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Adolescent Alcohol Consumption, Stress, and the Developing Brain

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ABSTRACT

Adolescence is a critical phase of brain development with significant cognitive and behavioral changes. Throughout the time period of adolescence, a multitude of changes in brain composition and cortical rewiring take place. As a result, adolescents are found to be more susceptible to ethanol during this time period especially during periods of significant ethanol consumption known as binge drinking. Binge drinking is defined as a BAC to above 0.08 g/dL due to repeated drinking episodes typically reached within a two-hour period. More specifically, binge drinking, and stress have both been known to alter the development and typical function of neurotransmitter circuits. Binge drinking during adolescence has also been labeled a major public health issue with global estimates of over 14% of individuals from the age of 15-18 drinking enough to reach binge levels over the past month (Chung et al., 2018). Not only is this drinking pattern prevalent in individuals ages 15-18, but 9.6% of individuals ages 12 to 17 reflected alcohol use in the past month with roughly half of those individuals' reporting periods of binge drinking (Kann et al., 2023). In fact, according to the National Institute on Alcohol Abuse and Alcoholism, individuals ages 14-20 are more likely to binge drink (*Underage Drinking | National Institute on Alcohol Abuse and Alcoholism (NIAAA)*, n.d.). Additionally, alcohol leads to deficits within educational attainment, and has been implicated in later development of alcohol use disorder. Not only has alcohol been known to make significant behavioral and cognitive changes during adolescence, but stress can also alter neuronal development, leading to compounding effects. Similar to ethanol consumption, anxiety disorders are also extremely prevalent in adolescents with an estimated 31.9% of 13-18 years olds suffering from an anxiety disorder characterized by consistent anxiety and fear for 6 months or more (Merikangas et al., 2010). Therefore, this project investigated the effects of ethanol consumption and stress on cortical circuit development, brain structure, and behavior as well as the interplay between stress and ethanol consumption. Specifically, of particular interest is the prefrontal cortex (PFC) as this brain region is one of the last to develop and plays an important role in executive functioning. This project was

divided into 3 parts 1) Cell count experiments to measure for alterations in brain structure, 2) Electrophysiological experiments to measure changes in neural circuitry in the prefrontal cortex (PFC) 3) Behavioral experiments to measure alterations in anxiety-like and drinking behavior as a result of ethanol consumption.

TABLE OF CONTENTS

LIST OF FIGURES iii

ACKNOWLEDGEMENTS v

Chapter 1 Introduction 1

Brain Development 1
Neurocircuitry Development 3
Animal Studies of Alcohol and the Developing Brain 5
Human Studies of Alcohol and the Developing Brain 7

Chapter 2 Methods 10

Animals 10
Adolescent Drinking in the Dark 10
Adolescent Double Drinking Behavior 12
Acute Stress Exposure (Forced Swim Test) 12
SST Cell Counts 12
Data Analysis, Statistics, and Figure Preparation 13

Chapter 3 Results 14

Chapter 4 Discussion 18

LIST OF FIGURES

Figure 1. The DID model and experimental timeline.....11

Figure 2. Mouse blood ethanol content sample.....11

Figure 3. Ethanol consumption in adulthood. 14

Figure 4. Ethanol consumption and acute stress exposure.....15

Figure 5. Adolescent DID does not alter the density of SST cells in the PL cortex.....17

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Chapter 1

Introduction

Brain Development

Alterations in both brain organization and structure are common throughout adolescence. Within humans, adolescence ranges from ages 10 to the age of 24. While unclear on the exact ages of adolescence within mice, most definitions define it as postnatal day (PND) 28–42 (Spear, 2000). The prefrontal cortex is one of the last to mature and is therefore susceptible to stress and ethanol exposure (Larsen & Luna, 2018). In fact, the delayed maturation of the brain leads to heightened novelty seeking behavior and risk taking (Steinberg et al., 2008). Brain development begins with the widely characterized process of neurulation followed by proliferation, cell migration, and synaptogenesis. Each of these steps results in the formation of distinct brain regions, the generation of neurons, and the formation of synapses (Tierney & Nelson III, n.d.). This synaptic growth is then followed by significant synaptic pruning. Synaptic pruning is the loss of synaptic connectivity prevalent during early brain development, and results in almost 50% of synaptic connections being eliminated. Within the frontal lobe, this process occurs into early adolescence within humans (Spear, 2013). Synaptic pruning is widely regarded as experience dependent, in which there is continuous modification as a result of one's environment as well as external factors. As a result, this process is easily influenced by both ethanol consumption and stress. Not only is pruning involved in the process of learning, but it is essential for the improvement of increasing the speed and precision of coordinated movements (Zuo et al.,

2005). While the loss of synaptic connections is common throughout development, brain regions often experience myelinated and white matter increases such as the prefrontal cortex (PFC).

Not only are there structural changes throughout this time period but functional changes are also common throughout adolescence. As white matter increases, the speed of electrical transmission does as well. In fact, the amount of white matter present in the brain is directly correlated with time spent studying or performing repetitive behaviors. In regard to the prefrontal cortex, myelination is associated with inhibitory control as well as improved cognition, showing its role in the importance of brain development (Caballero et al., 2020).

Overall, each of these changes reflect the overarching theme of increases in brain efficacy during adolescence(Spear, 2013). Cortical gray matter follows a similar pattern. Grey matter increases in childhood, peaks in early adolescence and declines in adulthood. While thinning in gray matter typically occurs well prior to adolescence in most brain regions, it continues into early adulthood in the PFC. Later development in the frontal regions is also evidenced through increases in focal activation of the PFC and in the ability to perform top-down processing tasks throughout development(Durston et al., 2006). While these changes occur within both males and females, it is important to note that there are significant differences in brain development. For example, by adulthood, males have a greater number of neurons and glial cells in the PFC (Markham et al., 2013).

In combination with development of the frontal cortex, reward areas experience significant changes throughout this time period. More specifically, throughout development, the brain experiences increased activation of the ventral striatum as well as increasing levels of dopamine. In fact, dopaminergic neurons during development are able to release more dopamine than those in adulthood(Galvan et al., 2006). While the prefrontal cortex develops at a slow rate,

various other brain regions have different developmental projections, including the limbic, sensorimotor, and frontal regions. Development occurs beginning with the limbic regions and ending with frontal regions that assist with higher order functioning(Sowell et al., 1999). The delay in maturation of frontal regions allows for advanced cognitive functions, including decision making, reasoning, creativity, and perseverance. As a result, this delay often contributes to risk taking common in adolescence (Casey et al., 2010). Throughout brain maturation, the PFC switches to greater focal activation of frontal regions and increasing involvement of these regions to other areas of the brain(Zuo et al., 2005).

Neurocircuitry Development

Along with structural and functional development, innervation by the gamma-aminobutyric acid (GABA) system develops throughout adolescence. Overall, GABAergic inhibitory inputs onto pyramidal neurons increase throughout adolescence along with increased neural connectivity. Differing GABA cells have varying molecular markers which can allow for cellular identification. While calretinin-expressing GABAergic neurons decrease throughout adolescence the number of parvalbumin (PV)-expressing neurons, the largest subpopulation of GABAergic neurons, increases in the mouse PFC(Caballero et al., 2020; Kilb, 2012). As a result, inhibitory inputs onto pyramidal cells gradually increase throughout adolescence (Cass et al., 2014).

Somatostatin (SST) cell density also increased in early adolescent mPFC of female mice with males showing a steady decline(Du et al., 2018). Other studies have also implicated SST-expressing interneurons in PFC function and the interaction between SST-expressing

interneurons and pyramidal neurons to be critical for PFC circuitry. Additionally, research from the Crowley laboratory has shown that alcohol alters SST neuronal excitability in adulthood, showing the importance of further investigation in adolescence. Additionally, SST cell often undergo developmental changes throughout adolescence; however, little is understood regarding their developmental trajectories in adolescence (Brockway & Crowley, 2020).

Other cell populations including glutamatergic connections in the PFC and the dopaminergic system in the PFC are continuing to develop throughout the adolescent time period. Dopaminergic innervation within the mPFC of rodent surges during adolescence is hallmarked by changes in the shape and density of dopaminergic fibers. These projections often peak during adolescence and result in amplified neural sensitivity to reward. In many cases, these dopaminergic neurons are capable of having an exclusive excitatory effect on GABAergic interneurons (Uytun, 2018). In combination with dopaminergic innervation, Glutamate N-methyl-D-aspartate (NMDA) receptors are required for proper development and further contribute to the excitatory/inhibitory balance in the PFC. Typically, Glutamate to NMDA receptor binding increases throughout adolescence until PND 28 in mice and declines into adulthood, indicating alterations in NMDA receptor dynamics such as receptor expression and sensitivity. While the PFC glutamatergic connections finish developing into adolescence, studies suggest that maturation continues until PND 56 (Ueda et al., 2015). As discussed previously, the dopaminergic system also undergoes many changes throughout the process of development. Specifically, the expression of dopaminergic receptors D1, D2, and D4 all increase throughout adolescence (Click or tap here to enter text.Tarazi & Baldessarini, 2000)Click or tap here to enter text.. Additionally, serotonergic connections are reached in early development and are the first to arrive within the prefrontal cortex. It is understood that the arrival of serotonin to these regions

leads to development of other monoamines such as dopamine. In the PFC, arrival occurs at postnatal week (PNW) 10-13 in humans (Whitaker-Azmitia, 2001). Additionally, high field (7 Tesla) Magnetic Resonance Spectroscopic Imaging suggested the balance between both glutamate and GABA develops throughout this adolescent time period (Perica et al., 2022). Additionally, for the majority of anxiety and neuropsychiatric disorders, the PFC is heavily implicated within this pathology. Specifically, peptides in the PFC such as somatostatin are implicated in neuropsychiatric diseases and the development of the PFC has been associated with behavioral changes within animal models within these diseases. As a result of the involvement of the PFC in psychiatric disorders and varying developmental changes within the brain (specifically in the prefrontal cortex), it is important to examine the impact of drug and alcohol use during this time period.

Animal Studies of Alcohol and the Developing Brain

Animal examinations of ethanol exposure have allowed for a greater understanding of the impact of ethanol consumption on brain circuits as well as different neuron populations. Different mouse models have revealed that apoptosis quickly follows ethanol exposure, resulting in widespread cell death. Further examination reflects that of the most damaged structures in the cerebral cortex with different damage to varying neuronal subtypes (Ikonomidou et al., 2000). Typically, pyramidal neurons of layer five are most susceptible to the death-inducing effects of ethanol when compared to neurons found in other cortical layers (Olney et al., 2002). Other studies have also shown the alcohol consumption during adolescence altered typical properties of

pyramidal neurons, inkling voltage sag and resting membrane potential (Click or tap here to enter text.Salling & Harrison, 2020) Click or tap here to enter text.. Additional changes in neuronal death are also commonly found in different subtypes of neurons. Specifically, while no change in neuronal cell death was found on parvalbumin interneurons from PND 2-PND 6, a substantial reduction of the same interneurons was seen after PND 7 (Granato, 2006). Despite parvalbumin interneurons experiencing a significant decline, this trend is not present for all other interneurons with others increasing and experiencing no change after ethanol exposure. In combination with cell death, ethanol can also delay neuronal development, keeping neuronal populations from undergoing typical development(Smiley et al., 2015). For example, intermittent alcohol exposure results in both reduced cholinergic markers in the orbitofrontal cortex as well as reduced dendritic spine density in the infralimbic cortex of the PFC (Boutros et al., 2014). Additionally, in combination with cholinergic markers, adolescent alcohol exposure has been shown to significantly alter dopamine receptor activity in adolescence. While typical adolescent brain development involves the stimulation of dopamine receptor 1, resulting in the evoked firing of pyramidal neurons, following adolescent alcohol exposure this is not the case (Trantham-Davidson et al., 2017).

While GABA potentiation is a direct result of ethanol consumption, other long-lasting consequences are often found. After ethanol consumption during adolescence, studies have found a decline in both gray matter and white matter integrity both lasting throughout adulthood, both of which being especially prominent within the prefrontal cortex(Pascual et al., 2014). Within rats, alcohol consumption has been shown to alter density of myelin. Specifically, within the anterior cingulate cortex, male rats experienced a significant reduction in myelin because of adolescent alcohol consumption. Additionally, ethanol consumption has been seen to have

resulted in structural differences with female rats that underwent ethanol consumption exhibiting smaller nodes of Ranvier (Tavares et al., 2019).

Not only has ethanol been shown to alter the integrity of both gray and white matter, it also has been shown to reduce functional connectivity between regions within the PFC (Broadwater et al., 2018). In addition to declines in brain volume and connectivity, ethanol consumption directly results in declines in cortical thickness as well as connectivity between brain regions with earlier ethanol exposure resulting in earlier thinning of the PFC(Broadwater et al., 2018; Vetreno et al., 2014).

In addition to neurological changes, there were also behavioral changes as a result of drinking throughout adolescence. For example, C57Bl/6J mice exposed to Drinking-in-the-Dark (DID), drank increasingly more as adults after consumption in adolescence(Moore et al., 2010). While some studies reflect these same results, others show that drinking during adolescence does not increase ethanol consumption into adulthood (Strong et al., 2010). Additional effects of ethanol consumption in adolescence include increased risk of anxiety and risk taking with males being more vulnerable to develop social anxiety related behaviors(Varlinskaya et al., 2015). Other effects of adolescent alcohol consumption include increased risky behavior seen in adolescent male rats exposed to alcohol via intragastric gavage (Boutros et al., 2014b).

Human Studies of Alcohol and the Developing Brain

In addition to animal models, many human studies have been conducted to evaluate changes in behavior, brain function, and brain structure as a result of ethanol consumption during adolescence. One study examined a group of individuals who consumed ethanol throughout

adolescence, showing that those who consumed ethanol experienced increased frontal, occipital, anterior cingulate cortex (ACC), and posterior cingulate cortex volumes compared with non-drinking controls. While there were increases in brain volume, heavy drinking adolescents also experienced significant reductions in the thalamus, middle temporal gyrus, inferior temporal gyrus, caudate, and brainstem (Doallo et al., 2014). Additional examinations of brain volume reflected that heavy adolescent drinkers experience significant reductions in gray matter volume. These reductions are seen in the neocortex, frontal cortex, and temporal cortex (Squeglia et al., 2015). Not only have studies reflected a reduction in gray matter, but others have shown that binge drinking adolescents also experience significant decreases in both white matter growth and integrity with decreases primarily in the corpus callosum and pons (Squeglia et al., 2015).

In combination with reductions in brain volume, ethanol resulted in a reduction in brain activation during reward tasks in a dose dependent manner. More specifically, individuals who consumed ethanol during adolescence experienced decreased activation on an MRI scan on a global scale with no significant differences in brain region(Cservenka et al., 2015). Other studies have also reflected the behavioral changes that are the result of binge drinking on a long term scale. To begin, early ethanol consumption has been largely implicated with deficits in memory largely in both short term memory, long term memory, and working memory (Carbia et al., 2017; Mahedy et al., 2018; Mota et al., 2013). In regard to short term memory, one study showed that heavy alcohol use during adolescence is directly associated with difficulties in verbal memory as well as short-term cognitive monitoring, implicating damage to areas involved such as the temporal mesial and dorsolateral prefrontal cortex (Mota et al., 2013).With long term memory, binge drinking during adolescence is associated with delayed recall on episodic memories that continued to remain stable after ethanol consumption (Carbia et al., 2017).

Adolescence is regarded as a critical period for brain development. Adolescent alcohol exposure has been associated with increased alcohol consumption in adulthood, indicating lasting consequences of adolescent drinking. While somatostatin (SST) neurons in the prelimbic cortex are vulnerable to binge drinking and have been shown to mediate levels of alcohol consumption in adult mice, there has been little examination of changes in prelimbic SST neurons following adolescent alcohol consumption. The goal of this project is to 1) examine the effect of adolescent alcohol consumption on mouse behavior and to 2) examine the effect of adolescent alcohol consumption on brain connectivity, structure, and function looking at changes in cell density.

Chapter 2

Methods

Animals

All experiments were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Both male and female SST-Cre Ai9 mice were used for both electrophysiology, double drinking, and cell count experiments. Male and female C57BL/6J mice were used for acute stress exposure experiments. At postnatal day (PND) 21 all mice were single-housed and moved into a reverse light cycle room. For all experiments mice were assigned to either an adolescent alcohol exposure condition or a control condition.

Adolescent Drinking in the Dark

For each experiment, the Drinking in the Dark (DID) paradigm was used. This protocol as described previously states that mice receive 20% ethanol in water on 3 consecutive days beginning 3 hours into the dark cycle for 2 hours (10am-12pm) (Rhodes et al., 2005) On the fourth day, mice receive ethanol for a four hour period (10am-2pm) as seen in figure 1A. Between each 4 day cycle, mice receive 3 days of abstinence from ethanol. Ethanol consumption was measured through weighing ethanol bottles before and after each drinking window. For all experiments, adolescent drinking began PND 28-30 and ended PND 52-54 (Figure 1B). All control mice were given H2O. In order to validate ethanol exposure, blood ethanol concentrations were taken via tail blood sample 30 minutes after the final binge session (Figure

2).

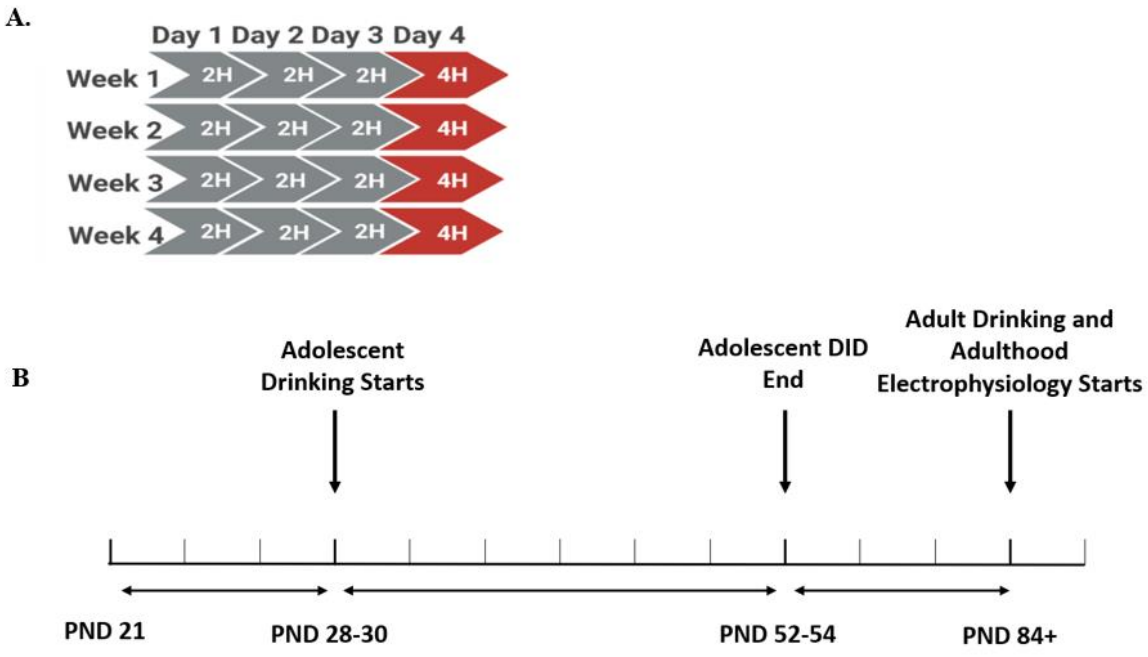

Figure 1. The DID model and experimental timeline. Graph A represents the DID paradigm in which mice are provided ethanol for 2 hours for 3 consecutive days followed by a 4 hour drinking period on the fourth day. Graph B reflects the experimental timeline for adolescent alcohol consumption as well as additional experiments.

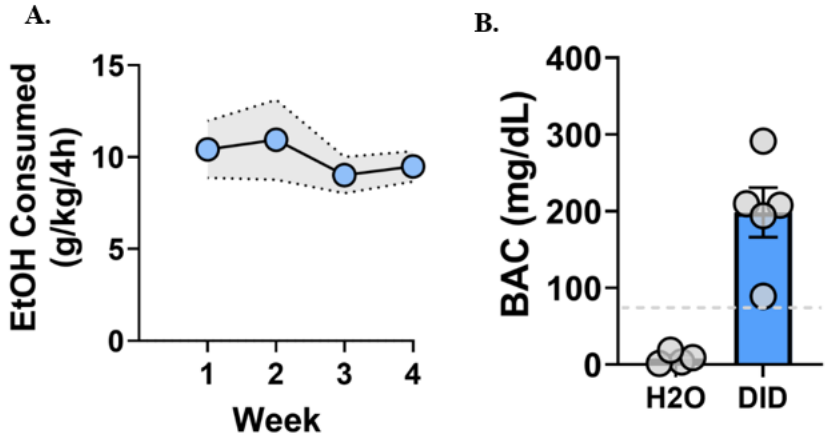

Figure 2. Mouse blood ethanol content sample. Graph A represents the amount of ethanol consumed per kilogram of body weight on the four hour binge period. Graph B represents mouse blood alcohol concentration after the binge period.

Adolescent Double Drinking Behavior

Double drinking behavior was assessed through either ethanol or water exposure to mice during adolescence PND (28-30) - PND (52-54) followed by ethanol exposure beginning on PND 84 for 4 consecutive weeks. Total ethanol consumption in g/kg was measured again during adulthood to assess whether drinking during adolescence led to increased ethanol consumption in adulthood.

Acute Stress Exposure (Forced Swim Test)

The effect of acute stress exposure on drinking with or without prior exposure to ethanol during adolescence was analyzed via forced swim test. Mice were either provided ethanol or water exposure to mice during adolescence PND (28-30) - PND (52-56). In adulthood, mice were exposed to ethanol again for an additional 3 weeks. During the second week of exposure on binge day (4 hour drinking period), 4 hours prior to binge, mice underwent a 6-minute forced swim stressor in which mice were placed in an inescapable glass beaker filled with 24-26℃ water. After the 6 minutes session, mice were removed from the container and dried. This paradigm was chosen as it has been shown previously to consistently increase ethanol consumption and will allow for the examination of the effects of stress on ethanol consumption (Becker et al., 2011)

SST Cell Counts

Twenty-four hours after the end of adolescent DID, mice were deeply anesthetized with Avertin (250 mg/kg) and perfused transcardially with phosphate buffered saline (PBS; pH 7.4) followed

by 4% paraformaldehyde (PFA; pH 7.4). Brains were removed and postfixed in PFA overnight. Brains were sectioned at 40 µm using a Leica vibratome (VS 1200, Leica). Brain slices were placed in 1:10000 DAPI (4′,6-diamidino-2-phenylindole) for 10 minutes and washed with phosphate buffered saline (PBS; pH 7.4). PL cortex-containing sections were mounted and a coverslip was added. SST+ cell counts in SST-Ai9 mice, expressing the fluorophore tdTomato exclusively in SST-expressing neurons, were quantified using ImageJ (National Institutes of Health, Bethesda, MD, United States). The PL cortex was delineated and SST+ were automatically quantified under matched criteria for size, circularity, and intensity consistently with our previously published work (Dao et al., 2020; Suresh Nair et al., 2022). The threshold to highlight all of the SST+ cells counted was set at 8.10±.01. Each ROI's total SST cell count was divided by the ROI area to give a total SST+ density value(Smith et al., 2020) 7-8 slices were analyzed per mouse.

Data Analysis, Statistics, and Figure Preparation

All data analysis was conducted with GraphPad Prism 7.0 (San Diego, CA). For analysis of all adolescent double drinking behavior experiments a 2-way ANOVA (factors: sex, drinking start age) was used. For forced swim and drinking experiments, a mixed effects model was used (factors: drinking start age, week of adulthood DID) comparing drinking across the 3 weeks. A 2-way ANOVA (factors: sex, adolescent DID condition) was used for SST cell counts. Total alcohol consumption (g/kg) across all 4 cycles of adolescent DID was correlated with SST cell density. Control mice were included in correlations with g/kg consumed = 0. Each correlation was reported as Pearson's r.

Chapter 3

Results

We first examined the effect that ethanol consumption in adolescence had on ethanol consumption in adults. Despite binge-like consumption of ethanol during adolescence, there are no apparent effects on ethanol consumption in adulthood. In figure 3A, no significant effect of sex ($F_{sex}(1, 29) = 3.310$; $p = 0.0792$) or age of first ethanol exposure ($F_{start\ age}(1, 29) = 3.966$; $p = 0.0559$), nor any interaction ($F_{sex \times start\ age}(1, 29) = 1.666$; $p = 0.2070$) were seen. In figure 3B, no significant differences are seen in total ethanol consumption over the adult drinking period although a main effect of sex was seen. Females consumed more alcohol during adulthood DID compared to males, regardless of adolescent alcohol consumption.

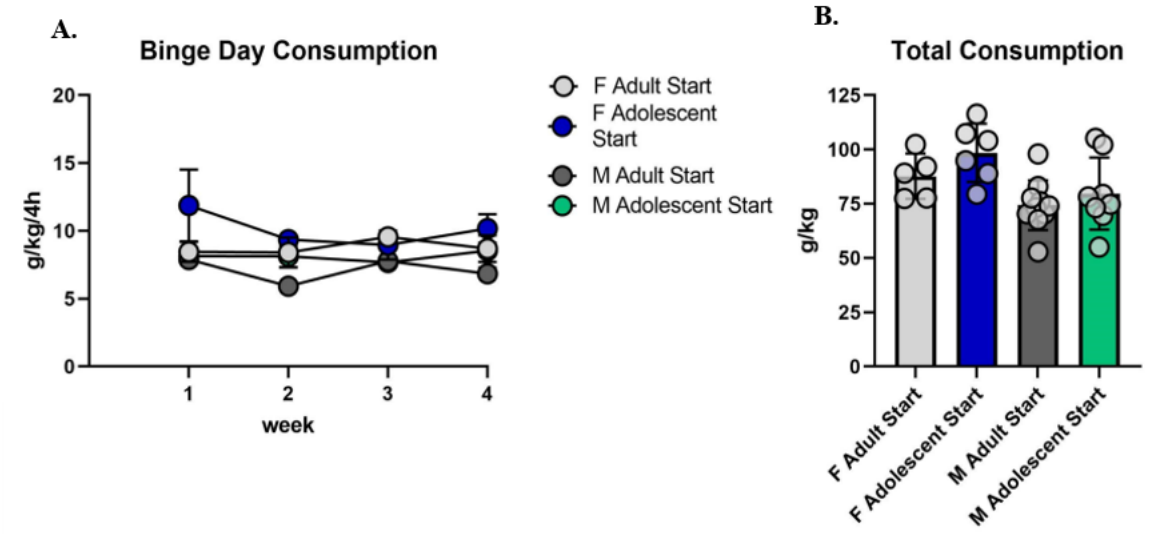

Figure 3. Ethanol consumption in adulthood. A. Tot**al** ethanol consumption per kilogram of body weight over the 4-hour binge period for the four weeks of ethanol consumption in adulthood. B. Total ethanol consumption per kilogram body weight over the entirety of the four-week drinking period.

In addition to double drinking behavior shown in figure 3, the effect of ethanol consumption during adolescence on stress induced drinking is shown in figure 4. Preliminary data indicates no effect of acute stress exposure on adult ethanol consumption with or without ethanol exposure during adolescence. Stress induced drinking was examined to look at potential stress reactivity induced by adolescent alcohol consumption. Figure 4B shows that there were no significant differences in ethanol consumption due to acute stress exposure ($t(5) = 0.03130$; $p = 0.9762$) with no significant difference remaining in the third week of drinking as seen in figure 3C ($t(5) = 0.5383$, $p = 0.6134$). Figure 4D shows that there is no significant change in ethanol consumption immediately after stress exposure with or without ethanol consumption during adolescence.

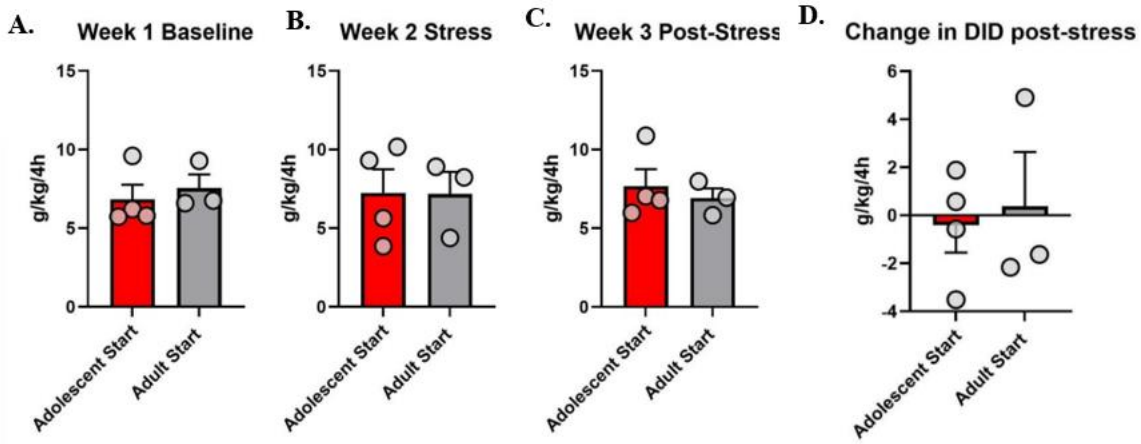

Figure 4. Ethanol consumption and acute stress exposure. A. Baseline ethanol consumption per kilogram of body weight over the 4-hour binge period. B. Ethanol consumption per kilogram of body weight over the 4-hour binge period after acute stress exposure (FST). C. Ethanol consumption per kilogram of body weight over the 4-hour binge period the week following the acute stress exposure. D. Change in ethanol consumption as a result of stress exposure for both mice that drank in adolescence and mice that drank in adulthood.

In combination with synaptic changes, we also investigated changes in the number of SST cells immediately following adolescent DID to gain an understanding between the microcircuitry and

total number of cells in the PL cortex. Representative images are shown in figure 5A. No effect on adolescence was found on the density of SST cells in the prelimbic cortex (figure 5B). We found no effect of adolescent DID on the density of SST cells in the PL cortex (Figure 5B, $F_{DID}(1, 20) = 0.02833$; $p = 0.8680$). There was no difference in SST cell density based on sex ($F_{sex}(1, 20) = 2.557$; $p = 0.1255$) and there was no interaction between sex and adolescent DID condition ($F_{sex}(1, 20) = 0.03393$; $p = 0.8557$). Additionally, there was no difference in cell density based on sex and no interaction between sex and adolescent DID conditions. Additionally, we correlated total alcohol consumption during adolescence with the number of PL SST cells to see if there was any association between drinking and SST cell density. There was no correlation found in either females $r(14) = 0.146$, $p = 0.619$) or males $r(10) = 0.072$, $p = 0.844$) (figure 5C & 5D).

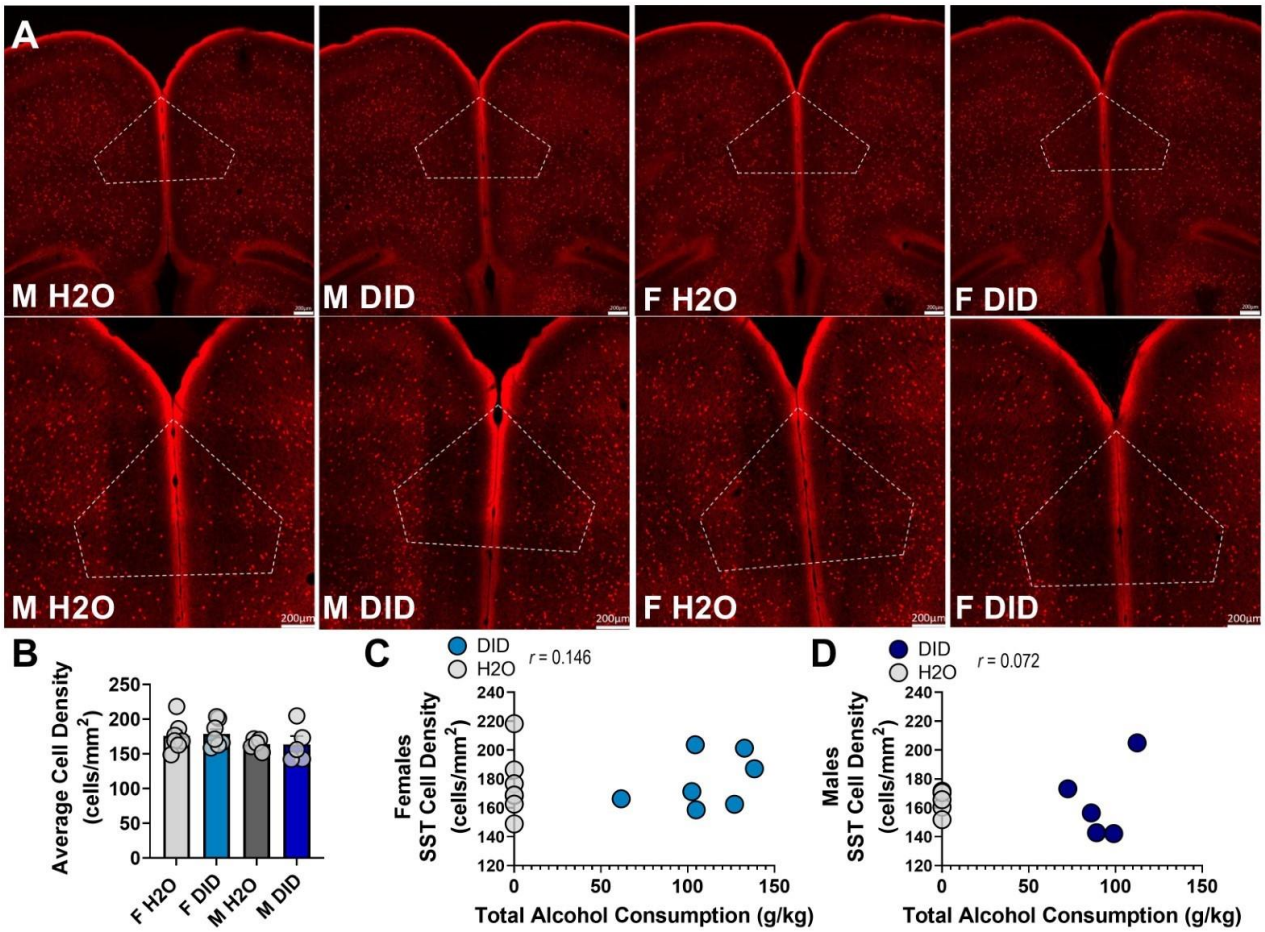

Figure 5. Adolescent DID does not alter the density of SST cells in the PL cortex. (A) Representative images of SST cells in the PL cortex of SST-Ai9 mice. Scale bars in the bottom right of each image represent 200um. (B) There was no effect of sex or adolescent DID condition on SST cell density in the PL cortex. (C-D) Total alcohol consumption was not correlated with SST cell density in female or male mice. Filled circles represent individual DID mice; white circles represent individual H2O mice.

Chapter 4

Discussion

Because excessive ethanol consumption is common in human adolescents during a significant portion of brain development, it is essential that we continue to understand the consequences of this consumption. We performed a variety of experiments to evaluate the behavioral and physiological impacts of binge ethanol consumption during adolescence. We found that 1) Early ethanol exposure during adolescence has no effect on adulthood ethanol consumption 2) No significant changes in binge ethanol consumption after stress are seen with or without prior consumption in adolescence 3) No significant changes in SST cell number are seen after drinking during adolescence. Despite no preliminary effect of acute stress exposure, it is important to note the differences in experimental paradigms commonly used to evaluate the effects of stress exposure. While acute stress exposure has not been found to reliably induce ethanol consumption, exposure to chronic variable stress during adolescence has been shown to significantly increase ethanol consumption in adulthood (Caruso et al., 2018). Additionally, all mice in acute stress exposure experiments were single housed, which is well documented to result in additional stress (Manouze et al., 2019). Therefore, it is important to consider potential stress effects that social isolation can have. Importantly, social isolation during adolescence has been shown to result in increased ethanol consumption in adulthood(Lopez et al., 2011; Lopez & Laber, 2015). While our research exhibited no effect of ethanol consumption on the impact of acute stress exposure on drinking in adulthood, it is important to consider the potential of a stress threshold that has already been reached as a result of social isolation, limiting the effectiveness of the acute stress exposure prior to ethanol consumption during adulthood. Another important consideration within our experiment was the sample size used. Because the power of the sample

was low, it is important to note that our results still need additional data to draw significant conclusions. Within our experimental paradigm, stress exposure began 4 hours prior to ethanol consumption as seen in prior experiments previous studies have exhibited a delayed effect of stress exposure on ethanol intake typically taking place in the weeks following stress exposure(Lowery et al., 2008). Despite this effect being seen with chronic stress exposure, within our experiment, this was not seen in the 1 week following stress exposure. In order to properly evaluate the effect of stress on ethanol consumption, additional validation is needed of stress exposure timing and downstream effects in the weeks following stress exposure. Specifically, cortisone levels can be recorded to evaluate effectiveness of stress exposure for single housed mice.

In addition to stress exposure, the literature is unclear regarding the effect of early ethanol exposure on later ethanol consumption during adolescence. While our paradigm reflected that early ethanol exposure through voluntary consumption resulted in no significant change in ethanol consumption in adulthood, other literature has opposed this conclusion. Previous work has shown that C57Bl/6J mice exposed to Drinking-in-the-Dark (DID) as adolescence (PND 25–45) showed increased drinking in adulthood (Moore et al., 2010). In addition, other examinations of drinking in adolescence have demonstrated increased drinking during adulthood with a primary effect in female rodents(Strong et al., 2010). Therefore, it is important to consider potential sex specific effects implicated in ethanol consumption as well as further investigate the widely understood belief that ethanol consumption during adolescence increases ethanol consumption in adulthood. It is also important to consider the potential of ethanol sensitivity in altering our results. Different labs have shown adolescent mice have increased sensitivity to the rewarding effects of ethanol and decreased sensitivity to the aversive effects compared to adult

mice(Hefner & Holmes, 2007). Additionally, one study showed that within adolescents' ethanol has rewarding effects particularly within high doses (Dickinson et al., 2009). While the adolescent mice that underwent ethanol exposure in this experiment did not exhibit increased ethanol consumption in adulthood, this may be the result of an increased preference to ethanol during adolescence compared to adulthood.

Similar to other studies, adolescent ethanol exposure did not change SST neuronal cell density within the prefrontal cortex (Koss et al., 2012). While there is no change in neuronal cell density, the changing properties of neurons and their receptors as a result of alcohol consumption during development may account for the lack of this change. It has been suggested that the GABA agonist properties of alcohol result in cell death (Olney et al., 2002). Regarding the SST neurons evaluated in our experiments, GABA has been found to act on these neurons. While there are few connections between SST neurons, other GABAergic interneurons such as parvalbumin (PV) have been shown to act on SST neurons. Because many of the properties of SST are altered during adolescence, the cell death response may be subsequently modified. Additionally, while there is commonly understood that significant ethanol consumption results in a loss of gray matter, it does not necessarily result in cell death. In fact, mice exposed to binge ethanol consumption during adolescence have been shown to have reductions of gray matter with an absence of cell death in the mPFC. Additionally, while no effects on SST cell number was found other research suggests that glial cell density is commonly altered by ethanol consumption (Koss et al., 2012).

With binge ethanol consumption playing a significant role in the United States, it is increasingly important to understand the behavioral and physiological impacts of ethanol consumption. Through the continued examination of brain development, sex differences, animal

and human models of alcohol consumption, potential therapeutics for alcohol use disorder can be developed and our understanding of the effects of alcohol consumption can be furthered. Additionally, further examination is important to understand the relationship between stress and ethanol consumption. Within clinical studies, both alcoholism and mood/anxiety disorders have been shown to be commonly found together with a common hypothesis for ethanol consumption surrounding the reduction of stress(Brady & Sonne, 1999; Pohorecky, 1991). Therefore, it is essential to continue to piece together the relationship between stress and ethanol consumption.

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ACADEMIC VITA

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EDUCATION

PENN STATE UNIVERSITY - SCHREYER HONORS COLLEGE, State College, Pennsylvania

Bachelor of Science in Biology (Neuroscience Focus) **August 2019 - April 2023**

- Neuroscience minor

PROFESSIONAL EXPERIENCE

PENN STATE UNIVERSITY, State College, Pennsylvania

Research Assistant (Crowley Lab) **December 2020-Present**

- Evaluated the role of somatostatin neurons in alcohol consumption and anxiety-like behavior in mouse models
- Ran drinking in the dark (placing alcohol bottles on at set time points) and performed mouse stereotaxic surgeries
- Performed mouse perfusions, running fluid through the mouse to clear its brain of blood before slicing
- Sliced/stained/imaged of brains to quantify neuronal density of brain slices
- Performed brain extractions for patch clamp electrophysiology and prepped electrophysiology solutions
- Co-author of 2 publications, including 1 review paper and 1 publication on the effects of altering somatostatin (SST) neurons in the bed nucleus stria terminalis (BNST) region of the brain on ethanol consumption and behavior
- Presenter at the Fall 2022 Eberly College of Science Poster exhibition and Penn State Department of Biology Student Research Showcase

Teaching Assistant **April 2021-Present**

- TA for Kinesiology 403 lab taught EMT skills to over 30 students
- Ran practice call scenarios, testing the students on a variety of different scenarios (heart attack/allergic reaction etc.)

COMPANY 20, State College, Pennsylvania

Emergency Medical Technician **January 2021-Present**

- EMT for Penn State EMS, working over 500 hours on the ambulance responding to calls/completing training
- Worked over 100 hours at Penn State sporting events as an EMT, including hockey, football, lacrosse, and soccer

UNIVERSITY OF DELAWARE, Newark, Delaware

Field Research Assistant (Wisser Lab) **June 2019 - August 2019**

- Investigated environmental adaptation and disease resistance of corn to create a genetic predictability model
- Recorded plant characteristics (height/leaf count/flowering time) and performed cross pollinations/DNA extractions

LEADERSHIP AND CAMPUS INVOLVEMENT

Events Coordinator (2021-2022) President (2022-2023), *REMOTE AREA MEDICAL* **September 2019-Present**

- Traveled between states (Maryland/Virginia/Pennsylvania/Ohio) to provide free medical services
- Made over 100 pairs of glasses, triaged patients, worked on dental floor, and setup/broke down the clinic
- Planned and organized Remote Area Medical clinic in PA, establishing a community host group
- Coordinated outside activities (rock climbing/COVID friendly studying groups/game days/movie nights/dinners)
- Ran biweekly workshops and recruited speakers (how to get into a lab at Penn State/getting involved as a medical volunteer/med school myths/how to crack the MCAT/medicine from the multicultural resource center)

Mentor, *SHO TIME* **September 2020-Present**

- Mentor for new students in the Penn State Schreyer Honors College
- Helped students get accustomed to college life and helped to organize the Sho Time orientation

Mentor, *PHYSIO CAMPS* **September 2020-Present**

- Created PowerPoints about human physiology presented to elementary schoolers (the heart/the brain/the lungs)

Member, *SERVE STATE* **January 2020-Present**

- Member of Penn State Thon organization dedicated to helping the Penn State community through volunteering
- Dedicated hours to make medical PowerPoints, cards for veterans, hospital quilts, etc.

Member, *PENN STATE CLUB GOLF* **September 2019-Present**

- Member of the Pennsylvania State club golf participating in club tournaments and events

AWARDS

- Eberly College of Science Research Scholarship
- Braddock Scholarship Award, Eberly College of Science, Penn State
- Academic Excellence Scholarship, Penn State
- Alma Newlin Scholarship Award for Science
- Delaware State Golf Association Scholarship Award
- Student Engagement Network Grant/Schreyer Honors College Research Grant