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Repeated Binge Pattern Ethanol Administration During Adolescence or Adulthood: Long-term Changes in Voluntary Ethanol Intake and Mesolimbic Dopamine Functionality in Male Rats

Antoniette Michelle Maldonado-Devincci
University of South Florida, antoniette.maldonado@gmail.com

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Repeated Binge Pattern Ethanol Administration During Adolescence or Adulthood:
Long-Term Changes in Voluntary Ethanol Intake and Mesolimbic Dopamine
Functionality in Male Rats

by

Antoniette M. Maldonado-Devincci

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Psychology
College of Arts and Sciences
University of South Florida

Major Professor: Cheryl L. Kirstein, Ph.D.
Toru Shimizu, Ph.D.
Mark Goldman, Ph.D.
Kristen Solomon, Ph.D.
Stanley Stevens, Jr., Ph.D.

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Dedication

I would like to dedicate this dissertation to my husband, Mario and my mother, Sylvia. Without you two in my life I would not be the woman I am today. Mario, your 'Grumpy' wife thanks you for loving me the way you do. You are completely supportive of me and my goals and aspirations. You are a rare gem, and by far one of the greatest men I know. Your respect and admiration for me and the method to my madness ensures our relationship will survive the distance that will soon come between us as we each chase our dreams. Our mutual respect for one another is the foundation for our marriage and the reason we make such a great team. I love you more than you will ever know and this will continue to grow as I follow the path to becoming an independent scientist. Mom, your "Mula" has shown this stubbornness and passion for education from prekindergarten to now. You are my rock and my very best friend. You know me better than I know myself. We really are made from the same mold and I have never questioned the love you have for me. In fact, it was the certainty of your love for me that was the foundation for my confidence in myself and life and has enabled me to pursue my goals. You are a phenomenal mother and I am the luckiest daughter to have such an amazing woman as my madre de mi vida.

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Abstract

Binge alcohol consumption is a rising concern in the United States, especially among adolescents as during this developmental period alcohol use is usually initiated and has been shown to cause detrimental effects on brain structure and function. These findings have been established through the use of binge models in animals, where animals are repeatedly administered high doses of ethanol typically over a period of three or four days. While such work has examined the effects of a four-day and repeated three-day binge, there has been almost no work conducted aimed at investigating the long-term behavioral and neurochemical and/or functional consequences of repeated binge pattern administration during adolescence relative to adulthood on later ethanol-induced behavior and neurochemistry in adulthood. The present set of experiments aimed to examine the dose-response and age-related differences induced by repeated binge pattern ethanol administration during adolescence or adulthood on voluntary ethanol consumption (Aim 1), changes in ethanol metabolism following ethanol pretreatment (Aim 2) and mesolimbic dopamine functionality (Aim 3) in adulthood. In both experiments, adolescent and adult male rats were intragastrically administered ethanol (0.5, 1.0 or 2.0 g/kg/ig) or isovolumetric water on postnatal days (PND) 28-31, PND 35-38 and PND 42-45 for adolescent rats and PND 60-64, PND 67-70 and PND 74-77 for adult rats. In both experiments all rats underwent fourteen days of abstinence (PND 46-59 or PND 78-91, respectively). Subsequently, in Experiment 1, all rats underwent voluntary ethanol consumption procedures, in which animals were exposed to 10% ethanol combined with decreasing saccharin

concentrations across days from PND 60-82 for adolescent-exposed rats and PND 92-114 for adult-exposed rats. Finally, on PND 83 and PND 115, respectively, all animals were challenged with 2.0 g/kg ethanol and trunk blood samples were collected at 60 and 240 minutes post-injection. Results indicate there was a significant increase in voluntary ethanol intake in adolescent ethanol-exposed rats pretreated with 2.0 g/kg relative to their adult ethanol-pretreated counterparts. Faster ethanol metabolism was observed in adolescent rats pretreated with 2.0 g/kg during adolescence relative to adolescent-exposed rats pretreated with 0.5 g/kg and adults pretreated with 2.0 g/kg. For Experiment 2, all rats underwent surgery (PND 60 for adolescent-exposed and PND 92 for adult-exposed rats). From PND 61-64 for adolescent-exposed and PND 93-96 for adult exposed rats, all animals underwent recovery from surgery. Finally, all rats underwent *in vivo* microdialysis on PND 65 for adolescent-exposed and PND 97 for adult-exposed rats, with K⁺ (100 mM) infused into the ventral tegmental area and accumbal dopamine overflow assessed in the nucleus accumbens septi. The results from Experiment 2 indicate lasting changes in mesolimbic dopamine functionality with a trend for decreased potassium-stimulated dopamine overflow in the nucleus accumbens septi in adolescent-ethanol pretreated rats and a trend for increased potassium-stimulated dopamine overflow in adult ethanol-pretreated rats. The results from the present set of experiments show the dose-dependent impact of binge-pattern ethanol exposure during adolescence on subsequent ethanol consumption and ethanol metabolism in adulthood. These findings indeed determine adolescence as a period of vulnerability to the long-term changes in ethanol consumption relative to similarly-exposed adult male rats. Importantly, the results of Experiment 2 indicate an alteration in the functionality of the mesolimbic pathway in adulthood following adolescent binge

pattern ethanol exposure, which demonstrates a long-term depression in mesolimbic dopamine functionality following adolescent binge pattern ethanol exposure.

Chapter 1: Background

Alcohol use in humans

Recent evidence has shown a high rate of experimentation with alcohol during adolescence. Alcohol is one of the most commonly abused psychoactive substances, and use is highly prevalent in adolescents (Bates & Labouvie, 1997; Windle, 1990). According to the 2009 Monitoring the Future study, 15% of 8th graders, 30% of 10th graders, and 44% of 12th graders reported current use of alcohol, defined as consumption of at least one alcoholic beverage in the past 30 days, and 5% of 8th graders, 16% of 10th graders, and 27% of 12th graders reported being drunk in the last 30 days (Johnston et al., 2009). Additionally, the time course from casual use to dependence on alcohol during adolescence is accelerated relative to adults who initiate use after the age of 21 (Clark et al., 1998). Importantly, it has been suggested that use of alcohol during the adolescent developmental period may render individuals at greater risk for developing dependence on alcohol (Andersen et al., 2002; Dewit et al., 2000; Hawkins et al., 1997; Rose et al., 2001) and to abuse alcohol as adults (Duncan et al., 1997). These data suggest that level of consumption of alcohol is high in adolescents and that initiation of use during this period can produce long-term changes in alcohol-related behaviors.

Binge drinking, often defined as the consumption of five or more drinks for men or four or more drinks for women on a single occasion (Wechsler et al., 1994), has been labeled the number one source of preventable morbidity and mortality for more than 6 million college students in the United States (Wechsler et al., 1995). In college students,

binge drinkers had almost a threefold greater increase in alcohol-related problems relative to non-bingers and almost an eight-fold increase relative to current abstainers, including school, relationship, job and legal problems (Sheffield et al., 2005). In human adolescents, binge drinking within the last two weeks has been reported to be 8% for 8th graders, 16% for 10th graders and 25% for 12th graders (Johnston et al., 2009). Furthermore, recent longitudinal data indicate about one-half of males and one-third of females that engaged in binge drinking during adolescence continued to engage in similar binge drinking patterns in adulthood (McCarty et al., 2004). These statistics are of concern, as evidence supports the notion that early exposure to alcohol may be a significant predictor of later alcohol consumption, dependence, and various psychiatric disorders (Grant et al., 2001; Hasin and Glick, 1988; Robin et al., 1998).

These data highlight the importance of systematically investigating the impact of early moderate and heavy binge drinking during this developmental period. Some work recently conducted in human adolescents found differences in verbal encoding strategies between binge drinking adolescents and non-binge drinking adolescents (Schweinsburg et al., 2010). Differences in white matter integrity have also been observed in adolescent binge drinkers, with greater reductions in white matter integrity reported for binge drinking adolescents relative to controls (McQueeney et al., 2009; Jacobus et al., 2009). One of the drawbacks to this work conducted in humans is that the binge drinkers in these experiments were self-selected and other individual differences aside from their binge drinking status may mediate the differences observed. Additionally, many human adults that consume alcohol experimented with it early in development (McCarty et al., 2004). In this light, animal models during adolescence are effective tools that can control for extraneous variables and can be used to

systematically investigate the impact of early binge ethanol exposure on long-term changes in neurobiological and neurobehavioral functioning.

Animal model of adolescence

Adolescence is a time of change that is marked by many factors, including the onset of puberty, hormonal changes, growth spurt, and increased interactions with peers (for review see Spear, 2000). In rodents, adolescence is generally accepted to occur from about postnatal day (PND) 28 to 42 (Spear & Brake, 1983) and last until approximately PND 55 (Ojeda & Urbanski, 1994). Adolescent rodents show increased novelty seeking (Stansfield et al., 2004; Stansfield & Kirstein 2006) and social interactions with peers (Primus & Kellogg, 1989; for review see Spear, 2000). In addition to behavioral changes, the adolescent brain is undergoing major organization and maturational changes during this developmental period (for review see Spear, 2000). For example, dopaminergic input to the prefrontal cortex is still developing (Kalsbeek et al., 1988; Rosenberg & Lewis, 1994), as are amygdalar projections to cortical areas (Cunningham et al., 2002). Limited data suggest that exposure to drugs of abuse during this time may alter normal developmental processes, rendering the brain more vulnerable to acquiring substance use disorders in adulthood (for review see Chambers et al., 2003; Smith, 2003). The need for an animal model to assess the effects of ethanol on development has been raised (Witt, 1994).

Effects of ethanol on behavior

Adults

Ethanol has been shown to produce different effects on behavior in adult animals that may be related to the rewarding and reinforcing or aversive properties of ethanol. Alcohol has biphasic effects on behavior (Lewis & June, 1990), and some studies have yielded mixed results using low and high doses of ethanol. In adult rats, high doses of

ethanol produce sedative/hypnotic effects on behaviors, such as motor coordination (White et al., 2002) and locomotor activity (e.g., Little et al., 1996) and appear to be aversive in a conditioned place preference (CPP) paradigm (van der Kooy et al., 1983). In contrast, low doses of ethanol have been shown to produce stimulatory effects of locomotor activity (Correa et al., 2003) when animals were separated into high and low responders to novelty (Hoshaw & Lewis, 2001). Adult animals are extremely sensitive to the depressant effects of ethanol (Little et al., 1996; Silveri & Spear, 1998; White et al., 2002) and ethanol clearance appears to take longer in adults relative to adolescent (Walker and Ehlers, 2009). All of these data demonstrate the complexity of the effects of ethanol on behavior in adult animals. High and low doses of ethanol have different effects on behavior, and prior exposure to ethanol can alter subsequent responding to ethanol. Novelty-related behaviors also appear to be related to ethanol's effects on behavior. Given that adolescents appear to be differentially sensitive to the effects of ethanol relative to adults, it is important to examine the long-term behavioral effects of ethanol during this developmental period.

Adolescents

Adolescent rats are especially sensitive to the effects of ethanol on a number of behavioral measures (for a review see Spear & Varlinskaya, 2005). Adolescents are reported to be less sensitive to the sedative/hypnotic and motor incoordinating effects of ethanol (Little et al., 1996; Silveri & Spear, 1998; White et al., 2002), to develop an ethanol-induced CPP more readily (Philpot et al., 2003), and to voluntarily consume more ethanol than adults (Doremus et al., 2005; Maldonado et al., 2008). Additionally, adolescent rats reach peak blood ethanol concentrations (Little et al., 1996) and develop tolerance to ethanol more rapidly than adults (Silveri & Spear, 1999). Together, these data suggest that adolescents experience more of the rewarding properties of ethanol

than adults, rendering them especially sensitive to the immediate and long-term effects of ethanol (Maldonado-Devincci et al., 2010a). Importantly, the effects of ethanol pretreatment during adolescence produce long-term behavioral alterations in novelty preference (Stansfield & Kirstein, 2007) and locomotor activity (Maldonado & Kirstein, 2005) in adulthood. All of these data indicate that adolescents and adults are differentially sensitive to the behavioral effects of ethanol and that ethanol can produce long-term changes in ethanol-related behaviors in adulthood, which may be mediated by ethanol's effects on the developing brain.

Research has shown that ethanol exposure during adolescence may result in negative consequences such as impaired spatial learning (Markwiese et al., 1998) and intermittent ethanol (3.0 g/kg for two consecutive days at 48 hr intervals) induced chronic neurobehavioral deficiencies (Pascual et al., 2007). Lower doses of daily ethanol may not induce these same effects (Acheson et al., 2001). Moreover, recent work has indicated that binge four-day ethanol administration has greater detrimental effects on brain structure and function in adolescent as compared with adult rats (Crews et al., 2000; Monti et al., 2005). Ethanol exposure during adolescence has also been reported to cause dose-dependent cognitive and behavioral impairments (Crews et al., 2006), however chronic intermittent ethanol exposure during adolescence does not induce similar effects (Silvers et al., 2003, 2006). Taken together, these data indicate the deleterious effects of repeated binge ethanol exposure altering normal adolescent brain and behavioral development.

Binge ethanol during adolescence

One of the most common methods used in investigating binge pattern ethanol consumption in rodents has been a four-day binge model, used particularly because of its similarity to a model of a “bender” for an alcoholic in humans (Nixon and Crews,

2002). Using this model of ethanol administration to produce tolerance and dependence to ethanol in adult rats, binge ethanol exposure decreased neurogenesis in the adult rat hippocampus (Nixon and Crews, 2002). Moreover, the four-day binge ethanol exposure model induced cognitive dysfunction in rodents, suggested to induce comparable problems in humans (Obernier et al., 2002). Alternatively, using a repeated three-day binge ethanol administration model, adolescent rats repeatedly administered ethanol over four weeks displayed significantly increased anxiety in a passive avoidance task (Popovic et al., 2004). While previous work has been conducted to examine the consequences of the four-day binge model (Gavaler et al., 1993; Obernier et al., 2002; Penland et al., 2002), others have examined the immediate consequences of repeated three-day binge ethanol administration (Popovic et al., 2004). There is little research aimed at investigating the long-term neurobehavioral consequences of repeated binge ethanol exposure during adolescence. The pattern of repeated binge ethanol administration during adolescence may serve as an approximate model of adolescent human patterns of binge alcohol drinking.

Some forms of adolescent ethanol exposure (e.g., ethanol vapor or constant voluntary access to ethanol) may not induce alterations in voluntary ethanol consumption in adulthood. When ethanol intake was assessed in adulthood, rats given voluntary access to unsweetened ethanol beginning in adolescence and extending into adulthood (PND 28-90) drank similar amounts as rats not given free-access to ethanol until adulthood (PND 71-90; Vetter et al., 2007). In another study, forced periadolescent (PND 30-40) exposure to ethanol vapor for 12 hours a day did not enhance sucrose sweetened ethanol drinking in adulthood (> PND 92; Slawecki and Betancourt, 2002). One of the key aspects hypothesized to increase ethanol intake in young adulthood is the pattern of adolescent ethanol exposure with repeated cycles of four consecutive

days of ethanol administration coupled with intermittent abstinence days during the adolescent exposure period. While adolescent rats exposed to ethanol every day did not show enhanced ethanol consumption in adulthood, rodents exposed to intermittent ethanol vapor during periadolescence exhibited a smaller conditioned taste aversion in adulthood as compared to those exposed to chronic ethanol vapor during periadolescence (Diaz-Granados and Graham, 2007). Intermittent exposure to ethanol enhanced ethanol consumption in adolescent rats relative to those given continuous access (Hargreaves et al., 2009). Repeated ethanol withdrawal may mediate the effects of intermittent ethanol exposure (Devaud and Alele, 2004). Given differences in behavioral responses to intermittent ethanol exposure were observed (Diaz-Granados and Graham, 2007; Hargreaves et al., 2009), but not when animals were exposed to chronic ethanol during adolescence (Slawecki and Betancourt, 2002; Vetter et al., 2007), it is likely the intermittent nature of the binge exposure induces behavioral changes observed in young adulthood.

Considering adolescence is a developmental period in which ethanol is initially consumed and may lead to greater alcohol consumption later in life (Grant et al., 2001; Hasin and Glick, 1998; McCarty et al., 2004; Robin et al., 1998), it is important to elucidate the impact of early ethanol exposure on the subsequent predisposition to drink later in life. It is possible early patterns of drinking (binge-drinking), rather than simple exposure to ethanol during adolescence, may play a crucial role in the development and continuation of ethanol use disorders into adulthood (Hill et al., 2000), which are likely mediated by its effects in the brain (e.g., the functionality of the mesolimbic dopamine pathway).

Effects of ethanol in the brain

Adults

Ethanol produces a number of neurochemical alterations in the adult brain that may be related to its rewarding and reinforcing as well as aversive properties. Among other neurochemical systems affected by ethanol, the mesolimbic dopamine system has been implicated in the effects of ethanol and other drugs of abuse mediating the rewarding effects associated with these drugs (Koob, 1992; Moghaddam & Bunney, 1989; Nakahara et al., 1989; Phillips et al., 1992; Wise & Rompre, 1989). Ethanol has been shown to increase activity of the mesolimbic dopamine pathway (Appel et al., 2004; Blomqvist et al., 1993; Engel et al., 1988, Imperato & Di Chiara, 1986; Mereu et al., 1984; Weiss et al., 1993) via activation of ventral tegmental area neurons (Gessa et al., 1985). Most studies demonstrate a dose-response relationship with low to moderate doses producing an increase in dopamine while higher doses produce a decrease in accumbal dopamine and dopamine activity (Williams-Hemby & Porrino, 1994). However, some studies have shown that administration of high doses of ethanol (i.e., 2-3 g/kg) elevate accumbal dopamine for up to 2 hours (Kohl et al., 1998). Rats will self-administer ethanol directly into the ventral tegmental area (Gatto et al., 1994) and pharmacological manipulation of dopamine neurotransmission modifies self-administration and preference of ethanol (Weiss et al., 1990; Samson et al., 1993; George et al., 1995; Panocka et al., 1995). Gonzales and colleagues (2004) suggest that cues rather than the actual pharmacological effects of ethanol consumption mediate initial increases in accumbal dopamine in animals previously treated with ethanol. Taken together, these studies imply that neurochemical differences within the nucleus accumbens septi influence the reinforcing nature of ethanol and result in a corresponding change in behavioral output, which may be dependent on cues

associated with previous exposure to ethanol.

Adolescents

Reward mechanisms in the brain, including alterations of the mesolimbic dopamine system, continue to undergo significant developmental changes during adolescence (Nakano et al., 1996; Spear, 2000; Teicher et. al., 1995). However, relatively little information is available related to changes induced by ethanol in the developing adolescent brain and how these changes may be associated with the differential sensitivity of adolescents to ethanol. Following repeated treatment with ethanol, periadolescent animals PND 25 exhibited a shift to the left in the temporal peak of stimulated dopamine relative to the effects of acutely administered ethanol (Philpot & Kirstein, 1998). Additionally, adolescent (PND 45) rats have greater basal dopamine levels and lack of change in DOPAC/DA turnover ratio relative to younger and older animals (Philpot & Kirstein, 2004). Expectancy theory indicates that behavior is modified based on past experience and the dopaminergic system is intimately involved in this process (for review see Goldman, 2002). Periadolescent rats showed a dramatic ethanol expectancy-induced increase in dopamine, exhibited as a significant increase in dopamine in the nucleus accumbens septi when rats received saline instead of an expected administration of ethanol (Philpot and Kirstein, 1998). This unique neurochemical profile in adolescent animals may be indicative of a lack of tolerance to the rewarding effects of ethanol. These specific age-related neurochemical patterns coupled with mesolimbic dopamine may be implicated in the rewarding effects of ethanol that is unique to adolescents. Although adolescents are less sensitive behaviorally to many of the effects of ethanol, when focusing on brain alterations, adolescents appear more sensitive to cortical and hippocampal neurotoxic alterations induced by ethanol. Swartzwelder and colleagues observed that adolescents suffered from more ethanol-

induced disruptions of hippocampal plasticity and memory (Swartzwelder et al., 1995a, b). In a hippocampal-dependent task, adolescents also appear to be more impaired in the Morris water maze to 1.0 or 2.0 g/kg ethanol (Markweise et al., 1998) and larger impairments in working memory were observed in adolescent animals exposed to repeated 5.0 g/kg ethanol every 48 hours (White et al., 2000). Crews and colleagues have also reported greater ethanol-induced neurotoxicity in adolescent animals (Crews et al., 2000, 2006). All of these data indicate that adolescent animals are uniquely sensitive to the effects of ethanol in the brain, with increased dopamine-related activity and greater hippocampal and cortical damage induced by ethanol. These alterations occurred during adolescence and resulted in long-term neuroadaptations, which appear to cause long-term changes in ethanol-associated behaviors.

Long-term neurobehavioral effects of ethanol exposure during adolescence

Adolescents are uniquely sensitive to the effects of ethanol, with less sensitivity expressed behaviorally, but greater neurotoxic effects observed in the brain. When animals were exposed to ethanol during preweaning (Hayashi & Tadokoro, 1985), or postweaning (Ho et al., 1989), later increases in preference for ethanol were observed. However others have reported no change in preference for ethanol later in life when preexposure occurred during adolescence (Kakihana & McClean, 1963; Parisella & Pritham, 1964; Tolliver & Samson, 1991). Exposure to ethanol during adolescence induced impairments in attention and memory (Slawecki et al., 2004) and fear conditioning (Bergstrom et al., 2006) in adulthood. Additionally, adolescent ethanol exposure enhanced anxiety- and depressive-like behaviors (Slawecki et al., 2004) and long-term tolerance in adulthood (Silvers et al., 2003). Exposure to ethanol during adolescence impaired spatial memory (Sircar & Sircar, 2005) and altered hippocampal-mediated neurophysiological function (Slawecki et al., 2001) in adulthood. Furthermore,

adolescent ethanol drinking altered stimulated ethanol-induced dopamine efflux in adulthood in alcohol preferring (P) rats (Sahr et al., 2004). Chronic ethanol exposure also increased basal dopamine levels in adulthood in male rats (Badanich et al., 2007). All of these data suggest that, indeed, adolescent ethanol exposure produces long-term behavioral and neurochemical alterations in anxiety and depressive-like behaviors and adaptations of hippocampal and dopamine systems. However, long-term alterations in mesolimbic functioning following binge ethanol exposure during adolescence have not been systematically investigated.

Functionality of the mesolimbic pathway: opioid system modulation and ethanol alterations

Dopamine is released in the cell body region in the ventral tegmental area and in the terminal region in the nucleus accumbens septi (Kalivas and Duffy, 1988). Somatodendritic and terminal dopamine release are calcium dependent, but somatodendritic release appears to be less dependent on the activity of action potentials (Cragg et al., 1997; Kalivas and Duffy, 1991). Dopamine release in the somatodendritic region of the ventral tegmental area, via back propagation, has been shown to be released similarly as in the accumbal terminal region, except that overall levels were lower, reuptake was slower and was insensitive to autoreceptor regulation (Cragg et al., 1997; Kalivas and Duffy, 1991; Kita et al., 2009). However, the time course for release in both regions is similar (Ford et al., 2010). Both terminal and somatodendritic release of dopamine are affected by neuronal firing in the ventral tegmental area, as changes in concentrations in potassium (K⁺) can readily alter dopamine overflow from the ventral tegmental area (Adell and Artigas, 2004). Dopamine release in the cell body region may be functionally different than that at the terminal region (Cragg and Greenfield, 1997; Kalivas and Duffy, 1991), as there is greater dopamine release at the terminal than at

the somatodendritic site (Cragg and Greenfield, 1997). These data indicate an intricate interaction between dopamine release from the cell body/somatodendritic region and the axonal/terminal region, both of which drugs of abuse can alter the functionality.

Dopamine activity in the nucleus accumbens septi can activate a long-loop negative feedback onto dopamine neurons in the ventral tegmental area via D1 and D2 receptors to regulate dopamine release in the somatodendritic and terminal areas (Adell and Artigas, 2004; Kohl et al., 1998; Rahman and McBride, 2000, 2001). There is also a short-loop feedback at the somatodendritic region that can regulate dopamine output from the ventral tegmental area, which occurs through autoinhibition of action potentials in the ventral tegmental area, which helps to regulate the tonic inhibitory tone in the ventral tegmental area (Wang, 1981; Yan et al., 1996). However, D2 autoreceptors in the somatodendritic region of the ventral tegmental area appear to have no action of autoinhibition of dopamine in the ventral tegmental area (Cragg and Greenfield, 1997). D2 receptor blockade does not alter motivational states, and D1 receptor activation is implicated in reinforcement (Shippenberg and Herz, 1987, 1988). D1 receptor activation is modulated by activity of μ opioid receptors in the nucleus accumbens septi, which are likely involved in the long-loop feedback to the ventral tegmental area (Adell and Artigas, 2004; Kohl et al., 1998; Rahman and McBride, 2000, 2001; Shippenberg and Herz, 1987, 1988). Other afferent mechanisms likely mediate differences in responses to dopamine release in each region, given the differences in D1 and D2 receptor modulation on different regions of mesolimbic functionality in the cell body and terminal regions, including the opioid peptidergic system.

Mu (μ) opioid receptors are present in high numbers in the ventral tegmental area, which are located primarily on non-dopaminergic neurons (Mansour et al., 1988; Svingos et al., 2001). Activation of μ opioid receptors in the ventral tegmental area acts

to inhibit the activity of GABAergic neurons, which results with greater somatodendritic release of dopamine in the ventral tegmental area (Chefer et al., 2009; Di Chiara and North, 1992; Johnson and North, 1992). Somatodendritic dopamine release may not be dependent on neuronal firing (Chefer et al., 2009). Mu opioid receptors induce a tonic tone on the ventral tegmental area (Herz, 1988; Spanagel et al., 1992), via decreasing GABAergic activity and in turn increasing dopamine overflow in the ventral tegmental area (Chefer et al., 2009). It is hypothesized that the action of μ opioid receptors to inhibit the activity of GABAergic neurons in the ventral tegmental area, induces a disinhibition of dopamine neurons, inducing action potentials in the ventral tegmental area on dopamine neurons, which facilitates dopamine release in somatodendritic and terminal fields in the mesolimbic pathway (Johnson and North, 1992). The proposed mechanism for this action is that μ opioid receptors are located presynaptically on afferent GABAergic nerve terminals, which when presynaptically activated decrease GABA release through inhibition of GABAergic activity, which in turn disinhibits ventral tegmental area dopamine neurons and thus induces facilitation of ventral tegmental area dopamine neuron firing (Bergevin et al., 2002).

Ethanol can excite dopamine neurons in the ventral tegmental area through inhibition of GABAergic neurons (Brodie et al., 1999), by acting on μ opioid receptors in the soma and dendritic regions of the ventral tegmental area (Xiao and Ye, 2008). Ethanol may stimulate release of endogenous opioid peptides, which in turn would activate μ opioid receptors in the ventral tegmental area to stimulate dopamine release (Mendez et al., 2001). Thus, ethanol can function as a μ opioid receptor agonist, drugs which are known to increase dopamine transmission in the mesolimbic pathway (Di Chiara and Imperato, 1988) and can serve as a reinforcer (Herz, 1988; Mucha and Herz, 1985). Following withdrawal from ethanol, the number of neurons responsive to

dopamine in the ventral tegmental area was reduced; suggesting the major action of ethanol was at the cell body of the dopamine neuron in the ventral tegmental area (Bailey et al., 1998). Ethanol increases dopamine output both at the somatodendritic and terminal regions when locally infused into the ventral tegmental area (Yan et al., 1996). In low-ethanol-drinking rats, δ opioid receptors presynaptically alter GABAergic activity in the ventral tegmental area (Margolis et al., 2008). During chronic ethanol consumption, μ opioid receptor expression is downregulated in the ventral tegmental area (Mendez et al., 2001), which decreases its ability to alter the GABAergic inhibitory tone on the ventral tegmental area. In contrast, during chronic ethanol consumption, δ opioid receptor expression is upregulated (Margolis et al., 2008). Thus across ethanol treatment, the opioid peptidergic system is intricately involved in the modulation of both somatodendritic and terminal dopamine release within the mesolimbic pathway.

Overview of the present set of experiments

The present set of experiments aimed to investigate the long-term behavioral and neurochemical functional impact of binge pattern ethanol exposure during adolescence relative to that in adulthood in male rats. Recent data from our laboratory indicates that adolescent male rats are more vulnerable, relative to females, to elevated ethanol consumption in adulthood following binge ethanol exposure during adolescence when compared their ethanol-naïve counterparts (Maldonado-Devincci et al., 2010b). Given intermittent exposure to ethanol during adolescence alters subsequent ethanol consumption to a greater degree relative to continuous exposure (Hargreaves et al., 2009), the present set of experiments aimed to further investigate these effects through the use of lower doses of ethanol exposure during pretreatment with the repeated four-day pattern of exposure. The present set of experiments further aimed to investigate the hypothesis of greater vulnerability to the long-term impact of intermittent exposure to

ethanol during adolescence by comparing similar patterns of treatment to animals exposed to intermittent ethanol in adulthood. Adolescent male rats showed greater changes in ethanol consumption relative to adult rats, indicating that adolescence is indeed a period of vulnerability to the long-term effects of ethanol treatment during this critical developmental period.

Previous work from our laboratory has shown alterations in the mesolimbic dopamine system in adulthood following chronic ethanol treatment during adolescence, manifested as greater basal dopamine levels in the nucleus accumbens septi in adolescent ethanol-treated rats relative to adolescent saline-treated rats (Badanich et al., 2007). These data indicate that during this critical period of neuronal development, there are alterations in the mesolimbic system (Maldonado-Devincci et al., 2010a). The present set of experiments aimed to investigate the long-term impact of binge pattern ethanol exposure on mesolimbic functionality in adulthood by infusing K⁺ (100 mM) to induce depolarization of dopaminergic neurons in the ventral tegmental area and assessing dopamine overflow in the nucleus accumbens septi in adolescent-exposed and adult-exposed male rats. If dopamine overflow is differentially altered in adolescent-exposed rats relative to adult-exposed rats, this would provide a mechanism for the enhanced vulnerability in behavioral changes in voluntary ethanol consumption in adolescent-ethanol-exposed rats.

Hypotheses

1. Consistent with previous data (Maldonado-Devincci et al., 2010b), it was expected there would be an increase in adulthood voluntary ethanol consumption in adolescent rats exposed to ethanol during adolescence relative to their ethanol-naïve counterparts.

2. Consistent with preliminary data from our laboratory (presented below), it was expected there would be dose-dependent changes in voluntary ethanol consumption in adolescent-exposed, but not adult-exposed male rats. It was expected there would be an increase in ethanol consumption in rats exposed to 1.0 and 2.0 g/kg ethanol during adolescence, but not to 0.5 g/kg ethanol during adolescence.
3. Faster ethanol clearance was expected in adolescent-ethanol pretreated rats relative to adult ethanol-pretreated rats when changes in ethanol pharmacokinetics were assessed in adulthood. There are conflicting results with regard to ethanol clearance rates between adolescent and adult rats, with no differences in ethanol clearance reported between adolescent and adult rats (Silveri and Spear, 1999), and more rapid ethanol clearance in adolescent relative to adult rats (Walker and Ehlers, 2009). The expected outcome in blood ethanol concentrations in rats exposed to ethanol during adolescence relative to adulthood was not clear.
4. Greater K⁺-induced elevations in extracellular accumbal dopamine overflow in adulthood following repeated intermittent exposure to ethanol during adolescence (PND 30-50) relative to animals exposed to intermittent ethanol beginning in adulthood (PND 60-80) were expected. Long-term increases in basal extracellular levels of dopamine have been observed in adulthood following chronic adolescent ethanol exposure (Badanich et al., 2007) and changes in dopamine overflow have been observed following repeated ethanol administration between the ages of PND 35 to PND 45, but not in older animals (Philpot et al., 2009).

Chapter 2: Methods and Materials

Subjects

One hundred and eighty-four male adolescent (n = 95) and adult (n = 89) Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN), were derived from established breeding pairs at the University of South Florida, Tampa and were used as subjects in Experiment 1. Seventeen adolescent (n= 9) and adult (n = 8) rats were used as subjects for Experiment 2. Litters were sexed and culled to 10 pups per litter (6 males and 4 females whenever possible) on postnatal day (PND) 1, with the day of birth designated as PND 0. Pups remained with their respective dams until PND 21, when pups were pair-housed with same-sex littermates. Females were used in other ongoing experiments in the laboratory. Animals were maintained on a 12:12 hour light: dark cycle (lights on at 0700 hr), in a temperature and humidity-controlled vivarium. Animals were allowed free access to food and water throughout the experiment. No more than one male pup per litter was used in any given condition. Animals were randomly assigned to conditions of 0.5, 1.0 or 2.0 g/kg ethanol (25% v/v in water; Pharmaco-Aaper, Shelbyville, KY) or an isovolumetric administration of water (Hunt et al., 2000; Nixon and Crews, 2002). Maintenance and treatment of the animals were within the guidelines for animal care by the National Institutes of Health (Public Health Service Policy on Humane Care and Use of Laboratory Animals, NIH, 2002).

Apparatus

Male rats were intragastrically administered water or 25% v/v ethanol in water via daily intubation using a 12-cm length of polyethylene tubing (PE-50; Becton Dickinson

and Company; Sparks, MD) attached to a 21.5 gauge needle and a disposable syringe (Hunt et al., 2001). The intubation volume was different depending on the dose of ethanol administered, as all ethanol doses were administered on a gram of ethanol per kilogram of body weight basis. This was achieved by multiplying the body weight of the animal by 0.00253 for the 0.5 g/kg dose, 0.00506 for the 1.0 g/kg dose and 0.01012 for the 2.0 g/kg dose. Assessment for voluntary intake of ethanol and water was performed with 500 mL glass bottles with double-ball bearing tips (Ancare Corporation, Bellmore, NY).

Aim 1: Binge pattern ethanol pretreatment and voluntary ethanol intake in adulthood and ethanol metabolism

The present experiment was conducted in five phases over a period of fifty-eight days. The first phase was handling, which occurred over two days. The second phase was repeated binge pattern ethanol treatment, which occurred over eighteen days. The third phase was abstinence, which occurred over fourteen days. The fourth phase was voluntary ethanol intake, which occurred over twenty-three days. The final phase was a challenge administration of ethanol (2.0 g/kg) and trunk blood collection. The methods are depicted in the table below.

Table 1: Methods for Aims 1 and 2

| Phase | | Adolescents | Adults |
|-------|--------------------------------------|-------------|------------|
| 1 | Handling | PND 26-27 | PND 58-59 |
| 2 | Repeated Binge Ethanol Treatment | PND 28-45 | PND 60-77 |
| 3 | Abstinence | PND 46-59 | PND 78-91 |
| 4 | Voluntary Ethanol Intake | PND 60-82 | PND 92-114 |
| 5 | Challenge and Trunk Blood Collection | PND 83 | PND 115 |

Handling

On PND 26-27 for adolescents and PND 58-59 for adults, all rats were handled for five min each. This involved transporting animals from the colony room to the

laboratory, where they were weighed and marked for identification purposes. Each animal was positioned in a vertical supine position with the maxillary area of the rat distended to mimic the procedures that occurred during drug or vehicle administration. This was repeated at least twice within the five-min period each day. Animals were also allowed to move freely about the hands and arms of the experimenter to acclimate animals to experimenter manipulation. Following the five minutes, the rats were returned to the homecage and returned to the colony.

Repeated binge pattern ethanol treatment

On PND 28-31, PND 35-38, and PND 42-45 for adolescent rats and PND 60-63, PND 67-70 and PND 74-77 for adult rats, animals were intragastrically administered ethanol (25% v/v ethanol diluted from 95% ethanol in water) or water using one of three doses (0.5, 1.0, or 2.0, g/kg/ig). Animals were administered either ethanol or an equivalent isovolumetric administration of water. Therefore, there was a control group for each dose (0.5, 1.0, and 2.0 g/kg/ig), with one group administered ethanol and the control group administered water equivalent in volume to that of the ethanol group. Using a repeated four-day binge-pattern administration, adolescent and adult rats were transported to the laboratory, weighed, and administered their respective ethanol dose or water. This procedure was repeated every 24 hours on treatment days between 0900-1200 hr during the light cycle. On PND 32-34 and PND 38-41 for adolescent rats and PND 64-66 and PND 71-73 for adult rats, animals were left undisturbed in the colony room, except for regular cage maintenance.

Abstinence

From PND 46-59 for adolescent rats and PND 78-91 for adult rats, all animals underwent abstinence. During this time all animals were left undisturbed in the colony room, except for regular cage maintenance.

Adulthood Voluntary Ethanol Intake

Beginning on PND 60 through PND 82 for adolescent-exposed and PND 92 through PND 114 for adult-exposed rats, all rats were assessed for voluntary ethanol intake using a limited access two-bottle choice paradigm. Fresh bottles were presented to all animals daily with one bottle containing a saccharin/ethanol or ethanol solution and the other bottle containing tap water. The saccharin/ethanol solutions were composed of decreasing concentrations of saccharin dissolved in 10% ethanol. Saccharin (Alta Aesar, Ward Hill, MA) was presented as weight/volume and ethanol was presented as volume/volume. The order of presentation of the saccharin/ethanol concentrations is shown in Table 2 below.

Table 2: Saccharin and Ethanol Concentrations for Ethanol Fading Procedures

| Saccharin Concentration with 10% Ethanol | Adolescents | Adults |
|---|--------------------|---------------|
| 0.4 | PND 60 | PND 92 |
| 0.3 | PND 61-62 | PND 93-94 |
| 0.2 | PND 63-64 | PND 95-96 |
| 0.1 | PND 65-66 | PND 97-98 |
| 0.05 | PND 67-69 | PND 99-101 |
| 0.025 | PND 70-72 | PND 102-104 |
| 0 | PND 73-82 | PND 105-114 |

The side of presentation of the saccharin/ethanol and water bottle was alternated daily to avoid development of a side preference. Bottles were weighed to the nearest 0.1 g before and after the 30-min access period. The difference in weight indicated the amount of ethanol consumed, and data were presented as grams of ethanol per kilogram of body weight (g/kg) for the 30-min session. Spillage was accounted for by placing saccharin/ethanol and water bottles in a similar holding cage unoccupied by a

rat. The difference calculated between the presentation and removal of the bottle from the holding cage accounted for spillage and was subtracted from the daily difference calculated for each rat.

On each day, beginning on PND 60 through PND 82 for adolescent-exposed rats and PND 92 through PND 114 for adult-exposed rats, animals were transported to the laboratory and weighed. Animals were placed in a holding cage with free access to food and water for 30-min to allow them to acclimate to the behavioral testing room. After a timed 30-min interval, the original water bottle was removed, and rats were simultaneously presented with the saccharin/ethanol bottle and a second bottle containing tap water. The bottles were previously weighed to the nearest 0.1 g (as indicated above), and were available to the animal for 30-min. After the 30-min access period, both bottles were removed and again weighed to the nearest 0.1 g. All animals were presented with the original water bottle and remained in the behavioral testing room for an additional 60-min, after which all rats were returned to the colony room. This procedure was repeated each day between 0900-1200 hr during the light cycle.

Aim 2: Challenge Injection and Blood Ethanol Levels

On PND 83 for adolescent-exposed rats and PND 115 for adult-exposed rats, all animals were administered a challenge injection of 2.0 g/kg ethanol via intraperitoneal administration. On PND 83 for adolescent-exposed and PND 115 for adult-exposed rats, animals were transported to the laboratory, weighed and each administered 2.0 g/kg ip ethanol. After 60-min post-injection half of the animals, and after 240 minutes post-injection the other half of the animals were decapitated and trunk blood samples (400 μ L) were collected into 600 μ L capacity heparanized blood collection tubes (BD Microtainer, Becton Dickinson and Company, Franklin Lakes, NJ). The samples were centrifuged at 8,000 rpm for 10-min for serum separation. The serum was transferred to

1500 μ L capacity microcentrifuge tubes (Fisherbrand, Thermo Fisher Scientific, USA), covered with Parafilm (Alcan Packaging, Menasha, WI) and stored at -80 degrees Celsius until analysis on the AM1 blood alcohol analyzer (Analox Instruments, Hammersmith, London).

Aim 3: Binge pattern ethanol pretreatment and mesolimbic dopamine functionality in adulthood

The present experiment was conducted in five phases over a period of forty days. The first phase was handling, which occurred over two days. The second phase was repeated binge pattern ethanol treatment, which occurred over eighteen days. The third phase was abstinence, which occurred over fourteen days. The fourth phase was surgery. The fifth phase was recovery. The final phase was *in vivo* microdialysis. The methods are depicted in the table below.

Table 3: Methods for Aim 3

| Phase | | Adolescents | Adults |
|-------|---|------------------|------------------|
| 1 | Handling | PND 26-27 | PND 58-59 |
| 2 | Repeated Binge Ethanol Treatment | PND 28-45 | PND 60-77 |
| 3 | Abstinence | PND 46-59 | PND 78-91 |
| 4 | Surgery | PND 60 | PND 92 |
| 5 | Recovery | PND 61-64 | PND 93-96 |
| 6 | Microdialysis | PND 64-65 | PND 96-97 |

Handling

For Experiment 2, handling was conducted as discussed above in Experiment 1.

Repeated binge pattern ethanol treatment

For Experiment 2, repeated binge pattern ethanol treatment was administered identically as discussed above in Experiment 1 at the dose of 2.0 g/g ethanol or an isovolumetric administration of water.

Abstinence

For Experiment 2, abstinence was conducted identically as discussed above in Experiment 1.

Surgery

On PND 60 for adolescent-exposed rats and PND 92 for adult-exposed rats, all animals underwent surgery. Animals were transported to the laboratory, weighed and administered an anesthetic dose of ketamine/xylazine cocktail (100 and 0.15 mg/kg/ip). Anesthesia was verified by the absence of a toe-pinch reflex. Once animals were anesthetized, the head was shaved to clear the surgical site. Animals were then mounted onto a stereotaxic instrument for surgery (MyNeuroLab, Leica Microsystems, Richmond, IL). To ensure the head was immobilized, supporting the animal's head, the ears were guided into the locking ear bars, then sliding the tooth bar between the teeth and finally tightening the nose bar the animal was securely mounted into the stereotaxic instrument. The surgery area was draped with a sterile surgical drape and all surgical instruments were sterilized prior to surgery via autoclave. With the head immobilized, an incision was made from right behind the center of the eyes to the center between the ears. The fascia was peeled back using sterile cotton swabs. The skin was held open by the use of sterile clips to ensure the surgery site is unobstructed. With the surgery site open, ensuring a 0.0 coordinate was observed between bregma and lambda, the skull was leveled.

With the skull level, five holes were drilled with sterile drill bits, one in the anterior left hemisphere, the lateral left hemisphere, the posterior left hemisphere, the anterior and posterior right hemisphere of the skull for skull screws to be implanted, one for the implantation of the guide canola (guide cannula for CMA 11; outer diameter 0.6 mm) into the nucleus accumbens septi and one ipsilaterally into the ventral tegmental area.

Relative to bregma, weight-based coordinates will be used to lower the guide cannulae directly above the anterior portion of the nucleus accumbens septi (A: 2.34; L: 0.69; V: -7.78; Philpot et al., 2001) and the ventral tegmental area (P: -5.49; L: 1.00; V: -7.19). The cannula was affixed to the skull with dental acrylic cement (Duralay, Bioanalytical Systems, West Lafayette, IN). The ventral coordinates were measured from the surface of the skull. One booster injection of the ketamine/xylazine cocktail was administered to the rat as needed. Animals were continuously monitored during recovery from anesthesia. Once animals fully recovered from anesthesia, they were returned to the colony singly housed following surgery.

Recovery

From PND 61-64 for adolescent-exposed rats or PND 93-96 for adult-exposed rats, animals were single housed for recovery. Each day, animals were transported to the laboratory and weighed. During this time, animals were exposed to the microdialysis bowl (Raturn Bowl, Bioanalytical Systems, West Lafayette, IN) and affixed with a removable rat harness, with one zip tie affixed around the ventral surface of the rat behind the forelegs, and one affixed around the neck and joined together on the dorsal section of the rat between the legs. For 120 min, animals were placed in the microdialysis bowl to acclimate rats to the Raturn bowl. Once animals were removed from the microdialysis, all animals were gently handled for 10-min. Following handling, animals were returned to the homecage and immediately returned to the colony. This procedure occurred daily for the four days of recovery.

Microdialysis

On PND 64 for adolescent-exposed rats and PND 96 for adult exposed rats, at 1730 hr, rats were transported to the laboratory, weighed, and fitted with a harness. Rats had the stylets for the guide cannulae removed and the microdialysis probes (CMA

11, 2 mm membrane, 240 mm ODS, 6 kDa MW cutoff) inserted into the nucleus accumbens septi and ventral tegmental area site and perfused continuously at a flow rate of 0.2 μL per min with artificial cerebrospinal fluid (aCSF: 136 mM NaCl, 3.7 mM KCl, 1.0 mM MgCl_2 , 1.2 mM CaCl_2 , 10 mM NaHCO_3 pH = 7.2) connected to a 1000 μL syringe pump (Baby Bee syringe pump, Bioanalytical Systems, West Lafayette, IN) and controller (Beehive, Bioanalytical Systems, West Lafayette, IN) after which animals were left overnight for at least 12 hours in the Return optical swivel system (Bioanalytical Systems, West Lafayette, IN).

On the next morning (PND 65 or PND 97, respectively), the flow rate was increased to 0.5 μL per min. Samples were collected into collection vials containing 1 μL of 10 mM hydrochloric acid to prevent enzymatic breakdown, which were stored on dry ice after the 10-min collection interval and then immediately stored at -80 degrees Celcius. The animals were left undisturbed for two hours to allow for equilibration after the increase in flow rate. Five μL volume samples were collected at 10-min intervals during the entire course of microdialysis. Baseline samples were collected for 6 samples. At the seventh sample, the pump connected to the ventral tegmental area was switched to a 500 μL Hamilton gastight syringe, through the use of a liquid switch (BAS Uniswitch Syringe Selector, Bioanalytical Systems, West Lafayette, IN), to a 100 mM K^+ solution at a flow rate of 0.5 μL per min for 20-min through reverse microdialysis (Tran-Nguyen et al., 1996). To maintain the osmolarity of the perfusate, the concentration of Na^+ will be changed to 39.7 mM. After the 20 min microinfusion, the liquid switch connected to the ventral tegmental area site was switched to perfuse the normal aCSF solution and samples continued to be collected for an additional 130-min.

After the microdialysis experiment was complete, all animals were euthanized, brains removed and flash frozen in 2-methylbutane and dry ice at -40 degrees Celcius. Brains were cut on a cryostat (Leica Microsystems, Bannockburn, IL) into 40 μm sections and thaw mounted on glass slides (Corning Incorporated, Corning, NY), stained with a Nissl stain (Acros Organics, Pittsburg, PA), and cover slipped with microscope glass covers (Corning Incorporated, Corning, NY) and Permount (Cole Parmer, Vernon Hills, IL). Histological verification was performed using light microscopy. The drop sites for each animal in the nucleus accumbens septi (Panel A) and ventral tegmental area (Panel B) are depicted in Figure 2.1.

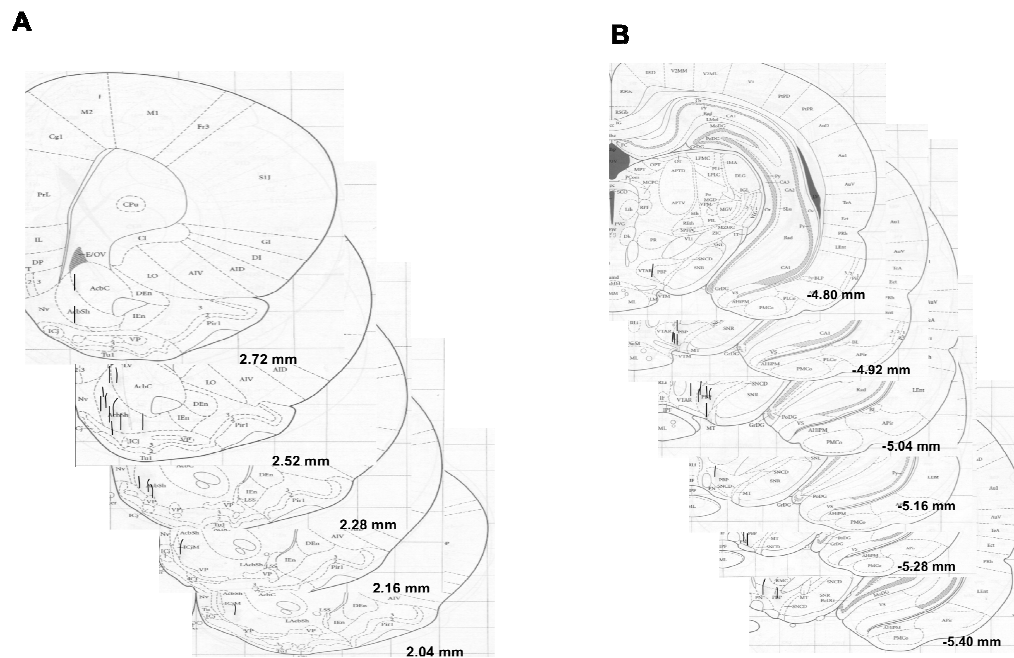


Figure 2.1: Histological verification of nucleus accumbens septi and ventral tegmental area drop sites. The number in each frame indicates the coordinates relative to bregma. Each line indicates the active portion of the microdialysis membrane for each animal in the nucleus accumbens septi (Panel A) and the ventral tegmental area (Panel B). Figure adapted from Paxinos and Watson, 2005.

Neurochemical analyses

All dialysis samples were analyzed via high performance liquid chromatography (HPLC) coupled with electrochemical detection set to oxidize dopamine (DA) and its' metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) at 700 mV (Bioanalytical Systems, West Lafayette, IN). A digital detector (Epsilon, Bioanalytical Systems, West Lafayette, IN) was used with a radial flow glassy carbon working electrode, referenced to a Ag/AgCl electrode. Dopamine and DOPAC were eluted with a mobile phase composed to 75 mM sodium phosphate, 1.4 mM octane sulfonic acid, 1mM EDTA and 10% v/v acetonitrile pHed to 2.9 set with an approximate flow rate of 60 μ L per min. Six μ L dialysis samples were injected onto a C-18 microbore column, 100 x 1 mm, 3 μ m ODS for peak separation (Bioanalytical Systems, West Lafayette, IN). The HPLC was calibrated with a standard curve using standards ranging in concentration from 0.1 to 50 nM for dopamine and 10 to 1000 nM for DOPAC (Philpot et al., 2009). Data were recorded and quantified by Chromgraph on a Dell Dimension 2100.

Design and Analyses

For Experiment 1, voluntary ethanol intake (g/kg) and ethanol preference data were analyzed separately for each dose using a three-factor mixed model design ANOVA with Pretreatment (2; Water, Ethanol), and Age (2; Adolescent, Adult) as between subjects factors and Ethanol Concentration/Days as a repeated measure for both ethanol Acquisition and Maintenance. Given the differences in voluntary ethanol intake within age between doses, data were transformed as a percent of control and data were analyzed using a three-factor mixed model design ANOVA with Age (2; Adolescent, Adult), Dose (3; 0.5, 1.0, 2.0 g/kg) and Ethanol Concentration/Days as a repeated measure for both ethanol Acquisition and Maintenance. When appropriate, post-hoc tests were used to isolate effects in the presence of an interaction (Newman-

Keuls and simple-effects). The level of significance was set at 0.05 (SuperAnova, Abacus Concepts, Berkeley, CA).

Blood ethanol concentration data were analyzed using a between-subjects design ANOVA with Pretreatment (2; Water, Ethanol), Dose (3; 0.5, 1.0 and 2.0 g/kg/ig) Age (2; Adolescent, Adult) and Time (2; 60 and 240 min) as between subjects factors. When appropriate, post-hoc tests will be used to isolate effects in the presence of an interaction (Newman-Keuls and simple-effects). The level of significance was set at 0.05 (SuperAnova, Abacus Concepts, Berkeley, CA).

For Experiment 2, basal dopamine levels were analyzed with a two-factor ANOVA as the mean of the last three baseline samples with Age (2; Adolescent, Adult) and Dose (2; 0.0, 2.0 g/kg) as between subjects factors. Time course analysis for dopamine, DOPAC and DOPAC/DA turnover data were transformed as a percent of baseline samples, and the time course in changes for dopamine, DOPAC concentrations were analyzed using a three-factor mixed model design ANOVA with Dose (3; 0.0, 2.0 g/kg) and Age (2; Adolescent, Adult) as between subjects factors and Time as a repeated measure. When appropriate, post-hoc tests were used to isolate effects in the presence of an interaction (Newman-Keuls and simple-effects). The level of significance was set at 0.05 (SuperAnova, Abacus Concepts, Berkeley, CA).

Chapter 3: Binge ethanol pretreatment during adolescence increases voluntary ethanol intake in adulthood

0.5 g/kg ethanol pretreatment

During acquisition of ethanol intake, age moderated voluntary ethanol intake regardless of the concentration of saccharin combined with 10% ethanol [$F(1, 55) = 12.47, p < 0.001$], an effect that changed across days [$F(5, 275) = 14.80, p < 0.001$]. Specifically, collapsed across saccharin/ethanol concentration, Adolescent ($M = .69$) voluntarily consumed more ethanol relative to Adults ($M = 0.58$). However, there were no clear concentration-dependent changes in voluntary ethanol intake across the ethanol acquisition phase (Figure 3.1, Panel A). During maintenance of ethanol intake on unsweetened 10% ethanol, Adolescent ($M = 0.61$) rats consumed significantly more ethanol than Adult ($M = 0.53$) rats [$F(1, 59) = 9.64, p < 0.005$]. Patterns of maintenance of voluntary ethanol intake across days did not systematically differ between adolescent and adult rats pretreated with 0.5 g/kg ethanol (Figure 3.1, Panel B).

When preference for the ethanol solution was assessed in rats previously treated with 0.5 g/kg ethanol, Age moderated consumption during acquisition of ethanol intake across days [$F(5, 295) = 5.59, p < 0.005$]. This effect was supported by a significant main effect of Age [$F(1, 59) = 4.67, p < 0.05$] and Days [$F(5, 295) = 8.19, p < 0.0005$]. As shown in Figure 3.2 (panel A), Adults showed a significantly high preference for ethanol during consumption of 0.2% saccharin/10% ethanol [$F(1, 62) = 16.30, p < 0.0005$] and 0.025% saccharin/10% ethanol [$F(1, 62) = 16.24, p < 0.0005$]. There was a trend for Adults to show a higher preference for the sweetened ethanol solution during

exposure to 0.4% saccharin/10% ethanol [$F(1, 62) = 3.26, p = 0.07$] and 0.3% saccharin/10% ethanol [$F(1, 62) = 2.80, p = 0.09$]. During maintenance on unsweetened 10% ethanol, Age moderated preference for the ethanol solution across days [$F(4, 240) = 2.57, p < 0.03$]. This effect was supported by a significant main effect of Days [$F(4, 240) = 4.36, p < 0.005$]. As illustrated in Figure 3.2, Panel B, Adults showed significantly greater preference for the ethanol solution relative to Adolescents on PND109-110 [$F(1, 62) = 4.36, p < 0.05$]. There was a trend for greater preference for ethanol in Adults relative to Adolescents on PND 105-106 [$F(1, 62) = 3.68, p = 0.059$]. This pattern was moderated by a trend for ethanol-pretreated rats to show a higher preference for ethanol relative to water-pretreated rats [$F(1, 50) = 2.85, p = 0.09$].

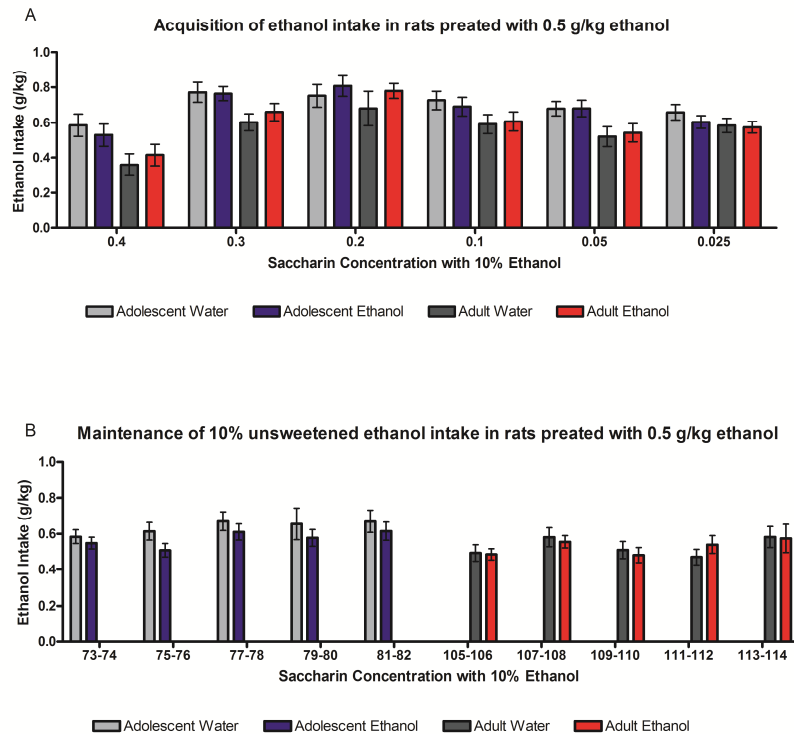


FIGURE 3.1: Acquisition and maintenance of ethanol intake in rats pretreated with 0.5 g/kg ethanol. Ethanol intake (g/kg/ 30-min) presented as mean \pm SEM. Panel A) Acquisition of ethanol intake across decreasing saccharin concentrations with 10% ethanol. Panel B) Maintenance of ethanol intake to unsweetened 10% ethanol in adolescent-pretreated rats (left) and adult-pretreated rats (right). Adolescent Water $n = 17$, Adolescent Ethanol $n = 17$, Adult Water $n = 14$, Adult Ethanol $n = 16$.

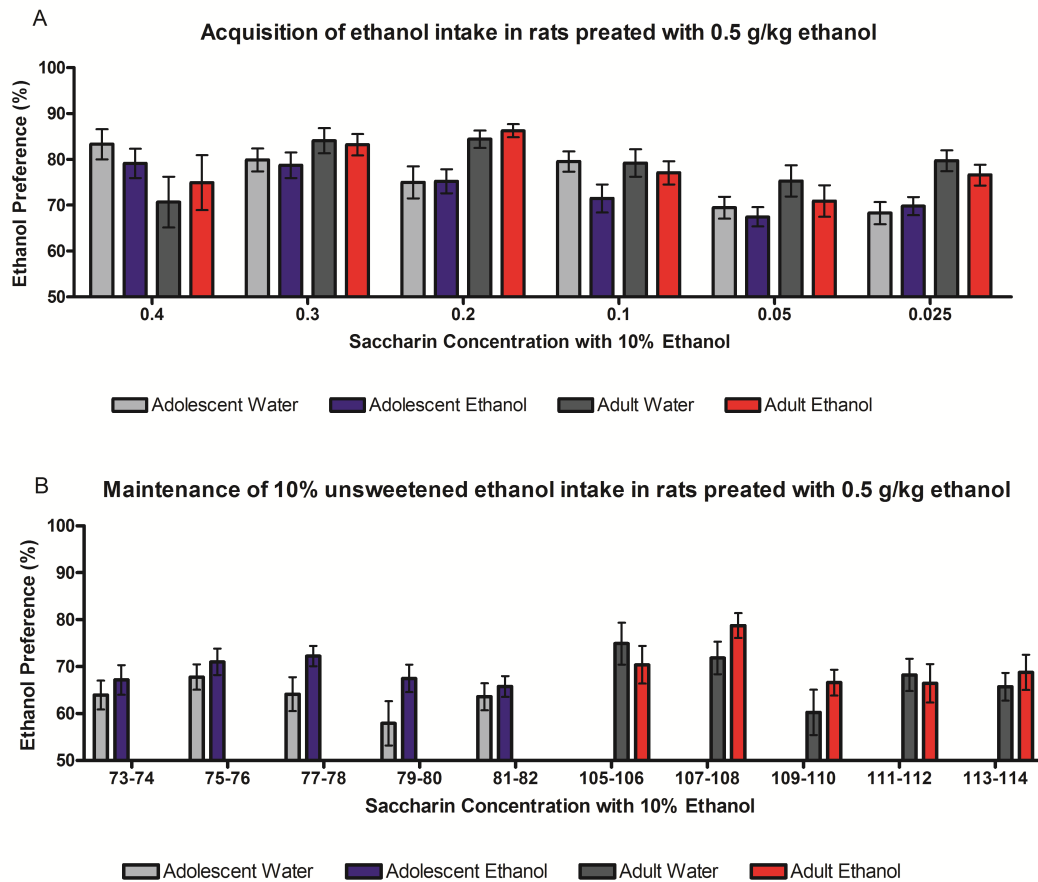


FIGURE 3.2: Acquisition and maintenance of ethanol preference in rats pretreated with 0.5 g/kg ethanol. Ethanol preference $\{[EtOH (ml)] / [EtOH (ml) + Water (ml)] * 100\}$ presented as mean \pm SEM. Panel A) Acquisition of ethanol intake across decreasing saccharin concentrations with 10% ethanol. Panel B) Maintenance of ethanol intake to unsweetened 10% ethanol in adolescent-pretreated rats (left) and adult-pretreated rats (right). Adolescent Water n = 17, Adolescent Ethanol n = 17, Adult Water n = 14, Adult Ethanol n = 16.

1.0 g/kg ethanol pretreatment

During acquisition of ethanol intake, there was a significant change in ethanol consumption across saccharin/ethanol concentration $[F(5, 250) = 10.07, p < 0.0005]$. As shown in Figure 3.3, Panel A, there was no significant change in acquisition of ethanol intake in adolescent ethanol-pretreated rats relative to their ethanol-naïve counterparts or their respective adult ethanol-pretreated counterparts. However, there was a trend for greater ethanol intake during acquisition in Adolescent relative to Adult

rats [$F(1, 50) = 2.89, p = 0.09$]. During maintenance of voluntary ethanol intake to unsweetened 10% ethanol, age and pretreatment significantly altered voluntary ethanol intake across days as supported by a significant three-way interaction of Age by Pretreatment by Days [$F(4, 204) = 2.47, p < 0.05$]. This was supported by a significant main effect of Age [$F(1, 51) = 16.59, p < 0.0005$]. On PND 75-76 [$F(1, 29) = 5.00, p < 0.05$] and PND 77-78 [$F(1, 29) = 4.99, p < 0.05$], Adolescent water-pretreated rats consumed more ethanol than their respective Adult counterparts. On PND 73-74 [$F(1, 25) = 5.66, p < 0.05$] and PND 81-82 [$F(1, 25) = 6.29, p < 0.05$], Adolescent ethanol-pretreated rats consumed significantly more ethanol than their respective Adult counterparts.

When preference for ethanol was assessed in rats pretreated with 1.0 g/kg water or ethanol during adolescence or adulthood, there were no significant differences in acquisition of sweetened voluntary ethanol intake (Figure 3.4, Panel A). However, there was a significant change in preference for ethanol across days as supported by a significant main effect of Saccharin/Ethanol concentration [$F(5, 270) = 6.43, p < 0.0005$]. During maintenance on unsweetened 10% ethanol, there were no significant differences in preference for ethanol between adolescent-pretreated and adult-pretreated rats, regardless of a previous history with water or ethanol exposure (Figure 3.4, Panel B).

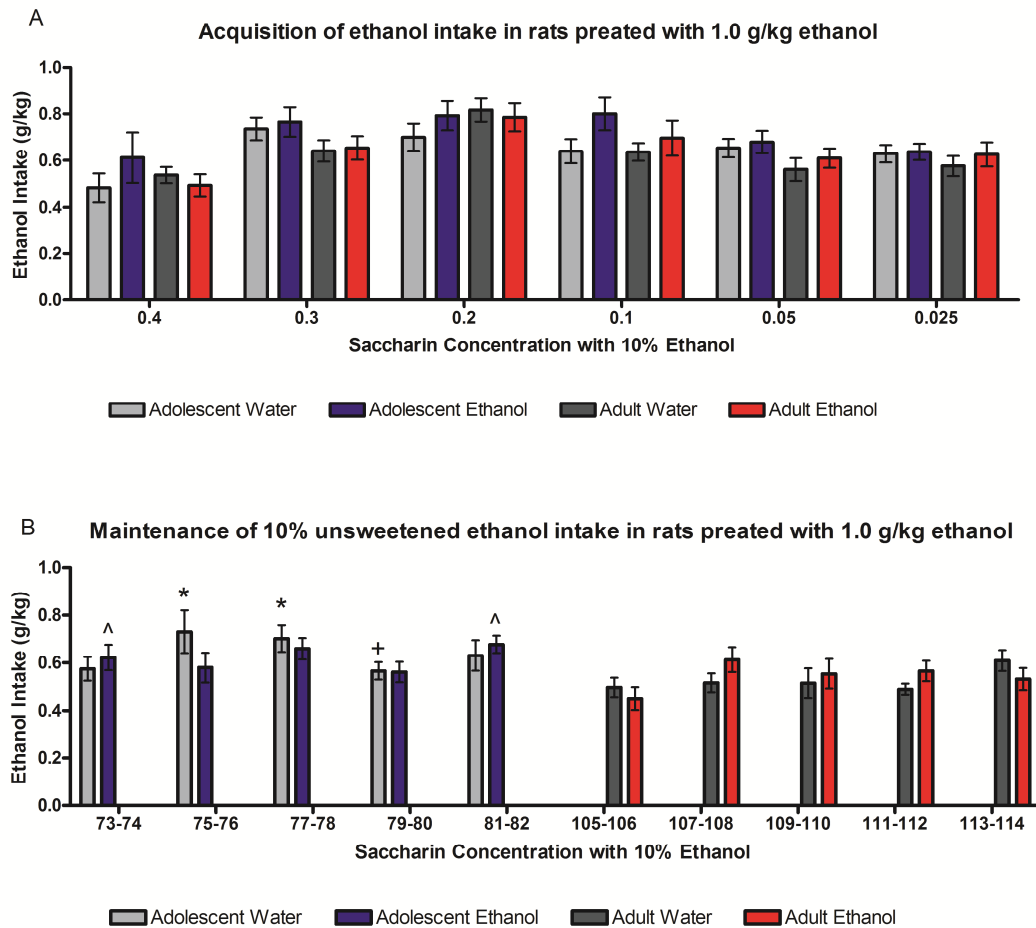


FIGURE 3.3: Acquisition and maintenance of ethanol intake in rats pretreated with 1.0 g/kg ethanol. Ethanol intake (g/kg/ 30-min) presented as mean +/- SEM. Panel A) Acquisition of ethanol intake across decreasing saccharin concentrations with 10% ethanol. Panel B) Maintenance of ethanol intake to unsweetened 10% ethanol in adolescent-pretreated rats (left) and adult-pretreated rats (right). Adolescent Water n = 15, Adolescent Ethanol n = 14, Adult Water n = 16, Adult Ethanol n = 13. * Adolescent water-treated rats significantly greater than Adult water-treated rats. ^ Adolescent ethanol-treated rats significantly greater than Adult ethanol-treated rats. + p < 0.10 Adolescent water-treated rats greater than Adult water-treated rats.

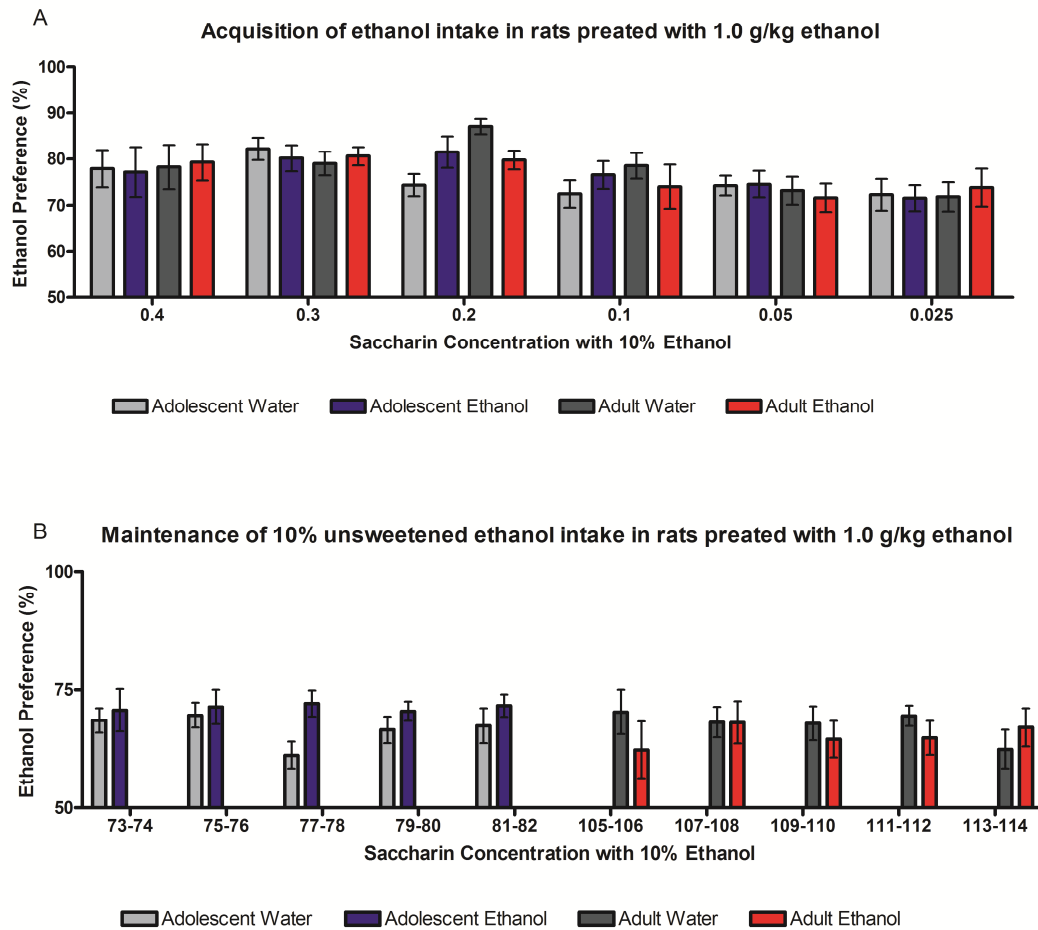


FIGURE 3.4: Acquisition and maintenance of ethanol preference in rats pretreated with 1.0 g/kg ethanol. Ethanol preference $\{[\text{EtOH (ml)}] / [\text{EtOH (ml)} + \text{Water (ml)}] * 100\}$ presented as mean \pm SEM. Panel A) Acquisition of ethanol intake across decreasing saccharin concentrations with 10% ethanol. Panel B) Maintenance of ethanol intake to unsweetened 10% ethanol in adolescent-pretreated rats (left) and adult-pretreated rats (right). Adolescent Water n = 15, Adolescent Ethanol n = 14, Adult Water n = 16, Adult Ethanol n = 13.

2.0 g/kg ethanol pretreatment

During acquisition to voluntary sweetened ethanol intake, Age [F (1, 56) = 9.742] and Pretreatment [F (5, 280) = 3.11, p < 0.05] altered voluntary sweetened ethanol intake across saccharin/ethanol concentration {Age by Saccharin/Ethanol concentration [F (5, 280) = 6.65, p < 0.0005] and Saccharin/Ethanol concentration [F (5, 280) = 9.46, p

< 0.0005]. Planned comparisons indicate pretreatment history and age moderated ethanol intake across saccharin/ethanol concentration (Figure 3.5, Panel A). During exposure to 0.4% saccharin/10% ethanol, Adolescent rats consumed significantly more ethanol relative to Adult rats [F (1, 57) = 14.79, $p < 0.0005$]. During exposure to 0.3% saccharin/10% ethanol, Adolescent ethanol-pretreated rats consumed significantly more ethanol than their adult ethanol-pretreated counterparts [F (1, 58) = 14.27, $p < 0.0005$]. When rats were given the opportunity to consume 0.1% saccharin/10% ethanol, Adolescent ethanol-pretreated rats consumed significantly more ethanol than controls [F (1, 58) = 8.02, $p < 0.05$]. At 0.5% saccharin/10% ethanol, Adolescent rats consumed significantly more ethanol relative to Adults, regardless of treatment history [F (1, 58) = 5.48, $p < 0.05$]. During maintenance on unsweetened 10% ethanol, Adolescent pretreated rats consumed significantly more ethanol relative to Adults [F (1, 55) = 11.90, $p < 0.05$] across Days [F (4, 220) = 2.91, $p < 0.05$]. Planned comparisons revealed Adolescent rats consumed significantly more ethanol relative to Adults on PND 78-80 [F (1, 58) = 21.13, $p < 0.0005$]. There was a trend for greater ethanol intake in Adolescent ethanol-pretreated rats relative to Adult ethanol-pretreated rats on PND 81-82 [F (1, 57) = 2.76, $p = 0.10$].

When ethanol intake was expressed as preference for the ethanol solution, there was a trend for greater ethanol preference for Ethanol-pretreated rats relative to Saline-pretreated rats [F (1, 57) = 3.23, $p = 0.07$] during acquisition of ethanol intake (Figure 3.6, Panel A). Ethanol preference changed across saccharin/ethanol concentrations during acquisition as supported by a significant main effect of Days [F (5, 285) = 3.49, $p < 0.01$]. During maintenance of ethanol consumption on unsweetened 10% ethanol (Figure 3.6, Panel B), Age and Pretreatment altered preference for ethanol across days as supported by a significant three-way interaction of Age by Pretreatment by Days [F

(4, 232) = 2.88, $p < 0.05$]. Posthoc analyses revealed a trend for a greater preference for ethanol in Adults on PND 107-108 relative to Adolescents on PND 73-74 [F (1, 29) = 2.96, $p = 0.09$].

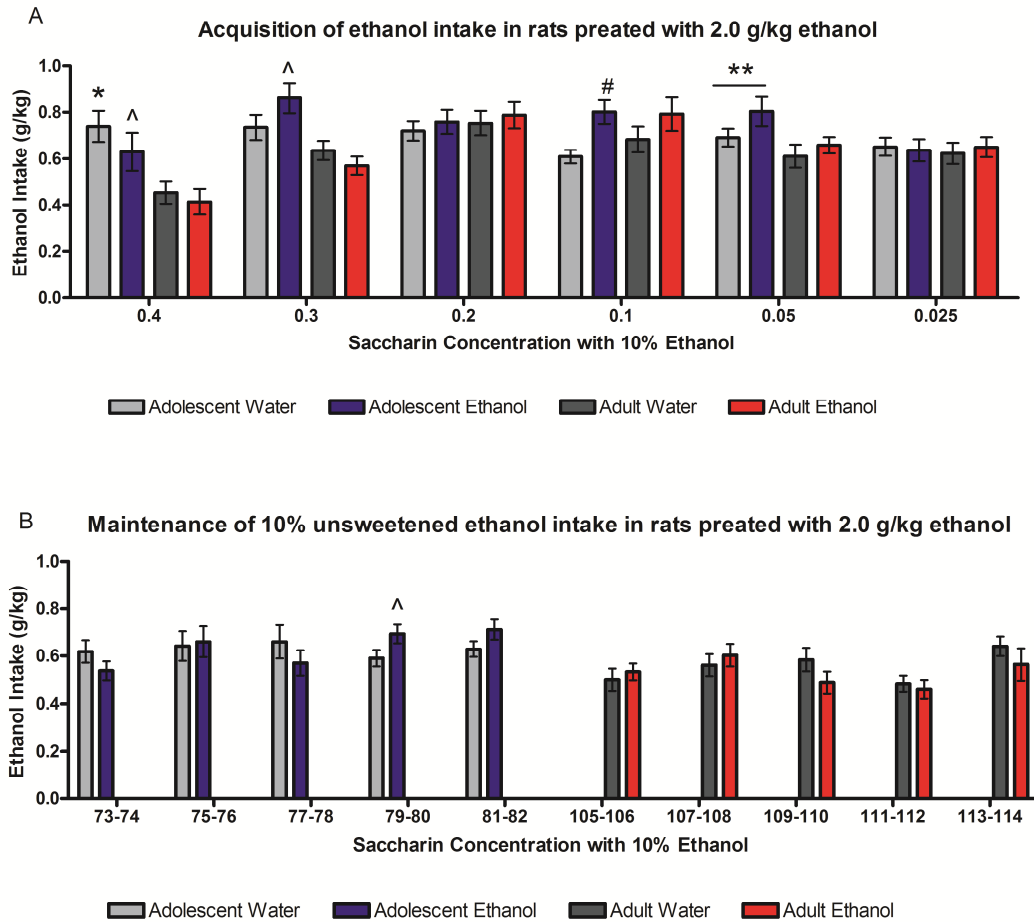


FIGURE 3.5: Acquisition and maintenance of ethanol intake in rats pretreated with 2.0 g/kg ethanol. Ethanol intake (g/kg/ 30-min) presented as mean +/- SEM. Panel A) Acquisition of ethanol intake across decreasing saccharin concentrations with 10% ethanol. Panel B) Maintenance of ethanol intake to unsweetened 10% ethanol in adolescent-pretreated rats (left) and adult-pretreated rats (right). Adolescent Water n = 16, Adolescent Ethanol n = 16, Adult Water n = 15, Adult Ethanol n = 15. * Adolescent water-treated rats significantly greater than Adult water-treated rats. ^ Adolescent ethanol-treated rats significantly greater than Adult ethanol-treated rats. # Adolescent ethanol-treated rats significantly greater than Adolescent water-treated rats. ** Adolescent significantly greater than Adult.

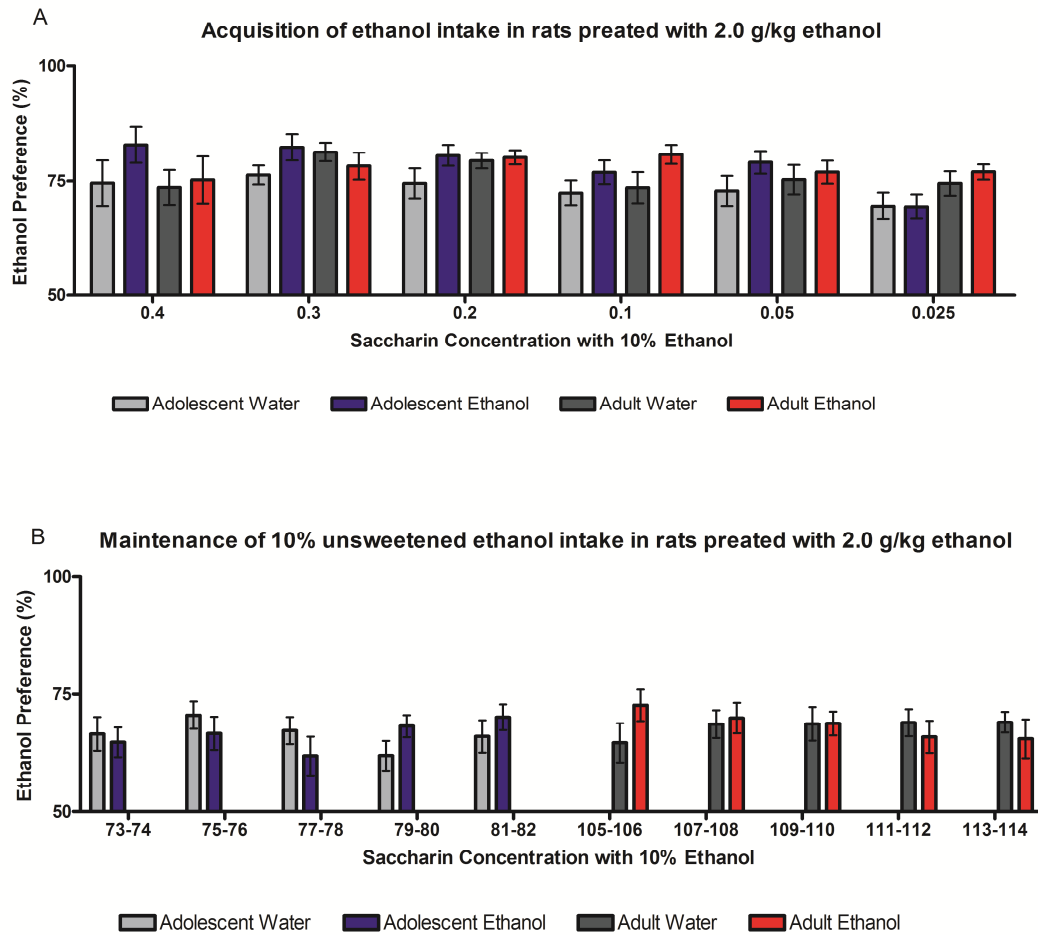


FIGURE 3.6: Acquisition and maintenance of ethanol preference in rats pretreated with 2.0 g/kg ethanol. Ethanol preference $\{[EtOH (ml)] / [EtOH (ml) + Water (ml)] * 100\}$ presented as mean \pm SEM. Panel A) Acquisition of ethanol intake across decreasing saccharin concentrations with 10% ethanol. Panel B) Maintenance of ethanol intake to unsweetened 10% ethanol in adolescent-pretreated rats (left) and adult-pretreated rats (right). Adolescent Water $n = 15$, Adolescent Ethanol $n = 14$, Adult Water $n = 16$, Adult Ethanol $n = 13$.

Dose comparisons (percent of control)

Given there were slight age differences in control animals within age {Figure 3.7; Adolescent $\{[F(10, 210) = 1.954, p < 0.05]$; Adult (Panel B) $[F(10, 210) = 0.88, p > 0.05]$ }, direct comparisons between ethanol dose and age were not possible with the raw g/kg data. Therefore, data were transformed relative to controls (100%) to

make direct Dose and Age assessments (Figure 3.8). During acquisition, within the ethanol-pretreated rats, Age and Dose significantly affected voluntary ethanol intake, regardless of saccharin/ethanol concentration as supported by a significant Age by Dose interaction [$F(2, 84) = 3.41, p < 0.05$]. Specifically, Adolescent rats pretreated with 2.0 g/kg ethanol showed significantly higher preference for ethanol relative to those pretreated with 0.5 g/kg and Adult rats pretreated with 2.0 g/kg.

During maintenance on unsweetened 10% ethanol, with data expressed as a percent of control (Figure 3.9), Age and Dose altered ethanol consumption relative to water controls across Days as supported by a significant Age by Dose by Days interaction [$F(8, 324) = 3.06, p < 0.005$]. There was a trend for Adult (PND 105-106) rats pretreated with 0.5 g/kg to consume more ethanol relative to Adolescent (PND 73-74) ethanol-pretreated rats [$F(1, 31) = 3.18, p = 0.08$]. On PND 107-108, Adult rats pretreated with 1.0 g/kg consumed significantly more ethanol relative to their Adolescent (75-76) ethanol-treated counterparts [$F(1, 25) = 7.80, p < 0.05$]. On PND 111-112, Adult rats pretreated with 0.5 g/kg consumed significantly more ethanol relative to their Adolescent (PND 79-80) counterparts [$F(1, 31) = 5.35, p < 0.05$]. On PND 79-80, Adolescent rats pretreated with 2.0 g/kg consumed significantly more ethanol relative to Adults on PND 111-112 [$F(1, 29) = 6.13, p < 0.05$] and relative to age-matched rats pretreated with 0.5 or 1.0 g/kg ethanol [$F(2, 42) = 8.07, p < 0.005$]. On PND 81-82, Adolescent rats pretreated with 1.0 g/kg [$F(1, 25) = 5.40, p < 0.05$] and 2.0 g/kg [$F(1, 29) = 4.30, p < 0.05$] consumed significantly more ethanol relative to similarly treated Adults on PND 113-114. On PND 81-82, Adolescent rats pretreated with 2.0 g/kg consumed significantly more ethanol than age-matched rats pretreated with 0.5 g/kg [$F(2, 42) = 4.03, p < 0.05$].

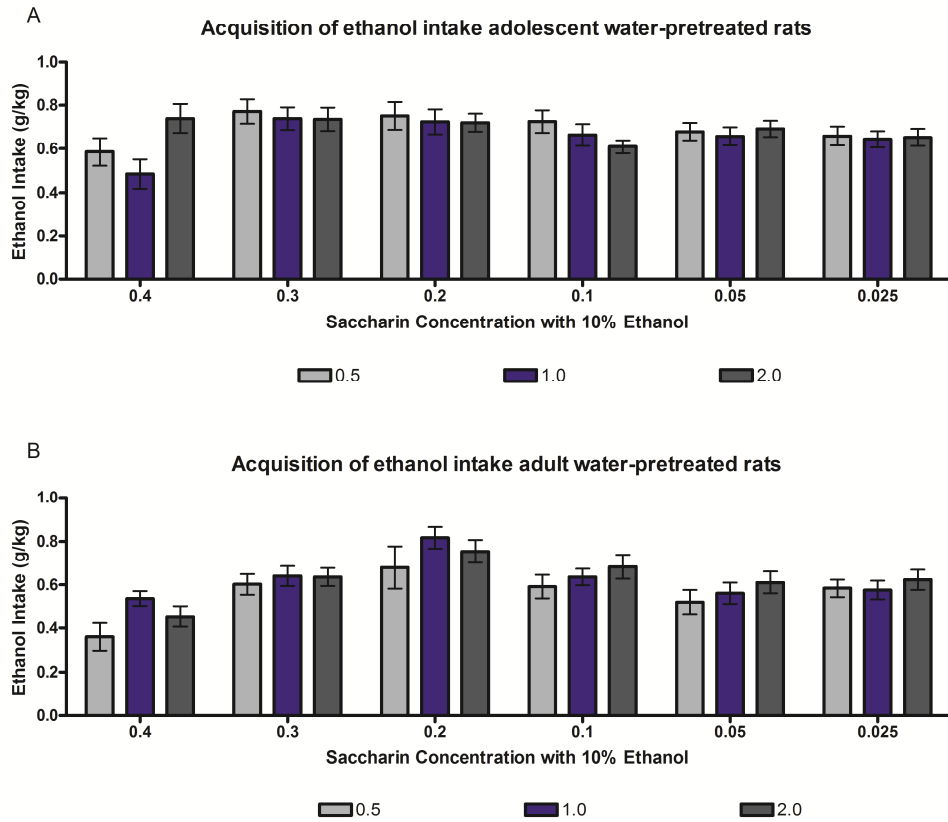


FIGURE 3.7: Acquisition of ethanol intake in water-pretreated rats. Ethanol intake (g/kg/30-min) presented as mean \pm SEM. Panel A) Adolescent rats pretreated with 0.5, 1.0 or 2.0 g/kg water during adolescence and assessed for voluntary ethanol intake in early adulthood. Panel B) Adult rats pretreated with 0.5, 1.0 or 2.0 g/kg water during early adulthood and assessed for voluntary ethanol intake in later adulthood. Adolescent 0.5 n = 17, Adolescent 1.0 n = 15, Adolescent 2.0 n = 16, Adult 0.5 n = 14, Adult 1.0 n = 16, Adult 2.0 n = 15.

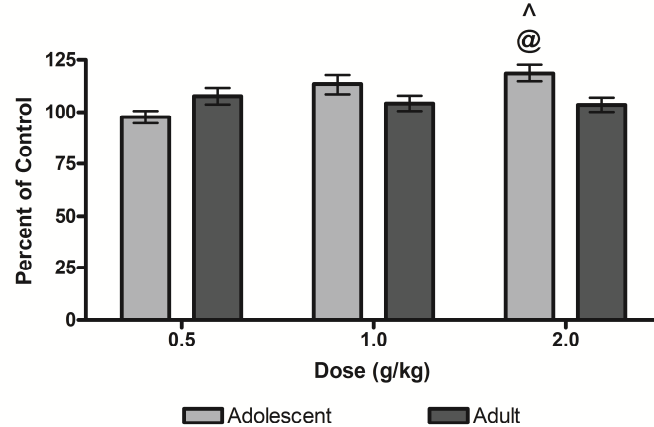


FIGURE 3.8: Acquisition of ethanol intake in ethanol-pretreated rats. Ethanol intake (%) presented as percent of corresponding water-treated rats as mean \pm SEM. Adolescent 0.5 n = 17, Adolescent 1.0 n = 15, Adolescent 2.0 n = 16, Adult 0.5 n = 14, Adult 1.0 n = 16, Adult 2.0 n = 15. @ Adolescent 2.0 g/kg significantly greater than 0.5 g/kg. ^ Adolescent ethanol significantly greater than Adult ethanol.

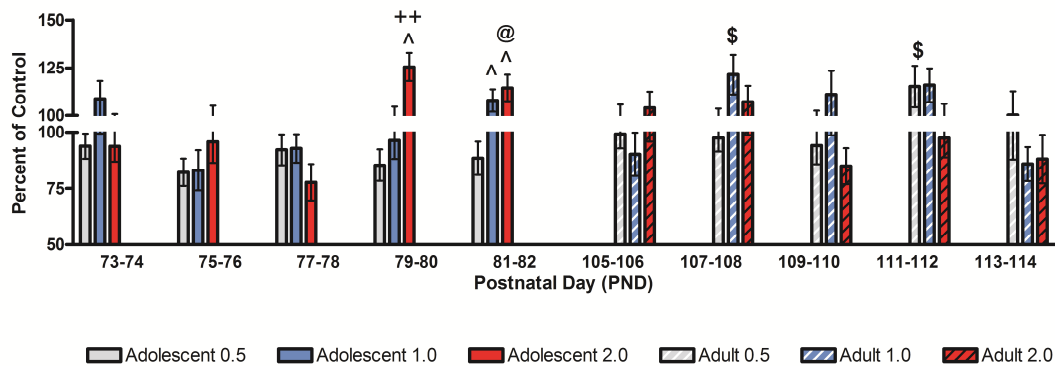


FIGURE 3.9: Maintenance of unsweetened 10% ethanol intake in ethanol-pretreated rats. Ethanol intake (%) presented as percent of corresponding water-treated rats as mean \pm SEM. The break in the y-axis is set to 100% to express the relative water-treated control. Adolescent 0.5 n = 17, Adolescent 1.0 n = 15, Adolescent 2.0 n = 16, Adult 0.5 n = 14, Adult 1.0 n = 16, Adult 2.0 n = 15. ^ Adolescent ethanol significantly greater than Adult ethanol. ++ 2.0 g/kg significantly greater than age-matched 0.5 and 1.0 g/kg. @ Adolescent 2.0 g/kg significantly greater than 0.5 g/kg. \$ Adult ethanol-treated rats significantly greater than corresponding Adolescent ethanol-treated rat.

Chapter 4: Binge ethanol pretreatment dose-dependently alters ethanol metabolism

Ethanol metabolism was assessed using time course changes in blood ethanol concentrations following a challenge injection (i.p.) of 2.0 g/kg when trunk blood samples were collected at 60-min and 240-min postinjection. Age and Dose significantly altered blood ethanol concentrations across time as supported by a significant three way interaction of Age by Dose by Time [$F(2, 149) = 3.153, p < 0.05$]. Specifically, Adult (PND rats pretreated with 2.0 g/kg ethanol showed significantly higher blood ethanol concentrations at 60-min relative to similarly treated Adolescent rats [$F(2, 76) = 3.30, p < 0.05$]. Adolescent rats pretreated with 0.5 g/kg had significantly higher blood ethanol concentrations at 60-min relative to rats pretreated with 2.0 g/kg [$F(2, 41) = 3.18, p = 0.05$]. There were no significant differences in blood ethanol concentrations between Age or Pretreatment Dose at 240-min postinjection. All groups showed significant reductions in blood concentrations from 60-min to 240-min [$F(1, 149) = 688.86, p < 0.005$].

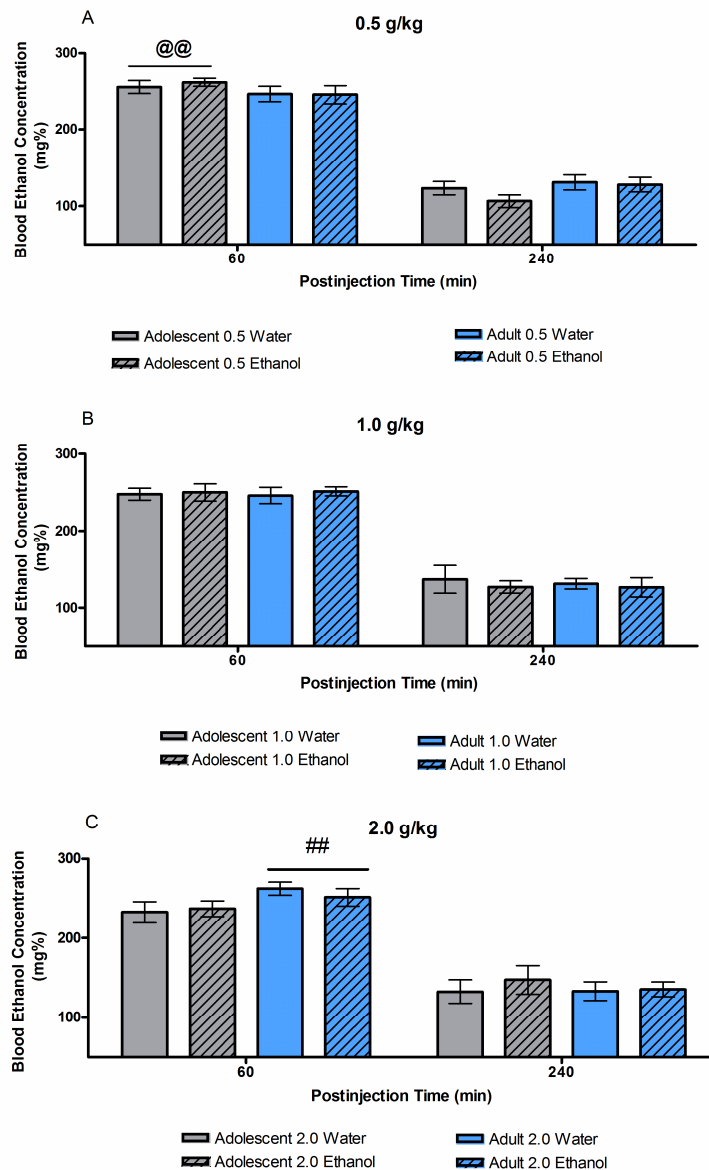


FIGURE 4.1: Blood ethanol concentrations across time in adolescent-exposed and adult-exposed rats. Data expressed as blood ethanol concentration (mg%) +/- SEM. A) Rats pretreated with 0.5 g/kg. Panel B) Rats pretreated with 1.0 g/kg. Panel C) Rats pretreated with 2.0 g/kg. Adolescent 0.5 Water 60-min n = 8; 240-min n = 9, Adolescent 0.5 Ethanol 60-min n = 8; 240-min n = 8, Adolescent 1.0 Water 60-min n = 8; 240-min, Adolescent 1.0 Ethanol 60-min n = 17; 240-min, Adolescent 2.0 Water 60-min n = 17; 240-min n = 7, Adolescent 2.0 Ethanol 60-min n = 6; 240-min n = 8, Adult 0.5 Water 60-min n = 7; 240-min n = 7, Adult 0.5 Ethanol 60-min n = 7; 240-min n = 9, Adult 1.0 Water 60-min n = 6; 240-min n = 7, Adult 1.0 Ethanol 60-min n = 7; 240-min n = 6, Adult 2.0 Water 60-min n = 5; 240-min n = 7, Adult 2.0 Ethanol 60-min n = 6; 240-min n = 7. @@ Adolescent 0.5 g/kg is significantly greater than Adolescent 2.0 g/kg. ## Adult is significantly greater than Adolescent.

Chapter 5: Binge ethanol pretreatment during adolescence does not alter mesolimbic dopamine functionality in adulthood

Potassium-stimulated dopamine in nucleus accumbens septi (nM)

Basal dopamine did not differ between adolescent and adult water-pretreated and ethanol-pretreated rats (Figure 5.1). Potassium-stimulated dopamine release was significantly affected by Pretreatment Dose and Age [$F(1, 12) = 5.09, p < 0.05$]. This effect was supported by a significant main effect of Time [$F(17, 204) = 2.23, p < 0.05$]. The changes in potassium-stimulated dopamine tended to be mediated by pretreatment with ethanol, with ethanol-pretreated rats showing a trend for greater potassium-stimulated dopamine in the nucleus accumbens septi [$F(17, 204) = 1.59, p = 0.08$]. Posthoc analyses did not reveal any significant changes in potassium-stimulated dopamine, however there were several trends for age and dose-dependent changes in potassium-stimulated dopamine (Figure 5.2). Adult rats pretreated with 2.0 g/kg ethanol showed a trend for greater potassium-stimulated dopamine relative to similarly pretreated adolescents [$F(1, 6) = 4.03, p = 0.09$]. Additionally, there was a trend for Adolescent water-pretreated rats to show greater potassium-stimulated dopamine relative to age-matched ethanol-pretreated rats [$F(1, 7) = 4.68, p = 0.06$].

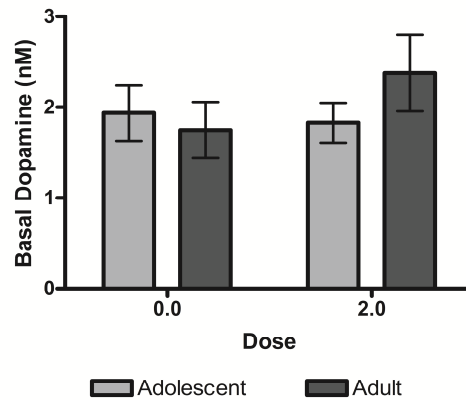


FIGURE 5.1: Basal dopamine levels are similar in adolescent and adult water-pretreated and ethanol-pretreated rats. Data presented at mean basal dopamine levels (nM) +/- SEM. Adolescent 0.0 n=4, Adolescent 2.0 n=5, Adult 0.0 n=4, Adult 2.0 n=4.

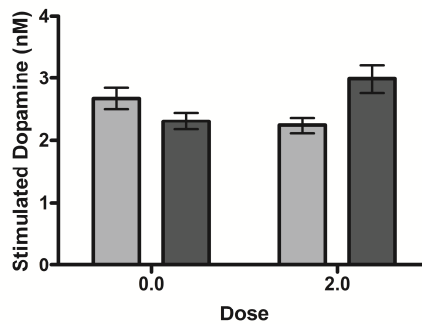


FIGURE 5.2: Potassium-stimulated dopamine in adolescent and adult water-pretreated and ethanol-pretreated rats. Data presented at mean potassium-stimulated dopamine levels (nM) +/- SEM. Adolescent 0.0 n=4, Adolescent 2.0 n=5, Adult 0.0 n=4, Adult 2.0 n=4.

Potassium-stimulated dopamine in nucleus accumbens septi (percent of baseline)

As depicted in Figure 5.3, when dopamine levels are expressed as a percent of basal dopamine, there were no significant changes in potassium-stimulated dopamine levels in Adolescent (Panel A) or Adult (Panel B) rats across time. However, there was a significant change in potassium-stimulated dopamine in the nucleus accumbens septi across time as supported by a significant main effect of Time [$F(28, 221) = 2.352, p < 0.05$].

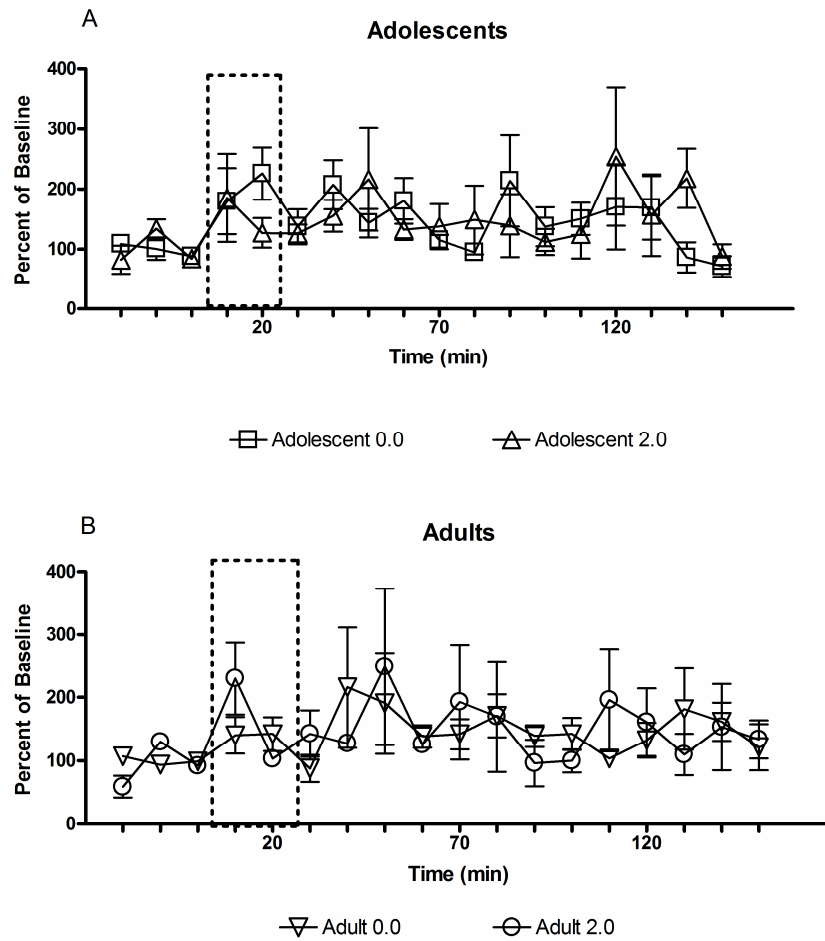


FIGURE 5.3: Time course in potassium-stimulated dopamine in adolescent and adult water-pretreated and ethanol-pretreated rats. Data presented at mean percent of baseline \pm SEM. The dashed box indicates the delivery of 100 mM potassium stimulation. Panel A) Adolescent water and ethanol pretreated rats. Panel B) Adult water and ethanol pretreated rats. Adolescent 0.0 $n=4$, Adolescent 2.0 $n=5$, Adult 0.0 $n=4$, Adult 2.0 $n=4$.

Potassium-stimulated DOPAC in nucleus accumbens septi (nM)

As depicted in Figure 5.4, when DOPAC levels are expressed as a percent of basal DOPAC, there were no significant changes in potassium-stimulated DOPAC levels in Adolescent (Panel A) or Adult (Panel B) rats across time.

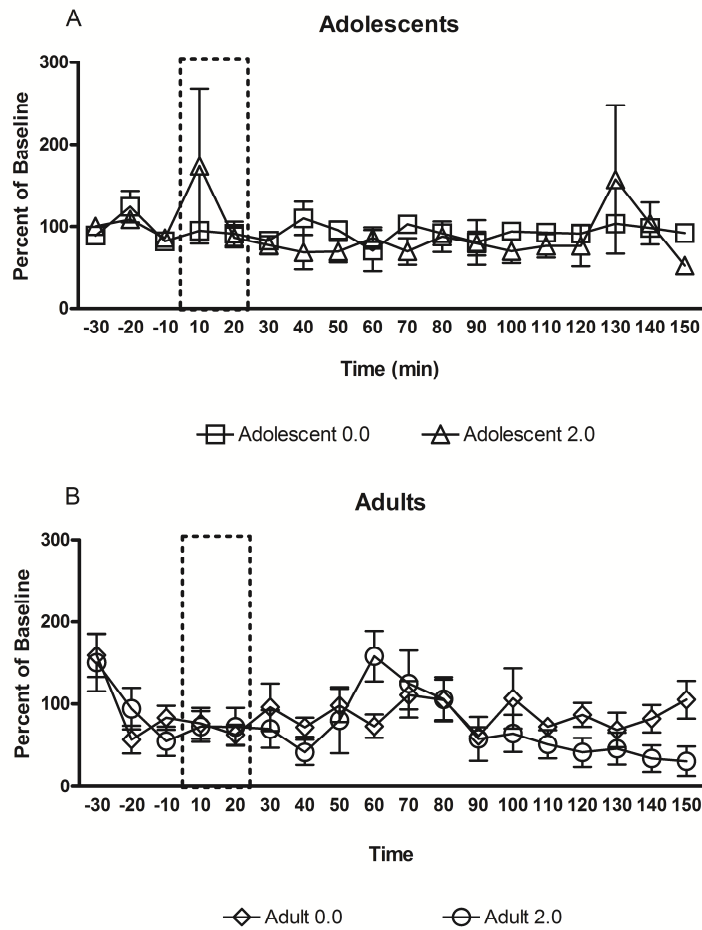


FIGURE 5.4: Time course in potassium-stimulated DOPAC in adolescent and adult water-pretreated and ethanol-pretreated rats. Data presented at mean percent of baseline +/- SEM. The dashed box indicates the delivery of 100 mM potassium stimulation. Panel A) Adolescent water and ethanol pretreated rats. Panel B) Adult water and ethanol pretreated rats. Adolescent 0.0 n=4, Adolescent 2.0 n=5, Adult 0.0 n=4, Adult 2.0 n=4.

Potassium-stimulated DOPAC/DA turnover in nucleus accumbens septi

As depicted in Figure 5.5, when DOPAC/DA levels are expressed as a percent of basal DOPAC/DA turnover levels, there were no significant changes in potassium-stimulated DOPAC/DA turnover levels in Adolescent (Panel A) or Adult (Panel B) rats across time. However, DOPAC/DA turnover did change across time as supported with a significant main effect of Time ($F(16, 221) = 2.75, p < 0.05$). There was also a trend for ethanol pretreatment to decrease DOPAC/DA turnover across time [$F(16, 221) = 1.58, p = 0.10$].

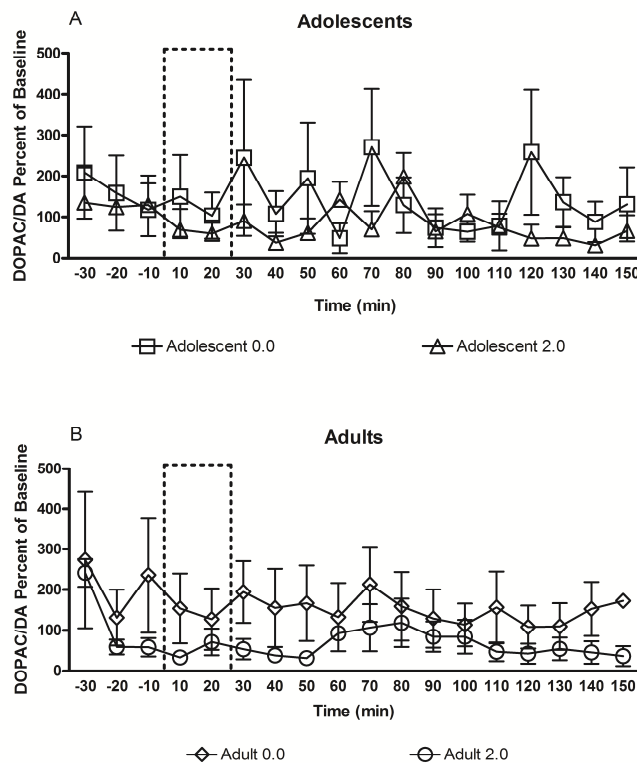


FIGURE 5.5: Time course in potassium-stimulated DOPAC/DA turnover in adolescent and adult water-pretreated and ethanol-pretreated rats. Data presented at mean percent of baseline \pm SEM. The dashed box indicates the delivery of 100 mM potassium stimulation. Panel A) Adolescent water and ethanol pretreated rats. Panel B) Adult water and ethanol pretreated rats. Adolescent 0.0 $n=4$, Adolescent 2.0 $n=5$, Adult 0.0 $n=4$, Adult 2.0 $n=4$.

Chapter 6: Discussion

Aim 1: Voluntary ethanol intake after water or ethanol pretreatment

Cognitive and behavioral dysfunctions have been reported following binge ethanol treatment during adolescence (Popovic et al., 2004; Obernier et al., 2002). Overall, data from the present set of experiments demonstrate that all rats exposed to a high dose of ethanol (2.0 g/kg ig) during adolescence are especially susceptible to enhanced ethanol consumption in young adulthood as compared to similarly ethanol-pretreated adult rats. Recent work supports the present findings of enhanced ethanol intake in adulthood following ethanol exposure during adolescence relative to animals that initiated ethanol exposure later in life (Holstein et al., 2011; Melendez, 2011; Metten et al., 2011; Sherrill et al., 2011; String et al., 2010). When adolescent mice were initiated on binge ethanol exposure during adolescence at ~PND 28, they showed sustained elevated ethanol intake relative to rats that initiated ethanol intake at ~PND 63 (Metten et al., 2011). Interestingly, when late adolescent mice were initiated on binge ethanol exposure at ~PND 42, these rats did not show long-term elevations in voluntary ethanol consumption relative to young adult mice, in fact these late adolescent mice showed decreased ethanol intake later in life relative to adult-ethanol-exposed mice (Metten et al., 2011). These data support the hypothesis of a critical period of exposure to ethanol to affect long-term changes in ethanol intake in adulthood and suggest a unique vulnerability to a specific pattern of ethanol exposure that induced long-term behavioral changes in adolescent high-dose ethanol-exposed rats.

It is important to note that not all forms of ethanol exposure (ethanol vapor or constant voluntary access to ethanol) during adolescence leads to changes in voluntary ethanol intake in adulthood. In rats that were given daily voluntary access to ethanol beginning during adolescence and extending into adulthood (PND 28-90), there were no significant differences in voluntary ethanol intake compared to rats that began voluntary ethanol consumption in adulthood (PND 71-90; Vetter et al., 2007). In another study, ethanol vapor exposure during periadolescence (PND 30-40) did not enhance sucrose sweetened ethanol consumption in adulthood (> PND 92; Slawecki and Betancourt, 2002). These data are consistent with recent findings indicating that daily access to ethanol can reverse the enhanced ethanol intake observed following intermittent exposure to ethanol in periadolescent rodents (Melendez, 2011). One of the key aspects hypothesized to increase ethanol intake in young adulthood in adolescent rats pretreated with the high 2.0 g/kg dose of ethanol was the pattern of adolescent ethanol exposure with repeated cycles of four consecutive days of ethanol administration coupled with intermittent abstinence days during the adolescent exposure period. Vetter and colleagues (2007) allowed animals ethanol access everyday beginning in adolescence through adulthood, with no ethanol-free days. Slawecki and Betancourt (2002) exposed adolescent male rats to ethanol for ten consecutive days, with no ethanol-free days. While adolescent rats exposed to ethanol every day did not show enhanced ethanol consumption in adulthood, rodents exposed to intermittent ethanol vapor during periadolescence exhibited a smaller conditioned taste aversion in adulthood as compared to those exposed to chronic ethanol vapor during periadolescence (Diaz- Granados and Graham, 2007). Intermittent exposure to ethanol enhanced ethanol consumption in adolescent rats relative to those given continuous access (Hargreaves et al., 2009). Given differences in behavioral responses to

intermittent ethanol exposure were observed (Diaz-Granados and Graham, 2007), but not when animals were exposed to chronic ethanol during adolescence (Slawecki and Betancourt, 2002; Vetter et al., 2007), it is likely the intermittent nature of the binge exposure used in the present set of experiments that induced the behavioral changes observed in response to ethanol in young adulthood in both male.

Recent work has shown that withdrawal severity is similar in adolescent relative to adult rats (Morris et al., 2010). However, others have shown that withdrawal severity is decreased in adolescents as compared to adults (Acheson et al. 1999). Adolescent mice show greater ethanol intake across repeated intermittent cycles of access to ethanol to consume binge quantities of ethanol during the adolescent developmental window (Holstein et al., 2011). Binge ethanol-exposed animals showed a behavioral profile of decreased conditioned taste aversion to binge ethanol relative to adult mice (Holstein et al., 2011), an effect that was dose-dependent to moderate doses of ethanol in rats (Schramm-Sapyta et al., 2010). This behavioral profile of greater ethanol intake and decreased aversion associated with ethanol firmly supports the hypothesis of differential effects of ethanol during adolescence relative to adulthood is due to decreased sensitivity to the aversive properties associated with ethanol (Holstein et al., 2011; Schramm-Sapyta et al., 2010). Consistent with this hypothesis, previous work shows adolescents are less sensitive to the aversive effects of ethanol and more sensitive to the rewarding/reinforcing/positive effects of ethanol relative to adults (Little et al., 1996; Philpot et al., 2003; Silveri & Spear, 1998; White et al., 2002). Therefore, it is not the withdrawal severity that likely mediates the lasting change in voluntary ethanol intake in adulthood in adolescent-pretreated rats exposed to 2.0 g/kg, however it is most likely the developmental change associated with a 'stamping in' of the rewarding

properties and a disassociation of the aversive properties associated with ethanol following intermittent adolescent ethanol pretreatment.

Recent work has assessed post-deprivation induced ethanol intake in adolescent relative to adult rats, and shown that adolescents show greater post-deprivation induced ethanol intake relative to adult rats (Schramm-Sapyta et al., 2010). In rats administered the high dose of ethanol, the alcohol deprivation effect of two weeks without ethanol administration between adolescence and adulthood could account for the greater ethanol consumption in young adulthood in adolescent ethanol-pretreated male rats (Fullgrabe et al., 2007; Siegmund et al., 2005). An additional explanation for the results obtained is the impact of repeated ethanol withdrawals during adolescent pretreatment on subsequent ethanol consumption in young adulthood. It is likely a similar phenomenon would have been observed during the voluntary ethanol intake portion of the experiment in the present work if rats had been given intermittent voluntary access to ethanol during the voluntary ethanol intake assessment. Indeed, we recently conducted an experiment in which adolescent and adult animals were intermittently exposed to binge ethanol during adolescence or adulthood and assessed for intermittent voluntary ethanol intake in adulthood with two days of maintenance on 10% ethanol followed by two days without access to ethanol, repeated for a total of three ethanol-free periods. These data strongly support the hypothesis of the intermittent nature of exposure to ethanol enhancing subsequent ethanol intake in adulthood in adolescent-exposed relative to adult-exposed rats (Appendix A; Michael, Maldonado-Devincci and Kirstein, in prep).

In previous work, when adolescent males were exposed to higher doses of binge ethanol (1.5, 3.0 and 5.0 g/kg) during adolescence and assessed for voluntary sweetened ethanol (0.5% saccharin/10% ethanol) intake in adulthood, there was a

dramatic increase in voluntary ethanol intake expressed relative to body weight (g/kg) and as a percent of control, regardless of pretreatment dose (Maldonado-Devincci et al., 2010b). The results from the present experiment are consistent with these previous findings in that there was enhanced ethanol intake in adolescent ethanol-pretreated rats exposed to 2.0 g/kg, however this effect was not nearly as robust. Interestingly, comparing the present findings to the previous work, there appears to be a threshold dose for enhanced ethanol intake to be observed in adulthood following binge ethanol exposure during adolescence between the dose range of 1.0 and 1.5 g/kg. The most notable changes in ethanol intake in adolescent ethanol-pretreated rats were relative to similarly-exposed adults and to a lesser degree relative to their water-treated counterparts during acquisition and maintenance of ethanol intake. One of the primary reasons for these different findings in the present work relative to the previous work using the same model of binge ethanol exposure was that in the present experiments rats were exposed to less-sweetened ethanol beginning during ethanol acquisition and the saccharin was faded completely from the solution during maintenance on unsweetened 10% ethanol. It has been suggested that ethanol is primarily consumed for its pharmacological actions, although as saccharin concentrations are decreased there is a potentially increased aversiveness associated with the taste of the ethanol solution when decreasing saccharin/ethanol concentrations are used (Slawecki et al., 1997). When ethanol solutions are adulterated with a sweetener, there is always the possibility that rats consume the ethanol not only for its pharmacological effects, but for a combination of the sweetener, which is an appetitive component of the ethanol solution in and of itself, and the pharmacological effects experienced from the ethanol consumption. Therefore, one of the assertions that can be made from the present findings, despite the overall lower level of ethanol intake compared to previous work

using a sweetened ethanol solution, was that the adolescent ethanol-pretreated rats exposed to 2.0 g/kg consumed ethanol later in life during the maintenance portion of the experiment for its pharmacological properties. Given adult rats are more sensitive to the sedative and hypnotic effects of ethanol relative to adolescent rats (Silveri and Spear, 1998), it is likely that increased sensitivity to ethanol's post-ingestive effects may serve to limit the amount of ethanol consumed by adult rats (Samson and Slawecki, 1997) upon each exposure to voluntary access to ethanol. In turn, the enhanced voluntary ethanol consumption in adolescent rats was observed at later time points during maintenance on unsweetened 10% ethanol.

Binge pattern ethanol exposure during adolescence was recently shown to permanently alter the functionality of the hypothalamo-pituitary-adrenal axis, by sensitizing this pathway to subsequent stress later in life (Przybycien-Szymanska et al., 2011). These authors conducted this work under the premise that exposure to ethanol is a stressor in and of itself, and therefore exposure to repeated binge ethanol (3.0 g/kg) during adolescence was essentially synonymous with exposure to intermittent stress during adolescence (Przybycien-Szymanska et al., 2011). Subsequently, when animals were exposed to binge ethanol in adulthood, these animals were exposed again to stress acutely or repeatedly (Przybycien-Szymanska et al., 2011). The present work exposed adolescent rats to repeated cycles of binge ethanol pretreatment during adolescence or adulthood with lower doses than those used in the work by Przybycien-Szymanska and colleagues (2011). Adolescent rats have been shown to be more sensitive to ethanol/stress interactions relative to adult rats (Brunell and Spear, 2005). Assessing the present work under the same notion as proposed by Przybycien-Szymanska and colleagues (2011), the long-term changes in increased ethanol intake in adolescent-exposed rats relative to adult-exposed rats may be the behavioral

manifestation of greater long-term alterations in functionality of the HPA axis manifested as enhanced ethanol intake, an effect that is likely mediated by the dose of ethanol pretreatment given that enhanced ethanol intake was most evident at the highest ethanol pretreatment dose. In previous work, when adolescent males underwent a 14 day alcohol deprivation effect they showed less than 200% increased over baseline drinking which declined to 100% over 4 days, and in response to forced swim stress adolescent males showed a maximal 150% increase above baseline drinking (Siegmund et al., 2005). Comparing the present data to that of Siegmund and colleagues (2005), during acquisition of ethanol intake, adolescent rats pretreated with 2.0 g/kg ethanol showed similar levels of ethanol intake compared to their respective controls. However, during maintenance, across days adolescent ethanol-pretreated rats showed an increase in voluntary ethanol intake to approximately 125% above controls. It is likely that repeated gavage during adolescence was a stressful procedure, and given that the stressed rats in the work by Siegmund and colleagues (2005) showed a blunted response to enhanced ethanol intake after an abstinence period, the present results are consistent with this previous work. This blunted, yet significant increase in voluntary ethanol intake in adolescent ethanol-pretreated rats to 2.0 g/kg relative to similarly pretreated adults supports the position of Przybycien-Szymanska and colleagues (2011) in a permanently altered HPA axis in adolescent ethanol-pretreated rats expressed behaviorally as an increase in voluntary ethanol consumption relative to similarly treated adults.

Recent work has shown that the rise in peripubertal gonadal hormones is essential to exhibit the enhanced ethanol intake in adulthood following binge ethanol exposure during adolescence (Sherrill et al., 2011). Considering adolescence is a developmental period in which ethanol is initially consumed and may lead to greater

alcohol consumption later in life (Grant et al., 2001; Hasin and Glick, 1998; McCarty et al., 2004; Robin et al., 1998), perturbations to this developing system via ethanol exposure coupled with the presence of gonadal hormones set the stage for lasting changes in behavioral response to ethanol. The results of the present set of experiments demonstrate the importance of elucidating the impact of early ethanol exposure on the subsequent predisposition to drink later in life. The present results indicate that the early patterns of binge ethanol exposure during adolescence play a crucial role in the development and continuation of enhanced ethanol consumption, which could be manifested in humans as the higher level of alcohol use disorders into adulthood (Hill et al., 2000) following binge alcohol consumption during development.

Aim 2: Ethanol metabolism after water or ethanol pretreatment

In general, the data from Aim 2 indicate that adolescent binge pattern ethanol treatment with the high dose of ethanol induced long-term changes in ethanol metabolism relative to similarly ethanol-pretreated adult rats. Consistent with previous work, adult rats administered a high dose of ethanol displayed elevated blood ethanol concentrations at the 60-min time point relative to similarly treated adolescent rats (Walker and Ehlers, 2009). This metabolic profile is intriguing given that all animals were adults (PND 83 for adolescent-exposed rats and PND 115 for adult-exposed rats) in the present experiment indicating long-term alterations in ethanol metabolism were present in adolescent-exposed relative to adult-exposed rats that underwent an abstinence period and were subsequently exposed to voluntary ethanol intake for approximately three weeks. Strong and colleagues (2010) suggest that ethanol metabolism is not different between adolescent and adult mice, given the similar relationship of elevated ethanol intake corresponding to higher blood ethanol concentrations in adolescent relative to adult mice. In contrast, recent work in rats

support the notion of faster metabolism of ethanol in adolescent relative to adult rats given that blood ethanol concentrations were decreased in adolescents relative to adults before ethanol dosing in a binge model of ethanol exposure aimed to equate blood ethanol concentrations in adolescent and adult rats (Morris et al., 2010). These findings are similar to previous work showing faster ethanol metabolism in adolescent relative to adult rats (Brasser and Spear, 2002). Chronic intermittent injections of high-dose ethanol during adolescence has been found to induce long-lasting tolerance to ethanol as measured by enhanced blood ethanol elimination rates in adulthood compared to saline-treated rats (Silvers et al., 2003). In general, faster ethanol metabolism was found in adolescent high-dose ethanol pretreated rats relative to similarly treated adult rats, suggesting long-term changes in ethanol pharmacokinetics mediating the enhanced ethanol intake observed in Aim 1. The enhanced ethanol intake observed in Aim 1 is likely attributed to differences in long-lasting tolerance in adolescent relative to adult rats.

The present data are inconsistent with recent work in mice (Holstein et al., 2011; Strong et al., 2010) and rats (Schramm-Sapyta et al., 2010) indicating that adolescent animals achieve higher blood ethanol concentrations relative to adult animals. Holstein and colleagues (2011) assessed blood ethanol concentrations immediately after the mice were given their daily access to ethanol. Schramm-Sapyta and colleagues (2010) assessed blood ethanol concentrations 15 min after ethanol administration of 1.0 g/kg (ip). These data may support the hypothesis that adolescent rats reach higher peak blood ethanol concentrations (Holstein et al., 2011; Schramm-Sapyta et al., 2010). The present work aimed to assess changes in ethanol metabolism in adolescent-exposed relative to adult-exposed rats using a time course analysis. Blood samples were not collected until 60-min after ethanol challenge, and it is likely that peak blood ethanol

concentrations were not assessed using this protracted time-scale in the present set of experiments.

Aim 3: Potassium-stimulated dopamine in the nucleus accumbens septi after water or ethanol pretreatment

Collectively, the present data show a trend for increased potassium-stimulated dopamine release in the nucleus accumbens septi in adult binge-pattern ethanol-pretreated rats and a trend for decreased potassium-stimulated dopamine release in the nucleus accumbens septi in ethanol-pretreated adolescent rats. These data suggest a developmental change following binge-pattern ethanol pretreatment on functionality of the mesolimbic dopaminergic pathway. Somatodendritic and axonal terminal release of dopamine are released in different quantities following potassium stimulated release in the ventral tegmental area and the nucleus accumbens septi, with greater dopamine released terminally in the nucleus accumbens relative to the cell body region in the ventral tegmental area (Irivani et al., 1996, Kalivas and Duffy 1991). Consistent with previous data, potassium-stimulated dopamine was increased in nucleus accumbens septi in adult rats pretreated with ethanol.

Previous research has shown dose-dependent differences due to adolescent (Crews et al., 2006) and adult (Matthews et al., 2002) ethanol exposure. Periadolescent rodents exposed to different doses of ethanol (1.0, 2.5, and 5.0 g/kg) exhibited decreased neural progenitor cell proliferation and neurogenesis that was directly proportional to the dose of ethanol administered (Crews et al., 2006). Other work in non-human primates has shown binge ethanol during adolescence decreased hippocampal neurogenesis (Taffe et al., 2010). These data support that high dose ethanol during adolescence can significantly alter long-term neurobiological response to ethanol pretreatment. Recent work has shown that binge ethanol during adolescence enhanced

microglial reactivity in hippocampus in young adulthood (McClain et al., 2011). It was suggested that repeated withdrawal from high dose ethanol treatment may exacerbate the greater reactivity of the microglia in hippocampus, to in turn induce a proinflammatory response in these ethanol pretreated rats (McClain et al., 2011). This proinflammatory response likely induces long-term damage in the brain during and following adolescent binge ethanol in animals administered approximately 5.0 g/kg (p.o.) over the four days of binge ethanol treatment (McClain et al., 2011). Astrocyte cell swelling upstream of the ventral tegmental area has recently been suggested to mediate ethanol-induced increases in dopamine in the nucleus accumbens septi (Adermark et al., 2010). Ethanol exerts an influence on a sodium/potassium transporter that can interact with astrocyte cell swelling (Adermark et al., 2010). This astrocyte cell swelling regulation of dopamine release in the nucleus accumbens septi was observed in adult animals (Adermark et al., 2010). Binge ethanol exposure during adolescence decreased dopaminergic and cholinergic gene expression in adulthood (Coleman et al., 2011). It is probable that in the present experiment the adolescent rats administered binge-pattern ethanol showed enhanced microglial and astrocyte activity in mesolimbic pathway, and in turn induced long-term proinflammatory activity that altered the responsivity of the mesolimbic pathway that resulted in different neurochemical responses to potassium-stimulated dopamine release in the mesolimbic pathway in adolescent relative to adult rats.

Recent work indicates there is an intricate interaction between glutamate, GABA and dopamine that modulates the mesocorticolimbic pathway (Yamaguchi et al., 2011). A new mesocorticolimbic pathway has been identified indicating glutamatergic projections originate from the A10 region (which includes the ventral tegmental area) and projects to the prefrontal cortex and the nucleus accumbens septi that follows along

the same pathways as the dopaminergic-only and GABAergic-only pathways originating from the A10 region and projecting to these same structures (Yamaguchi et al., 2011). This newly identified pathway contains subpopulations of glutamatergic-only projections and another subpopulation coexpressing glutamatergic and dopaminergic projections (Yamaguchi et al., 2011). Previous work has shown neurons co-expressing vesicular glutamate transporter-2 (VGLUT-2) and tyrosine hydroxylase immunostaining decrease across ontogeny, indicating colocalization of glutamate and dopamine is present in high numbers early in develop and this decreases across maturity (Berube-Carriere et al., 2009). During adolescence there is a dramatic increase in the innervation of glutamatergic regulation from cortical structures to the mesolimbic pathway that can regulate activity within this pathway (Brenhouse et al., 2008). There are glutamatergic projections to both the ventral tegmental area and the nucleus accumbens septi that develop during adolescence and reciprocating projections to the prefrontal cortex that regulate activity of the mesocorticolimbic pathway (Kalsbeek et al., 1988). Therefore, in the present set of experiments it is likely that ethanol administration during adolescence altered the normal ontogenetic pruning of these projections and altered the infrastructure of the mesocorticolimbic pathway that would have normally developed in the absence of drug pretreatment. It is likely this pruning had already occurred in the adult animals that were administered ethanol in early adulthood, and therefore showed the trend for increased potassium-stimulated dopamine release relative to their water-treated counterparts, a trend that was decreased in adolescent ethanol-pretreated rats.

Recent work has shown that behavioral responsivity to repeated ethanol during adolescence relative to adulthood is inversely related to extracellular glutamate levels in the nucleus accumbens septi, with adults that showed greater behavioral sensitization showed a decrease in accumbal glutamate levels in response to a challenge

administration of ethanol (Carrara-Nascimento et al., 2011). This inverse relationship of behavioral responsivity inversely related to neurochemical responsivity are similar to the findings from the present set of experiments, with adolescent rats that were administered ethanol during adolescence exhibited greater ethanol intake in adulthood, but a trend for lower potassium-stimulated dopamine, while the opposite was observed in adult ethanol-pretreated rats showing lower levels of voluntary ethanol intake and a trend for greater potassium-stimulated dopamine release. Recently, increased activity of Lyn kinase in the ventral tegmental area has been shown to blunt ethanol-induced dopaminergic output to the nucleus accumbens septi (Gibb et al., 2011). Phasic, but not tonic release of dopamine was found to be regulated by this protein kinase activity (Gibb et al., 2011). The ontogenetic profile of Lyn kinase is not well understood to date, but it is interesting to speculate that locally the activity of the ventral tegmental area is altered during adolescence when ethanol is administered and likely an overactivation of these molecular targets induced the blunted potassium-induced dopamine release in the nucleus accumbens observed in the present set of experiments.

Pretreatment and challenge with binge ethanol induced similar dopaminergic responsivity in the nucleus accumbens, regardless if ethanol pretreatment occurred during adolescence relative to adulthood (Pascual et al., 2009). However, basal dopamine levels were elevated in binge ethanol-pretreated rats when pretreatment occurred during adolescence (Pascual et al., 2009). These findings are consistent with previous work from our laboratory using intraperitoneal injections of ethanol following ethanol pretreatment during adolescence (Badanich et al., 2007). The present work is not consistent with these previous findings (Badanich et al., 2007; Pascual et al., 2009) demonstrating a lack of significant alteration in basal dopamine levels in rats that were pretreated with ethanol relative to those pretreated with water. It is likely due to the

route of ethanol administration during pretreatment in the present experiment, where rats were administered ethanol via intragastric gavage. In the present work it is likely peak blood ethanol concentrations were lower and delayed during ethanol pretreatment relative to ethanol administered via intraperitoneal injection (Livy et al., 2003; Walker and Ehlers, 2009) and no changes in basal dopamine levels following ethanol pretreatment during adolescence were observed. Recent work has shown that dopamine activity is lower in adolescent relative to adult rats following presentation of unexpected, nonsocial stimuli (Robinson et al., 2011). Neuronal firing in the ventral tegmental area is decreased following chronic ethanol administration after withdrawal signs have ceased (Bailey et al., 1998) and dopamine levels are decreased during withdrawal from ethanol (Rossetti et al., 1992). Peak dopamine in response to ethanol was increased in adolescent relative to adult rats (Philpot et al., 2009). These data suggest that during the four-day ethanol treatment adolescent rats showed greater dopamine release in response to ethanol (Philpot et al., 2009) and the repeated withdrawal from ethanol during the intermittent off days during ethanol treatment likely induced greater withdrawal effects in adolescent relative to adult rats (Wills et al., 2009) and exacerbated the decrease in dopamine levels, in turn altering the normal functioning of the mesolimbic pathway during adolescent ethanol treatment. In previous work, binge ethanol pretreatment did not alter DOPAC levels in adolescent or adult rats following ethanol challenge (Pascual et al., 2009). The present data are consistent and expand previous work, demonstrating long-term neuronal adaptations in mesolimbic dopamine functionality specific to ethanol pretreatment, rather than in response to pharmacological responsivity to an ethanol challenge, as these effects were observed following potassium stimulation delivered locally into the ventral tegmental area and dopamine overflow assessed in the nucleus accumbens septi, when all animals were adults.

Summary

Together the data from the present set of experiments indicate that adolescent ethanol pretreatment using a binge pattern of exposure to a high dose of ethanol induces long-term changes in behavior, pharmacokinetics and neurochemistry that is different from similar adult exposure. Behaviorally, adolescent pretreatment with a high dose of ethanol during adolescence increased voluntary ethanol intake in adulthood relative to similarly treated adults. It is important to note these differences in voluntary ethanol intake were observed during acquisition of ethanol intake and during maintenance on an unsweetened ethanol solution. These data indicate that adolescent ethanol-pretreated animals consumed ethanol for its pharmacological properties as opposed to the appetitive nature of a sweetener that was faded completely from the solution. The second major finding from this series of experiments indicates long-lasting changes in ethanol metabolism in adolescent pretreated animals that were exposed to 2.0 g/kg during adolescence. This effect was dose-dependent in that decreased blood ethanol concentrations were observed at 60 min postinjection relative to age-matched animals pretreated with 0.5 g/kg during adolescence. The animals pretreated with 2.0 g/kg during adolescence also showed decreased blood ethanol concentrations at 60 min relative to similarly treated adults. These changes in ethanol metabolism likely mediate the enhanced ethanol intake observed in adolescent ethanol-pretreated rats. Finally, long-term changes in non-pharmacological functionality of the mesolimbic dopaminergic pathway were altered following binge pattern ethanol pretreatment, with animals that were pretreated with 2.0 g/kg ethanol during adolescence showing a trend for decreased potassium-stimulated dopamine overflow in the nucleus accumbens septi relative to controls and a trend for increased potassium-stimulated dopamine overflow in adult rats pretreated with 2.0 g/kg ethanol. Together these data indicate an intricate dissociation

between behavior and ethanol metabolism and mesolimbic functionality following binge-pattern ethanol pretreatment during adolescence to a high dose of ethanol. These long-lasting changes likely mediate the enhanced ethanol-seeking and relapse behavior observed in humans given higher doses of ethanol are needed to achieve similar blood ethanol concentrations and likely greater dopaminergic responsivity in individuals with a history of high alcohol use.

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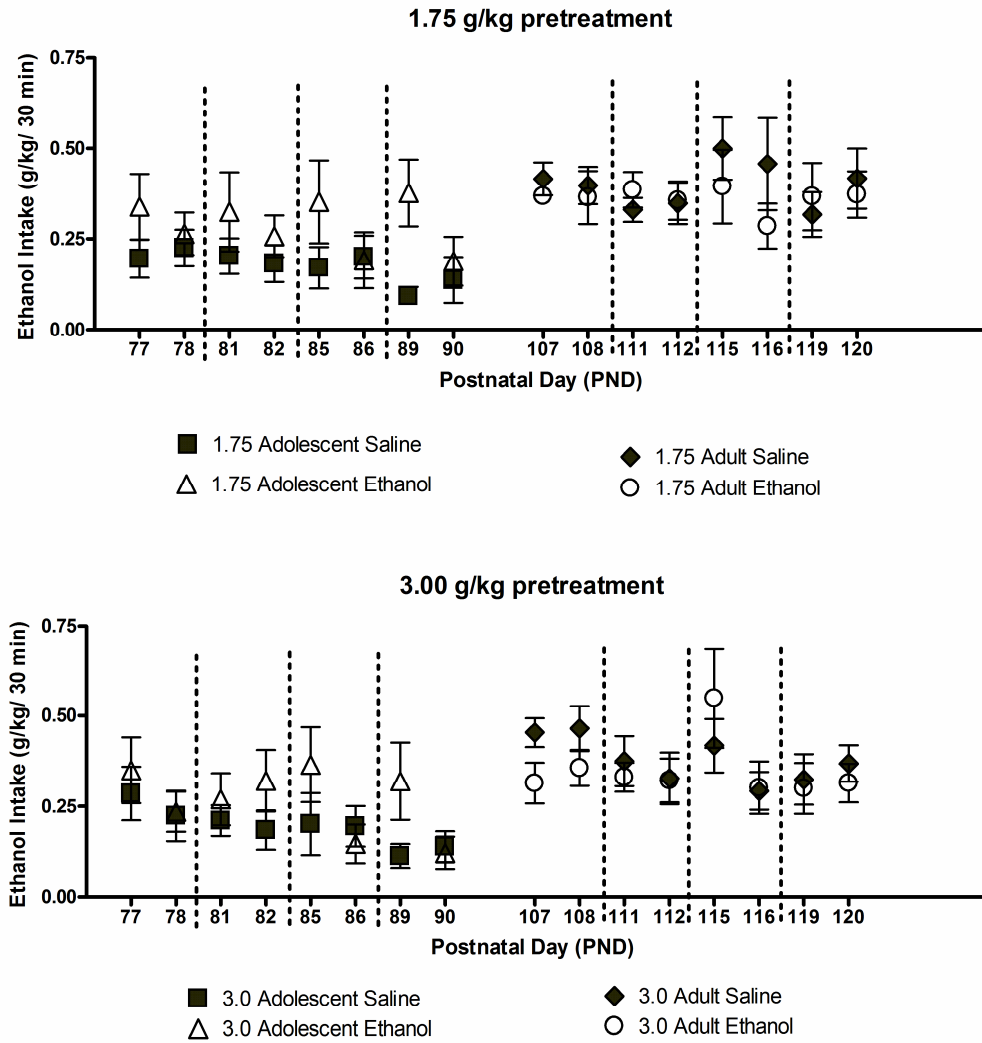
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Appendices

Appendix A: Supplemental Figure

Figure A1: Alcohol deprivation-induced ethanol consumption in male rats.



Data presented as mean ethanol intake (g/kg) +/- SEM for adolescent and adult-ethanol pretreated rats with 1.75 g/kg (Panel A) or 3.0 g/kg (Panel B) using a four-day repeated binge model. The dotted vertical bars indicate the ethanol-free period between ethanol access periods. Adolescents show greater post-deprivation relative increase in ethanol-intake following voluntary access to ethanol relative to their saline-pretreated counterparts (Michael, Maldonado-Devincci and Kirstein, in prep).