in rodents, models such as social defeat stress, social isolation, and unstable housing have all been developed with ethological significance preserved. In models of social stress, long-term effects of the stressor are observed to persist into adulthood, including increased anxiety-like and depression-like behavior, altered adrenal sensitivity, enlarged adrenal glands, social avoidance, altered prefrontal cortex, subsequent amygdala activity, and altered hippocampal morphology and function (Schmidt, Sterlemann, and Muller, 2008; Schmidt et al., 2007; McCormick, Green, and Barnes, 2013; McEwen, 2007; McEwen and Milner, 2007; McEwen and Morrison, 2013).

Social stress has been characterized as an important factor in the onset of anxiety disorders and depression in humans (McEwen, 2000; Schmidt et al., 2010b; Kendler et al., 2002,

2006). Interestingly, a large percentage of patients diagnosed with major depression disorder also have HPA axis hyperactivity and elevated cortisol levels, the primary glucocorticoid in humans (Holsboer, 1999, 2000). Along with increased susceptibility for developing anxiety and affective disorders, socially stressed adolescent rodents exhibit behaviors associated with the development of alcohol use disorders. Rodents that underwent a chronic social stress procedure through adolescence have shown changes in self-administration of alcohol, alcohol consumption, acquisition of alcohol conditioned place preference and reinstatement of alcohol seeking behavior (Lopez et al., 2011; Lopez and Laber, 2015; Skelly et al., 2015; Song et al., 2007; Funk et al., 2005). The importance of the period of stress exposure, either adolescence or adulthood, is further reinforced by a preclinical study in C57BL/6J mice. When social isolation was applied during adolescence, the stress group consumed significantly more alcohol than the non-stressed control group. However, when the same stress procedure was applied during adulthood, alcohol consumption was not altered and both groups consumed similar levels of alcohol (Lopez et al., 2011). This age dependent effect indicates that adolescents have a window of vulnerability to the effects of social stress that can lead to increased alcohol consumption post-stress exposure.

Studying this relationship between chronic social stress and alcohol use in preclinical rodent studies has limitations. In rodents, significant differences in male and female consumption of alcohol after prolonged stress exposure during adolescence has brought into question some models of social stress, such as social isolation and social reordering, as effective models across genders (Schmidt et al., 2010a). Human studies suggest that this difference between males and females may be due to a sexual dimorphism in the stress response system, in particular the resistance to negative-feedback of cortisol in women (Young and Altemus, 2004). Notably, women are twice as likely as men to develop a major depression, a disorder in which

chronic stress significantly increases the risk (Kendler et al., 2002, 2006). Studies with male rats indicate that chronic social isolation during adolescence not only significantly increases alcohol intake in adulthood, but also produces binge-like drinking behavior in adulthood (Skelly et al., 2015). However, social isolation has mixed results in both rats and mice on alcohol intake post-adolescent stress (Moriya et al., 2015; Butler et al., 2014). Also, chronic social isolation has proved to be more applicable females compared to males, while social grouping is more stressful in males (Blanchard et al., 2001). Similarly, social defeat, a commonly used chronic social stress model that utilizes a same-sex dominant aggressor, has proved to not be applicable to both males and females. Male often aggress to establish social dominance, forming a hierarchy with a dominant male and subordinate male, however, females display low aggression towards other females and are not stressed by the social defeat protocol (Palanza et al., 2001).

The importance of variability of the stressor presented within a stress procedure has proven to be important when a single stress model is used in both males and females, while preventing habituation to the stress procedure. Chronic variable stress (CVS) is a model that exposes the animal to different physical stressors with varying durations of each stressor. CVS is not a social stress procedure, but instead alternates between physical mild stressors. These include, but are not limited to, physical restraint, forced swim, slanted wire floor, and continuous light cycle (Lopez et al., 2011). This model was initially shown to have lasting effects on the development of the HPA axis and its ability to regulate future responses to stress (Flak et al., 2009). It has been shown to produce lasting changes in the responsiveness to commonly abused drugs (Lepsch et al., 2005; Molina et al., 1995). This model is particularly interesting because it resembles the stress that a typical adolescent human would experience in terms of variability in

stressor, exposure time, and exposure duration (Casey et al., 2010). However, this stress procedure is not social in nature, therefore, may not be entirely ethologically relevant stressor. One chronic social stress model that has shown promise for applicability with both male and female mice is social instability stress, in this stress procedure the changing of group housing composition periodically disrupts the established social hierarchy (Schmidt, Sterlemann, and Muller, 2008; Flak et al., 2009). This stressor has been shown to increase basal corticosterone levels, enhance adrenal size, and decrease hippocampal mineralocorticoid receptors in female stressed mice (Schmidt et al., 2010b). Similar acute and persistent effects have also been noted in male mice, as well as decreased anhedonic behavior and anxiety-like behavior. Further in male mice, decreased expression of hippocampal glucocorticoid and mineralocorticoid receptors and increased levels of vasopressin have been observed following this social stress procedure (Schmidt 2008). Drug treatments that have been proven effective for treating anxiety disorders in humans have been effective in reversing the anxiety-like behaviors observed after these social stress procedures, supporting the validity of these models in studying human affect-related pathology (Scharf et al., 2013; Schmidt et al., 2007; Belzung and Lemoine, 2011). However, with social instability stress alone, these results have not been consistently observed in males and females when social instability stress is applied during adolescence (Bourke and Gretchen, 2011).

Chronic Variable Social Stress (CVSS)

The limitations of some commonly used chronic social stress protocols have led to the development of the chronic variable social stress protocol. This model aims to reduce

habituation, ensure that the stress is inescapable, and be applicable to both sexes (Caruso, McClintock, and Cavigelli, 2014; Caruso, Kamens, and Cavigelli, under review; Hodges and McCormick, 2015). Chronic variable social stress (CVSS) is a protocol developed for adolescent mice which alternates between a period of individual housing (3 days) and social reorganization (pairing with unfamiliar non-littermate cage mates for 4 days) (Caruso, McClintock, and Cavigelli, 2014; Caruso, Kamens, and Cavigelli, under review). Male and female mice exposed to CVSS exhibit anxiety-like behavior measured by elevated plus maze (EPM) and reduced locomotion (Caruso, Kamens, and Cavigelli, under review). These effects show that this model of chronic social stress has the same effect on males and females which persist into adulthood. Using inbred mice, which lack genetic variability, this model of chronic variable social stress can be used to compare sex specific effects on other behaviors related to stress-associated disorders, such as alcohol consumption.

Objective

In this study, we utilize the chronic variable social stress (CVSS) protocol to observe the effects of social stress during adolescence on late-adolescent anxiety-like behavior, early adulthood depression-like behavior, and alcohol consumption in BALB/cJ inbred mice. The BALB/cJ strain was chosen because it exhibits high anxiety-like behavior and neuroendocrine stress reactivity (Cavigelli et al., 2013). To reduce the effect of individual differences in physiology due to genetic variability between animals, often seen in outbred strains, inbred mice are used (Caruso, McClintock, and Cavigelli, 2014). BALB/cJ mice have a low alcohol preference ratio, therefore, increased alcohol consumption may be easily observed between stress

and control groups (McClearn, Wilson, and Meridith, 1970). Individual variations in fear-related behaviors are balanced among stress (CVSS) and control (CON) groups by initial ultrasonic vocalization (USV) testing and performance in the novel physical experiment. The EPM experiment and scores of physical coat state are used to examine anxiety-like behavior in late-adolescence. The Drinking in the Dark (DID) protocol is used to measure alcohol consumption in late-adolescence, which models binge-like drinking in rodents. To observe persistent effects of the CVSS procedure in adulthood, the Social Interaction experiment is utilized to measure social anxiety-like behavior. We hypothesize that chronic variable social stress during adolescence will increase late-adolescent alcohol consumption and later affect-related behavior in late-adolescence and early adulthood.

Chapter 2

Materials and Methods

Animals

Male and female adult BALB/cJ mice were obtained from The Jackson Laboratory to breed the experimental subjects. Once born, each litter of mice was housed with the female parent until weaning on postnatal day (PND) 21. Experimental housing included a small red tube for environmental enrichment and free access to food and water.

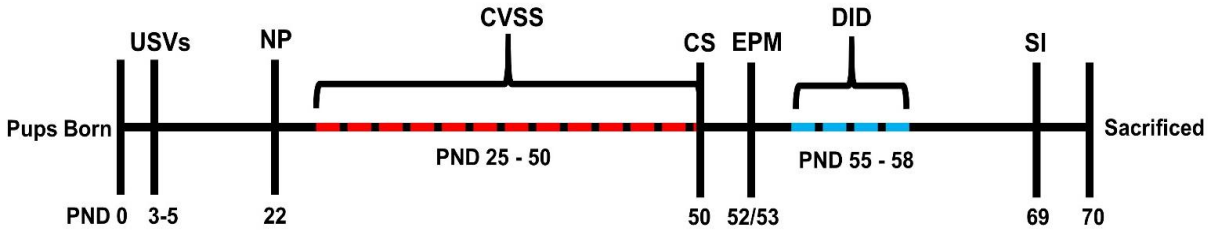

Figure 2.1 Experimental Timeline
Experimental timeline from PND 0-70, including CVSS and behavioral testing. Ultrasonic vocalizations (UVSs), Novel Physical (NP), Chronic Variable Social Stress (CVSS), Coat Score (CS), Elevated Plus Maze (EPM), Drinking in the Dark (DID), Social Interaction (SI). Animal handling approved: IACUC # 46958

Ultrasonic Vocalization (USV)

USVs were recorded to determine an index of fear-related behavior on PND 3-5. This measure allowed us to control for individual differences in fear-related behavior during the experiment. Mice were separated in groups of high or low USVs based on a median split for each litter. High and low mice were divided into experimental conditions in order to balance fear-related behavior temperament across conditions. To record USVs, pups were placed individually into a test box (empty mouse cage-bottom without bedding) with the ultrasonic recording device. USVs are recorded for 2 minutes on each test day. Avisoft SASLab Lite was used to visualize UVSs which were coded by trained observers.

Novel Physical Test

The Novel Physical test is an animal model of social inhibition behavior. This test is used to index the temperament of individual animals as either inhibited (shy) or non-inhibited (less socially latent). Inhibited temperament in the Novel Physical test has been shown to be an

important factor in determining the likelihood of prolonged corticosterone release in a novel situation (Cavigelli et al., 2007). On PND 22-23, each mouse was recorded for latency to interact with a novel object, as well as locomotor activity in a novel environment in a dark behavioral room. Each mouse was transferred to the test arena by a familiar red tube to reduce handling/novelty immediately prior to testing. Each mouse was placed in an open field arena (61cm x 61cm) for 5 minutes with novel mouse-size objects in each corner. After the mouse was placed into the test arena, a camera recorded the activity of the animal during the test. Locomotor activity and latency to approach a novel object was collected.

Chronic Variable Social Stress Paradigm (CVSS)

Mice were either subjected to the chronic variable social stress (CVSS) paradigm from PND 26- 50, or control conditions. All (20 male and 17 female) mice were assigned either to the control group (CON) or stress group (CVSS). Effort was made to balance both groups by sex and temperament determined by USV behavior. CON mice remained with same-sex littermates (3/cage) and were left undisturbed with the exception of normal handling. Mice in the stress condition alternated between social isolation (3 days) and re-housing (4 days). CVSS mice were single housed during social isolation period and housed 3/cage during re-housing procedure. All mice, including the CON group, were weighed once a week. Throughout the duration of the stress procedure, all mice were monitored for signs of fighting by visual observance of wounding and coat state. All mice had free access to food and water throughout the CVSS procedure.

Physical Coat State

On PND 50, physical coat state of all mice was scored. The state of the animals' coat was a composite score of 7 individual body areas; including the head, neck, back, abdomen, forepaws, hind-paws, and tail. The score ranged from a 0, indicating a well-groomed area with shiny fur and smooth coat, 0.5 for slightly tousled fur, and 1 for observances of matted, dirty, or clumped fur indicating the poorest state of coat. The scoring of the coat status is a method of determining the animals' self-grooming behavior. A deterioration in coat status has been characterized as an indicator of stress following CVSS (Caruso, Kamens, and Cavigelli, under review).

Elevated Plus Maze (EPM)

The EPM is used to measure anxiety-like behavior in rodents. Inhibition of the rodent to venture out onto the open arms and spend more time in the closed arms indicates a rodent with higher levels of anxiety-like behavior (Kalueff and Tuohimaa, 2005). The EPM experiment was performed on PND 52 and 53. All testing occurred during the animals' dark cycle with infrared and red lights for optimal tracking of the animal by on overhead camera. The EPM is made up of two closed arms (30 x 5 cm) and two open arms (30 x 5 x 14.5 cm), connected in a plus sign shape, elevated 42 cm off the ground. The mice were placed in the center of the EPM and were allowed to explore the open and closed arms of the maze for 5 minutes. During this test, time spend in the open arms, closed arms, center, and total locomotion was recorded by an overhead video camera. The AnyMaze® tracking system recorded these measurements using a two-paw (85% of the animal's body) entry to indicate time spent in a zone, with a preprogrammed

protocol to specify the zones of interest. After each mouse was tested, the maze was cleaned with 30% ethanol.

Alcohol Consumption during DID

On PND 55-58, all mice were subjected to the Drinking in the Dark (DID) paradigm, an animal model of binge-like alcohol consumption. This procedure was designed to take advantage of the animals' peak ingestion period (3 hours into the dark cycle) in order to facilitate high alcohol consumption to achieve relevant blood ethanol concentrations (BECs greater than 0.08% or 80mg/dL) (Rhodes et al., 2005; NIAAA, 2004). All mice were single housed on PND 53, immediately after EPM testing to acclimate to the housing conditions. All mice were weighed on the first day of DID. The DID protocol includes 4 days of exposure to 20% ethanol in one serological pipette fitted with a ball-bearing drinking spout. Water was removed during the 2 and 4 hour exposure periods and returned after each DID session, mice had free access to food throughout the duration of the test. Day 1-3 exposure occurred 3 hours into the dark cycle for 2 hours (13:00 – 15:00) and day 4 for 4 hours (13:00 – 17:00). Day 4 consumption was used to determine alcohol consumption for DID, as this 4 hour period has been shown to facilitate the highest consumption and BEC in mice (Rhodes et al., 2005). Alcohol consumption was calculated as g/kg for each mouse. After DID was completed, mice were returned to cages with their original cage-mates.

Social Interaction

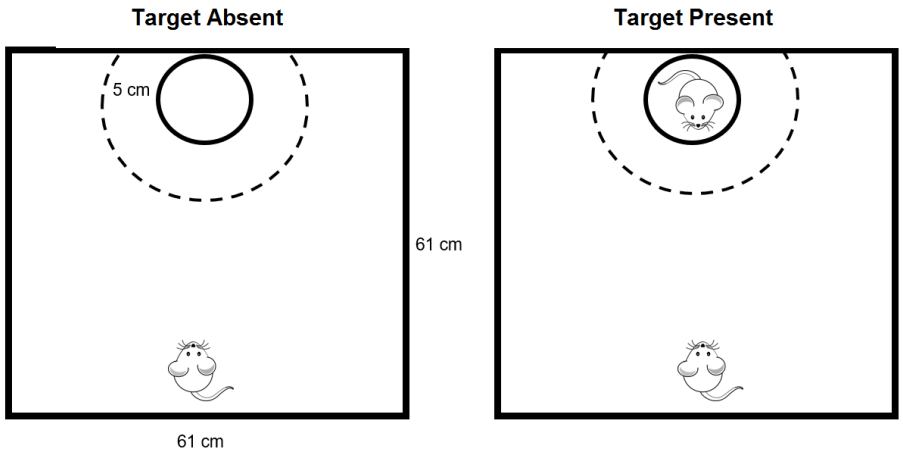

Figure 2.2 Social Interaction Arena
Social Interaction testing arena (61x61cm), dotted lines indicate Interaction Zone (5cm) space around wired mesh cage. Target present trial included a same-sex, same-age mouse in the wired mesh cage.

The Social Interaction test takes advantage of a social interaction between two mice that may naturally occur, making it ethologically relevant. The conflicting motivations that may lead a mouse to avoid or approach a novel social interaction may be descriptive of social depression-like disorders, indicating an depression-like behavior in rodents that is social in nature (Brodkin et al., 2004). Social interaction was measured in an open field arena (61 x 61 x 30 cm) covered with a plastic transparent lid, with a wired cage at the far end (10cm diameter). The wired cup is used to house a target mouse of the same sex and strain during the target-present testing. The space (5 cm) surrounding the wired mesh cage was designated as the interaction zone. The lighting conditions included a combination of infrared and red lights for optimal video tracking of the mice in the arena.

On PND 69, all animals were brought into the testing room and allowed to acclimate for one hour. Each mouse was tested in two trials, target absent and target present, before returning

to their original cage conditions. In each trial, mice were free to explore the arena for 2.5 minutes. In the first trial, the test mouse was allowed to explore the arena and the empty wire mesh cage (target absent). Between trials, the testing mouse was removed and a target mouse placed in the wired mesh cage. In the second trial, the test mouse was placed in the arena and the wired mesh cup containing the target mouse (target present). Total locomotion and time spent in the interaction zone was recorded for each trial by overhead video camera. Anymaze® tracking system recorded these measurements with a preprogrammed protocol for zone reference.

Data Analysis

Two-way or repeated measures analysis of variance (ANOVA) was performed that included the possible independent variables of stress condition, sex, age (PND) and trial (target absent/present). This allowed us to determine if these factors influence the dependent variables body weight, percent time spent on open arms, physical coat score, alcohol consumption (g/mg) and time spent in the interaction zone (s). Any significant effect ($\alpha < 0.05$) was followed up with a Tukey's HSD post hoc analysis.

Chapter 3

Results

Ultrasonic Vocalization (USV)

USVs were recorded for all mice (17 females and 20 males) on PND 3 through 5. A median-split of the average number of calls for each litter designated high and low callers. Those above the median within the litter were high callers, indicating relatively higher fear-related behavior, while those below the median were low callers. The animals were assigned to either CVSS or CON balancing high and low callers among groups.

Body Weight

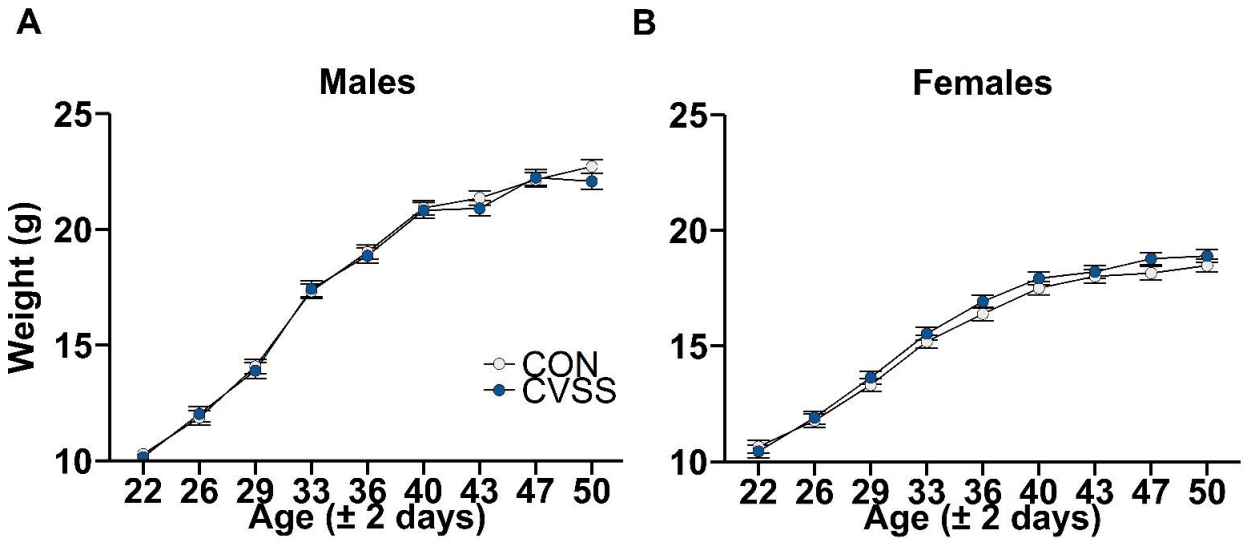

Figure 3.1 Body weight of all animals through adolescence.
Mean body weight for (A) males and (B) females between both control (CON) and stress (CVSS) groups. Data (Mean ± SEM)

The weight of each mouse was recorded on cage-change days (PND 22, 26, 29, 33, 36, 40, 43, 47, and 50) in order to minimize handling outside of normal procedures. A two-way repeated measures analysis of variance (ANOVA) with age, sex, and stress group as independent variables was used to examine body weight. There was no significant difference between CON and CVSS animals. There was a significant difference in weight between males and females ($F_{1,32} = 57.4$, $p<0.001$), such that males weighed more than females, and a significant effect of age ($F_{8,264} = 1869.8$, $p<0.001$) as expected. There was also a significant age x sex interaction ($F_{8,264} = 75.6$, $p<0.001$), such that males were associated with higher mean weights than females after PND 33 (Figure 3.1).

Novel Physical

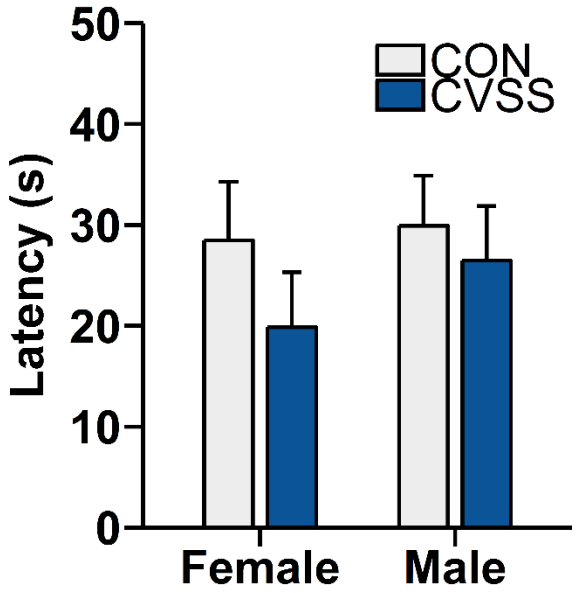

Figure 3.2 Latency to approach in Novel Physical test.
Mean latency to approach the first novel object for control (CON) and stress (CVSS) animals. Data (Mean ± SEM)

The Novel Physical test was conducted on PND 22 and 23 to measure the latency to approach a novel physical object. This test provided an index and initial screening for socially-inhibited behaviors in all mice prior to the chronic variable social stress procedure. There was no significant difference in latency to approach the novel physical object(s) between CVSS and CON animals (Figure 3.2). Further, there were no significant interactions present between stress group and sex. These results indicate that mice did not differ in latency to approach a novel physical object.

Physical Coat State

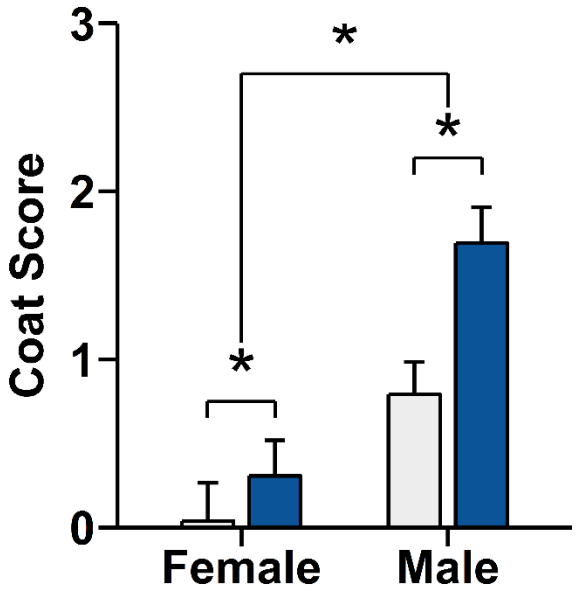

Figure 3.3 Coat score.
Mean coat score on PND 50 in control (CON) and stress (CVSS) animals. Data (Mean ± SEM), *p < 0.05.

The coat status was scored by visual observation on PND 50. There was a significant difference in coat score between CON and CVSS animals ($F_{1,32} = 8.5$, $p<0.01$), such that CVSS animals scored higher (1.0 ± 0.1, n=18) than CON animals (0.41 ± 0.15, n=19)indicating a

deteriorated physical coat status (Figure 3.3). There was also a significant difference between males (1.2 ± 0.1, n=20) and females (0.2 ± 0.2, n=17) with males scoring higher than females ($F_{1,32}$ = 22.0, p<0.001). There was no interaction between sex and stress group. These results indicate that the CVSS group as well as males of both groups had significantly higher scores on coat status, descriptive of neglecting the activity of self-grooming.

Elevated Plus Maze (EPM)

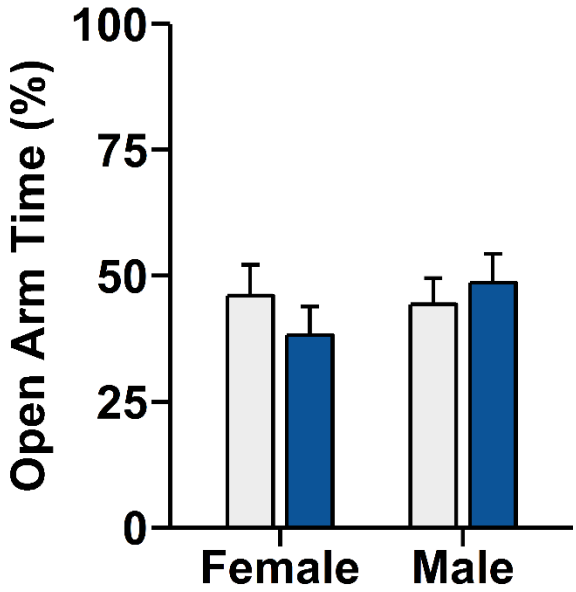

Figure 3.4 Percent of time spent on the open arms of Elevated Plus Maze (EPM).
Mean percent time on open arms on EPM for control (CON) and stressed (CVSS) animals. Data (Mean ± SEM)

The EPM was used to measure anxiety-like behavior in late-adolescent animals (PND 52 and 53). The results of this test indicate that there is no significant difference between CON and CVSS animals on the percent of total time spent on the open arms (Figure 3.4). There were no

differences between males and females, as well as no interaction of sex and stress group. These results indicate that anxiety-like behavior was not influenced by CVSS group.

Alcohol Consumption during DID

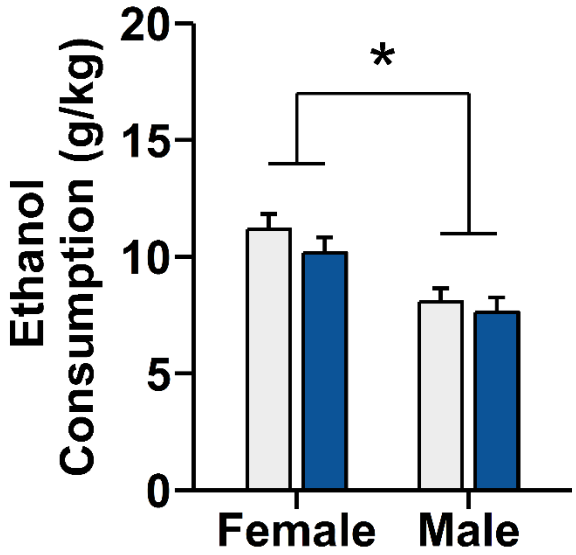

Figure 3.5 Ethanol consumption during Drinking in the Dark (DID).
Mean ethanol consumption measured in DID procedure for control (CON) and stress (CVSS) animals. Data (Mean ± SEM), *p < 0.05.

In late-adolescence (PND 55-58) all mice were tested in the DID paradigm. There were no significant differences in alcohol consumption between CON and CVSS animals on the Day 4 DID session (Figure 3.5). There was a significant difference between males and females ($F_{1,32}$ = 18.0, p<0.001) such that females (10.7 ± 0.47 g/kg, n=17) had higher levels of alcohol consumption than males (7.8 ± 0.43 g/kg, n=20). There was no interaction between sex and stress group.

Social Interaction

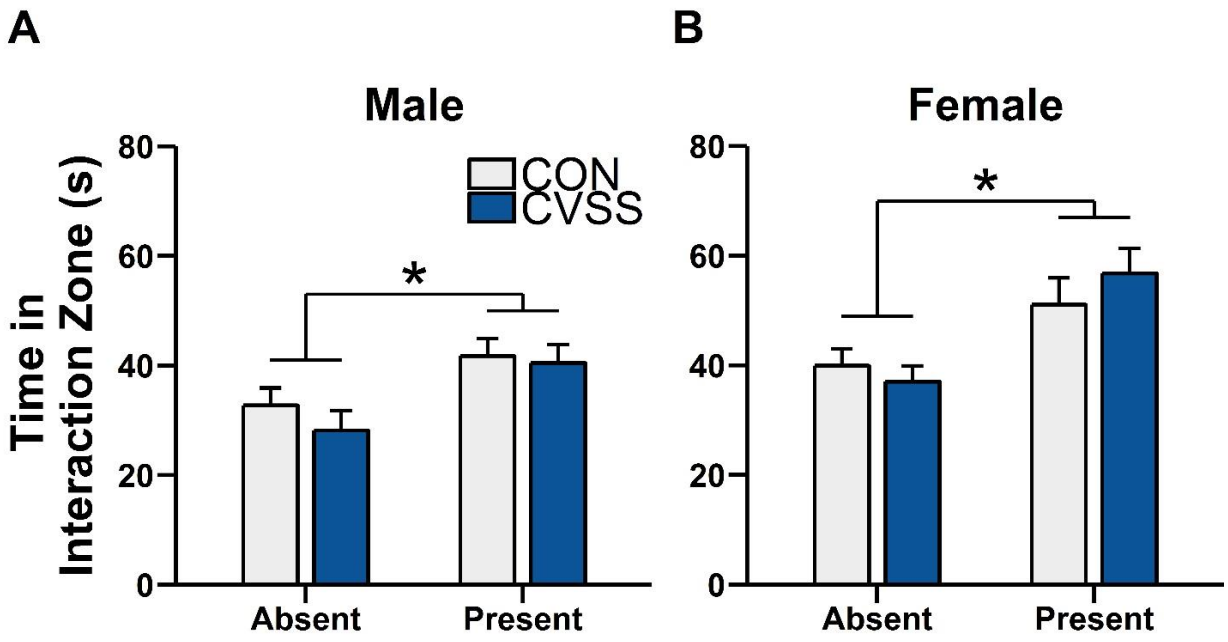

Figure 3.6 Amount of time spent in the interaction zone of the Social Interaction test. Mean time spent in the interaction zone of (A) male and (B) female control (CON) and stress (CVSS) animals for each trial. Two way ANOVA of time spent in the interaction zone, using trial as an independent variable (target mouse absent or present). Data (Mean ± SEM), *p < 0.05.

All mice were tested in the Social Interaction test on PND 70. Data were analyzed with a two-way ANOVA to consider differences in time spent in the interaction zone and total distance traveled, with trial (target present or target absent) as an independent variable. As shown in Figure 3.6, there was a significant effect of trial ($F_{1,32} = 28.7$, $p<0.001$) where animals spent more time in the interaction zone when the target mouse was present (47.4 ± 2.0 s, n=37) than when the target was absent (34.4 ± 1.6 s, n=37). There was a significant difference between males and females on time spent in the interaction zone ($F_{1,32} = 5.6$, $p<0.05$) indicating females spent more time in the interaction zone (44.8 ± 2.1 s, n=17) than males (37.0 ± 1.97 s, n=20).

There was no significant difference between CVSS and CON mice in time spent in the interaction zone.

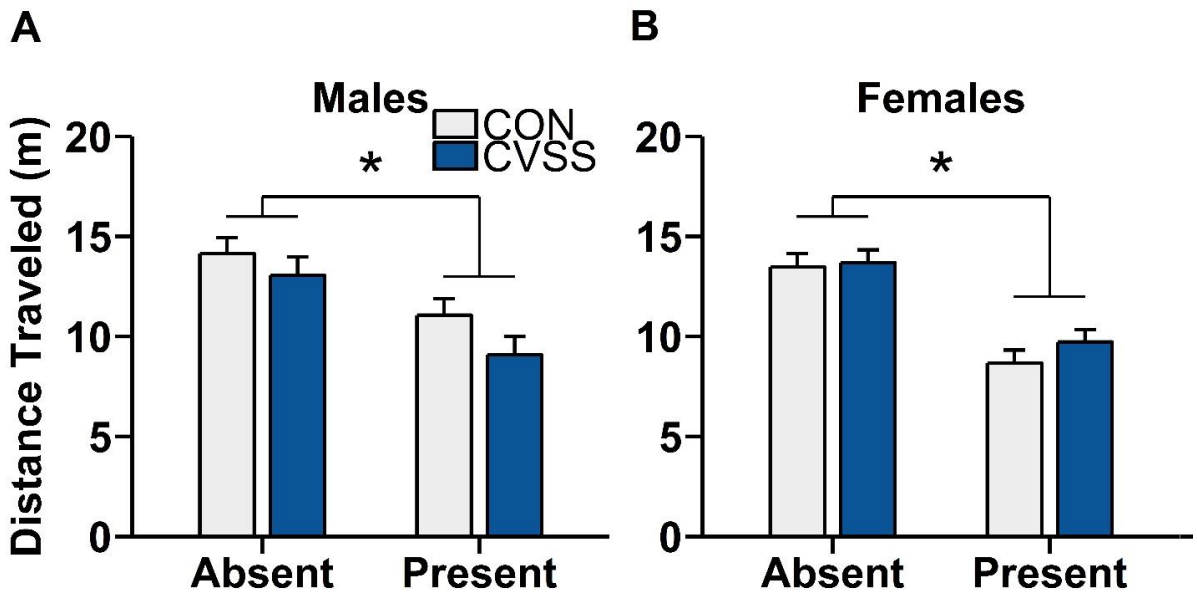

Figure 3.7 Total distance traveled in the Social Interaction test arena.
Mean distance traveled of (A) male and (B) female control (CON) and stress (CVSS) animals for each trial. Two way ANOVA of time spent in the interaction zone, using trial as an independent variable (target mouse absent or present). Data (Mean ± SEM), *p < 0.05.

As shown in Figure 3.7, there was an effect of trial on total distance traveled ($F_{1,31}$ = 83.8, p<0.001) such that all mice were more active when a target mouse was absent (13.6 ± 0.4 m, n=37) than present (9.7 ± 0.4, n=37). There were no significant differences in total distance traveled between sexes or stress groups.

Chapter 4

Discussion

Our findings suggest that the chronic variable social stress procedure (CVSS) did not induce anxiety-like behavior measured by the EPM or the Social Interaction test compared to control (CON) animals. The CVSS paradigm also did not induce changes in alcohol consumption in stressed BALB/cJ mice, indicated by the lack of difference between CVSS and CON animals in alcohol consumed on Day 4 of the DID procedure. However, males in the CVSS group were observed to have a degraded physical coat state, relative to females in the CVSS group and all CON mice. The reduction in grooming behavior in CVSS males may be associated with the deleterious effects of chronic variable social stress. These results collectively do not support the original hypothesis that chronic variable social stress during adolescence would induce changes in affect-related behavior in late adolescence or adulthood and alcohol consumption in late adolescent mice.

Weight

The results of mean body weight over time indicate that the chronic variable social stress procedure did not induce changes in weight gain in stressed mice. The weight gain in all mice were similar to weight gain observed in BALB/cJ mice (Normative data provided by The Jackson Laboratory MPD 2017). Other studies using a chronic social stress paradigm of social rehousing have also found that despite increased anxiety-like behavior and corticosterone levels in stressed mice, there were no differences in weight gain between conditions (Schmidt et al., 2010a, 2010b). Chronic social isolation during adolescence produced similar findings in

C57BL/6J mice (Lopez et al., 2011). BALB/cJ mice did not show a difference in weight gain between CVSS and CON groups during or after the chronic variable social stress procedure during adolescence in earlier data from our lab (Caruso et al., under review; Caruso et al., in prep). Collectively, changes in body weight gain may not be a reliable indicator of stress in mice following chronic stress.

Physical Coat State

Male CVSS mice did exhibit effects of stress indicated by a deteriorated physical coat state. This effect has also been observed in mice with increased anxiety-like behavior on EPM after stress (Ducottet and Belzung, 2005; Hua-Cheng et al., 2010). Other chronic mild stress procedures have found that this deteriorated coat state can persist several weeks after discontinuation of the stressor (Mitchell and Redfern, 2005; Willner, 2005). One study showed that mice subjected to chronic mild stress had markedly deteriorated coat states, as well as a reduction in cell proliferation in dentate gyrus, an important part of the hippocampus thought to be involved in formation of new episodic memories and novel environment exploration (Alonso et al., 2004). Supporting the validity of deterioration of physical coat state as an indicator of stress, the study used drugs to specifically target important components of the stress response system, corticotrophin-releasing factor (CRF-1) receptor antagonist, vasopressin (V1b) receptor antagonist, and a clinically used antidepressant fluoxetine. Administration of CRF-1 and V1b receptor agonists, as well as fluoxetine treatment, reversed the effects of the chronic mild stress procedure and improved the physical coat state in stressed mice, as well as prevention of reduced granule cell neurogenesis (Alonso et al., 2004). In a similar study, the persistent degradative

effects of chronic mild stress on physical coat state were improved in stressed mice treated with a common antidepressant medication fluoxetine and vasopressin receptor antagonist SSR149415, or Nelivaptan, relative to stressed mice treated with a vehicle injection. The improvement in physical coat state was dependent on dosage, as stressed mice improved more dramatically with a higher dose of SSR149415, 10mg/kg vs 30 mg/kg (Griebel et al., 2002). In studies with a chronic social stress procedure, mice showed deteriorated coat states during stress procedure that persisted well after the discontinuation of the stressor (Boleij et al., 2014; Schmidt, Sterlemann, and Muller, 2008). Chronic stress has altered physical coat state in BALB/c mice, and antidepressant administration was protective against the effect, suggesting that a deterioration of coat state is an indicator of stress (Yalcin and Belzung, 2008). Previously, male CVSS mice did not exhibit depression-like behavior measured in forced-swim test, or Sucrose preference test (Caruso, Kamens, and Cavigelli, under review). This suggests that physical coat state is not a reliable measure of depression-like behavior following CVSS, although, it is an indicator of stress.

Elevated Plus Maze (EPM)

Performance on EPM was similar between both CVSS and CON groups, indicating that the stress procedure did not induce anxiety-like behavior. The stressed and non-stressed animals spent approximately the same amount of time on the open arms. We expected to find that CVSS mice would spend less time on the open arms, exhibiting aversion or avoidance of open spaces, thus this finding does not support the hypothesis. Anxiety-like behavior on the EPM has been classically characterized as an accurate method for detecting anxiety-like behavior in chronically

stressed animals by exploiting rodent fears of open and unprotected spaces. Importantly, administration of known anti-anxiety drugs in rodents has been shown to increase time spent in the open arms or reduce anxiety-like behavior (McCormick, Green, and Barnes, 2013; Hogg, 1996; Pellow et al., 1985). The EPM has been used to model anxiety-like behavior in studies using a chronic social stress paradigms in mice and rats. Adolescent exposure to chronic social instability stress has been shown to decrease the time spent in the open arms of the EPM in rodents, indicating increased anxiety-like behavior (McCormick et al., 2008; Schmidt et al., 2007, 2010a). The effect of chronic social stress on time spent in the open arms has been shown to affect both males and females indiscriminately in most cases, with the exception of decreased open arm time in dioestrus females compared to oestrous females (McCormick et al., 2008). However, one important distinction between EPM experimentation in this study and the aforementioned is the period of time in the animals' life cycle in which the experiment was conducted. When examining the effects of adolescent chronic social stress, EPM procedure is typically performed in adulthood (PND 60) or later in order to determine effects on anxiety-like behavior that persist well after the stressor is administered, therefore, few studies have examined adolescent behavior on the EPM (McCormick and Green, 2013). In our study, EPM testing took place during late-adolescence (PND 52-53). While the difference between these two periods of the life cycle is roughly one week, displays of anxiety-like behavior can be distinctly different and even opposite between groups that only differ in age (Doremus and Spear, 2004; Spear, 2000). In a separate cohort of BALB/cJ mice that underwent the same CVSS procedure, all mice spent roughly 5% of time on the open arms when tested in adulthood (PND 61-63), while all mice in this study spent roughly 50% of their time on the open arms when measured in adolescence (Caruso et al., in prep). Using a similar paradigm to model anxiety-like behavior in

rodents, differences in this behavior between adult and adolescent mice have been observed. Tested on the elevated-zero maze, which utilizes a continuous outer ring with closed and open quadrants, adolescent mice exhibited more open quadrant entries than adult mice (Wilking et al., 2012). In rats, findings of age dependent performance on the EPM supports these results. Adolescent rats exhibited high levels of open arm entries and time spent on the open arms, whereas adults showed a striking reduction in these measures (McCormick et al., 2008). Other studies have mixed results, suggesting adolescent mice exhibit more or less anxiety-like behavior measured on EPM (Doremus et al., 2006). The difference in time spent in the open arms between adolescent and adult rodents may be due in large part to the increased risk-taking and reward seeking behavior seen in adolescent mice, as well as adolescent humans (Spear, 2000).

Another important difference that may explain the lack of anxiety-like behavior observed on EPM are strain differences in behavior on EPM. BALB/cJ have been characterized physiologically and behaviorally as a stress sensitive and anxiety-prone strain of mice, shown to display differences in depressive- and anxiety-like behavior as well as sensitivity to antidepressants (Kalueff and Tuohimaa, 2005a). Studies that have compared non-stressed C57BL/6J, DBA/2J, and BALB/cJ strains of mice have indicated that when tested in adulthood on EPM, BALB/cJ strain spent the least amount of time on open arms, between 5-10% of total time on EPM. Also supporting the results of our study, after an unpredictable chronic mild stress paradigm, BALB/cJ mice did not differ significantly in time spent on the open arms between stressed and non-stressed groups when measured in adulthood (Mineur et al., 2006). Inbred BALB/cJ mice have been shown to exhibit higher risk assessment than various other inbred mouse strains when assessed on the EPM (Ducottet and Belzung, 2005). Collectively, this suggests that BALB/cJ adolescent mice have high levels of exploratory-like behavior during

adolescence and venture onto the open arms infrequently during adulthood, suggesting a potential limitation of the EPM for assessing anxiety-like behavior by measures of time spent on closed and open arms in this strain and during adolescence regardless of strain. In previous work, adult CVSS mice have shown anxiety-like behavior on EPM, indicated by reduced time spent in the open arms and reduced novelty-induced locomotor activity (Caruso, Kamens, and Cavigelli, under review; Caruso et al., in prep). While these results indicate that CVSS did not alter affect-related behavior on EPM in late-adolescent mice, CVSS does alter performance on the EPM in adult mice.

Alcohol Consumption during DID

The results of this study suggest that the chronic variable social stress did not induce changes in alcohol consumption, indicated by a lack of difference between CVSS and CON mice. One important distinction between our study and prior work that has examined the effect of stress during adolescence is the strain of mouse used. The most frequently used strain in alcohol drinking studies is the C57BL/6J. This strain consumes a large amount of alcohol in the DID paradigm when compared to 11 other inbred strains of mice. Also, the BECs achieved by BALB/cJ mice were much lower than C57BL/6J mice (Rhodes et al., 2007). One study in particular found that the BALB/cJ strain of mouse had a preference for alcohol roughly 25% that of the C57BL/6J mouse strain (Crabbe et al., 2008). Together, these findings suggest a possible limitation of using BALB/cJ mice to observe the effects of chronic stress on alcohol consumption, as well as comparing our results to previous findings. Another important difference is the age in which the mice were exposed to the alcohol. Alcohol consumption and

preference of adult mice is more frequently measured than adolescent alcohol consumption, following social stress (Juaez and Vazquez-Cortes, 2003; Lopez et al., 2011; Lopez and Laber, 2015). Previous work examining the difference in adolescent and adult binge-like alcohol consumption using the DID procedure found that adolescent C57BL/6J mice consumed more alcohol (Holstein et al., 2011). Importantly, this higher level of binge-like drinking in adolescence may be due to a reduced sensitivity to the aversive effects of alcohol (Spear and Varlinskaya, 2005; Vetter-O'Hagen, Varlinskaya, and Spear, 2009; Holstein et al., 2011). This evidence suggests that, when interpreting the results of this study, any changes due to chronic variable social stress procedure during adolescence may be masked by high levels of adolescent alcohol consumption. However, it is also likely that CVSS does not alter alcohol consumption in adolescent mice, but instead induce effects evident only in adulthood. Our lab has data to suggest that CVSS alters binge-like alcohol consumption in adult BALB/cJ mice (Kamens et al., unpublished). This suggests that CVSS does alter alcohol consumption; however, these effects may be specific to adulthood alcohol consumption.

The results also indicate that females consume more alcohol than males, regardless of condition, on Day 4 DID. Voluntary alcohol consumption in mice has been found to differ between males and females, which supports our findings of a significant effect of gender on binge-like alcohol consumption (Tambour et al., 2008). Female C57BL/6J mice have been shown to voluntarily consume more alcohol than males during limited access alcohol exposure, where all mice had access to both alcohol and water for a 2 hour period (Lopez et al., 2011; Rhodes et al., 2005). This elevated alcohol consumption in females is supported by prior research with BALB/cJ mice when examining choice paradigms for alcohol (Rhodes et al., 2007).

Social Interaction (SI)

The results of the Social Interaction test indicated that adult (PND 70) CVSS mice did not exhibit social avoidance, a measure of depression-like behavior after stress in rodents (Haller et al., 2003). CVSS and CON mice did not differ significantly on the time spent in the interaction zone, both when a target mouse was absent and present in the wired-mesh cage. There was a significant effect of trial, where all mice were observed to spend more time in the interaction zone when a target mouse was present. The difference in time spent in the interaction zone between the first trial and the second trial with an addition of a social target mouse suggest that all mice exhibited some degree of social interaction. Females spent more time than males in the interaction zone in both trials, suggesting that females interact more with social targets than males. All mice traveled more distance when the target was absent than when the target was present, indicating that mice exhibited reduced activity/locomotion when a social target was present during the Social Interaction test. Combined, however, these results do not support the hypothesis that chronic variable social stress during adolescence would induce lasting effects on social behavior in adulthood. These results contradict previous work that suggests rodents that underwent adolescent social stress during adolescence exhibit social avoidance measured by time in the interaction zone (Vidal et al., 2007; Haller et al., 2003; Leveleki et al., 2006).

Previously noted, BALB/cJ mice have been shown to exhibit higher levels of risk assessment relative to other strains (Ducottet and Belzung, 2005). In a comparison of neuroendocrine and behavioral responses between C57BL/6J and BALB/c mice through emotional performance on behavioral tasks, BALB/c inbred mice displayed higher levels of risk assessment and exploration of novel testing environment despite exhibiting higher corticosterone responsiveness to stress (Brinks et al., 2007). This suggests that adolescent BALB/cJ inbred

mice, while physiologically stressed, may not exhibit altered social interaction in the Social Interaction test. Previous work supports these results, finding that adult CVSS mice spent more time in the interaction zone when the target was present than when the target was absent and females spent more time in the interaction zone than males (Caruso et al., in prep). Additionally, adult CVSS males spent less time in the interaction zone and traveled less distance than CON males. This suggests that CVSS reduced novelty-induced locomotion in the Social Interaction test and reduced social interaction in adult CVSS males (Caruso et al., in prep).

Conclusion

Differences in behavior, stress-responsivity, and alcohol preference in BALB/cJ mice as well as between adolescents and adults may have limited our ability to detect effects of CVSS on affect-related behavior and alcohol consumption. CVSS did not affect anxiety-like behavior, social interaction, or binge-like alcohol consumption in adolescent BALB/cJ inbred mice. Although, CVSS did alter behavior in adult mice in previous work in our lab. In adult mice, CVSS reduced locomotion/activity in Social Interaction and EPM, altered alcohol consumption, and induced anxiety-like behavior on EPM (Caruso, Kamens, and Cavigelli, under review; Caruso et al., in prep; Kamens et al., unpublished). These findings suggest that the effects of adolescent stress on affect-related behavior, alcohol consumption, and exploratory behavior in a novel environment are delayed until adulthood. Delayed effects on locomotion response to novel environments have been found in rats following chronic social stress during adolescence (Kabbaj et al., 2002). Other studies utilizing chronic social stress have found delayed effects, reporting

that these effects are delayed likely because further brain development is required before these effects can emerge (Mathews et al., 2008; McCormick, Smith, and Mathews, 2008).

To summarize, we report our findings on the effect of chronic variable social stress during adolescence on changes in affect-related behavior and binge-like alcohol consumption in late adolescence, in BALB/cJ mice. Collectively, the findings of this study suggest that the CVSS paradigm did not induce anxiety-like behavior in late-adolescent CVSS mice. Furthermore, alcohol consumption between CVSS and CON mice was not significantly different when measured in late-adolescence. Although adolescence may provide a window of vulnerability to chronic social stressors, our results suggest that changes in affect-related behavior and alcohol consumption may only be evident in adulthood. Chronic social stress during adolescence has resulted in delayed effects in hippocampal morphology, cognitive ability, and HPA axis functions in laboratory animals (Isgor et al., 2004). In order to further investigate these delayed physiological changes, future research directions might look more closely at the potential mechanisms that are involved in the maturation of the adolescent brain and underlie the delay in behavioral changes following chronic variable social stress.

Appendix

Data Analysis

Weight				
ANOVA	numDF	denDF	F-Value	*p*-value
Age	8	264	1869.8	<0.0001
Sex	1	32	57.4	<0.0001
StressGroup	1	32	0.12	0.74
Age:Sex	8	264	75.7	<0.0001
Age:StressGroup	8	264	1.0	0.41
Sex:StressGroup	1	32	0.6	0.44
Age:Sex:StressGroup	8	264	1.0	0.46

Tukey's HSD	Sex	Mean Weight (g)	SE	df	Lower.CL	Upper.CL	Group
Age = 22	Male	10.2	0.21	32	9.71	10.72	A
	Female	10.6	0.23	32	10.00	11.10	A
Age = 26	Male	11.9	0.21	32	11.44	12.44	A
	Female	11.8	0.23	32	11.30	12.39	A
Age = 29	Male	13.9	0.21	32	13.48	14.48	A
	Female	13.48	0.23	32	12.93	14.02	A
Age = 33	Male	17.4	0.21	32	16.87	17.88	A
	Female	15.4	0.23	32	14.82	15.91	B
Age = 36	Male	18.9	0.21	32	18.43	19.44	A
	Female	16.6	0.23	32	16.11	17.21	B
Age = 40	Male	20.9	0.21	32	20.37	21.37	A
	Female	17.7	0.23	32	17.17	18.26	B
Age = 43	Male	21.1	0.21	32	20.62	21.63	A
	Female	18.1	0.23	32	17.57	18.66	B
Age = 47	Male	22.2	0.21	32	21.69	22.70	A

	Female	18.5	0.23	32	17.92	19.01	B
Age = 50	Male	22.39	0.21	32	21.89	22.90	A
	Female	18.7	0.23	32	18.14	19.23	B
	Results are averaged over the levels of: StressGroup Confidence level used: 0.95 Conf-level adjustment: sidak method for 2 estimates significance level used: alpha = 0.05						

Novel Physical: Latency					
ANOVA	DF	Sum Sq	Mean Sq	F value	Pr(<F)
Sex	1	169.9	169.9	0.65	0.43
StressGroup	1	312.9	312.9	1.19	0.28
Sex:StressGroup	1	60.2	60.2	0.23	0.64
Residuals	32	8425.0	263.28		

Coat Score						
ANOVA	DF	Sum Sq	Mean Sq	F value	Pr(<F)	
Sex	1	8.78	8.78	21.99	<0.0001	
StressGroup	1	3.4	3.4	8.52	<0.01	
Sex:StressGroup	1	0.92	0.92	2.29	0.14	
Residuals	32	12.78	0.40			
Tukey's HSD	Mean	SE	Df	Lower.CL	Upper.CL	Group
CON	0.42	0.15	32	0.07	0.76	A
CVSS	1.00	0.15	32	0.65	1.35	B
Tukey's HSD	Mean	SE	Df	Lower.CL	Upper.CL	Group
Male	1.24	0.14	32	0.90	1.58	A
Female	0.17	0.16	32	-0.20	0.54	B

EPM: Percent Time Open Arms					
ANOVA	DF	Sum Sq	Mean Sq	F value	Pr(<F)
Sex	1	166.2	166.25	0.58	0.45
StressGroup	1	15.2	15.15	0.05	0.82
Sex:StressGroup	1	333.9	333.94	1.17	0.29
Residuals	32	9128.8	285.28		

DID: Alcohol Consumption						
ANOVA	DF	Sum Sq	Mean Sq	F value	Pr(<F)	
Sex	1	61.50	61.50	17.96	<0.0001	
StressGroup	1	4.47	4.47	1.31	0.26	
Sex:StressGroup	1	0.70	0.70	0.20	0.66	
Residuals	32	109.58	3.43			
Tukey's HSD	Mean (g/kg)	SE	Df	Lower.CL	Upper.CL	Group
Male	7.85	0.43	32	6.82	8.87	A
Female	10.67	0.47	32	9.57	11.78	B

Social Interaction: Time Spent in Interaction Zone						
ANOVA	numDF	denDF	F-Value	*p*-value		
Trial	1	31	28.7	<0.0001		
Sex	1	32	5.60	0.0242		
StressGroup	1	32	0.59	0.45		
Trial:Sex	1	31	1.19	0.28		
Trial:StressGroup	1	31	1.39	0.25		
Sex:StressGroup	1	32	0.43	0.52		
Trial:Sex:StressGroup	1	31	0.33	0.57		
Tukey's HSD	Mean (s)	SE	Df	Lower.CL	Upper.CL	Group
Male	36.98	1.97	31	32.36	41.60	A
Female	44.83	2.08	31	39.94	49.72	B
Tukey's HSD	Mean (s)	SE	Df	Lower.CL	Upper.CL	Group
Absent	34.38	1.56	32	30.71	38.05	A
Present	47.43	2.05	31	42.61	52.2	B

Social Interaction: Total Distance Traveled						
ANOVA	numDF	denDF	F-Value	*p*-value		
Trial	1	31	83.8	<0.0001		
Sex	1	32	0.95	0.34		
StressGroup	1	32	0.91	0.35		
Trial:Sex	1	31	0.06	0.82		
Trial:StressGroup	1	31	1.04	0.32		
Sex:StressGroup	1	32	3.13	0.09		
Trial:Sex:StressGroup	1	31	1.13	0.30		
Tukey's HSD	Mean (m)	SE	Df	Lower.CL	Upper.CL	Group
Absent	13.62	0.38	32	12.72	14.51	A
Present	9.69	0.39	31	8.76	10.61	B

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Academic Vita

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Education

The Pennsylvania State University, Anticipated Graduation May 2017

Eberly College of Science**,** Biology – Neuroscience

Schreyer Honors College**,** Department of Biobehavioral Health

Recent Work Experience

Bellefonte EMS, Bellefonte, PA, 16823

Emergency Medical Technician – B **2015 – Present**

- Covered overnight 12hr shifts operating as a basic-life support unit.
- Provided pre-hospital treatment in a variety of health-related emergency situations.
- Trained new EMT volunteers.
- Implemented strict schedule-coordination between overnight shifts, school, volunteering, research, and shadowing obligations.

The Pennsylvania State University, University Park, PA 16802

Senior Learning Assistant, General Biochemistry 401 **Jan 2017 – May 2017**

- Assisted in facilitating learning both in BMB401 lectures and through office hours.
- Co-led the BMB398B supplemental course and assisted in breaking-down complex concepts with peer-involved learning, with ~30 students.
- Further developed tutoring skills by guiding practice of material.

Learning Assistant, General Biochemistry 401 **Aug 2016 – Dec 2016**

- Acted as a resource for students both during lecture and through email communication.
- Led the BMB 398B supplemental review course to assist students to achieve understanding of novel and often complex biochemical relationships.
- Awarded senior lecture position for Spring 2017 semester.

Proctor/Grader, Department of Mathematics **Aug 2016 – Dec 2016**

- Presided over semester and final examinations through coordination with the Mathematics Department.
- Enforced academic integrity during examinations consisting of ~30 to 100 students.
- Coordinated with instructors on exam preparation, operations and debriefing activities.

Research & Publications

Department of Biobehavioral Health, Behavioral Neurogenetics Laboratory

Research Assistant **2015 – 2017**

- Worked extensively on scientific investigation through the completion of an Honors Thesis, including literature review and behavioral testing.
- Assisted in leading summer experimentation and guiding assistants in proper technique.
- Presented work by poster at Penn State Undergraduate Research Exhibition.

Department of Biology, Huck Institute of the Life Sciences, Monshausen Laboratory

Research Assistant **Spring 2016**

- Investigated the root integrity of Arabidopsis mutant variants with possible applicability to modern agricultural practice.
- Gained valuable insight into plant physiology, anatomy and socio-economic and environmental impact associated with changes from normal.
- Analyzed root angle data consisting dependent on 2 mutant phenotypes in 3 different growing-medium densities.

Community Service Involvement

Mount Nittany Medical Center, State College, PA 16803

Emergency Department Volunteer **2016 – 2017**

- Provided comfort through positive patient interaction during transport to and from medical testing.
- Coordinated relieving nurses of some nursing orders including running medical information, patient discharge and retrieving meals.
- Trained new emergency department volunteers and oriented them to the hospital.

University Health Services, University Park, PA 16802

Clinic Volunteer/Intern **2016 – 2017**

- Conducted patient intakes and updating patient information including pertinent vitals, NOI/MOI, and discussing symptoms.
- Assisted in training new volunteers.
- Ensured a positive experience by providing excellent service as the first individual the patient has contact with at the clinic.

Honors, Scholarships & Grants

- **Schreyer Honors College, May 2017**
  - Recognized for academic excellence and scholarly pursuit

- **Erickson Discovery Grant,** Summer 2016
    - Sizeable grant award towards thesis project and research discovery
- **Dean's List, 2013 – 2017**
    - Recognized for semester GPA $\geq$ 3.5
- **George E., Jr. and Elizabeth S. Sperling Scholarship,** 2017
    - Scholarship award for Senior Learning Assistant position in Eberly College of Science
- **Baynard D. and Ethel M. Kunkle Scholarship,** 2016 & 2017
- **Dr. Mary. M. Finn and Robert F. Pruner Sr. Trustee Scholarship in Science,** 2016 & 2017
- **Behrend Honors Program, 2015**
    - Recognized for academic excellence