Characterization of Adolescent and Adult Ethanol Sensitization

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ABSTRACT

Rebekah Stevenson: Characterization of Adolescent and Adult Ethanol Sensitization

(Under the direction of Dr. Clyde Hodge)

Alcoholism is a serious health problem that affects the lives of millions of people worldwide. People that first experiment with alcohol as adolescents are at a greater risk to become alcoholics. The adolescent brain may be particularly vulnerable to drug-induced neuroadaptations. Behavioral sensitization is a method that uses repeated drug exposure to induce neurobiological changes that are thought to model the changes taking place during addiction. Sensitization to ethanol has not been studied in adolescents and is an important tool to aid in the understanding of ethanol-induced neuroadaptations that occur during development. The research described in this dissertation entailed the study of the dose response and time course of ethanol sensitization in adolescent and adult mice. The results indicate that adolescent mice are less sensitive to ethanol sensitization than adult mice. The neurobiological mechanisms mediating ethanol sensitization have not been fully characterized. One type of glutamate receptor, the metabotropic glutamate receptor subtype 5 (mGluR5), is involved in drug reinforcement, relapse, and reward processes, although it has not been studied in adolescent ethanol sensitization. Results of the research described in this dissertation showed that mGluR5 is not involved in adolescent ethanol sensitization, while it is critical in adult ethanol sensitization. This indicates that mGluR5 might underlie the differential response to ethanol sensitization in adolescent and adult mice. Finally, this research was designed to determine whether the differential response to ethanol sensitization makes the adolescent mice more susceptible to subsequent

ethanol intake. The results show that, following ethanol sensitization, the adolescent mice do not show increased ethanol intake, while the adult mice demonstrate a significant increase in ethanol intake and preference. Overall, this dissertation shows for the first time that adolescent mice are less sensitive to ethanol sensitization than adult mice. This difference in sensitization, however, does not appear to underlie the adolescent vulnerability to alcoholism that has been observed in humans.

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CHAPTER I: GENERAL INTRODUCTION

ADOLESCENCE

Adolescence (age 12-20 years in humans) is a developmental period characterized by numerous physical, behavioral, and cognitive changes. The neurobiological systems that underlie these behavioral and cognitive changes mature at different rates, which leaves the adolescent with the difficult task of balancing increases in emotional and hormonal drive with increases in decision-making abilities (Steinberg 2005). Behaviorally, adolescents spend an increased amount of time engaged in social interaction with peers, taking part in risky behaviors, and exploring novel situations (Primus and Kellogg 1989; Spear 2000b). Cognitively, adolescents develop abstract thinking skills, show improvements in executive functions and reasoning, and begin to develop adult-levels of decision making (Keating 2004). Interestingly, evidence shows that adolescents take part in dangerous activities despite knowing the risks involved, and this discrepancy is due to social and emotional influences (Martin et al. 2002; Slovic 1987). Adolescence appears to be a unique period of competing social, emotional, and intellectual influences.

THE ADOLESCENT BRAIN

During adolescence, the mesolimbic dopamine system and the prefrontal cortex undergo numerous changes (Crews et al. 2007; Spear 2000b). Dopamine receptors are overexpressed in both limbic and prefrontal cortical regions, and this increase is followed by massive pruning of dopamine receptors in the limbic system compared to the prefrontal cortex (Kalsbeek et al. 1988; Lewis 1997; Rakic et al. 1994). This leads to an

overall dominance of the mesocortical dopaminergic pathway compared to the mesolimbic pathway. Overall, the prefrontal cortex takes much longer than the limbic system to develop during adolescence (Bourgeois et al. 1994; Huttenlocher 1979). Glutamatergic synapses show a burst at puberty, followed by pruning after puberty (Huttenlocher 1984; Insel et al. 1990; Rakic et al. 1994). The result of synapse pruning is more focal activity in the prefrontal cortex, which can be viewed as more efficient information processing (Durston et al. 2006). It is likely that the developing mesocortical dopamine system modulates the synaptic pruning of the prefrontal cortex, such that rewarding stimuli are able to create stronger synaptic connections in the prefrontal cortex (Davey et al. 2008). This could underlie the known vulnerability for drug use during adolescence to lead to drug abuse problems in adulthood (Grant and Dawson 1998).

ADOLESCENCE AND DRUG ABUSE

The behavioral and neurobiological changes that take place during this developmental stage cause the adolescent to be particularly vulnerable to experimenting with drugs of abuse and to subsequent drug-induced neuroadaptations (Crews et al. 2007; Spear 2002). In fact, seventy-five percent of twelfth graders have experimented with alcohol in their lifetime, and almost thirty percent of these adolescent drinkers have consumed five or more drinks in the last two weeks (O'Malley et al. 1998). Symptoms of alcohol dependence can emerge at an accelerated rate in adolescents compared to adults (Clark et al. 1998; Pollock and Martin 1999). It is known that people who start drinking during adolescence are four times more likely to become alcohol dependent as adults (Grant 1998). Altogether, it seems that adolescence is a period of unique susceptibility to drug and alcohol intake and to future drug and alcohol problems.

Similar to the clinical findings in human adolescents, studies in rodents have shown that adolescents and adults are differentially sensitive to the effects of ethanol. Adolescent rodents (age 28 – 42 days) are more sensitive to the effects of acute and chronic ethanol on measures of locomotor stimulation, anxiety, ataxia, spatial memory, binge-induced brain damage, conditioned place preference, and social interaction as compared to adult rats (Crews et al. 2000; Hefner and Holmes 2007; Markwiese et al. 1998; Philpot et al. 2003; Rajendran and Spear 2004; Varlinskaya and Spear 2002; Yttri et al. 2004). By contrast, other studies have shown that adolescent rodents are less sensitive than adult rats to the sedative and motor-impairing effects of ethanol, to ethanol withdrawal-induced anxiety, and analgesia (Doremus et al. 2003; Hefner and Holmes 2007; Silveri and Spear 1998; Varlinskaya and Spear 2002; White et al. 2002). These differences in the sensitivity of adolescents to ethanol are important because it has been shown that a decreased response to acute alcohol challenge during adolescence is a potent predictor of future alcoholism (Schuckit 1993; 1994). At the present time, it remains unknown precisely what mechanisms underlie differences in sensitivity between adolescent and adult rodents. Identifying the neurobehavioral adaptations that underlie differential sensitivity during development is important to understanding how alcohol exposure early in life predisposes people to subsequent development of addiction.

ALCOHOLISM

Alcoholism is a disease that affects the lives of most people. Over 7% of the United States population is alcoholic which leads to annual costs of more than $180 billion (Harwood et al. 1998). Clinically, alcoholism is defined in the DSM-IV as an impaired ability to control the drinking of alcohol. This impairment manifests itself as craving, a lack of ability to stop drinking once drinking has begun, physical dependence,

tolerance, preoccupation with alcohol consumption, lack of interest in other life activities besides drinking alcohol, and continued alcohol use despite physical and psychological problems. Withdrawal symptoms include autonomic hyperactivity, tremor, nausea or vomiting, anxiety, and seizure, while tolerance is defined as a need for more alcohol in order to obtain the same level of intoxication.

Addiction to ethanol and other drugs of abuse arises from an interaction between various genetic, environmental, and neurobiological factors (Goldstein and Volkow 2002). Although the mechanisms underlying addiction have not been fully characterized, it is well known that dopamine (DA) is a key neurotransmitter which modulates addiction through the mesocorticolimbic pathway (Volkow et al. 2002). This pathway is critical in integrating inputs from sensory systems, emotional state, memory, and attention systems, and keeping these systems in a balance. Ethanol exerts its effects on numerous neurotransmitter receptors (ie $GABA_A$ and NMDA) throughout this pathway. It is postulated that changes to the signaling along this path by repeated ethanol exposure can lead to ethanol dependence (Kiianmaa et al. 2003). This pathway is characterized by dopaminergic neuronal projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), and this has been shown to be important for the reinforcing and rewarding properties of drugs of abuse (Everitt and Wolf 2002). Ethanol interacts with this pathway by causing an increase in dopamine in the NAc due to increased firing of the VTA DA neurons. Animal studies using self-administration and knockout mice have shown that this interaction seems critical to the subjective rewarding effects of ethanol (Weiss and Porrino 2002).

ALCOHOLISM AND GLUTAMATE

Studies have begun to show the importance of the glutamate system for the behavioral effects of ethanol and alcoholism (Krystal et al. 2003). Glutamate is a

prominent neurotransmitter in the cerebral cortex and in limbic areas of the brain, suggesting a key role of glutamate transmission in the process of addiction. It is known that glutamatergic neurotransmission modulates dopaminergic neurotransmission in the mesocorticolimbic pathway (Tsai and Coyle 1998).

Much of this research has focused on the NMDA glutamate receptor, as it is known that this receptor has a high affinity site for ethanol (Grant and Lovinger 1995). NMDA receptors are unique in that they are ligand-gated ion channels that also have voltage dependency. This means that in order for the channel to allow ions through, glutamate must bind and the membrane must be depolarized. Depolarization removes the Mg^{2+} block of the receptor, allowing both Na^{+} and Ca^{2+} to flow into the cell. The Ca^{2+} that enters the cell can modulate gene transcription and protein expression, which are critical to the function of NMDA receptors (Morgan and Curran 1988). NMDA receptors consist of 2 variable subunits which determine where the receptor is expressed and the precise function of the receptor (Krystal et al. 2003).

The exact interaction of ethanol with NMDA receptors is not known, although it appears that when given acutely, ethanol binds to a hydrophobic pocket on the receptor (Peoples and Weight 1995). This binding inhibits the influx of Ca^{2+} into the cell, which is critical to ethanol's effects (Wirkner et al. 1999). Chronic ethanol exposure leads to an increase in NMDA receptor number and function in brain regions known to be important in addiction, namely the cerebral cortex, striatum, thalamus, and hippocampus (Tsai and Coyle 1998). This upregulation in receptor number is important to the withdrawal syndrome, a key feature of alcoholism. An increase in glutamate release is noticed during withdrawal, and this increase is directly related to the increase in NMDA receptor number and function (Rossetti and Carboni 1995; Tsai and Coyle 1998). Treatment with MK-801, an NMDA receptor antagonist, reduces many of the physical signs of

withdrawal, along with reducing the increase in glutamate release (Grant et al. 1992; Tsai and Coyle 1998).

Pathways that lead to feelings of reward from drugs of abuse are implicated in the process of addiction. NMDA receptors are known to be expressed throughout the mesocorticolimbic pathway, with high areas of expression in the hippocampus, frontal cortex, nucleus accumbens, striatum, and amygdala (Cotman and Monaghan 1986; Hodge and Cox 1998). NMDA receptors can affect the release of dopamine along this pathway when ethanol is administered. Ethanol inhibits NMDA receptors, which in turn inhibit GABAergic interneurons, which then leads to a disinhibition of the forebrain glutamatergic neurons, leading to an augmentation of dopamine release (Nestler et al. 1993).

METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5 (mGluR5)

Another glutamate receptor that has recently been shown to be critical for ethanol's effects is the metabotropic glutamate receptor subtype 5 (mGluR5). Metabotropic glutamate receptors (mGluRs) are a class of G-protein coupled receptors that are divided into three groups based on sequence homology, agonist pharmacology, and the signal transduction cascade initiated at the receptor. Group I mGluRs (mGluR1 and mGluR5) are positively coupled to phospholipase C, which activates signaling through diacylglycerol (See Figure 1). Group II (mGluR2/3) and Group III (mGluRs 4, 6, 7, and 8) mGluRs inhibit adenylyl cyclase activity, and thus inhibit signaling through cyclic- AMP (Conn and Pin 1997).

MGluR5 are expressed abundantly in the nucleus accumbens and ventral tegmental area, where they interact with dopamine to generate locomotor activity (Swanson and Kalivas 2000; Vezina and Kim 1999). MGluR5 knockout mice show no cocaine-induced enhancement of locomotor activity and do not self-administer cocaine,

indicating that mGluR5's interact with dopamine signaling in the nucleus accumbens to effect drug reward (Chiamulera et al. 2001). It has also been shown that mGluR5 and D1R interact in the striatum to modify signal transduction pathways (Voulalas et al. 2005). Thus, mGluR5's have the potential to be involved in many of the rewarding properties of drugs and in addiction.

Ethanol is known to modulate mGluR activity based on *in vitro* and *in vivo* studies. In cultured Purkinje neurons, ethanol inhibits the burst activity mediated by mGluRs (Netzeband and Gruol 1995). Ethanol also has been shown to inhibit mGluR5 function in *Xenopus* oocytes (Minami et al. 1998). Behavioral studies have shown that antagonism of mGluR5 reduces ethanol self-administration and blocks the discriminative stimulus properties of ethanol (Backstrom et al. 2004; Besheer and Hodge 2004; Hodge et al. 2006; Olive et al. 2005; Schroeder et al. 2005). Overall, glutamatergic signaling through mGluR5's appears to be critical for ethanol's rewarding properties.

Figure 1. Glutamate Signaling Pathways.

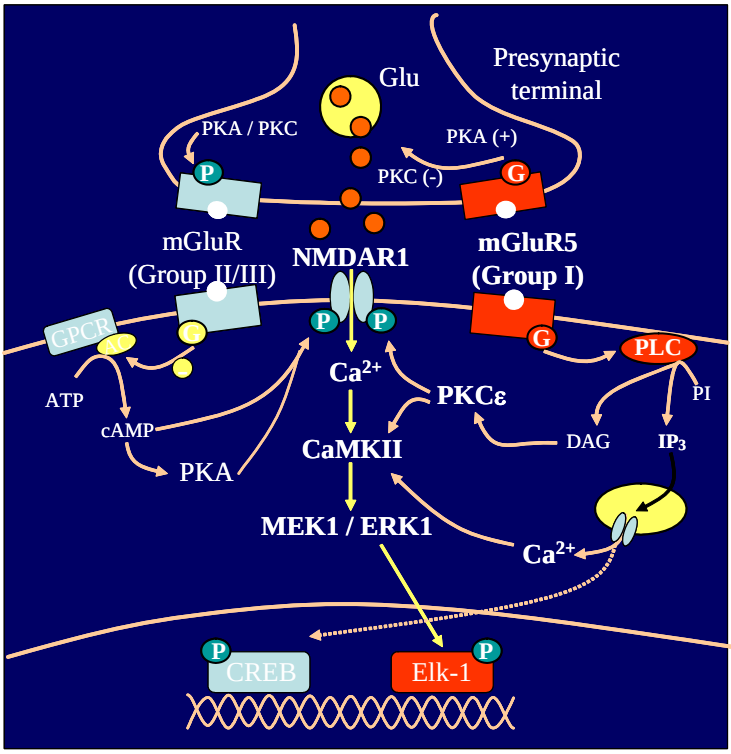

Figure 1. Intracellular signaling following the activation of glutamate receptors. Activation of the mGluR5's can activate numerous downstream targets, including Ca^{2+}, CaMKII, and $ERK_{1/2}$. These signaling molecules can effect long-term changes in gene expression and neuronal function.

LOCOMOTOR SENSITIZATION

Behavioral sensitization is a model that can be used to study the neuroadaptations that occur following repeated drug exposure. Sensitization is typically shown as a progressive increase in locomotor activity following repeated administration of a single dose of a drug of abuse. For example, repeated administration of psychostimulants leads to an increase in locomotor activity, which is thought to be analogous to increases in anxiety and paranoia seen in human stimulant abusers (Pierce and Kalivas 1997). One theory of behavioral sensitization proposes that drug craving sensitizes with repeated use, which makes the study of sensitization extremely important in understanding the mechanism of addiction (Robinson and Berridge 1993). Research has shown that sensitization is mediated by an interconnected network of mesocorticolimbic brain regions (i.e., VTA, nucleus accumbens, prefrontal cortex, amygdala, and thalamus) and neurotransmitter systems (i.e., dopamine, glutamate, and GABA) that all undergo alterations during the adolescent developmental period (Kalivas 1995; Spear 2000a; Vezina and Kim 1999). Thus, studying sensitization during adolescence may elucidate specific age-dependent mechanisms by which drugs alter brain and behavioral functions. Also, since these neural systems play key roles in alcohol and drug reinforcement during adulthood (Koob 2000; McBride et al. 1999), age-dependent differences in sensitization may influence the increased probability of developing dependence in adulthood.

Figure 2. Neurocircuitry of Locomotor Sensitization.

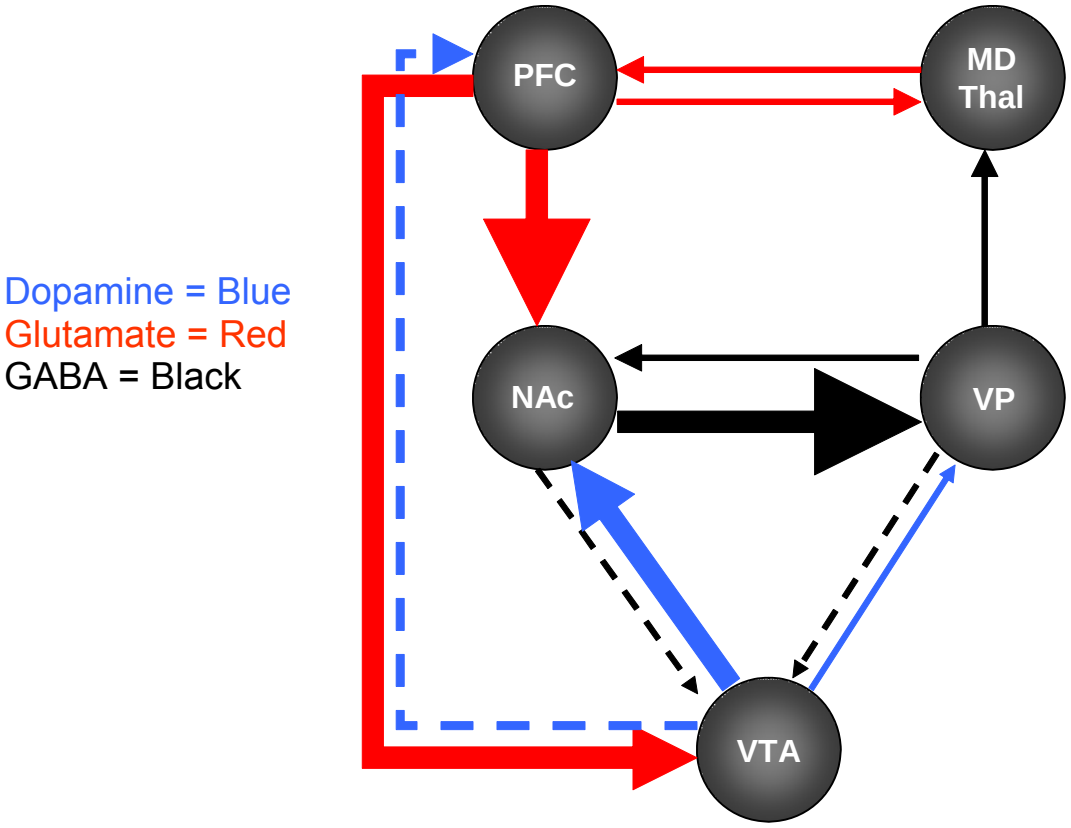

Figure 2. Neurocircuitry changes induced by sensitization. Proposed changes that occur due to locomotor sensitization in adult mice, adapted from Pierce and Kalivas 1993. Locomotor sensitization occurs following an increase in dopaminergic neurotransmission from the VTA to the NAc (bold blue arrow), along with increases in glutamatergic neurotransmission from the PFC to the NAc (bold red arrow). Dashed lines indicate a decrease in neurotransmission. VTA=ventral tegmental area; NAc=Nucleus Accumbens; PFC=Prefrontal Cortex; VP=Ventral Pallidum; MD Thal=Medial dorsal Thalamus

ETHANOL LOCOMOTOR SENSITIZATION

Like other drugs of abuse, ethanol is known to be a locomotor stimulant at low doses (up to 2.5 g/kg). Since the rewarding properties of drugs are thought to be positively correlated with locomotor activation (Wise and Bozarth 1987), studying ethanol sensitization has the potential to identify adaptations that may influence reward (Phillips et al. 1997). Ethanol sensitization has been primarily studied in adult mice. Researchers use various protocols to induce ethanol sensitization, with all involving repeated administration of ethanol over a number of days. The dose of ethanol used to induce locomotor sensitization commonly ranges from 1.5 g/kg to 3.5 g/kg, given for anywhere between 4 and 21 days (Broadbent and Harless 1999; Broadbent and Weitemier 1999; Fish et al. 2002; Itzhak and Martin 2000; Lessov et al. 2001; Meyer and Phillips 2003; Miquel et al. 2003; Quadros et al. 2003). The time course of the development of ethanol sensitization has been studied in adult DBA/2J mice, with the mice developing sensitization after three ethanol exposures (Lessov et al. 2001). Ethanol sensitization has been shown to last up to 29 days after the final ethanol treatment, indicating that lasting neurobiological changes occur during sensitization (Lessov and Phillips 1998).

Studies have shown that baclofen, the GABA(B) agonist, and 7-nitroindazole, an inhibitor of neuronal nitric oxide synthase, and a corticotropin releasing factor-1 receptor antagonist all prevent the expression of ethanol sensitization (Broadbent and Harless 1999; Fee et al. 2007; Itzhak and Martin 2000). Furthermore, the glutamatergic NMDA receptor antagonist MK-801, the mGluR5 antagonist MTEP, and the AMPA antagonist GYKI 52466 all inhibit ethanol sensitization (Broadbent et al. 2003; Broadbent and Weitemier 1999; Kotlinska et al. 2006) indicating a definitive role for glutamatergic signaling in the development of ethanol sensitization. Thus, studying ethanol sensitization in adolescent and adult mice might lead to insights in the glutamatergic mechanisms that mediate addiction-related behaviors.

DBA/2J MICE

The DBA/2J inbred strain of mice show strong locomotor activation to ethanol and display conditioned place preference to ethanol (Cunningham et al. 1992; Phillips et al. 1994). Although DBA/2J mice will not orally self-administer ethanol due to a taste aversion, they do self-administer ethanol intravenously and intragastrically, indicating that ethanol is rewarding (Grahame and Cunningham 1997; Risinger et al. 1998); Cunningham et al., 2005). Furthermore, it has been shown by our lab that DBA/2J mice will self-administer ethanol following ethanol sensitization, indicating that pre-exposure to ethanol will induce ethanol drinking in these mice (Camarini and Hodge 2004). Finally, ethanol induces stronger excitation from ventral tegmental dopamine neurons in DBA/2J mice than in C57BL/6J mice, which might underlie the behavioral effects of ethanol in DBA/2J mice (Brodie and Appel 2000). Overall, DBA/2J mice offer an excellent model to study the neurobiological effects of ethanol.

EXPERIMENTAL DESIGN / HYPOTHESES

Adolescence in humans and rodents appears to be a unique period of sensitivity to drugs of abuse, including ethanol. Studying ethanol sensitization offers a method to assess the neuroadaptations that occur due to ethanol exposure during adolescence. It is hypothesized that adolescent mice are less sensitive to the dose response and time course of ethanol sensitization. As glutamate neurotransmission is involved in adolescent development, alcoholism and ethanol sensitization, the study of the glutamate mGlu5 receptor in adolescent and adult ethanol sensitization may provide insight into the mechanisms mediating differential adolescent sensitivity to ethanol. It is hypothesized that mGluR5 is involved in adult, but not adolescent, ethanol sensitization. Ethanol self-administration following ethanol sensitization can be utilized to determine whether the neuroadaptations that occur during ethanol sensitization in adolescence are

relevant to the adolescent vulnerability to future alcoholism. Therefore, it is hypothesized that adolescent mice self-administer more ethanol following ethanol sensitization than adult mice. Overall, these studies have the potential to extend the current knowledge of neurobiological differences between adolescent and adult mice and provide new insight into the mechanisms by which adolescent alcohol use increases the probability of alcoholism in adulthood (Figure 3).

Figure 3: Dissertation Overview

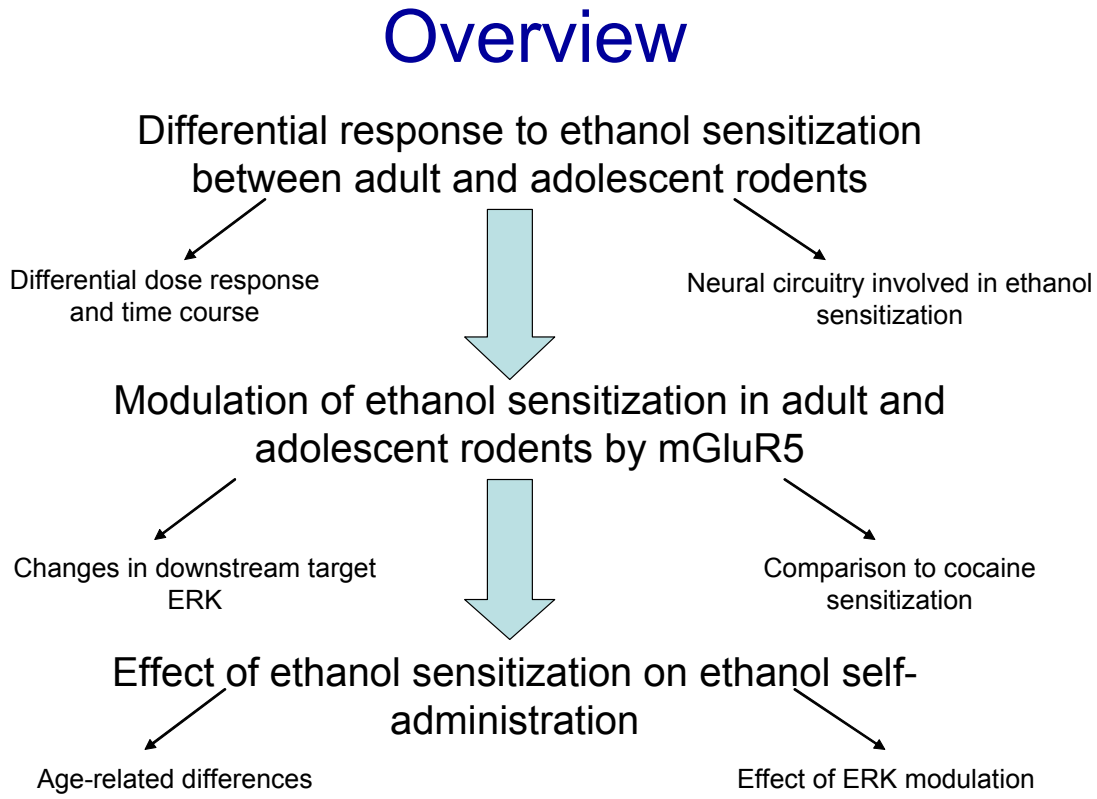

Figure 3: Dissertation Overview. The experiments in this dissertation will first determine the dose response and time course to ethanol sensitization in adolescent and adult mice. The role of the mGlu5 receptor in ethanol sensitization will be determined. Finally, the hypothesis that ethanol sensitization during adolescence leads to an increase in subsequent ethanol self-administration will be tested.

CHAPTER II: COMPARISON OF ETHANOL LOCOMOTOR SENSITIZATION IN ADOLESCENT AND ADULT DBA/2J MICE

INTRODUCTION

Adolescence is a critical period of development during which children and young animals undergo adaptive changes in behavior and neurobiological systems that bring about the transition into adulthood. Behavioral changes include spending an increased amount of time engaged in social interaction with peers, taking part in risky behaviors, and exploring novel situations, while neurobiological changes include remodeling in the cortex and mesolimbic regions such that glutamatergic and GABAergic neurotransmission is reduced and dopaminergic neurotransmission is increased (Spear 2000a).

The behavioral and neurobiological adaptations that take place during this developmental stage cause the adolescent to be particularly vulnerable to experimenting with drugs of abuse and to subsequent drug-induced neuroadaptations (Crews et al. 2007; Spear 2002). The study of ethanol exposure during the adolescent period is important because it is known that people who start drinking during adolescence are four times more likely to become alcohol dependent as adults (Grant 1998). However, the mechanism(s) underlying this finding remain to be fully characterized.

Studies in rodents have shown that adolescents and adults are differentially sensitive to the effects of acute and chronic ethanol. Adolescent rodents are more sensitive to the effects of acute and chronic ethanol on measures of locomotor stimulation, anxiety, ataxia, spatial memory, conditioned place preference, and social interaction as compared to adult rats (Hefner and Holmes 2007; Markwiese et al. 1998;

Philpot et al. 2003; Rajendran and Spear 2004; Varlinskaya and Spear 2002; Yttri et al. 2004). By contrast, other studies have shown that adolescent rodents are less sensitive than adult rats to the sedative and motor impairing effects of ethanol, to ethanol withdrawal induced anxiety, and analgesia (Doremus et al. 2003; Hefner and Holmes 2007; Silveri and Spear 1998; Varlinskaya and Spear 2002; White et al. 2002). These differences in the sensitivity of adolescents to ethanol are important because it has been shown that a decreased response to acute alcohol challenge during adolescence is a potent predictor of future alcoholism (Schuckit 1993; 1994).

One model of neurobehavioral adaptations that occur following chronic ethanol exposure is locomotor sensitization. Sensitization is typically defined as a progressive increase in locomotor activity following repeated administration of a drug of abuse (Kalivas and Stewart 1991). The process of sensitization is thought to produce enduring adaptive changes in brain and behavioral function that may underlie components of addiction (Kalivas et al. 1998; Robinson and Berridge 2000). Research has shown that sensitization is mediated by an interconnected network of mesocorticolimbic brain regions (i.e., VTA, nucleus accumbens, prefrontal cortex, amygdala, and thalamus) and neurotransmitter systems (i.e., dopamine, glutamate, and GABA) (Kalivas 1995; Vezina and Kim 1999). These brain regions and neurotransmitter systems all undergo alterations during the adolescent developmental period (Kalivas 1995; Spear 2000a; Vezina and Kim 1999). Thus, sensitization models are useful tools to determine if adolescent vulnerability to addiction involves differential sensitivity to neurobehavioral changes that occur with repeated drug use.

Various protocols have been used to induce ethanol locomotor sensitization, all of which involve repeated administration of ethanol over a number of days. The dose of ethanol used to induce locomotor sensitization commonly ranges from 1.5 g/kg to 2.5 g/kg, administered for 4 to 21 days (Broadbent and Harless 1999; Broadbent and

Weitemier 1999; Fish et al. 2002; Itzhak and Martin 2000; Lessov et al. 2001; Meyer and Phillips 2003; Miquel et al. 2003; Quadros et al. 2003). In adult DBA/2J mice, sensitization develops after three ethanol exposures and persists up to 68 days after the final ethanol treatment (Fish et al. 2002; Lessov et al. 2001). These studies indicate that long-lasting neurobiological changes occur during sensitization. Importantly, ethanol locomotor sensitization has not been studied in adolescent rodents.

The present study was designed to examine potential developmental differences in sensitivity to the neurobehavioral adaptations that occur during the induction of ethanol sensitization. Given the differential behavioral responses to ethanol in adolescents and adults, this study sought to fully characterize ethanol dose response and time course for sensitization in both adolescent and adult mice.

MATERIALS AND METHODS

Animals. Male 3-week old (adolescent) and 8-week old (adult) DBA/2J mice (Jackson Laboratories, Bar Harbor, ME) were housed in groups (4 animals per cage) in standard Plexiglas cages with food (Purina Rodent Chow) and water available *ad libitum*. The colony was maintained at 27^{o}C on a 12-hour light/dark cycle, with the lights on at 10pm. The behavioral experiments were conducted during the dark portion of the cycle. Mice were handled and weighed daily for 1-week prior to, and for the duration of, the experiment. Animals were under continuous care and monitoring by the Division of Laboratory Animal Medicine at UNC-Chapel Hill, and all procedures were carried out in accordance with the *NIH Guide to Care and Use of Laboratory Animals* (National Research Council, 1996) and Institutional guidelines.

Behavioral Apparatus. The locomotor activity (horizontal distance traveled, cm) of adolescent and adult mice was measured in eight covered Plexiglas chambers (30 cm^2, Med Associates, Georgia, VT). Two sets of 16 pulse-modulated infrared

photobeams were located on opposite walls to record ambulatory movements in the X-Y (horizontal) plane. All software settings were the same for adult and adolescent mice. The activity chambers were computer-interfaced (Med Associates) for data sampling at 100-millisecond resolution.

Behavioral Procedures. Mice were adapted to the colony and to handling for 1-week (adolescents=P28; adults=P63). On locomotor testing days, mice were taken in the home cage to the testing room at least 30-minutes prior to the session to habituate to the testing room. The first two days of the each experiment were habituation days (H1 and H2). On these days, all mice received an intraperitoneal (IP) injection of saline and were immediately placed in the locomotor chamber for the 10 minute session.

Experiment 1a: Acute Locomotor Activity. Adolescent and adult mice received an IP injection of 0, 1.0, 1.5, 2.0, or 2.5 g/kg ethanol (n=8 per age group per ethanol dose) and were immediately placed in the locomotor chamber for the 10 minute session.

Experiment 1b: Sensitization Dose Response. Following the acute locomotor session on day 1 (D1), mice received the assigned ethanol dose (0, 1.5, 2.0, 2.5, or 3.0 g/kg IP) once daily for nine days (D2-D10) in the home cage. On day 11 (D11), the mice were tested for locomotor sensitization. Mice were injected with 0, 1.0, 1.5, 2.0, or 2.5 g/kg ethanol (IP) and placed in the locomotor chamber for 10 minutes (Lessov and Phillips 1998).

Experiment 2a: Sensitization Time Course. On day 1 (D1), the mice received an IP injection of 0, 2.0, or 2.5 g/kg ethanol (n=8 per age group per ethanol dose per length of treatment) and were immediately placed in the locomotor chamber for 10 minutes. For the following days (D2-D6 or D2-D10) mice received the assigned ethanol dose (0, 2.5, or 3.0 g/kg IP) once daily and were returned to the home cage. On day 7 or 11 (D7 or D11), the mice were tested for locomotor sensitization. Mice were injected with 2.0 or 2.5 g/kg ethanol (IP) and placed in the locomotor chamber for 10 minutes.

Experiment 2b. Mice received ethanol (2.0 g/kg) on D1, followed by daily (D2-14) treatment with ethanol (2.5 g/kg; n=8 per age group). On day 15, mice were tested for locomotor sensitization to ethanol (2.0 g/kg).

Experiment 3: Blood Ethanol Determination. Tail blood was collected from adolescent and adult mice at 10, 60, and 180 minutes after an initial ethanol (2.0 g/kg) injection (D1; n=6-8 per age group). Mice were then treated with ethanol (2.5 g/kg) for the following nine days (D2-D10). Tail blood was collected again on day 11 (D11) of ethanol (2.0 g/kg; n=6-8 per age group) administration at 10, 60, and 180 minutes post-injection. Individual blood samples were centrifuged and 5 µL of plasma from each sample was analyzed to determine blood ethanol concentration using an AM1 Alcohol Analyzer (Analox Instruments, Lunenburg, MA).

Drugs. Ethanol (95% w/v) was diluted in saline (0.9%) to a concentration of 20% (v/v) and injected at different volumes to achieve the appropriate dosage (i.e., 2.0 and 2.5 g/kg). Control animals received 0.9% saline.

Behavioral Measures and Data Analysis. Horizontal distance traveled (in centimeters) during the 10-minute session was calculated from the number of photobeam breaks and presented as mean ± SEM. The distance traveled on habituation days 1 and 2 was compared between adolescent and adult mice using an unpaired t-test. Statistical significance was defined as $p \leq 0.05$ in all experiments.

Experiment 1a. Acute Locomotor Activity. The total distance traveled (cm) after an acute injection of saline or ethanol was examined using 2-way ANOVA with age (adolescent and adult) and ethanol dose as factors. Post-hoc Tukey tests were used to determine between group differences.

Experiment 1b. Sensitization: Dose Response. Distance traveled (cm) was analyzed for the adolescents and adults using three-way repeated measure (RM) ANOVA, with age (adolescent and adult), day (D1 and D11), and ethanol dose as

factors. Significant interactions were followed with analysis by lower order (e.g., two-way) ANOVA where appropriate. Sensitization was defined as activity on day 11 being significantly greater than activity on day 1 within an ethanol dose, as determined by post-hoc Tukey tests. This within group definition of sensitization was applied because it was observed that groups of adolescent and adult mice treated repeatedly with saline and given acute ethanol (1.0, 1.5, 2.0, or 2.5 g/kg) on day 11 displayed an equivalent locomotor response to the mice treated with acute ethanol on day 1 (data not shown). The data were presented as mean (+/- SEM).

In order to determine whether the magnitude of sensitization to ethanol 2.5 g/kg differed in the adolescents and adults, the locomotor activity from day 11 was expressed as a percent increase from day 1 activity. An unpaired t-test was used to compare the magnitude of sensitization between the age groups.

In order to determine if degree of sensitization was influenced by acute response to ethanol, a linear regression analysis was conducted comparing locomotor response to acute ethanol (D1) versus the sensitization test day (D11) for two doses of ethanol (2.0 and 2.5 g/kg) within each age group.

Experiment 2a. Sensitization: Time Course. Groups of mice were evaluated for distance traveled (cm) following treatment with ethanol (2.0 or 2.5 g/kg) using four-way RM ANOVA, with age (adolescent and adult), ethanol dose, treatment duration (7 or 11 days) and test day (acute and sensitization) as factors. Significant interactions were followed with analysis by lower order (e.g., two-way) ANOVA where appropriate. Sensitization was defined as activity on day 7 or day 11 (D7 or D11) being significantly greater than activity on day 1 (D1).

Experiment 2b. The mice treated with ethanol 2.0 g/kg for 15 days were analyzed using RM two-way ANOVA, with age (adolescent and adult) and treatment day (D1 or D15) as factors. Sensitization was defined as activity on day 15 being significantly

greater than activity on day 1 within an age group, as determined by post-hoc Tukey tests. The data were presented as mean (+/- SEM).

Experiment 3. Blood Ethanol Clearance. The BEC data were analyzed using 3-way RM ANOVA, with age (adolescent and adult), day (D1 and D11), and time (10, 60, and 180 minutes) post-ethanol injection as factors. Significant interactions were followed with analysis by lower order (e.g., two-way) ANOVA in order to determine whether the BEC following acute ethanol and following chronic ethanol treatment was responsible for age-dependent differences in sensitization. Post-hoc Tukey tests were used to extract group differences.

RESULTS

Basal Activity and Response to Acute Ethanol. Since adolescent mice are differentially sensitive to acute effects of ethanol as compared to adults (Hefner and Holmes 2007), we first examined basal locomotor activity and response to acute ethanol (1.0 – 2.5 g/kg). On the habituation days, no differences in locomotor activity were observed between the adolescent and adult groups ($p=0.29$; Adolescents: 3011 +/- 234 cm; Adults: 2670 +/- 210 cm). Adolescent and adult mice showed equal saline-induced locomotor activity but different locomotor response to acute ethanol treatment (Figure 4). Two-way ANOVA showed that adolescent mice were more sensitive to the acute locomotor activating effects of ethanol as compared to adults. There was a significant main effect of age [$F(1, 61)=11.01$, $p=0.002$], a significant main effect of ethanol dose [$F(4, 61)=10.05$, $p<0.001$], and a significant interaction [$F(4, 61)=3.02$, $p=0.024$]. In the adolescents, ethanol doses of 1.5, 2.0, and 2.5 g/kg significantly increased locomotor activity (Figure 4). Overall, these results indicate that adolescent DBA/2J mice are more sensitive than adult mice to the acute locomotor activating effects of ethanol in a dose-dependent manner.

Figure 4. Acute Locomotor Response to Ethanol.

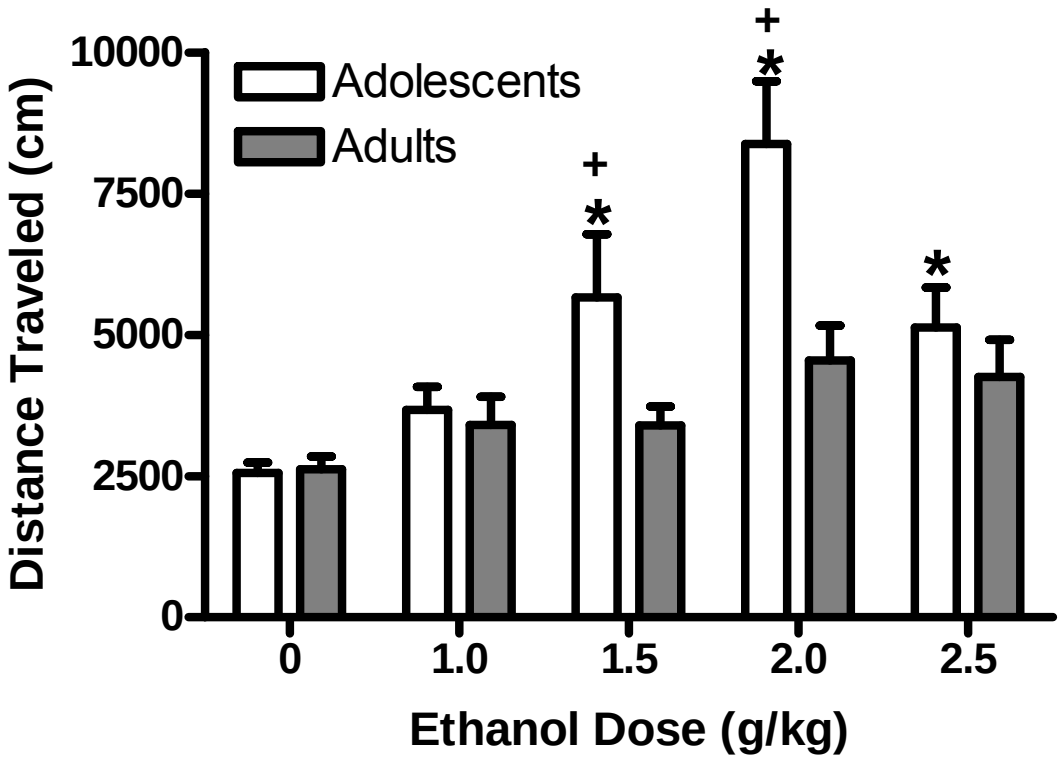

Figure 4. Acute Locomotor Response to Ethanol. DBA/2J adolescent (open bars) and adult (filled bars) locomotor response (distance traveled, cm, mean +/- SEM) to administration of ethanol (0 - 2.5 g/kg) during the 10-minute session. * indicates significant increase in distance traveled compared to ethanol 0 g/kg, $p<0.05$. + indicates significant increase in distance traveled compared to adult mice, $p<0.05$.

Sensitization: Dose Response. Following acute ethanol treatment, adolescent and adult mice were tested for locomotor sensitization (ethanol 0 – 2.5 g/kg). Locomotor sensitization was defined as a significant increase in locomotor activity on day 11 compared to day 1 within each dose, as determined by post-hoc Tukey tests. Three-way ANOVA of age X ethanol dose X day revealed significant main effects of the between subject variables age [$F(1, 56)=9.68$; $p=0.003$] and ethanol dose [$F(4, 56)=48.81$; $p<0.001$]. A significant main effect was also noted for the within subject factor day [$F(1, 56)=148.43$; $p<0.001$], along with a significant day X age interaction [$F(1, 56)=4.31$; $p<0.05$], a significant day X dose interaction [$F(4, 56)=38.32$; $p<0.001$], and a significant day X age X dose interaction [$F(4, 56)=5.88$; $p=0.001$]. Due to the three-way interaction, locomotor activity was analyzed separately for adolescent and adult mice in order to examine age-dependent sensitization. Overall, adolescent mice appeared to be less sensitive to ethanol sensitization as shown by lack of response to doses of ethanol that induced sensitization in adult mice (1.5 and 2.0 g/kg; Figure 4). Within the adolescents, there was a significant main effect of dose [$F(4, 29)=25.00$, $p<0.001$], a significant main effect of treatment day [$F(1, 29)=38.59$, $p<0.001$], and a significant interaction [$F(4, 29)=19.91$, $p<0.001$]. Sensitization was only observed at the 2.5 g/kg ethanol dose (Figure 5A; $p<0.001$). In the adults, there was also a significant main effect of dose [$F(4, 28)=21.98$, $p<0.001$], a significant main effect of treatment day [$F(1, 28)=129.89$, $p<0.001$], and a significant interaction [$F(4, 28)=17.88$, $p<0.001$]. The adults showed sensitization at ethanol doses of 1.5, 2.0, and 2.5 g/kg (Figure 5B; $ps<0.001$). These results indicate that the adolescent mice are less sensitive than the adult mice to ethanol sensitization, as they require a higher dose of ethanol (2.5 g/kg) to exhibit locomotor sensitization.

Figure 5. Ethanol Sensitization: Dose Response.

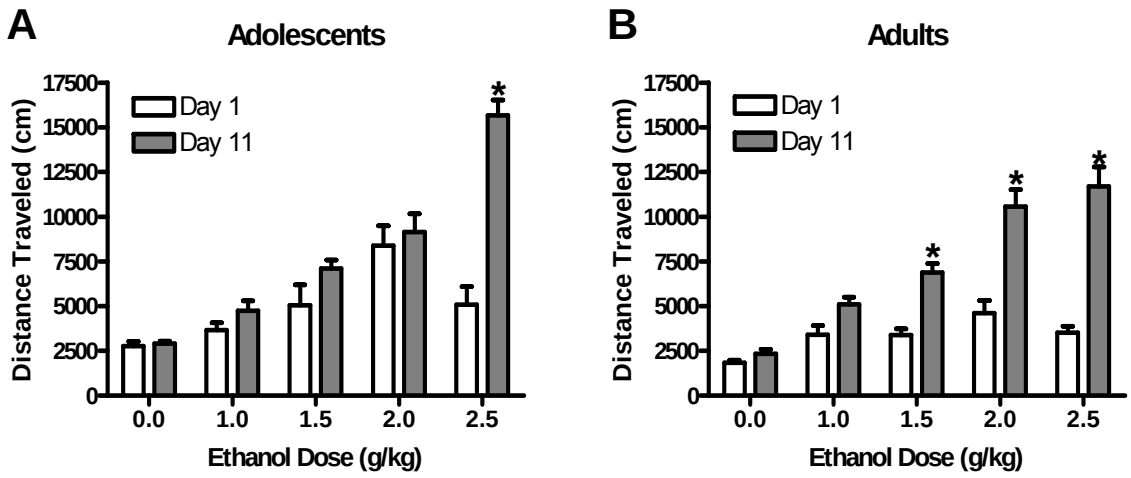

Figure 5. Ethanol Sensitization: Dose Response. **A.** Adolescent mice locomotor response (distance traveled, cm, mean +/- SEM) during 10 minute test sessions on day 1 and day 11 following administration of ethanol (0 – 2.5 g/kg). * indicates significant increase in distance traveled on day 11 compared to day 1, $p<0.05$. **B.** Adult mice locomotor response (distance traveled, cm, mean +/- SEM) during 10 minute test sessions on day 1 and day 11 following administration of ethanol (0 – 2.5 g/kg). * indicates significant increase in distance traveled on day 11 compared to day 1, $p<0.05$.

In order to determine if the magnitude of sensitization to ethanol (2.5 g/kg) was greater in the adolescents than in the adults, the locomotor activity from day 11 was expressed as a percentage of the locomotor activity from day 1 (data not shown). Comparison of the adolescent and adult level of sensitization did not differ ($p=0.53$; Adolescents: 438.9 +/- 118 % increase; Adults: 357.3 +/- 48 % increase). These data indicate that the adolescent and adult mice display sensitization to ethanol 2.5 g/kg to the same degree.

Sensitization: Time Course. In order to further assess age-dependent differences, we next examined the effect of different ethanol treatment durations on the induction of locomotor sensitization to ethanol (Figure 6). Sensitization was defined as a significant increase in distance traveled on the final day of treatment (day 7 or 11) as compared to a single acute treatment (day 1). The time course of the induction of ethanol sensitization was evaluated after ethanol (2.0 and 2.5 g/kg) tests in order to compare response to doses that demonstrated differential age-dependent sensitivity (shown in Figure 5).

Four way ANOVA comparing age x dose x treatment duration x test day identified significant main effects for the between-subjects factors of age [$F(1,54)=5.84$; $p=0.02$] and ethanol dose [$F(1,54)=15.89$; $p<0.001$]. There was no main effect of treatment duration and no interactions between age, dose, and duration. A significant main effect was also identified for the within-subject factor test day [$F(1,54)=181$; $P=0.001$]. Analysis of the two-way interactions showed that the main effect of test day (i.e., acute vs. sensitization test) was dependent on age [$F(1,54)=9.44$; $p=0.003$] and dose [$F(1,54)=59.44$; $p<0.001$]. Three-way interaction terms showed that the effect of test day (i.e., acute vs. sensitization test) was dependent on the level of age and dose [$F(1,54)=12.9$; $p=0.001$] as well as treatment duration and dose [$F(1,54)=4.07$; $p<0.05$].

Based on these significant interactions, the sensitization data was analyzed separately for each dose and duration.

For mice treated with ethanol (2.0 g/kg) for 7 days, two-way RM ANOVA revealed a significant main effect of test day [$F(1, 31)=12.44$; $p=0.003$] and a significant test day X age interaction [$F(1, 31)=6.75$; $p=0.02$]. Multiple comparisons showed that, overall, activity on day 7 was higher than activity on day 1 ($p=0.004$) and that this increase was dependent on an increase in locomotor activity in the adults on day 7 as compared to day 1 ($p<0.001$). These data show that the adults show locomotor sensitization to ethanol (2.0 g/kg) after 7 days of treatment, while the adolescents do not show sensitization at this time point (Figure 6A). For the 11 day time course of ethanol (2.0 g/kg), two-way RM ANOVA showed a significant main effect of test day [$F(1, 30)=8.32$; $p<0.02$] and a significant test day X age interaction [$F(1, 30)=26.46$; $p<0.001$]. Post-hoc comparisons showed that, overall, locomotor activity on day 11 was significantly greater than activity on day 1 ($p<0.02$), and this effect was caused by a significant increase in the adult group on day 11 compared to day 1 ($p<0.001$; Figure 6A). On day 1, the adolescents showed significantly more locomotor activity than the adults ($p<0.001$), while on day 11 the adults were more active than the adolescents ($p<0.02$). These results indicate that the adult group displayed sensitization to ethanol (2.0 g/kg) following 11 days of treatment while the adolescent group did not show sensitization. Furthermore, the adolescents displayed the expected greater acute locomotor activation to ethanol (2.0 g/kg), while the adults responded to a greater extent on the sensitization test day than the adolescents.

For ethanol (2.5 g/kg) after 7 days of treatment, two-way ANOVA showed only a significant main effect of day [$F(1, 31)=78.15$; $p<0.001$], indicating that all mice displayed significantly increased activity on day 7 compared to day 1 ($p<0.001$). Similarly, ANOVA of treatment for 11 days with ethanol (2.5 g/kg) showed a significant main effect of day

[F(1, 30)=108.85; p<0.001], indicating that all mice displayed sensitization (Figure 6B). Taken together, these data show that regardless of duration of treatment, adolescent mice do not exhibit locomotor sensitization to ethanol (2.0 g/kg) while adult mice do exhibit sensitization to this dose of ethanol. Both adolescent and adult mice display locomotor sensitization to ethanol (2.5 g/kg).

The time course was extended to 15 days for the ethanol (2.0 g/kg) in order to investigate whether treatment for a longer period of time would elicit sensitization in the adolescent group. Two-way ANOVA revealed a significant main effect of age [F(1, 12)=11.94; p=0.005], a significant main effect of test day [F(1, 27)=22.38; p<0.001], and a significant interaction [F(1, 27)=7.94; p<0.02]. Post-hoc Tukey tests showed no difference between day 15 and day 1 within the adolescents (p=0.23), while there was a significant increase in activity on day 15 in the adults (p<0.001). These results indicate that the adolescent mice did not demonstration locomotor sensitization following 15 days of treatment with ethanol (2.0 g/kg), while the adult group did show sensitization. On the acute test day 1, the adolescents were significantly more active than the adults (p<0.001), while the age groups were not different on day 15 (p=0.86; data not shown).

Overall, the time course experiment shows that adolescent mice do not display sensitization to ethanol (2.0 g/kg) with up to 15 days of ethanol exposure, while the adult mice show sensitization following only 7 days of exposure. Both adolescent and adult mice exhibit sensitization to ethanol (2.5 g/kg) with only 7 days of exposure. These results indicate that age-dependent ethanol sensitization is not effected by the duration of treatment but is mediated by the dose of ethanol.

Figure 6. Ethanol Sensitization: Time Course.

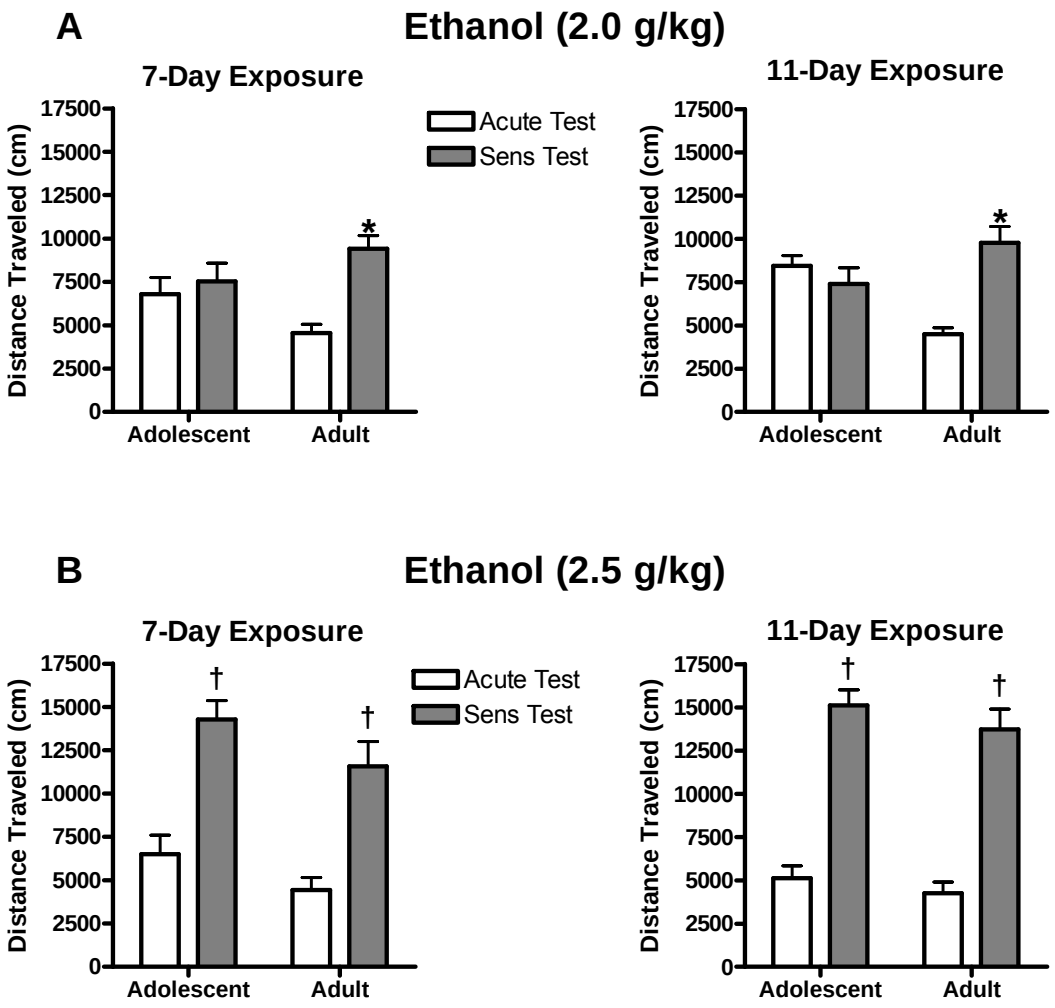

Figure 6. Ethanol Sensitization: Time Course. **A.** Adolescent and adult locomotor response (distance traveled, cm, mean +/- SEM) following administration of ethanol 2.0 g/kg during 10 minute test sessions on day 1 (acute test; open bars) and the final day (7 or 11; sensitization test; filled bars). * indicates significant increase in distance traveled compared to day 1, $p<0.05$. **B.** Adolescent and adult locomotor response (distance traveled, cm, mean +/- SEM) following administration of ethanol 2.5 g/kg during 10 minute test sessions on day 1 (acute test; open bars) and the final day (7 or 11; sensitization test; filled bars). * indicates significant increase in distance traveled compared to day 1, $p<0.05$. † indicates an overall significant increase from acute test for the two ages combined, $p<0.05$.

Correlation: Acute X Sensitized Locomotor Response. In order to examine the possibility that the acute locomotor response to ethanol was predictive of the degree of locomotor activation after repeated treatment, a linear regression comparing day 1 (acute) activation and day 11 (sensitized) activation was performed in the adolescents and adults treated with either ethanol (2.0 or 2.5 g/kg). There was no significant correlation between acute locomotor activation and sensitized locomotor activation in the adolescents or the adults at either ethanol dose (ps>0.1; data not shown). These data indicate that the acute locomotor response to ethanol (2.0 or 2.5 g/kg) does not affect the magnitude of the sensitized locomotor response to repeated ethanol treatment in adolescent or adult mice.

Blood Ethanol Concentration. In order to examine whether differential age-dependent sensitization might be mediated by differences in ethanol clearance, an analysis of blood ethanol concentration (BEC; mg/dL) was conducted for sensitization treatment with ethanol (2.0 g/kg). Importantly, this dose represents an ethanol dose at which the adolescent mice did not develop sensitization while the adult mice developed sensitization. The BEC was measured in adolescents and adults on day 1 (representing acute ethanol treatment) at 10, 60, and 180 minutes post-ethanol treatment. The 10 minute time point was examined to assess the BEC at a time point corresponding to the end of the locomotor behavior session, while the 60 and 180 minute time points were examined to assess the clearance of ethanol from the blood. The BEC was also measured in adolescents and adults on day 11 (corresponding to the sensitization test day) in order to examine any groups differences in ethanol clearance after repeated ethanol treatment.

The three-way RM ANOVA of BEC revealed a significant main effect of the between subject factor treatment day [$F(1, 21)=40.88$; $p<0.001$], a significant main effect of the within subject factor time post ethanol injection [$F(2, 42)=586.47$; $p<0.001$], a

significant time X age interaction [$F(2, 42)=3.69$; $p<0.05$], and a significant time X day interaction [$F(2, 42)=12.32$; $p<0.001$]. In order to assess whether differences in BEC on day 1 or day 11 were responsible for differences in locomotor activity of adolescents and adults, the BEC data were analyzed separately for day 1 and day 11.

A two-way RM ANOVA of BEC on day 1 of ethanol (2.0 g/kg) treatment showed a main effect of age [$F(1,6)=14.54$; $p=0.008$], a main effect of time (minutes) post-ethanol administration [$F(2,12)=345.43$; $p<0.001$], and a significant interaction [$F(2,11)=15.09$; $p<0.001$]. Within the adolescents and adults, the post-hoc Tukey test revealed that the BEC at 60 minutes was significantly less than the BEC at 10 minutes, while the BEC at 180 minutes was significantly less than the BEC at 60 minutes (Figure 6A; $ps<0.002$). These data are indicative of ethanol clearance from the blood. Within the time points, the adolescents were significantly different than the adults only at 60 minutes post-ethanol injection ($p<0.001$). These data indicate that the adolescents had cleared more ethanol from the blood than the adults at 60 minutes after ethanol (2.0 g/kg) administration on day 1.

The two-way RM ANOVA on day 11 revealed no significant main effect of age [$F(1,5)=0.08$; $p=0.79$], a significant main effect of time post-ethanol injection [$F(2,10)=184.06$; $p<0.001$], and no significant interaction [$F(2,10)=0.21$; $p=0.81$]. For all mice, the BEC at 60 minutes was significantly less than the BEC at 10 minutes, while the BEC at 180 minutes was significantly less than the BEC at 60 minutes (Figure 6B; $ps<0.005$), indicating ethanol clearance from the blood over time.

In order to examine the development of metabolic tolerance in the mice, the BEC at each time point was compared between day 1 and day 11. The two-way ANOVA showed a significant main effect of day [$F(1, 27)=50.25$; $p<0.001$], a significant main effect of time [$F(2, 49)=582.54$; $p<0.001$], and a significant interaction [$F(2, 49)=14.59$; $p<0.001$]. Post-hoc Tukey tests indicated that the BEC at 10 and 60 minutes post

ethanol injection differed on day 1 and day 11 (ps<0.001). This data is indicative of metabolic tolerance to ethanol following repeated administration of ethanol, which has been shown previously in adult and adolescent rats (Chester et al. 2005; Silvers et al. 2003; Varlinskaya and Spear 2007).

Overall, these data show that the adolescent mice clear more ethanol from the blood than adult mice following acute administration of ethanol (2.0 g/kg), but this effect diminishes following repeated administration of ethanol. All of the mice show lower BEC to ethanol following repeated ethanol administration, indicating the development of metabolic tolerance. Importantly, adolescent and adult mice have equivalent BEC at the 10 minute time point, which corresponds to the time of the locomotor session during which the age groups display differential ethanol-induced locomotor activity.

Figure 7. Blood Ethanol Clearance.

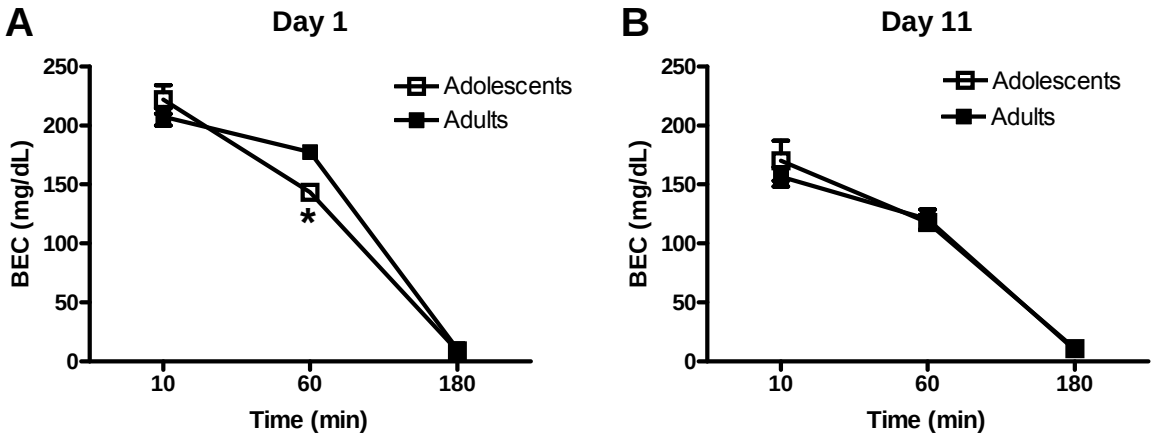

Figure 7. Blood Ethanol Clearance. **A.** BEC (mg/dL, mean +/- SEM) in adolescent and adult DBA/2J mice on day 1, 10, 60, and 180 minutes following administration of ethanol 2.0 g/kg. **B.** BEC (mg/dL, mean +/- SEM) in adolescent and adult DBA/2J mice on day 11, at 10, 60, and 180 minutes following administration of ethanol 2.0 g/kg. * indicates significant difference between age groups, $p<0.05$.

DISCUSSION

Adolescence is a time period marked by an increase in risk-taking behavior, which has been shown to lead to experimentation with drugs of abuse such as ethanol (Spear 2000a). The effects of ethanol on the maturing adolescent brain are not fully characterized at this time. Studies have shown that adolescent rodents are more or less sensitive than adults to ethanol, depending on the behavior being measured. It has been proposed that these differences in sensitivity might underlie the propensity for ethanol intake during adolescence to lead to alcoholism later in life (Spear and Varlinskaya 2005). The present study extends the previous findings to ethanol locomotor sensitization and shows that adolescent DBA/2J mice are less sensitive than adult mice to ethanol-induced neurobehavioral adaptations.

Adolescent DBA/2J mice showed an enhanced locomotor response to acute administration of ethanol 1.5 and 2.0 g/kg compared to the adults. This is in agreement with a recent report in adolescent C57BL/6J mice that showed an increase in locomotor activity after 1.5 g/kg ethanol administration during the first 10 minutes of testing (Hefner and Holmes 2007). These findings are significant because it has been shown in humans that heavy drinkers are more sensitive to the acute stimulant effects of ethanol than light drinkers (King et al. 2002).The acute activating effects of ethanol involve numerous neurotransmitter systems, including mesolimbic dopamine signaling, metabotropic and ionotropic glutamate receptors, GABA receptors, and opioid receptors (Blednov et al. 2004; Demarest et al. 1998; Kalivas 1995; Meyer and Phillips 2003; Pastor et al. 2005; Vezina and Kim 1999). The differences observed in the adolescent response to acute ethanol are possibly due to the fact that these neurotransmitter systems are not fully developed in the adolescent (Spear 2000a). The undeveloped neurotransmitter systems of the adolescent mice could perhaps be similar to the neurotransmitter systems following sensitization in adult mice. However, this explanation seems unlikely when it is

considered that the adolescents showed an enhanced acute response to ethanol 2.5 g/kg while they also displayed sensitization to this dose.

Repeated administration of ethanol in adult mice leads to an increase in locomotor activation that is markedly greater than the acute locomotor response, known as ethanol sensitization (Phillips et al. 1994). It has been suggested that the neural adaptations which underlie locomotor sensitization might occur in the same brain regions which underlie drug reward and craving (Pierce and Kalivas 1997; Robinson and Berridge 1993; Vanderschuren and Kalivas 2000). Interestingly, ethanol sensitization has not been studied in adolescents. In the present study, the highest dose of ethanol (2.5 g/kg) tested was required to produce locomotor sensitization in the adolescent mice, while multiple ethanol doses (1.5, 2.0, 2.5 g/kg) produced locomotor sensitization in the adult mice. Moreover, the time course study showed that even with a longer exposure time, the adolescent mice did not develop sensitization to ethanol (2.0 g/kg). These results indicate that adolescent DBA/2J mice are less sensitive to ethanol-induced locomotor sensitization than adult mice. The finding that adolescents are less sensitive to ethanol sensitization is significant because it has been shown in humans that sons of alcoholics, a group at high risk for developing alcoholism, are differentially sensitive to the physiological effects of ethanol when given repeated ethanol treatments (Newlin and Thomson 1991). Perhaps blunted sensitivity to the neuroadaptations that occur during the induction of ethanol sensitization in adolescents may be one factor that contributes to the epidemiological observation that adolescent alcohol use is associated with increased risk of abuse in adulthood (Grant and Dawson 1998).

One possible factor that could explain the difference in ethanol sensitization observed in this study is that the blood ethanol concentrations differ between the adolescent and adult groups. For example, adolescent C57BL/6J mice show higher initial BEC than the adults following ethanol (3.0 g/kg) injection but significantly lower

BEC by 90 minutes post-injection, which suggests more rapid ethanol clearance in adolescent mice (Hefner and Holmes 2007). To address this possibility, we evaluated BEC in adolescent and adult mice following injection of the dose of ethanol (2.0 g/kg) that produced differential age-dependent locomotor sensitization. The results show no differences in BEC between the age groups at the 10-minute time point either on day 1 or on day 11. This time point is critical because it corresponds to the length of the locomotor session during which the age-groups display differential locomotor activity, indicating dissociation between the BEC and locomotor activity. The results also extend previous findings in adolescent C57BL/6J mice and Sprague Dawley rats by showing that adolescent DBA/2J mice clear acute ethanol (2.0 g/kg) from the blood faster than adults at 60 minutes post-ethanol administration (Hefner and Holmes 2007; Little et al. 1996). On day 11 of the experiment, when adult mice show locomotor sensitization but adolescent mice do not, no differences in BEC between the age groups are apparent at any time point. These data indicate dissociation between BEC and locomotor sensitization. These data suggest that the age-dependent differential sensitization to ethanol (2.0 g/kg) observed in the present study cannot be attributed to differential BEC.

Another possible explanation for the differential sensitization observed in adolescent mice in this study is that the increased acute response to ethanol (1.5 and 2.0 g/kg) affected sensitization. That is, the enhanced acute response on day 1 prevented an increase in locomotor activity from occurring on day 11. However, previous studies have shown that both the neural mechanism and genetic correlates of acute locomotor activation are unrelated to those that underlie ethanol sensitization (Broadbent et al. 1995; Phillips et al. 1995). Furthermore, no correlation between acute locomotor activity and sensitized locomotor activity was observed in the present study, indicating that the acute response on day 1 to ethanol was not predictive of the sensitized response on day 11. The possibility also exists that the acute response to ethanol was

at the ceiling of locomotor activity, so that no further increase in activity could be observed following repeated ethanol administration. This can be examined directly by observing the amount of time the animal was ambulatory in the chamber. In this study, the adolescents were ambulatory on day 1 and day 11 for less than 5 minutes of the total 10 minutes that they were in the locomotor chamber (data not shown). This indicates that the ceiling of locomotor activity had not been reached during the session, as the mice had greater than 5 minutes to display enhanced locomotor activity. Together, these data confirm that the acute response to ethanol in the adolescents does not underlie their lack of ethanol sensitization.

Previous studies have suggested that increased sensitivity to locomotor sensitization in adults is a marker for increased likelihood of drug dependence (Robinson and Berridge 1993). One might predict, therefore, that adolescents would be more sensitive to ethanol sensitization based on human studies showing that ethanol intake during adolescence increases the likelihood of alcoholism in adulthood (Grant 1998). However, adolescent rodents are known to respond differently to ethanol than adults (see Introduction), which means predicted response patterns in adult rodents may not apply to adolescent rodents. In the present study, adolescents were found to be less sensitive to ethanol sensitization, which corresponds to a previous study showing that adolescents were less sensitive to the sedative properties of ethanol (Silveri and Spear 1998). Interestingly, adolescents are more sensitive to ethanol's inhibition of NMDA-mediated excitation and long-term potentiation (Swartzwelder et al. 1995a; 1995b). The NMDA receptor antagonist MK-801 has been shown to block ethanol sensitization at higher doses in DBA/2J adult mice (Broadbent and Weitemier 1999; Meyer and Phillips 2003). One possible explanation for the lack of ethanol sensitization seen in adolescent mice is that over the course of the development of sensitization, ethanol is more potently inhibiting the NMDA receptor, which effectively attenuates sensitization. However, at the

higher ethanol dose of 2.5 g/kg, the adolescents develop sensitization, which makes this explanation unlikely.

Overall, this is the first study to examine ethanol sensitization in adolescents, and the findings show that adolescent DBA/2J mice are less sensitive to ethanol sensitization than adult mice. This effect is not due to the enhanced acute locomotor response to ethanol in the adolescents or to differences in BEC. These data suggest that blunted sensitivity to ethanol-induced neurobehavioral adaptations during adolescence may be one factor that contributes to increased risk of abuse in adulthood.

CHAPTER III: MPEP INHIBITS THE INDUCTION OF ETHANOL SENSITIZATION IN ADULT, BUT NOT ADOLESCENT, DBA/2J MICE

INTRODUCTION

Adolescence is a critical developmental period during which alcohol or cocaine use in humans is often initiated (Johnston LD 2005). It is known that people who start drinking during adolescence are four times more likely to become alcohol dependent as adults (Grant 1998). Animal studies have shown that adolescents and adults are differentially sensitive to the effects of many drugs of abuse, including ethanol and cocaine. These differences in sensitivity occur in the absence of any differences in the brain availability of the drug, and instead seem to be related to an altered sensitivity of the neurobiological effects of the drugs (Spear 2000a; Spear and Brake 1983).

Studies have shown that adolescent rodents are more sensitive to the effects of ethanol on measures of locomotor stimulation, anxiety, ataxia, spatial memory, conditioned place preference, and social interaction as compared to adult rodents (Hefner and Holmes 2007; Markwiese et al. 1998; Philpot et al. 2003; Rajendran and Spear 2004; Varlinskaya and Spear 2002; 2006; Yttri et al. 2004). By contrast, adolescent rodents are less sensitive than adult rodents to the sedative and motor impairing effects of ethanol, to ethanol withdrawal induced anxiety, and analgesia (Doremus-Fitzwater and Spear 2007; Doremus et al. 2003; Hefner and Holmes 2007; Silveri and Spear 1998; Varlinskaya and Spear 2002; White et al. 2002). These differences in the sensitivity of adolescents to ethanol are important because it has been shown that a decreased response to acute alcohol challenge during adolescence is a potent predictor of future alcoholism (Schuckit 1993; 1994).

Similarly, studies assessing adolescent responses to cocaine have shown that adolescent rats are less sensitive than adults to cocaine induced locomotor stimulation but show the same cocaine-induced conditioned place preference (Campbell et al. 2000; Laviola et al. 1995). Overall, adolescent rodents appear to be more or less sensitive to the effects of psychomotor stimulants, depending on which behavioral paradigm is tested (Frantz et al. 2007).

One model of neurobehavioral adaptations that occur following chronic drug exposure is locomotor sensitization. Sensitization is typically defined as a progressive increase in locomotor activity following repeated administration of a drug of abuse (Kalivas and Stewart 1991). The process of sensitization is thought to produce enduring adaptive changes in brain and behavioral function that may underlie components of addiction (Kalivas et al. 1998; Robinson and Berridge 2000). Research has shown that sensitization is mediated by an interconnected network of mesocorticolimbic brain regions (i.e., VTA, nucleus accumbens, prefrontal cortex, amygdala, and thalamus) and neurotransmitter systems (i.e., dopamine, glutamate, and GABA) (Kalivas 1995; Vezina and Kim 1999). These brain regions and neurotransmitter systems all undergo alterations during the adolescent developmental period (Kalivas 1995; Spear 2000a; Vezina and Kim 1999). Thus, sensitization models are useful tools to determine if adolescent vulnerability to addiction involves differential sensitivity to neurobehavioral changes that occur with repeated drug use.

Recently, we have shown that adolescent DBA/2J mice are less sensitive to ethanol sensitization than adult mice (Stevenson et al. 2007). Several studies of cocaine sensitization have shown that adolescent rodents display less, similar, or even greater cocaine-induced sensitization compared to adults, depending on the rat/mouse strain and injection procedure employed in the study (Camarini et al. 2007; Collins and Izenwasser 2002; Frantz et al. 2007; Laviola et al. 1995; Niculescu et al. 2005). The

discrepancies in the results of these studies indicate that the maturation of the neurotransmitter systems involved in sensitization may differ between rat and mouse strains. The present study was designed to evaluate the mechanism underlying sensitization to both ethanol and cocaine in adolescent and adult DBA/2J mice, a strain that has shown differential age-dependent sensitivity to both ethanol and cocaine sensitization (Camarini et al. 2007; Stevenson et al. 2007).

Evidence indicates that glutamate signaling is an important component of both ethanol and cocaine sensitization in adult animals (Broadbent et al. 2003; Broadbent and Weitemier 1999; Kotlinska et al. 2006; Vanderschuren and Kalivas 2000). Metabotropic glutamate receptors (mGluRs) are a class of G-protein coupled glutamate receptors. The group I mGluR, mGluR5, is expressed abundantly in the nucleus accumbens and ventral tegmental area, brain regions which are known to be involved in locomotor sensitization (Kalivas 1995; Romano et al. 1996; Vezina and Kim 1999).

Ethanol has been shown to inhibit mGluR5 function in *Xenopus* oocytes (Minami et al. 1998), while behavioral studies have shown that antagonism of mGluR5 reduces ethanol self-administration and blocks the discriminative stimulus properties of ethanol (Backstrom et al. 2004; Besheer and Hodge 2004; Olive et al. 2005; Schroeder et al. 2005). It has recently been shown that the mGluR5 antagonist, MTEP, inhibits the expression of ethanol sensitization in adult mice (Kotlinska et al. 2006). However, the role of mGluR5 in the induction of ethanol sensitization has not been studied in adolescent or adult mice.

Evidence indicates that mGluR5 is involved in many of the behavioral effects of cocaine. The mGluR5 knockout mice lack cocaine-induced locomotor stimulation and the mGluR5 antagonist MPEP blocks acute cocaine-induced locomotor stimulation (Chiamulera et al. 2001; Herzig and Schmidt 2004; McGeehan et al. 2004). Furthermore, mGluR5 antagonism attenuates cue-induced cocaine seeking, cocaine

self-administration, and the conditioned rewarding effects of cocaine (Backstrom and Hyytia 2006; Kenny et al. 2005; McGeehan and Olive 2003). By contrast, recent evidence indicates that the mGluR5 antagonist MTEP does not alter the expression of cocaine sensitization in adult rats (Dravolina et al. 2006). The role of mGluR5 in adolescent cocaine sensitization remains unexplored.

Although it has been shown that glutamate receptors, along with GABA and dopamine receptors, are involved in ethanol sensitization, little is known about the intracellular molecular events that take place to bring about the long term changes associated with locomotor sensitization (Broadbent and Harless 1999; Broadbent et al. 2005). Activation of group I mGluR's is known to upregulate phosphorylation of the MAP kinase $ERK_{1/2}$ (Choe and Wang 2001a; 2001b). Evidence is emerging that the activity of the $ERK_{1/2}$ is modulated in reward-associated brain regions in response to drugs of abuse, including ethanol (Berhow et al. 1996; Kalluri and Ticku 2002; Tsuji et al. 2003; Valjent et al. 2000; Valjent et al. 2004). The $ERK_{1/2}$ pathway is activated when phosphorylated by MEK1/2, which causes $ERK_{1/2}$ to phosphorylate gene transcription factors such as CREB and Elk-1. The regulation of gene transcription by the $ERK_{1/2}$ allows it to mediate long-term changes in behavioral functions (Grewal et al. 1999; Qi and Elion 2005; Sweatt 2004; Thomas and Huganir 2004; Wang et al. 2007). The ability of $ERK_{1/2}$ to modulate long-term neurobiological changes in response to drug administration makes it an intriguing molecular target for the induction of locomotor sensitization. In fact, recent evidence shows that an inhibitor of the ERK pathway, SL327, inhibits the induction of cocaine and amphetamine sensitization (Valjent et al. 2006). Although it is known that chronic exposure to ethanol vapor reduces $ERK_{1/2}$ phosphorylation, the effect of ethanol sensitization on phophorylated $ERK_{1/2}$ has not been studied (Sanna et al. 2002).

The present study was designed to examine the role of mGluR5 in the induction of ethanol and cocaine sensitization in adolescent and adult DBA2/J mice using the receptor antagonist MPEP. The role of the downstream signaling kinase $ERK_{1/2}$ in the induction of ethanol sensitization was examined using immunohistochemistry.

MATERIALS AND METHODS

Animals. Male 3-week old (adolescent) and 8-week old (adult) DBA/2J mice (Jackson Laboratories, Bar Harbor, ME) were housed in groups (4 animals per cage) in standard Plexiglas cages with food (Purina Rodent Chow) and water available *ad libitum*. The colony was maintained at 27^{o}C on a 12-hour light/dark cycle, with the lights on at 10pm. The behavioral experiments were conducted during the dark portion of the cycle. Mice were handled and weighed daily for 1-week prior to, and for the duration of, the experiment. Animals were under continuous care and monitoring by the Division of Laboratory Animal Medicine at UNC-Chapel Hill, and all procedures were carried out in accordance with the *NIH Guide to Care and Use of Laboratory Animals* (National Research Council, 1996) and institutional guidelines.

Behavioral Apparatus. The locomotor activity (horizontal distance traveled, cm) of adolescent and adult mice was measured in eight covered Plexiglas chambers (30 cm^2, Med Associates, Georgia, VT). Two sets of 16 pulse-modulated infrared photobeams were located on opposite walls to record ambulatory movements in the X-Y (horizontal) plane. All software settings were the same for adults and adolescent mice. The activity chambers were computer-interfaced (Med Associates) for data sampling at 100-millisecond resolution.

Experimental Procedures. Mice were adapted to the colony and to handling for 1-week (adolescents=P28; adults=P63). On locomotor testing days, mice were taken in the home cage to the testing room at least 30-minutes prior to the session to habituate to

the testing room. The first two days of the each experiment were habituation days (H1 and H2). On these days, all mice received a pretreatment intraperitoneal (IP) injection of saline and were placed back into their home cages. Thirty minutes after the pretreatment injection, mice were given an IP injection of saline and were immediately placed in the locomotor chamber for the 10 minute session.

Experiment 1: Effect of MPEP on Ethanol Sensitization. On day 1, adolescent and adult mice were given a pretreatment injection of saline and returned to their home cages. Thirty minutes later, mice were injected with ethanol (0, 2.0, or 2.5 g/kg) and immediately placed in the locomotor chamber for 10 minutes (n=8 per age group per pretreatment per ethanol dose).

On days 2-10, mice were given a pretreatment injection of MPEP (0 or 30 mg/kg) 30-minutes prior to an injection of ethanol (0, 2.5, or 3.0 g/kg) in the home cage. No locomotor testing was performed on these days.

On day 11, mice were given a pretreatment injection of saline and returned to the home cage. Thirty minutes later, mice were injected with ethanol (0, 2.0, or 2.5 g/kg) and were immediately placed into the locomotor chamber to be tested for locomotor sensitization.

Experiment 2: MPEP Dose Response. On day 1, adult mice were given a pretreatment injection of saline and returned to their home cages. Thirty minutes later, mice were injected with ethanol (2.0 g/kg) and immediately placed in the locomotor chamber for 10 minutes (n=8 per MPEP dose).

On days 2-10, mice were given a pretreatment injection of MPEP (0, 1, 10, or 30 mg/kg) 30-minutes prior to an injection of ethanol (2.5 g/kg) in the home cage. No locomotor testing was performed on these days.

On day 11, mice were given a pretreatment injection of saline and returned to the home cage. Thirty minutes later, mice were injected with ethanol (2.0 g/kg) and were immediately placed into the locomotor chamber to be tested for locomotor sensitization.

Experiment 3: Immunohistochemical Analysis – pERK$_{1/2}$. Following the locomotor test session on day 11, all mice in experiment 1 (see Table 1: Experimental Design) were deeply anesthetized with pentobarbital (100 mg/kg IP) and transcardially perfused with 0.1 M phosphate buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde. The skulls were postfixed overnight, and then rinsed and stored in PBS at 4°C. Brains were sectioned at 40 µm and stored (-20°C) in cryoprotectant until immunohistochemistry processing.

For immunohistochemistry, the tissue was washed in PBS, the endogenous peroxidase activity was blocked in 0.6% H_2O_2, and antigen retrieval was performed at 70°C for 30 min (Antigen Retrieval Citra, BioGenex). Sections were blocked in PBS/0.1%triton-x/4% horse serum for 30 minutes and incubated at +4°C overnight in primary polyclonal antibody to p-ERK$_{1/2}$ (1:400; Cell Signaling Technology, Inc, Danvers, MA). The following day, sections were washed in PBS and incubated in secondary antibody for one hour (Dako EnVision Kit, Dako). Immunoreactivity was detected with nickel-enhanced diaminobenzidine (Dako EnVision Kit) as a chromagen. Sections were counterstained with toluidine blue, mounted, dried and coverslipped with Cytoseal. For consistency of staining across subjects, brain tissue from all groups was processed simultaneously.

Immunoreactivity was quantified using Bioquant image analysis software (Bioquant Nova Advanced Image Analysis; R&M Biometric, Nashville, TN). The image was background corrected and normalized to preset light levels to ensure consistent data collection. Cell count measurements were calculated from a brain region and divided by the area of the region and expressed as cells/mm^2. For the dentate gyrus, cells were

counted manually and expressed as the average number of cells/dentate. All data was acquired by a researcher blind to group condition from 4 sections/brain region/animal and averaged to obtain a single value per subject.

TABLE 1: Experimental Design

Day	Hab 1 & 2	1	2 - 10	11
Location	Locomotor Chamber	Locomotor Chamber	Homecage	Locomotor Chamber
Saline	Saline	Saline	Saline/ Saline	Saline
MPEP30	Saline	Saline	MPEP 30mg/kg / Saline	Saline
Acute E2	Saline	Saline	Saline/ Saline	Ethanol 2.0 g/kg
Acute E2.5	Saline	Saline	Saline/ Saline	Ethanol 2.5 g/kg
Chronic E2	Saline	Ethanol 2.0 g/kg	Saline/ Ethanol 2.5 g/kg	Ethanol 2.0 g/kg
MPEP/ E2	Saline	Ethanol 2.0 g/kg	MPEP 30mg/kg / Ethanol 2.5 g/kg	Ethanol 2.0 g/kg
Chronic E2.5	Saline	Ethanol 2.5 g/kg	Saline/ Ethanol 3.0 g/kg	Ethanol 2.5 g/kg
MPEP/ E2.5	Saline	Ethanol 2.5 g/kg	MPEP 30mg/kg / Ethanol 3.0 g/kg	Ethanol 2.5 g/kg

Experiment 4: Effect of MPEP on Cocaine Sensitization. Mice were allowed to habituate to the locomotor chambers during two 1-hour sessions (Habituation days 1 and 2). In both the adolescent and adult mice, total distance traveled on the second habituation session did not differ among groups. The initiation of cocaine sensitization was measured every other day, for a total of 5 cocaine exposures over 9 days. On these days (Cocaine days 1-5), mice were pretreated with MPEP (0, 10, or 30 mg/kg, IP) and returned to the home cage. 30-minutes later, mice were given an injection of saline or cocaine (10 mg/kg, IP) and immediately placed in the locomotor chamber for 1 h. 24 hours following the fifth cocaine exposure, mice were tested for the expression of cocaine sensitization. On this day (Catania et al.), mice were given an injection of saline 30 minutes prior to an injection of cocaine (10 mg/kg, IP), and placed in the locomotor chamber for 1 h.

Drugs. Ethanol (95% w/v) was diluted in saline (0.9%) to a concentration of 20% (v/v) and injected (IP) at different volumes to achieve the appropriate dosage (i.e., 2.0 and 2.5 g/kg). Cocaine hydrochloride (Sigma-Aldrich) was dissolved in 0.9% saline. The mGluR5 antagonist MPEP [2-methyl-6-(phenylethynyl)-pyridine] was dissolved in 0.9% saline. Cocaine and MPEP were injected IP at a volume of 10 mL/kg.

Behavioral Measures and Data Analysis. Horizontal distance traveled (in centimeters) during the 10-minute session was calculated from the number of photobeam breaks and presented as mean ± SEM. Statistical significance was defined as $p \leq 0.05$ in all experiments.

Experiment 1: Effect of MPEP on Ethanol Sensitization. Distance traveled (cm) was analyzed separately for adolescents and adults at each ethanol dose (0, 2.0, or 2.5 g/kg) using three-way RM ANOVA of the between groups factors pretreatment (MPEP 0 or 30 mg/kg) and ethanol treatment (0 or 2.0 g/kg) and the within group factor sensitization (locomotor activity on day 1 vs day 11). Significant interactions were

followed with lower order (e.g. two-way) ANOVA where appropriate. Sensitization was defined as activity on day 11 being significantly greater than activity on day 1 within an ethanol dose, as determined by post-hoc Tukey tests. This within group definition of sensitization was applied because it was observed that groups of adolescent and adult mice treated repeatedly with saline and given acute ethanol (2.0 or 2.5 g/kg) on day 11 displayed an equivalent locomotor response to the mice treated with acute ethanol on day 1 (data not shown). The data were presented as mean (+/- SEM).

Experiment 2: MPEP Dose Response. Distance traveled (cm) on sensitization test day 11 was analyzed using one-way ANOVA, with MPEP dose (0, 1, 10, or 30) as the factor. Post-hoc Tukey's test was used to determine between group differences. The data were presented as mean (+/- SEM).

Experiment 3: Immunohistochemical Analysis - $pERK_{1/2}$. Data were presented as the average cells/mm^2 within adolescents and adults at each ethanol dose, and analyzed using one-way ANOVA for each age group.

Experiment 4: Effect of MPEP on Cocaine Sensitization. Locomotor activity was determined by the total distance traveled (cm). In order to correspond with maximal receptor occupancy after IP injection of MPEP in mice (Anderson et al. 2003), locomotor activity during the first 15 min of the session was analyzed and presented. Open field activity was determined by the ratio of distance traveled in the center of the open field to total distance traveled in the 15-min interval. Acquisition of sensitization and open field activity were analyzed using two-way repeated measures analysis of variance (RM ANOVA), with one factor repetition (treatment day). Tukey post hoc tests were used to extract significant main effects. Sensitization was defined as significantly greater activity on day 5 relative to day 1. The magnitude of sensitization was determined by the difference of total activity on Day 5 from Day 1, and analyzed using one-way ANOVA.

Activity on the Test Day was analyzed using one-way ANOVA. Statistical significance was declared at $p \leq 0.05$.

RESULTS

Effect of MPEP on Ethanol Sensitization. It has been shown previously that the mGluR5 antagonist MTEP blocks the expression of ethanol sensitization (Kotlinska et al. 2006). In order to determine the role of mGlu5 receptor in the induction of ethanol sensitization, another mGluR5 antagonist, MPEP, was used in both adolescent and adult DBA/2J mice at ethanol doses (2.0 and 2.5 g/kg) for which the two age groups have been shown to display differential ethanol sensitization (Stevenson et al. 2007). Locomotor sensitization was defined as a significant increase in distance traveled on day 11 compared to the acute response on day 1. MPEP was given on the intervening days (2-10) 30 minutes before ethanol (0, 2.5, or 3.0 g/kg), during the induction phase of ethanol sensitization (Figure 8).

In the adolescents treated with ethanol (2.0 g/kg), three-way RM ANOVA of the between subject factors pretreatment (MPEP 0 or 30 mg/kg) and ethanol treatment (0 or 2.0 g/kg) and the within subject factor sensitization (day 1 vs day 11) revealed only a significant effect of ethanol treatment [$F(1, 23)=190.67$; $p<0.001$]. This result indicates that ethanol caused a significant increase in locomotor activity but did not lead to sensitization (Figure 8A). For ethanol (2.5 g/kg), a dose at which adolescents do display ethanol sensitization (Stevenson et al. 2007), the three-way RM ANOVA again revealed a significant between subject main effect of ethanol treatment [$F(1, 22)=163.86$; $p<0.001$], a significant main effect of the within subject factor sensitization [$F(1, 22)=111.87$; $p<0.001$], and a significant sensitization X ethanol treatment interaction [$F(1, 22)=105.08$; $p<0.001$]. These results indicated that all mice treated with ethanol

were more active on day 11 than on day 1 and that MPEP did not alter locomotor activity (Figure 8B).

In the adults treated with ethanol (2.0 g/kg), three-way RM ANOVA revealed a significant between subject main effect of pretreatment [$F(1, 24)=4.46$; $p<0.05$], a significant between subject main effect of ethanol treatment [$F(1, 24)=69.99$; $p<0.001$], and significant within subject effects of sensitization [$F(1, 24)=35.52$; $p<0.001$], sensitization X pretreatment [$F(1, 24)=6.77$; $p<0.02$], and sensitization X ethanol treatment [$F(1, 24)=30.11$; $p<0.001$]. Follow-up two-way RM ANOVA of the ethanol treated group showed a significant main effect of treatment day [$F(1, 12)=35.14$; $p<0.001$] and a significant day X MPEP pretreatment interaction [$F(1, 12)=5.06$; $p=0.04$]. In the control animals receiving saline pretreatment, activity on day 11 was significantly greater than activity on day 1 ($p<0.001$). In the group receiving MPEP (30 mg/kg) pretreatment, activity on day 11 was also significantly greater than activity on day 1 ($p=0.02$). On day 11, the control group was significantly more active than the MPEP treated group ($p<0.01$). Together, these data indicate that MPEP treatment significantly blunted the sensitization response on day 11 (Figure 8C).

In the adults at the ethanol (2.5 g/kg) dose, three-way RM ANOVA revealed significant main effects of the between subject factors pretreatment [$F(1, 25)=5.85$; $p<0.03$], ethanol treatment [$F(1, 25)=133.12$; $p<0.001$], and pretreatment X ethanol treatment [$F(1, 25)=4.35$; $p<0.05$], and significant main effects of the within subject factors sensitization [$F(1, 25)=118.65$; $p<0.001$], sensitization X pretreatment [$F(1, 25)=9.99$; $p<0.01$], sensitization X treatment [$F(1, 25)=96.86$; $p<0.001$], and sensitization X pretreatment X treatment [$F(1, 25)=8.47$; $p<0.01$]. Follow-up two-way RM ANOVA of the ethanol treated group showed a significant main effect of pretreatment [$F(1, 12)=5.35$; $p=0.039$], a significant main effect of day [$F(1, 12)=109.41$; $p<0.001$], and a significant interaction [$F(1, 12)=9.38$; $p=0.01$]. In both the control and MPEP pretreated

groups, activity on day 11 was significantly greater than activity on day 1 ($p<0.001$). On day 11, the control group was significantly more active than the MPEP group ($p=0.001$). Mice that received ethanol 0 g/kg throughout the experiment showed no differences on day 1 and day 11, and MPEP treatment in this group had no effect on locomotor behavior. These data show that pretreatment with MPEP (30 mg/kg) significantly blunts the induction of ethanol sensitization (Figure 8D). This effect is only present in the adult mice and occurs for ethanol (2.0 and 2.5 g/kg) sensitization.

Figure 8. MPEP Blunts Ethanol Sensitization in Adults only.

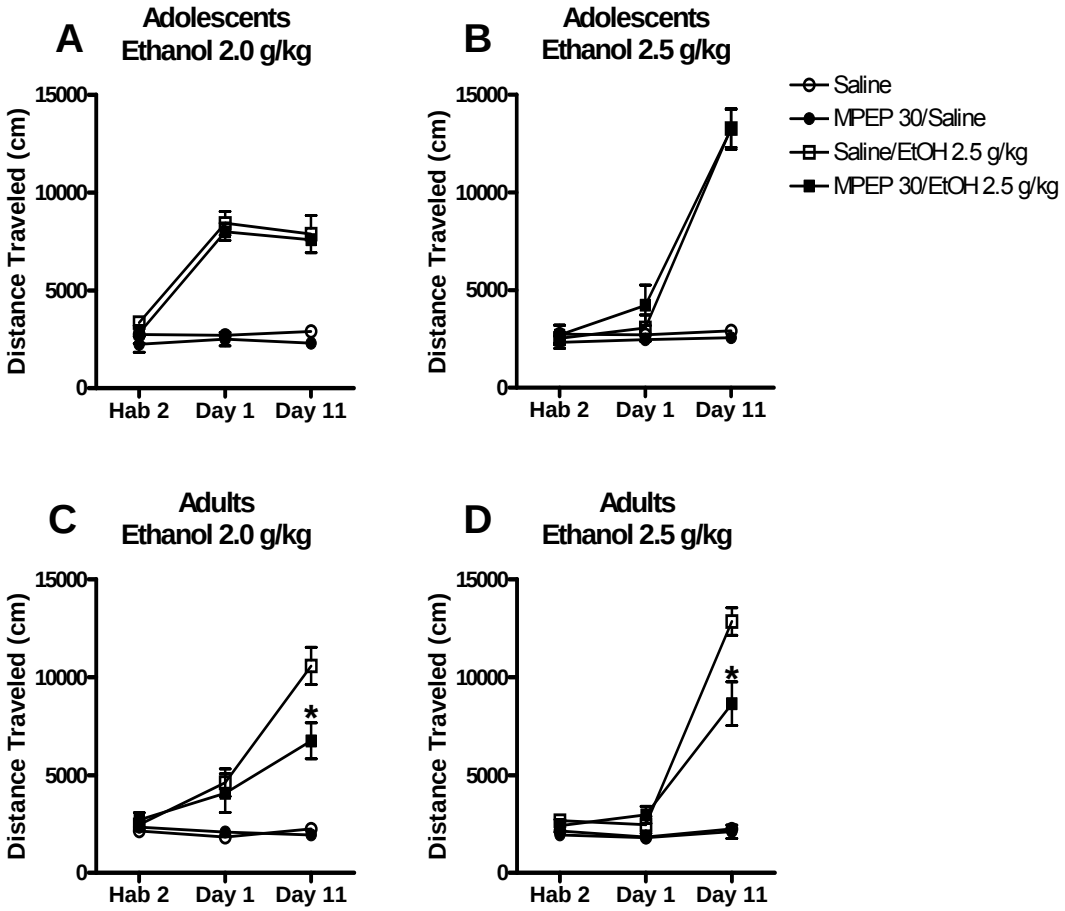

Figure 8. MPEP blunts ethanol sensitization in adult DBA/2J mice only. **A.** Distance traveled (cm, +/- SEM) during the 10-minute locomotor session in adolescent DBA/2J mice treated with ethanol 0 or 2.0 g/kg on day 1 and day 11. Groups were pretreated with MPEP (0 or 30 mg/kg) 30-minutes before ethanol 0 or 2.5 g/kg on days 2-10 in the homecage. **B.** Distance traveled (cm, +/- SEM) during the 10-minute locomotor session in adolescent DBA/2J mice treated with ethanol 0 or 2.5 g/kg on day 1 and day 11. Groups were pretreated with MPEP (0 or 30 mg/kg) 30-minutes before ethanol 0 or 3.0 g/kg on days 2-10 in the homecage. **C.** Distance traveled (cm, +/- SEM) during the 10-minute locomotor session in adult DBA/2J mice treated with ethanol 0 or 2.0 g/kg on day 1 and day 11. Groups were pretreated with MPEP (0 or 30 mg/kg) 30-minutes before ethanol 0 or 2.5 g/kg on days 2-10 in the homecage. *=significantly different than the saline/ethanol group, $p<0.05$. **D.** Distance traveled (cm, +/- SEM) during the 10-minute locomotor session in adult DBA/2J mice treated with ethanol 0 or 2.5 g/kg on day 1 and day 11. Groups were pretreated with MPEP (0 or 30 mg/kg) 30-minutes before ethanol 0 or 3.0 g/kg on days 2-10 in the homecage. *=significantly different than the saline/ethanol group, $p<0.05$.

MPEP Dose Response. In order to determine the dose response curve for MPEP's blunting of ethanol sensitization, adults were given pretreatment with MPEP doses of 0, 1, 10, and 30 mg/kg and treated for sensitization to ethanol (2.0 g/kg; Figure 9). The one-way ANOVA of locomotor activity on day 11 showed a significant between group difference [$F(3, 27)=5.31$; $p=0.005$]. Post-hoc analysis showed that the group treated with MPEP (30 mg/kg) was significantly less active than the groups treated with MPEP (0 and 1 mg/kg; $p<0.04$). These results indicate that pretreatment with only MPEP 30 mg/kg significantly blunts the sensitized locomotor response on day 11, while MPEP (1 and 10 mg/kg) do not significantly alter ethanol sensitization.

Figure 9. MPEP Dose Response on Sensitization Test Day 11.

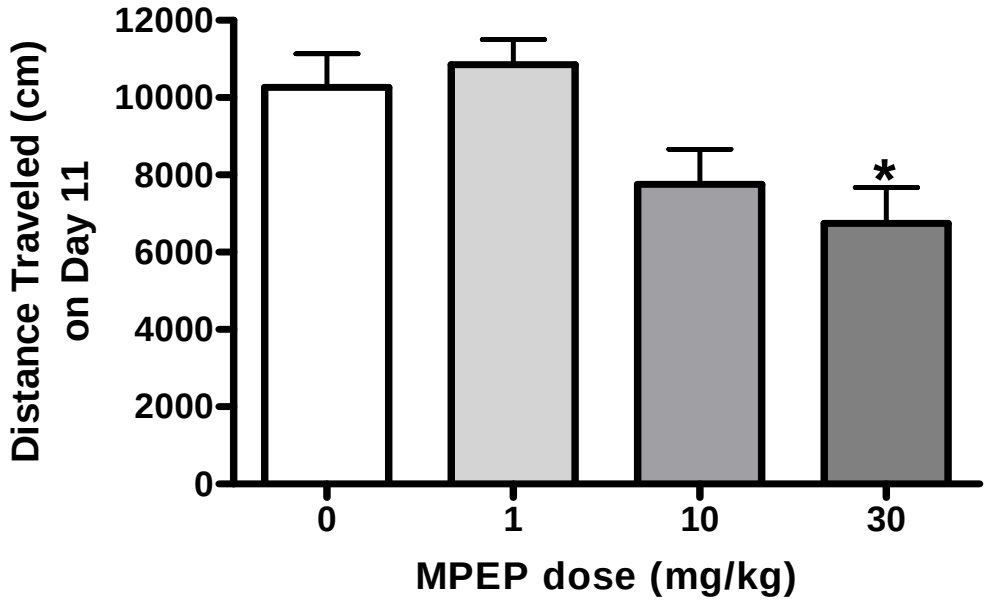

Figure 9. MPEP dose-dependently blunts ethanol sensitization in adult DBA/2J mice. Distance traveled (cm, +/- SEM) in adult DBA/2J on test day 11. Mice were treated with ethanol 2.0 g/kg on day 1, followed by treatment with MPEP 0, 1, 10 or 30 mg/kg and ethanol 2.5 g/kg on days 2-10. When tested on day 11 with ethanol 2.0 g/kg, there was a significant difference between the saline/ethanol group and the MPEP 30/ethanol group, *=$p<0.05$.

Immunohistochemical Analysis – $pERK_{1/2}$. To determine a potential mechanism underlying the induction of ethanol sensitization and the blockade of sensitization by the mGluR5 antagonist MPEP, an analysis of the phosphorylated (i.e., activated) form of $ERK_{1/2}$ was conducted using immunohistochemistry (Summarized in Table 2). Analysis of the adolescent mice showed that acute ethanol (2.0 and 2.5 g/kg) significantly increased the number of $pERK_{1/2}$ positive cells in the central amygdala [$F(7, 48)=17.98$; $p<0.001$], as compared to all other treatment groups (Figure 10A & B; $p<0.04$). However, tolerance to this effect was observed in the mice treated chronically with ethanol (sensitization group), as this group did not differ from saline controls ($p>0.97$). The only other significant difference between groups was in the dentate gyrus of the hippocampus [$F(7, 51]=8.41$; $p<0.001$]. Mice treated with acute ethanol (2.0 and 2.5 g/kg), chronic ethanol (2.0 and 2.5 g/kg), and MPEP (30 mg/kg) with ethanol (2.0 or 2.5 g/kg) all had significantly less $pERK_{1/2}$ than the saline control group (Figure 11A & B; $p<0.05$). No significant changes in $pERK_{1/2}$ were found in the other brain regions examined (Table 2).

In the adults, similar effects were found between groups in the central amygdala [$F(7, 54)=22.3$; $p<0.001$] and in the dentate gyrus [$F(7, 53)=19.4$; $p<0.001$]. As in the adolescents, acute ethanol (2.0 and 2.5 g/kg) significantly increased $pERK_{1/2}$ in the central amygdala compared to all other groups (Figure 10C & D; $p<0.001$), while acute and chronic ethanol (2.0 and 2.5 g/kg) significantly inhibited $pERK_{1/2}$ in the dentate gyrus compared to the saline and MPEP control groups (Figure 11C & D; $p<0.02$). Also, significant between group differences were found for the nucleus accumbens shell [$F(7, 49)=2.26$; $p<0.05$], the lateral septum [$F(7, 51)=2.47$; $p<0.03$], and the lateral habenulum [$F(7, 54)=3.28$; $p=0.006$]. The group treated with chronic ethanol (2.0 g/kg) showed a significant increase in $pERK_{1/2}$ positive cells in the nucleus accumbens shell (Figure 12C; $p<0.02$) and a significant decrease in the lateral habenulum ($p=0.01$). Overall,

these results showed that ethanol treatment altered the number of $pERK_{1/2}$ positive cells in certain brain regions, but there is no link between ethanol sensitization and $pERK_{1/2}$ in the adolescent or adult mice. That is, $pERK_{1/2}$ does not consistently change in groups that sensitize (adolescent ethanol (2.5 g/kg) and adult ethanol (2.0 and 2.5 g/kg)) compared to groups that do not sensitize (adolescent ethanol (2.0 g/kg)).

Figure 10. PERK1/2 in the Central Amygdala.

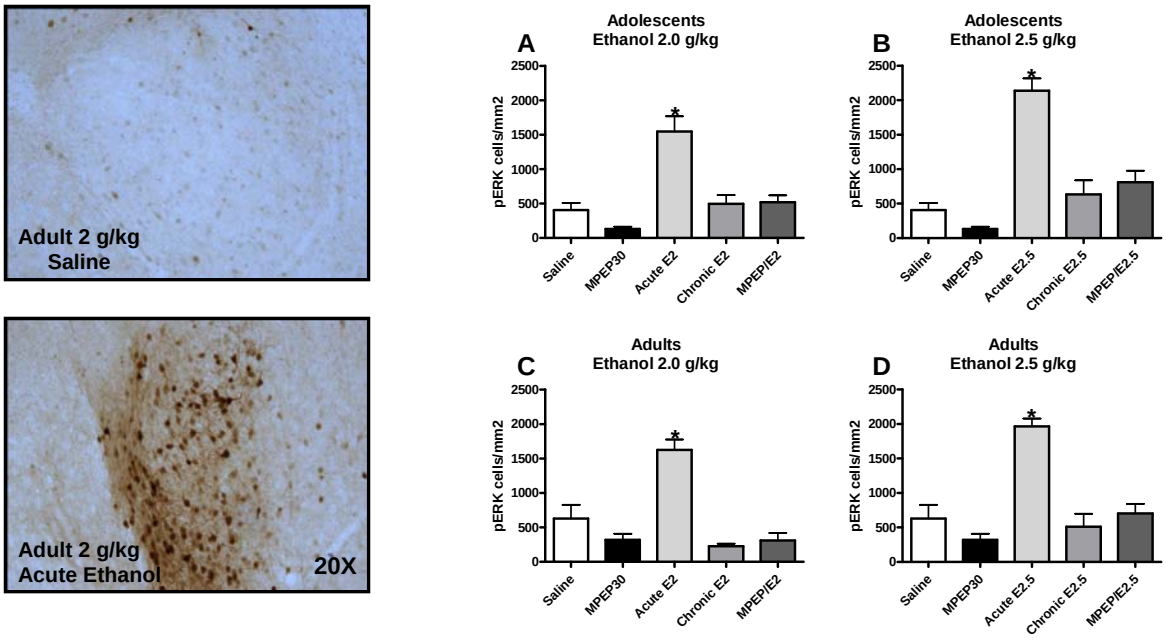

Figure 10. pERK1/2 expression in the Central Amygdala is increased by acute ethanol treatment. In the adolescents and adults, pERK1/2 expression is increased by acute ethanol (2.0 or 2.5 g/kg). This effect is absent following treatment with chronic ethanol (2.0 or 2.5 g/kg). Photos are representative samples from the groups. *=Significantly different than saline, $p<0.05$.

Figure 11. PERK$_{1/2}$ in the Dentate Gyrus.

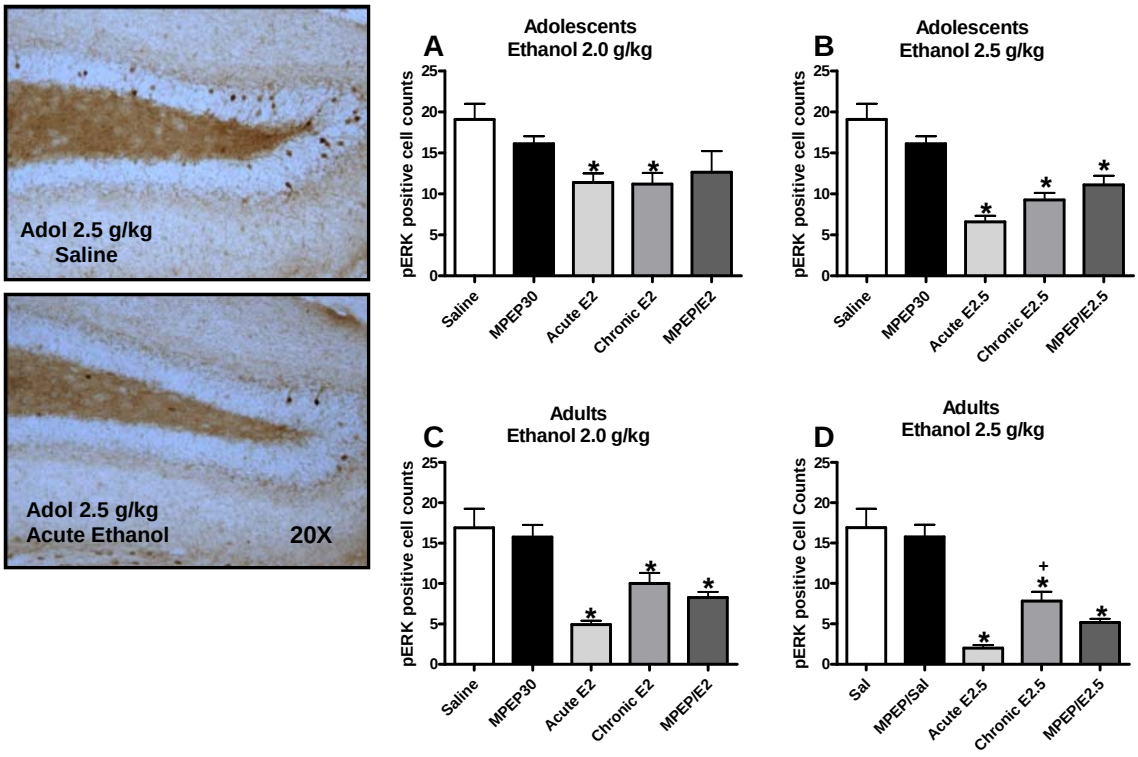

Figure 11. pERK1/2 expression in the Dentate Gyrus is decreased by both acute and chronic ethanol treatment. In the adolescents and adults, pERK1/2 expression is reduced by acute and chronic ethanol (2.0 or 2.5 g/kg). In the adults, chronic treatment with ethanol (2.5 g/kg) produces tolerance to the acute ethanol induced reduction in pERK1/2. Treatment with chronic MPEP (30 mg/kg) and ethanol (2.0 or 2.5 g/kg) does not alter the reduced expression of pERK1/2 caused by ethanol treatment alone. Photos are representative samples from the groups. *=Significantly different than saline, $p<0.05$. +=Significantly different than acute ethanol, $p<0.05$.

Figure 12. PERK$_{1/2}$ in the Nucleus Accumbens Shell.

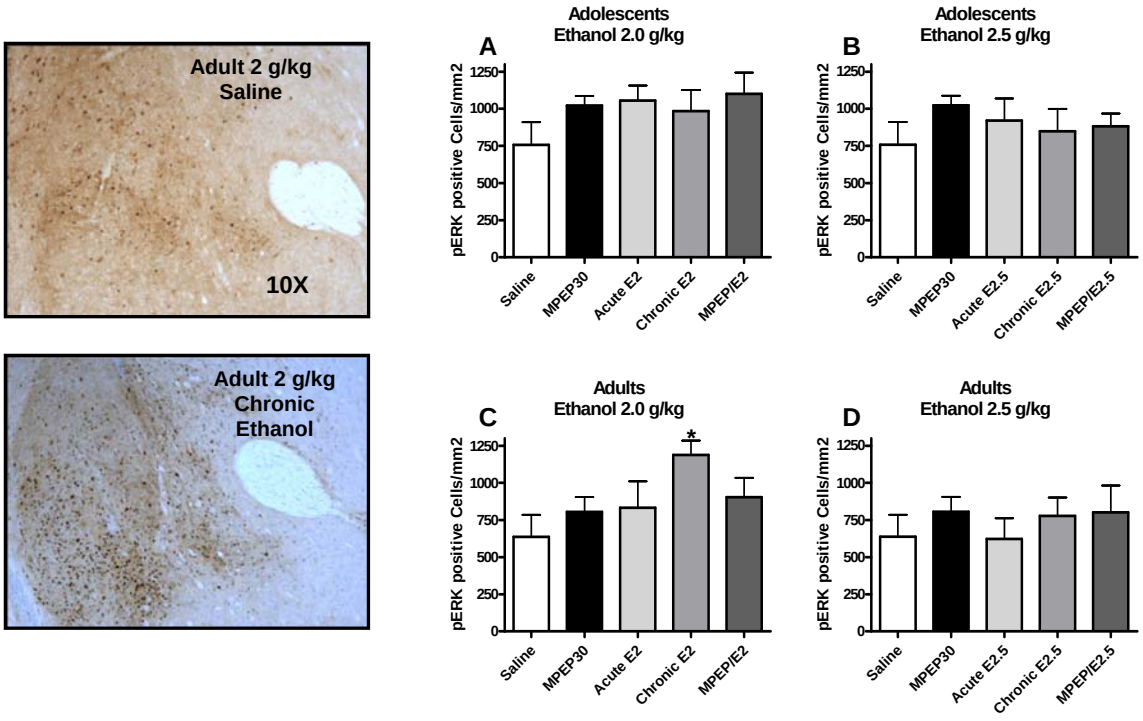

Figure 12. pERK1/2 is increased in the Nucleus Accumbens shell in an age and dose dependent manner. pERK1/2 is increased in the adult mice treated with chronic ethanol (2.0 g/kg) only. pERK1/2 expression returns to baseline levels following Inhibition of ethanol sensitization with MPEP (30 mg/kg). The ethanol 2.5 g/kg dose does not effect pERK1/2 expression. In the adolescents, pERK1/2 expression does not change due to any treatment with ethanol or MPEP. *=Significantly different compared to saline treated group, $p<0.05$.

TABLE 2: PERK$_{1/2}$ Immunohistochemistry Across the Brain.

Adolescent			EtOH 2 g/kg			EtOH 2.5 g/kg		
Brain Regions	Saline	MPEP	Acute EtOH	Sens EtOH	MPEP/EtOH	Acute EtOH	Sens EtOH	MPEP/EtOH
Nuc. Accumbens								
Shell	757.4+/-153.7	1022.8+/-65	1057.3+/-99.8	983.9+/-142.9	1101.4+/-143.2	920.2+/-149.1	848.8+/-150.9	882.8+/-85.5
Core	664.8+/-81.3	930.3+/-42.1	820.8+/-62.1	634.6+/-114	832.7+/-76.6	509.8+/-99.3	540.5+/-79.6	529.4+/-68.7
Prefrontal Cortex								
Medial	329.9+/-36.9	285.4+/-57.8	308.6+/-88.1	335+/-94.2	276.3+/-49.7	490.5+/-39.6	324.3+/-55.4	304+/-52
Amygdala								
Central	405.5+/-106.1	132.9+/-29.7	**1545.4+/-220.2***	449.9+/-128.8	519.7+/-100.9	**2137.2+/-175.8***	631.9+/-206.5	807.8+/-169.5
Basolateral	171.3+/-44	110.7+/-38	150.7+/-68.1	122+/-32.8	83.5+/-23.5	207.5+/-51	222.1+/-51.4	185.2+/-26.1
Hippocampus								
Dentate Gyrus	19.1+/-1.9	16.1+/-0.9	**11.4+/-1.1***	**11.2+/-1.3***	12.6+/-2.6	**6.6+/-0.8***	**9.3+/-0.8***	**11.1+/-1.1***
Thalamus								
PVN	1080.8+/-53.4	782.8+/-67.8	1247.8+/-138.3	894.1+/-139.4	927.1+/-165.2	1428+/-124.8	1238.2+/-84.6	1257.7+/-93.6
Hypothalamus								
PVN	2138.6+/-227.4	1942+/-169.2	2031.5+/-152.6	1677+/-167.5	1884.8+/-206.4	2228+/-182.2	1879.7+/-174	2132.7+/-244.4
Lateral Septum	1234.1+/-201.7	572.6+/-183.3	759+/-182.9	578.6+/-204.6	501.5+/-180.6	962.3+/-250.1	936.1+/-230.2	723.9+/-161.3
Lateral Habenula	1757.2+/-272.4	872+/-148.3	1082.2+/-297.2	964.3+/-241.9	791.4+/-167.6	1304.2+/-187.1	1158.3+/-149.9	1577+/-268.4

TABLE 2: Continued.

Adult			EtOH 2 g/kg			EtOH 2.5 g/kg		
Brain Regions	Saline	MPEP	Acute EtOH	Sens EtOH	MPEP/EtOH	Acute EtOH	Sens EtOH	MPEP/EtOH
Nuc. Accumbens								
Shell	508.2+/-81.1	805.6+/-100.1	832.7+/-179	**1188.8+/-97.5***	905.4+/-128.1	622.7+/-138.4	777.3+/-124.6	801+/-182.4
Core	482.9+/-90.5	640.5+/-83.3	546.3+/-133.3	814.8+/-74.5	723.4+/-90.5	348.7+/-89.9	451.8+/-46.1	583.8+/-95.2
Prefrontal Cortex								
Medial	338.7+/-58	422.3+/-94	376.7+/-32.6	426.6+/-65.6	332.5+/-24.6	497.7+/-22.1	373.7+/-57	341.3+/-36.7
Amygdala								
Central	701.1+/-209	302.7+/-86.3	**1624.8+/-149.2***	228.7+/-34.3	311.4+/-109.2	**1964.3+/-113.6***	509.7+/-189.3	702.2+/-139.8
Basolateral	277.6+/-54.5	258.4+/-55.9	290+/-114.5	200.9+/-100.8	158.9+/-42.2	179.1+/-32.8	206+/-45	233+/-63.6
Hippocampus								
Dentate Gyrus	16.9+/-2.3	15.8+/-1.5	**4.9+/-0.5***	**10+/-1.3***	**8.3+/-0.7***	**2+/-0.4***	**7.8+/-1.1*#**	**5.2+/-0.5***
Thalamus								
PVN	1299.9+/-366.1	921.7+/-164.4	1223.6+/-122.7	1114.8+/-164.1	1251.5+/-161.1	1538.4+/-133.4	1231+/-93.1	1211.9+/-78
Hypothalamus								
PVN	1738.4+/-139.4	1999.7+/-213	2484.4+/-382	2169.8+/-257.6	2352.7+/-228.4	2353.5+/-228.4	2210.6+/-176.8	2056.9+/-134.7
Lateral Septum	935+/-209.8	828.5+/-194.3	723.6+/-253.6	343.7+/-99.5	401+/-121.6	1285.2+/-263.1	764.2+/-223.8	766.2+/-154.6
Lateral Habenula	1488.3+/-107.8	1216.2+/-200.9	1181.4+/-198.8	**608.2+/-156.6***	1037.8+/-216.4	1609.6+/-125	1066.2+/-137.3	1143.7+/-180.6

TABLE 2: Phosphorylated $ERK_{1/2}$ in various brain regions following ethanol sensitization in adolescent and adult mice. Adolescent and adult DBA/2J mice were treated according to the Experimental Design Table 1. The number of phosphorylated $ERK_{1/2}$ pixels/mm^2 is shown for each brain region examined. Significant changes within a region are shown in bold. * denotes groups that are significantly different compared to the saline control, $p<0.05$. # denotes groups that are significantly different compared to acute ethanol 2.5 g/kg, $p<0.05$.

Effect of MPEP on Cocaine Sensitization. In the adolescent group, MPEP had no effect on the acquisition of cocaine sensitization (Figure 13). There was a significant main effect of cocaine treatment [$F(4,80)=45.80$, $p<0.001$], with greater distance traveled on each day as compared to the first day of cocaine exposure, $ps<0.001$. This data pattern indicates the acquisition of cocaine sensitization. The main effect of MPEP dose and the interaction were not significant. The magnitude of sensitization was also not altered by MPEP pretreatment. In the adult group, 30 mg/kg MPEP altered the acquisition pattern of cocaine sensitization (Figure 13C). There was a significant main effect of MPEP treatment [$F(2,84)=3.46$, $p=0.05$], a significant main effect of cocaine exposure [$F(4,84)=73.69$, $p<0.001$], and a significant interaction [$F(8,84)=3.66$, $p=0.001$].

Each MPEP treatment group showed acquisition of cocaine sensitization as evidenced by greater locomotor activity on each day relative to the first cocaine exposure ($ps<0.001$). However, 30 mg/kg MPEP reduced activity relative to saline treatment on the first and second exposure to cocaine ($ps<0.04$). The magnitude of cocaine sensitization was not altered by MPEP pretreatment. Together these data suggest that while cocaine sensitization was observed in each group and the magnitude of sensitization was similar across groups, 30 mg/kg MPEP blunted the initial locomotor activation induced by cocaine.

When tested for cocaine sensitization in the absence of any pretreatment, there were no significant differences in distance traveled among the adolescent groups or among the adult groups (Figure 13B & D). This data indicates that blockade of mGluR5 by 30 mg/kg MPEP during the initiation of cocaine sensitization did not alter the expression of cocaine sensitization.

Figure 13. Effect of MPEP on Cocaine Sensitization.

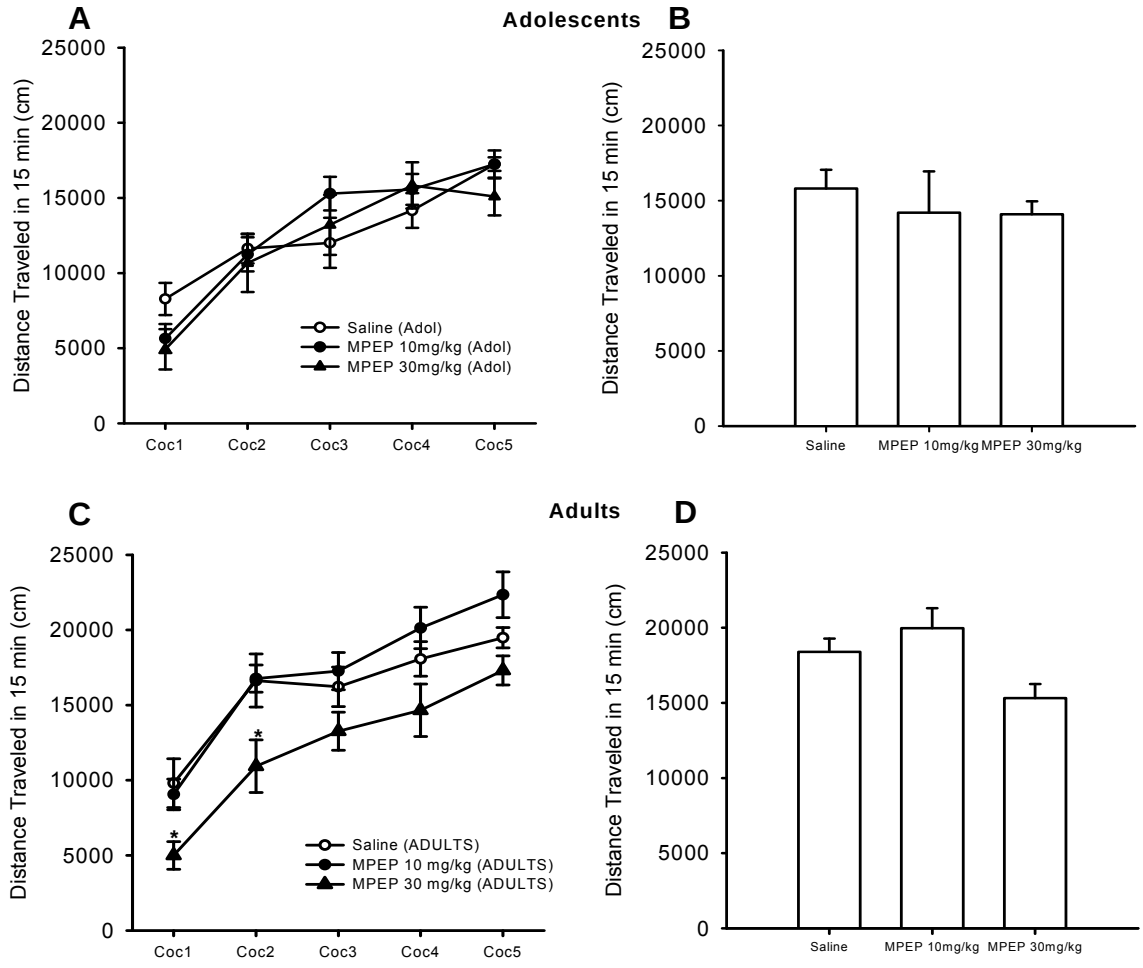

Figure 13. MPEP blunts the acute locomotor response to cocaine in adult mice only. **A.** Distance traveled (cm, +/- SEM) in the first 15-minutes of the locomotor session on treatment days 1,3,5,7, and 9 in adolescent DBA/2J mice. Mice were pretreated with MPEP (0, 10, or 30 mg/kg) 30-minutes prior to treatment with cocaine 10 mg/kg. **B.** Distance traveled (cm, +/- SEM) in the first 15-minutes of the locomotor session on test day 10 following saline pretreatment 30-minutes prior to treatment with cocaine 10 mg/kg in adolescent mice. **C.** A. Distance traveled (cm, +/- SEM) in the first 15-minutes of the locomotor session on treatment days 1,3,5,7, and 9 in adult DBA/2J mice. Mice were pretreated with MPEP (0, 10, or 30 mg/kg) 30-minutes prior to treatment with cocaine 10 mg/kg. *=significantly different compared to saline pretreated group, $p<0.04$. **D.** Distance traveled (cm, +/- SEM) in the first 15-minutes of the locomotor session on test day 10 following saline pretreatment 30-minutes prior to treatment with cocaine 10 mg/kg in adult mice.

DISCUSSION

Adolescent DBA/2J mice are less sensitive than adult mice to ethanol sensitization (Stevenson et al. 2007). However, the mechanisms mediating ethanol sensitization in adolescent or adult mice have not been fully characterized. This study sought to determine the role of the metabotropic glutamate receptor subtype 5, along with the downstream kinase $ERK_{1/2}$, in ethanol sensitization in adolescent and adult mice. Furthermore, the role of mGluR5 in cocaine sensitization in adolescent and adult mice was examined. The results show that the mGluR5 antagonist MPEP blunts ethanol sensitization in adult mice only, and that both sensitization and the blunting of sensitization by MPEP occur in the absence of modulation of $ERK_{1/2}$ phosphorylation. MPEP effected acute stimulant properties of cocaine in adult mice only, with no effect in adolescents.

Previous research has demonstrated that the mGluR5 antagonist MTEP blocks the expression of ethanol sensitization (Kotlinska et al. 2006). Kotlinska et al. initiated sensitization by administering ethanol 2.4 g/kg 4 times, at 3-day intervals. They then administered MTEP 15-minutes before an expression test for sensitization on day 14 and showed that the mice receiving MTEP did not display sensitization. The present study differed from the Kotlinska et al. study by administering MPEP, along with ethanol, on the days when sensitization was developing (days 2-10), but not on the test day, in order to determine the role of mGluR5 in the induction of sensitization. Evidence has emerged that glutamatergic transmission is necessary for the induction of sensitization to cocaine, amphetamine, and opioids, indicating a common glutamatergic mechanism for locomotor sensitization to all drugs of abuse. Together, these studies indicate that mGluR5 is important to both the induction and the expression of ethanol sensitization in adult mice.

A growing body of literature indicates that mGluR5 is involved in the neural and behavioral effects of ethanol (see Introduction). MGluR5 is also known to interact with the NMDA receptor, with research showing that mGluR5 agonists potentiate, while mGluR5 antagonists reduce, NMDA-mediated responses (Attucci et al. 2001; Awad et al. 2000). NMDA receptors have been shown to be involved in many of ethanol's effects, including ethanol sensitization (Broadbent and Weitemier 1999; Kotlinska et al. 2006). In fact, Kotlinska et al. showed that MTEP potentiates the effect of MK-801 on the blockade of the expression ethanol sensitization. Further, MPEP increases the loss of righting reflex induced by both the NMDA receptor antagonist ketamine and ethanol (Sharko and Hodge 2008). Taken together, these data indicate that MPEP may blunt the induction of ethanol sensitization by effecting ethanol's interaction with both mGluR5 and NMDA receptors.

A potential limitation to the present study is the possibility that MPEP is having effects on receptors other than mGluR5. In vitro studies have shown that high concentrations (>20μM) of MPEP can inhibit NMDA receptors (O'Leary et al. 2000). Plasma MPEP levels in mice 30-minutes after treatment with 10 mg/kg MPEP were 0.7 μM, which makes it highly unlikely that levels in the brain following 30 mg/kg MPEP would be sufficient to affect NMDA receptors directly (Anderson et al. 2003). MPEP has also been reported to inhibit norepinephrine (NE) uptake by the norepinephrine transporter (Netzeband and Gruol 1995) and to have effects similar to the standard NET inhibitor desipramine in the locus coeruleus (Heidbreder et al. 2003). However, this report could not rule out whether the in vivo effects of MPEP on NET inhibition were mediated by a direct interaction between MPEP and NET or through an mGluR5 mediated pathway. In fact, MPEP has been shown to mediate extracellular NE levels in the cortex via mGluR5 blockade (Page et al. 2005). Page et al. compared the effectiveness of MPEP to the effectiveness of the newly discovered, more selective

mGluR5 antagonist, MTEP (Cosford et al. 2003). MPEP and MTEP were found to have similar effectiveness in altering cortical NE levels (Page et al. 2005). At this time, there is no data linking blunting of ethanol sensitization with NET inhibition. Therefore, more experiments could be done to fully determine the link between MPEP and NET.

The present study shows that mGluR5 activity is involved in ethanol sensitization in adults, and that activity at mGluR5 is not involved in adolescent sensitization. Evidence that adolescent mice are less sensitive to ethanol sensitization than adult mice suggests that adolescent sensitization might involve a different mechanism than adult sensitization (Stevenson et al. 2007). In fact, adolescent rodents are differentially sensitive to numerous effects of ethanol compared to adults, and this difference in sensitivity seems to be related to the ongoing development of neuronal systems in the adolescent brain (for review, see (Spear and Varlinskaya 2005)). Previous studies examining the effect of ethanol on mGluR5 have all been conducted in adult animals, so it remains unknown what effects ethanol may be having at the mGluR5 in adolescents, if any. Studies have shown that adolescent rodents are more sensitive to the inhibition of NMDA receptors by ethanol in the hippocampus (Swartzwelder et al. 1995a; 1995b). Combined with the fact that NMDA receptor antagonists inhibit ethanol sensitization and that mGluR5 receptors increase the ethanol-induced inhibition of NMDA receptors, one might predict that MPEP would be more effective at preventing ethanol sensitization in adolescent rodents. However, little is known about differences in age-dependent interactions between ethanol and glutamate receptors in other brain regions. Further studies need to be conducted to determine the precise interactions between ethanol, NMDA receptors, and mGluR5 receptors before the present results can be explained.

One goal of the present study was to examine the role of the MAP kinase $ERK_{1/2}$ in ethanol sensitization. Blockade of $ERK_{1/2}$ by SL327 has been shown to prevent the induction (or acquisition) of cocaine and amphetamine sensitization (Valjent et al. 2006).

Although the current study revealed changes in $ERK_{1/2}$ related to ethanol treatment (central amygdala and dentate gyrus), no correlation was discovered between changes in $pERK_{1/2}$ and the induction of ethanol sensitization to both doses of ethanol. The increase in $pERK_{1/2}$ in the nucleus accumbens shell in the ethanol (2.0 g/kg) sensitized adult group, but not the MPEP + ethanol treated adult group or the adolescent group, is intriguing. The nucleus accumbens has been identified as a region critically important for the induction and expression of ethanol sensitization (Kalivas and Stewart 1991). Recently, it has been demonstrated that cocaine sensitization causes an increase in $pERK_{1/2}$ in the nucleus accumbens (Mattson et al. 2005; Yoon et al. 2007). The present data add to this growing literature of a role for $ERK_{1/2}$ signaling in the nucleus accumbens playing an essential role in locomotor sensitization to certain doses of drugs of abuse. The lack of an increase in $pERK_{1/2}$ following ethanol (2.5 g/kg) sensitization could be explained by an interaction between the dose of ethanol administered and the time course of activation of $pERK_{1/2}$. It has been shown that ethanol dose-dependently alters $pERK_{1/2}$ in mouse cortex (Kalluri and Ticku 2002). In the present study, it is possible that an effect of ethanol (2.5 g/kg) sensitization on $pERK_{1/2}$ might occur at a different time point than for ethanol (2.0 g/kg). Without a full time course of the activation of $ERK_{1/2}$ following ethanol sensitization, the immunohistochemistry data remain inconclusive for the role of $pERK_{1/2}$ in ethanol sensitization.

The present study found that adolescent and adult mice both displayed cocaine sensitization, and that cocaine sensitization was not altered by MPEP. Recent studies comparing cocaine sensitization in adolescent and adult rats have shown conflicting results (Camarini et al. 2007; Frantz et al. 2007). The Camarini study was performed in DBA/2J mice and found that adolescent mice displayed sensitization while adult mice did not. The finding that adult mice did not show cocaine sensitization is unusual, as cocaine sensitization in adult mice has been shown repeatedly. The Frantz study

showed that adolescent rats were less sensitive to cocaine sensitization, which we did not observe in the present study. The difference between the two studies is the species employed (i.e. mice vs rats) and the age of the animals (P28-43 vs P37-52). It is likely that development of key neurotransmitter systems such as dopamine and glutamate is different in the two rodent species and at the two different ages.

The finding that acute cocaine-induced locomotor activity was inhibited by MPEP in the adult mice is in line with previous studies (Chiamulera et al. 2001; Herzig and Schmidt 2004; McGeehan et al. 2004). The lack of an effect of mGluR5 antagonists on the induction and expression of cocaine sensitization is also in agreement with previous reports in adult rodents (Dravolina et al. 2006; Herzig and Schmidt 2004). McGeehan et al. (2004) observed that MPEP did not inhibit the acute stimulant properties of high doses of the very selective and potent dopamine reuptake inhibitor, GBR12909. High doses of GBR12909 cause larger and longer-lasting increases in dopamine release in the nucleus accumbens, and it is possible that the locomotor activity induced by these changes in dopamine release are not susceptible to mGluR5 regulation (McGeehan et al. 2004). Cocaine sensitization also leads to similarly increased dopamine levels in the nucleus accumbens, which may explain why mGluR5 is able to block acute cocaine-induced locomotor activity, but is ineffective at blocking the sensitized locomotor response.

The differential effects of MPEP on ethanol and cocaine sensitization are likely due to the different mechanisms of action of the drugs and to the different sensitization methods employed for each drug. As previously discussed, ethanol acts on numerous receptor systems, including directly on mGluR5, whereas cocaine blocks the reuptake of dopamine, serotonin, and norepinephrine (Minami et al. 1998; Ritz et al. 1987). Chronic ethanol self-administration leads to an increase in mGluR5 mRNA in the nucleus accumbens, while chronic psychomotor stimulant injections lead to a decrease in

mGluR5 mRNA and an increase in mGluR1 mRNA in the nucleus accumbens (Mao and Wang 2001). As it has been shown that cocaine sensitization is mediated by mGluR1 but not mGluR5, it seems that these two mGlu receptors are playing different roles in sensitization depending on the drug (Dravolina et al. 2006). It is not surprising that ethanol and cocaine sensitization involve different receptor systems, as it has been shown that the closely related drugs amphetamine and cocaine induce sensitization through different mechanisms (Vanderschuren and Kalivas 2000).

Psychostimulant sensitization is known to be influenced by drug and environmental pairings (Crombag et al. 2000; Robinson et al. 1998). It has been shown that much higher doses of cocaine or amphetamine are required to induce locomotor sensitization when the drug is given intravenously in the home cage rather than in a separate context, such as a locomotor chamber (Browman et al. 1998a; 1998b). However, ethanol sensitization can be expressed robustly when the drug is not paired with the locomotor chamber until the final test day (Fee et al. 2007). Studies from our laboratory have revealed that ethanol sensitization often does not occur when ethanol is paired with the locomotor chambers repeatedly (unpublished observations). In fact, it appears that ethanol sensitization is partially mediated by the novelty of the testing context (Meyer et al. 2005). It was shown that If mice are tested again on the day following the test day (similar to day 11 in the present experiments), the mice show much less activity because the environment is not novel anymore, but they still show sensitization. The authors proposed that this could be related to stress in a novel environment, as they have shown previously that restraint stress could cross sensitize with ethanol (Roberts et al. 1995). Another group has shown that MPEP administration during social defeat stress blocks the expression of amphetamine sensitization in mice, but has no effect on amphetamine-induced amphetamine sensitization (Yap et al. 2005).

Therefore, it is possible that MPEP, a known anxiolytic, blocks ethanol sensitization by modifying the stress response to the “novel” environment of the locomotor chamber.

The lack of an effect of MPEP on cocaine-induced acute locomotor activity and ethanol sensitization in adolescent mice is intriguing. It has been shown that mGluR5 mRNA expression remains about the same from P21 through adulthood, so it seems unlikely that the expression of mGluR5 underlies the age-dependent differences in sensitization and in the response to MPEP (Catania et al. 1994). To our knowledge, the present study is the first study of MPEP in adolescent rodents. Therefore, it is not known if MPEP has similar effects on mGluR5 or similar metabolism in adolescent rodents as have been shown for adult rodents. Studies of depression in adolescents have revealed that adolescents do not respond to tricyclic antidepressants in the same way as adults. Tricyclic antidepressants inhibit reuptake of norepinephrine and serotonin. A review of the literature has shown that although the NE transporter is fully expressed before the onset of adolescence, adult levels of innervation and NE synthesis are not reached until mid-adolescence, and this is likely the reason adolescents do not respond to tricyclic antidepressants (Bylund and Reed 2007). In regard to glutamate, it has been shown that numerous excitatory synapses are being pruned during early adolescence (Spear 2000a). This could mean that, although mGluR5 expression is not different in adolescents compared to adults, the location and activity of mGlu5 receptors differs from adults and causes them to respond differently to drugs such as MPEP. More studies on MPEP and adolescents are necessary to fully address this possibility.

The present study suggests that the mGluR5 antagonist MPEP blunts ethanol sensitization in adult mice while having no effect in adolescent mice. The intracellular pathway by which MPEP is blunting ethanol sensitization does not appear to involve $ERK_{1/2}$ signaling. This study is the first to show that ethanol sensitization is mediated by different mechanisms in adolescent and adult DBA/2J mice.

CHAPTER IV: EFFECT OF ETHANOL SENSITIZATION ON SUBSEQUENT ETHANOL SELF-ADMINISTRATION IN ADOLESCENT AND ADULT DBA/2J MICE

INTRODUCTION

Adolescence is a critical period of development during which children and young animals undergo adaptive changes in behavior and neurobiological systems that bring about the transition into adulthood. Behavioral changes include spending an increased amount of time engaged in social interaction with peers, taking part in risky behaviors, and exploring novel situations, while neurobiological changes include remodeling in the cortex and mesolimbic regions such that glutamatergic and GABAergic neurotransmission is reduced and dopaminergic neurotransmission is increased (Spear 2000a).

The behavioral and neurobiological adaptations that take place during this developmental stage cause the adolescent to be particularly vulnerable to experimenting with drugs of abuse and to subsequent drug-induced neuroadaptations (Crews et al. 2007). In these adolescents, alcohol exposure could disrupt the formation of neural networks and lead to increased drinking later in life (Spear 2002). It is known that people who start drinking during adolescence are four times more likely to become alcohol dependent as adults (Grant 1998). However, the mechanism(s) underlying this finding remain to be fully characterized.

Studies in rodents have shown that adolescents and adults are differentially sensitive to the effects of acute and chronic ethanol exposure. Adolescent rodents are more sensitive to the effects of ethanol on measures of acute locomotor stimulation, anxiety, ataxia, spatial memory, conditioned place preference, and social interaction as

compared to adult rats (Hefner and Holmes 2007; Markwiese et al. 1998; Philpot et al. 2003; Rajendran and Spear 2004; Varlinskaya and Spear 2002; Yttri et al. 2004). By contrast, other studies have shown that adolescent rodents are less sensitive than adult rats to the sedative and motor impairing effects of ethanol, to ethanol withdrawal induced anxiety, and analgesia (Doremus et al. 2003; Hefner and Holmes 2007; Silveri and Spear 1998; Varlinskaya and Spear 2002; White et al. 2002). These differences in the sensitivity of adolescents to ethanol are important because it has been shown that a decreased response to acute alcohol exposure during adolescence is a potent predictor of future alcoholism (Schuckit 1993; 1994).

One model of neurobehavioral adaptations that occur following chronic ethanol exposure is locomotor sensitization. Sensitization is typically defined as a progressive increase in locomotor activity following repeated administration of a drug of abuse (Kalivas and Stewart 1991). The process of sensitization is thought to produce enduring adaptive changes in brain and behavioral function that may underlie components of addiction (Kalivas et al. 1998; Robinson and Berridge 2000). Research has shown that sensitization is mediated by an interconnected network of mesocorticolimbic brain regions (i.e., VTA, nucleus accumbens, prefrontal cortex, amygdala, and thalamus) and neurotransmitter systems (i.e., dopamine, glutamate, and GABA) (Kalivas 1995; Vezina and Kim 1999). These brain regions and neurotransmitter systems all undergo alterations during the adolescent developmental period (Kalivas 1995; Spear 2000a; Vezina and Kim 1999). We have shown that adolescent mice are less sensitive to ethanol sensitization compared to adult mice (Stevenson et al. 2007). Thus, sensitization models are useful tools to determine if adolescent vulnerability to addiction involves differential sensitivity to neurobehavioral changes that occur with repeated drug use.

Ethanol sensitization leads to an increase in subsequent ethanol self-administration in adult DBA/2J mice (Camarini and Hodge 2004; Lessov et al. 2001). It was shown that ethanol sensitized DBA/2J mice consumed more ethanol than both non-sensitized mice and mice with one prior ethanol exposure. Moreover, sons of alcoholics sensitize to the effects of repeated alcohol on motor activity, while the sons of non-alcoholics show tolerance (Newlin and Thomson 1991). Furthermore, adolescent and adult rats that are bred for high alcohol consumption display locomotor activation to ethanol, while low consuming rats do not (Rodd et al. 2004). Sensitization to cocaine and amphetamine also leads to enhancement of self-administration of these drugs (Covington and Miczek 2001; Pierre and Vezina 1998; Vezina 2004; Vezina and Kim 1999). These studies indicate a relationship between locomotor sensitization and drug self-administration in adult rodents. However, it is not known whether ethanol sensitization in adolescence will lead to subsequently greater ethanol self-administration in adulthood.

Although previous data from our laboratory suggests that the $ERK_{1/2}$ pathway is not involved in ethanol sensitization, it has been shown that chronic ethanol exposure followed by withdrawal leads to long-lasting changes in $ERK_{1/2}$ phosphorylation (Sanna et al. 2002). Evidence is emerging that the activity of $ERK_{1/2}$ is modulated in reward-associated brain regions in response to drugs of abuse, including ethanol (Berhow et al. 1996; Kalluri and Ticku 2002; Tsuji et al. 2003; Valjent et al. 2000; Valjent et al. 2004). The $ERK_{1/2}$ pathway is activated when phosphorylated by MEK1/2, which causes $ERK_{1/2}$ to phosphorylate gene transcription factors such as CREB and Elk-1. The regulation of gene transcription by the $ERK_{1/2}$ allows it to mediate long-term changes in behavioral functions (Grewal et al. 1999; Qi and Elion 2005; Sweatt 2004; Thomas and Huganir 2004; Wang et al. 2007). The ability of $ERK_{1/2}$ to modulate long-term neurobiological

changes in response to drug administration makes it an intriguing molecular target for the long-term changes induced by locomotor sensitization.

The present study sought to determine whether the neurobiological changes induced by repeated ethanol treatment during adolescence can lead to enhanced ethanol intake during adulthood. Adolescent and adult DBA/2J mice were treated for ethanol sensitization and were subsequently tested for ethanol intake using a sucrose fading, two-bottle choice procedure. In a separate experiment, mice were treated with the $ERK_{1/2}$ inhibitor SL327 during sensitization and were then tested for ethanol intake to observe the long-lasting effects of $ERK_{1/2}$ modulation on ethanol behaviors.

MATERIALS AND METHODS

Animals. Male 3-week old (adolescent) and 8-week old (adult) DBA/2J mice (Jackson Laboratories, Bar Harbor, ME) were housed in groups (4 animals per cage) in standard Plexiglas cages with food (Purina Rodent Chow) and water available *ad libitum*. The colony was maintained at 27^{o}C on a 12-hour light/dark cycle, with the lights on at 10pm. The behavioral experiments were conducted during the dark portion of the cycle. Mice were handled and weighed daily for 1-week prior to, and for the duration of, the experiment. Animals were under continuous care and monitoring by the Division of Laboratory Animal Medicine at UNC-Chapel Hill, and all procedures were carried out in accordance with the *NIH Guide to Care and Use of Laboratory Animals* (National Research Council, 1996) and institutional guidelines.

Behavioral Apparatus. The locomotor activity (horizontal distance traveled, cm) of adolescent and adult mice was measured in eight covered Plexiglas chambers (30 cm^2, Med Associates, Georgia, VT). Two sets of 16 pulse-modulated infrared photobeams were located on opposite walls to record ambulatory movements in the X-Y (horizontal) plane. All software settings were the same for adults and adolescent mice.

The activity chambers were computer-interfaced (Med Associates) for data sampling at 100-millisecond resolution.

Experimental Procedures. Mice were adapted to the colony and to handling for 1-week (adolescents=P28; adults=P63). On locomotor testing days, mice were taken in the home cage to the testing room at least 30-minutes prior to the session to habituate to the testing room. The first two days of the each experiment were habituation days (H1 and H2). On these days, all mice received a pretreatment intraperitoneal (IP) injection of saline and were placed back into their home cages. Thirty minutes after the pretreatment injection, mice were given an IP injection of saline and were immediately placed in the locomotor chamber for the 10 minute session.

Experiment 1a: Ethanol Sensitization. Adolescent and adult mice received an IP injection of 0, 2.0, or 2.5 g/kg ethanol (n=11-12 per age group per ethanol dose) and were immediately placed in the locomotor chamber for the 10 minute session. Following the acute locomotor session on day 1 (D1), mice received the assigned ethanol dose (0, 2.5, or 3.0 g/kg IP) once daily for nine days (D2-D10) in the home cage. On day 11 (D11), the mice were tested for locomotor sensitization. Mice were injected with 0, 2.0, or 2.5 g/kg ethanol (IP) and placed in the locomotor chamber for 10 minutes

Experiment 1b: Two-Bottle Choice. Immediately following the locomotor session on day 11, all mice were separated into individual cages. The mice were given 2.5 weeks to acclimate to single housing. On day 17 of single housing, the water bottles were removed from the cage top and replaced with two plastic 50-ml graduated drinking bottles. Ethanol self-administration was induced using a sucrose fading procedure. On the first four days, mice were given one bottle with a solution containing 10% sucrose/5% ethanol and one bottle containing water. On the next four days, mice were given access to one bottle containing 5% sucrose/5% ethanol solution and water. During the final four days, mice were given access to 5% ethanol (no sucrose) and

water. The bottles were weighed daily to measure fluid intake, and the location of the solutions was alternated each day to control for side preferences.

Experiment 2a: Effect of SL-327 on Ethanol Sensitization. On day 1, adolescent and adult mice were given a pretreatment injection of 15% DMSO (dimethyl sulfoxide) and returned to their home cages. Thirty minutes later, mice were injected with ethanol (0 or 2.0 g/kg) and immediately placed in the locomotor chamber for 10 minutes (n=8 per age group per pretreatment per ethanol dose). On days 2-10, mice were given a pretreatment injection of SL327 (0 or 30 mg/kg) 30-minutes prior to an injection of ethanol (0 or 2.5 g/kg) in the home cage. No locomotor testing was performed on these days. On day 11, mice were given a pretreatment injection of 15% DMSO and returned to the home cage. Thirty minutes later, mice were injected with ethanol (0 or 2.0 g/kg) and were immediately placed into the locomotor chamber to be tested for locomotor sensitization.

Experiment 2b. Two-Bottle Choice. Immediately following the locomotor session on day 11, all mice were separated into individual cages. The mice were given 2.5 weeks to acclimate to single housing. On day 17 of single housing, the water bottles were removed from the cage top and replaced with two plastic 50-ml graduated drinking bottles. Ethanol self-administration was induced using a sucrose fading procedure. On the first four days, mice were given one bottle with a solution containing 10% sucrose/5% ethanol and one bottle containing 10% sucrose. On the next four days, mice were given access to one bottle containing 5% sucrose/5% ethanol solution and one bottle containing 5% sucrose. During the final four days, mice were given access to 5% ethanol and water (no sucrose). The bottles were weighed daily to measure fluid intake, and the location of the solutions was alternated each day to control for side preferences.

Drugs. For the sensitization experiments, ethanol (95% w/v) was diluted in saline (0.9%) to a concentration of 20% (v/v) and injected (IP) at different volumes to

achieve the appropriate dosage (i.e., 2.0 and 2.5 g/kg). The MEK/$ERK_{1/2}$ inhibitor SL327 (Tocris, Ellisville, MO) was dissolved in 15% DMSO. SL327 was injected IP at a volume of 10 mL/kg. For the two-bottle choice experiments, ethanol (95% w/v) was diluted in distilled water to a concentration of 5% (v/v). Sucrose (w/v; 5 or 10%) was dissolved in the ethanol solution or in distilled water alone.

Behavioral Measures and Data Analysis. Horizontal distance traveled (in centimeters) during the 10-minute session was calculated from the number of photobeam breaks and presented as mean ± SEM. Statistical significance was defined as $p \leq 0.05$ in all experiments.

Experiment 1a: Ethanol Sensitization. Distance traveled (cm) was analyzed separately for adolescents and adults using one-way repeated measure (RM) ANOVA, with day (D1 and D11) as the factor. Sensitization was defined as activity on day 11 being significantly greater than activity on day 1 within an ethanol each ethanol dose, as determined by post-hoc Tukey tests. The data were presented as mean (+/- SEM).

Experiment 1b. Two-Bottle Choice. Ethanol intake (g/kg) was determined from daily measurements of bottle weight and averaged across the four days of access to each solution. Ethanol preference (%) was determined by dividing ethanol solution intake by total fluid intake. The intake and preference of the age groups were analyzed separately using two-way repeated measure (RM) ANOVA, with sensitization treatment (ethanol 0, 2.0, or 2.5 g/kg) and ethanol drinking solution as factors. Post-hoc Tukey's test was used to extract group differences. The data were presented as mean (+/-SEM).

In order to determine if degree of sensitization was related to ethanol intake (g/kg), a linear regression analysis was conducted comparing the degree of sensitization (sensitization index: (day 11 activity/day 1)*100) for the two doses of ethanol (2.0 and 2.5 g/kg) within each age group to the average ethanol intake (g/kg) of each solution.

Experiment 2a: Effect of SL327 on Ethanol Sensitization. Distance traveled (cm) was analyzed separately for adolescents and adults using two-way repeated measure (RM) ANOVA, with pretreatment (SL327 0 or 30 mg/kg) and day (D1 and D11) as factors. Sensitization was defined as activity on day 11 being significantly greater than activity on day 1 within a treatment group, as determined by post-hoc Tukey tests. The data were presented as mean (+/- SEM).

Experiment 2b: Two-Bottle Choice. Ethanol intake (g/kg) was determined from daily measurements of bottle weight and averaged across the four days of access to each solution. Ethanol preference (%) was determined by dividing ethanol solution intake by total fluid intake. The intake and preference of the age groups were analyzed separately using three-way repeated measure (RM) ANOVA, with the between subject factors pretreatment (vehicle or SL327) and sensitization treatment (ethanol 0 or 2.0 g/kg), and the within subject factor ethanol drinking solution. Post-hoc Tukey's test was used to extract group differences. Significant differences were examined using lower order ANOVA, where appropriate. Planned comparisons were used to compare intake of the vehicle/ethanol group versus the SL327/ethanol group. The data were presented as mean (+/-SEM). Statistical significance was set at $p<0.05$.

In order to determine if degree of sensitization was related to ethanol intake (g/kg), a linear regression analysis was conducted comparing the degree of sensitization (sensitization index: (day 11 activity/day 1)*100) for the vehicle and SL327 pretreated ethanol sensitization groups, within each age group, to the average ethanol intake (g/kg) of each solution.

RESULTS

Ethanol Sensitization. As we have previously reported, adolescent mice did not display ethanol sensitization to ethanol (2.0 g/kg) but they did sensitize to ethanol (2.5

g/kg). Adults showed sensitization to ethanol (2.0 and 2.5 g/kg; Figure 14; Stevenson et al., 2007). In the adolescents, treatment with ethanol (2.5 g/kg) caused a significant increase in locomotor activity on day 11 compared to day 1 [$F(1, 9)=6.42$; $p=0.03$]. For the adults, treatment with ethanol (2.0 and 2.5 g/kg) induced a significant increase in locomotor activity on day 11 compared to day 1 [$F(1, 10)=37.85$; $p<0.001$][$F(1, 11)=96.58$; $p<0.001$].

Figure 14. Ethanol Sensitization.

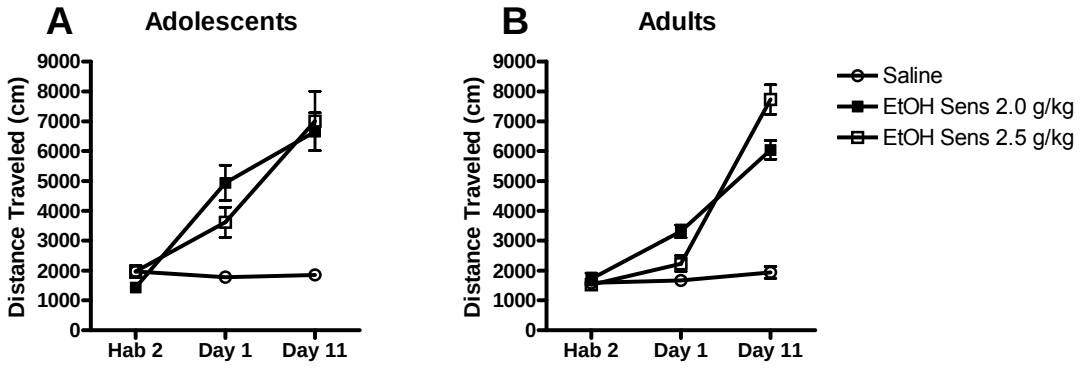

Figure 14. Ethanol sensitization in adolescent and adult DBA/2J mice. **A.** Distance traveled (cm, +/- SEM) following treatment with saline on habituation day 2 and ethanol (0, 2.0, and 2.5 g/kg) on days 1 and 11 in adolescent mice. Ethanol 2.5 g/kg elicited significant sensitization. **B.** A. Distance traveled (cm, +/- SEM) following treatment with saline on habituation day 2 and ethanol (0, 2.0, and 2.5 g/kg) on days 1 and 11 in adult mice. Ethanol 2.0 and 2.5 g/kg elicited significant sensitization.

Two-Bottle Choice. The mice were not treated for the 17 days following ethanol sensitization in order to allow the adolescent mice to become adults. At this time, the sucrose fading procedure began (Figure 15). Analysis of ethanol intake in the adolescent mice showed only a main effect of sucrose concentration in the drinking solution [$F(2, 60)=114.32$; $p<0.001$]. This effect showed that the adolescents drank significantly more sucrose 10%/ethanol 5 % than sucrose 5%/ethanol 5% and ethanol 5%, and more sucrose 5%/ethanol 5% than ethanol 5% ($p<0.001$). The data for ethanol preference showed the same pattern; that is, only a significant main effect of drinking solution [$F(2, 59)=132.07$; $p<0.001$]. The lack of an effect of sensitization treatment indicates that ethanol sensitization did not influence subsequent ethanol intake or preference in the adolescent mice.

In the adult mice, two-way RM ANOVA showed the same pattern as in the adolescents. A significant main effect of drinking solution was found [$F(2, 62)=76.92$; $p<0.001$], indicating that the adult mice drank more solution when the sucrose concentration was higher. Ethanol preference showed the same pattern of results in the adults, with only a significant main effect of drinking solution [$F(2, 62)=69.21$; $p<0.001$]. Therefore, there was no effect of ethanol sensitization on ethanol intake or preference in adolescent or adult mice.

In order to determine whether the degree of sensitization in each mouse correlated with ethanol intake, a linear regression was performed (Figure 16). The sensitization index was calculated by determining the percentage increase in locomotor activity on day 11 compared to day 1, with 100% being an equal amount of activity on each day. The linear regression showed that the degree of sensitization at either ethanol dose (2.0 or 2.5 g/kg) was not correlated with ethanol intake in the adolescent or adult mice.

Figure 15. Ethanol Intake and Preference.

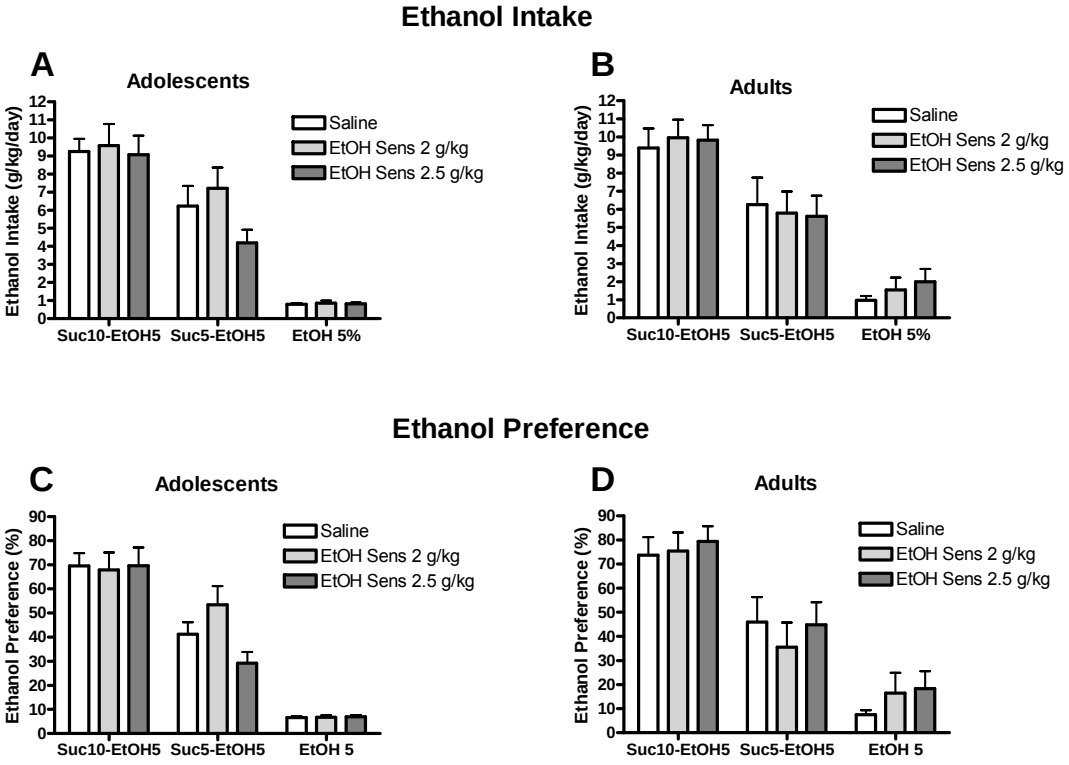

Figure 15. Ethanol sensitization does not affect subsequent ethanol intake or preference in adolescent or adult DBA/2J mice. **A.** Ethanol intake (g/kg) in the sucrose fading procedure in adolescent mice previously treated for sensitization with ethanol (0, 2.0, or 2.5 g/kg). **B.** Ethanol intake (g/kg) in the sucrose fading procedure in adult mice previously treated for sensitization with ethanol (0, 2.0, or 2.5 g/kg). **C.** Ethanol preference (%) in the sucrose fading procedure in adolescent mice previously treated for sensitization with ethanol (0, 2.0, or 2.5 g/kg). **D.** Ethanol preference (%) in the sucrose fading procedure in adult mice previously treated for sensitization with ethanol (0, 2.0, or 2.5 g/kg).

Figure 16. No Correlation Between Ethanol Intake and Sensitization.

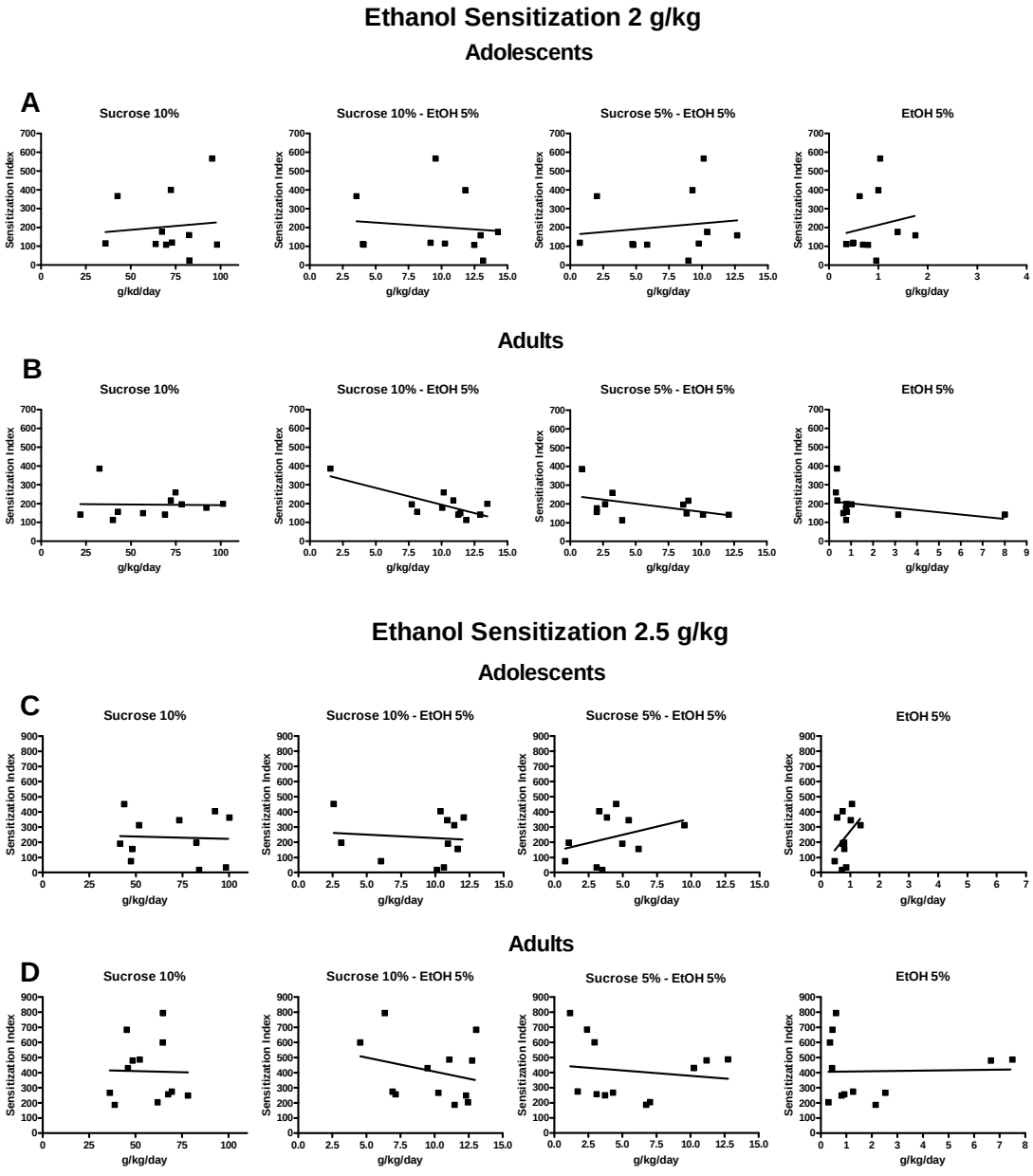

Figure 16. No correlation between ethanol intake and level of ethanol sensitization in adolescent or adult DBA/2J mice. **A.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.0 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adolescent mice. **B.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.0 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adult mice. **C.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.5 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adolescent mice. **D.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.5 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adult mice.

Effect of SL327 on Ethanol Sensitization. Results in Figure 17 indicate that SL327 30 mg/kg did not alter ethanol sensitization in adolescent or adult DBA/2J mice. Two-way RM ANOVA of the adolescents revealed no significant effects, indicating that no sensitization developed in the adolescents. In the adults, two-way RM ANOVA showed a significant main effect of day [$F(1, 13)=77.49$; $p<0.001$], indicating that the adults developed sensitization. Overall, SL327 had no effect on ethanol sensitization in adolescent or adult mice at the dose and pretreatment time used in this study.

Figure 17. SL327 Does Not Alter Ethanol Sensitization.

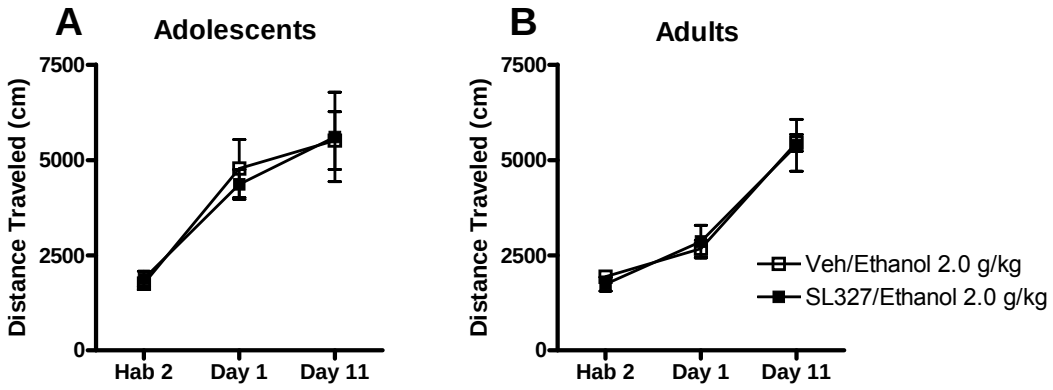

Figure 17. SL327 (30 mg/kg) does not alter ethanol sensitization in adolescent or adult DBA/2J mice. A. Distance Traveled (cm, +/- SEM) following saline treatment on habituation day 2 and ethanol treatment (2.0 g/kg) on days 1 and 11 in adolescent mice. Mice were treated with SL327 (0 or 30 mg/kg) 30-minutes prior to ethanol (2.5 g/kg) on days 2-10. B. Distance Traveled (cm, +/- SEM) following saline treatment on habituation day 2 and ethanol treatment (2.0 g/kg) on days 1 and 11 in adult mice. Mice were treated with SL327 (0 or 30 mg/kg) 30-minutes prior to ethanol (2.5 g/kg) on days 2-10.

Two-Bottle Choice. As in the previous two-bottle choice experiment, the mice were not treated for the 17 days following ethanol sensitization in order to allow the adolescent mice to reach adulthood. The sucrose fading procedure was altered in order to more closely match that of Camarini and Hodge, 2004. For the adolescent group, three-way RM ANOVA of ethanol intake revealed only a significant main effect of the within-subject factor ethanol solution [$F(1, 27)=118.96$; $p<0.001$], indicating that the mice drank less ethanol solution as the sucrose was faded out (Figure 18). Similarly, analysis of the preference data showed only a main effect of ethanol solution [$F(1, 27)=69.54$; $p<0.001$]. There were no effects of SL327 pretreatment of ethanol treatment on ethanol intake of preference in the adolescent mice.

Due to the main effect of ethanol solution on ethanol intake and preference, the data were analyzed separately for each solution. In the analysis of sucrose 10%/ethanol 5% preference, a main effect of pretreatment was revealed [$F(1, 27)=4.90$; $p<0.04$]. This indicated that adolescent mice receiving pretreatment with SL327 during sensitization preferred s10/e5% more than vehicle pretreated mice. Planned comparison t test of s10/e5% preference revealed that SL327/ethanol treated mice preferred the solution significantly more than vehicle/saline treated mice ($p=0.006$). No other significant differences were discovered.

In the adult mice, three-way RM ANOVA of ethanol intake revealed significant main effects of the within subject factor ethanol solution [$F(1, 27)=61.32$; $p<0.001$] and the between subject factors pretreatment [$F(1, 27)=8.53$; $p=0.007$] and ethanol treatment [$F(1, 27)=9.59$; $p=0.005$]. Analysis of ethanol preference in the adults revealed significant main effects of the within subject factor ethanol solution [$F(1, 27)=43.13$; $p<0.001$] and the between subject factors pretreatment [$F(1, 27)=6.58$; $p=0.016$] and ethanol treatment [$F(1, 27)=17.73$; $p<0.001$]. Together, these data indicate that

treatment with SL327 or with ethanol (2.0 g/kg) during ethanol sensitization caused an increase in ethanol intake and preference in the adult mice.

Due to the main effect of drinking solution on intake and preference, the data were analyzed separately for each solution. Analysis of s10/e5% intake showed a significant main effect of pretreatment [$F(1, 27)=4.82$; $p<0.04$] and a significant main effect of ethanol treatment [$F(1, 27)=5.09$; $p<0.04$]. Adult mice pretreated with SL327 or treated with ethanol drank significantly more ethanol solution than vehicle or saline treated mice ($p<0.04$). Preference of s10/e5% was significantly increased by treatment with ethanol [$F(1, 27)=12.79$; $p=0.001$]. Intake of s5/e5% showed similar effects, with a significant main effect of pretreatment [$F(1, 27)=7.63$; $p=0.01$] and treatment [$F(1, 27)=10.96$; $p=0.003$]. Preference for s5/e5% also revealed a significant main effect of pretreatment [$F(1, 27)=6.67$; $p<0.02$] and treatment [$F(1, 27)=16.24$; $p<0.001$]. Finally, analysis of ethanol (5%) preference revealed a significant main effect of ethanol treatment [$F(1, 27)=5.17$; $p<0.05$]. Together, these data indicate that pretreatment with SL327 or treatment with ethanol leads to an increase in ethanol solution intake and preference in adult mice. Planned t-test was used to examine differences in intake and preference between the vehicle/ethanol group and the SL327/ethanol group. Intake of s10/e5% and intake and preference of s5/e5% was significantly greater in the SL327/ethanol group compared to the vehicle/ethanol group ($ps<0.04$). These planned comparisons show that the combination of SL327 and ethanol during ethanol sensitization has an additive effect on increasing future ethanol intake and preference.

In order to examine whether the level of ethanol sensitization correlated with ethanol intake, a linear regression was performed (Figure 19). The results indicate that the level of sensitization did not correlate with ethanol intake in the adolescent or adult mice.

Figure 18. Ethanol Intake and Preference.

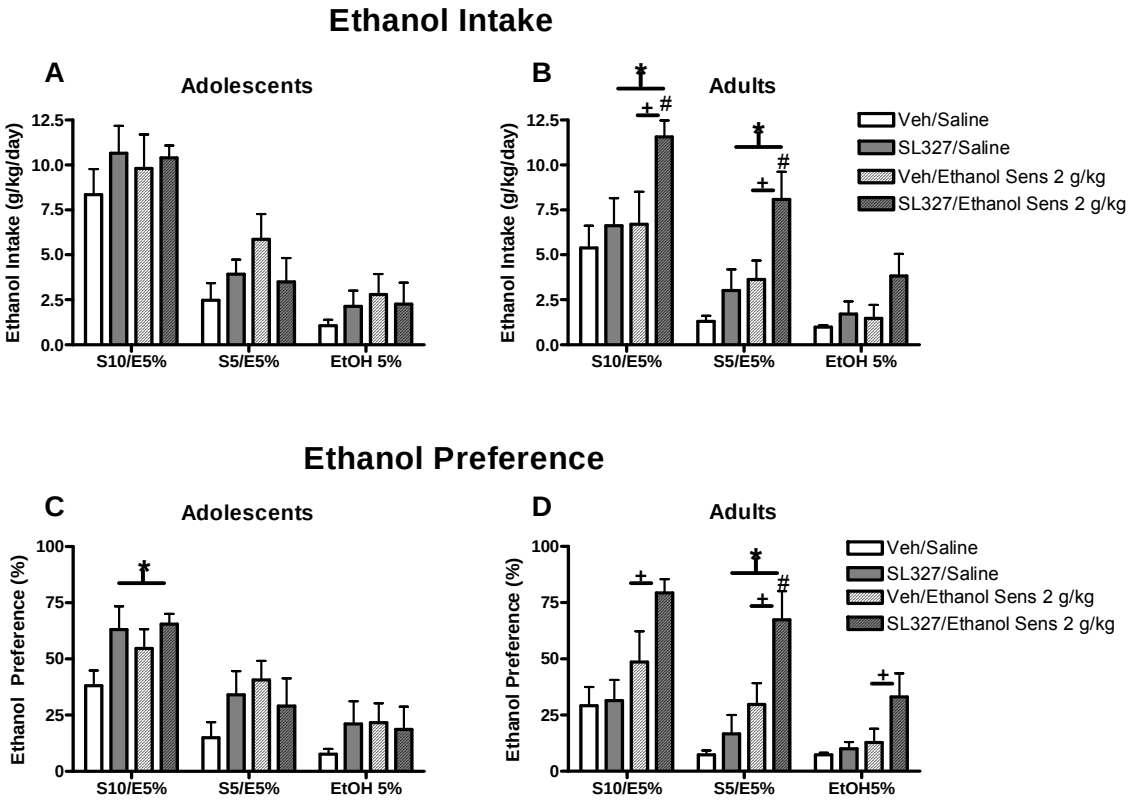

Figure 18. Ethanol sensitization and SL327 treatment increase subsequent ethanol intake and preference in adult DBA/2J mice only. **A.** Ethanol intake (g/kg) in the sucrose fading procedure in adolescent mice previously treated with SL327 (0 or 30 mg/kg) and ethanol (0 or 2.0 g/kg). **B.** Ethanol intake (g/kg) in the sucrose fading procedure in adult mice previously treated with SL327 (0 or 30 mg/kg) and ethanol (0 or 2.0 g/kg). **C.** Ethanol preference (%) in the sucrose fading procedure in adolescent mice previously treated with SL327 (0 or 30 mg/kg) and ethanol (0 or 2.0 g/kg). **D.** Ethanol preference (%) in the sucrose fading procedure in adult mice previously treated with SL327 (0 or 30 mg/kg) and ethanol (0 or 2.0 g/kg). *=significant main effect of SL327 pretreatment, $p<0.05$. +=significant main effect of ethanol treatment, $p<0.05$. #=significant effect of SL327/ethanol compared to vehicle/ethanol by t-test; $p<0.05$.

Figure 19. No Correlation Between Ethanol Intake and Sensitization.

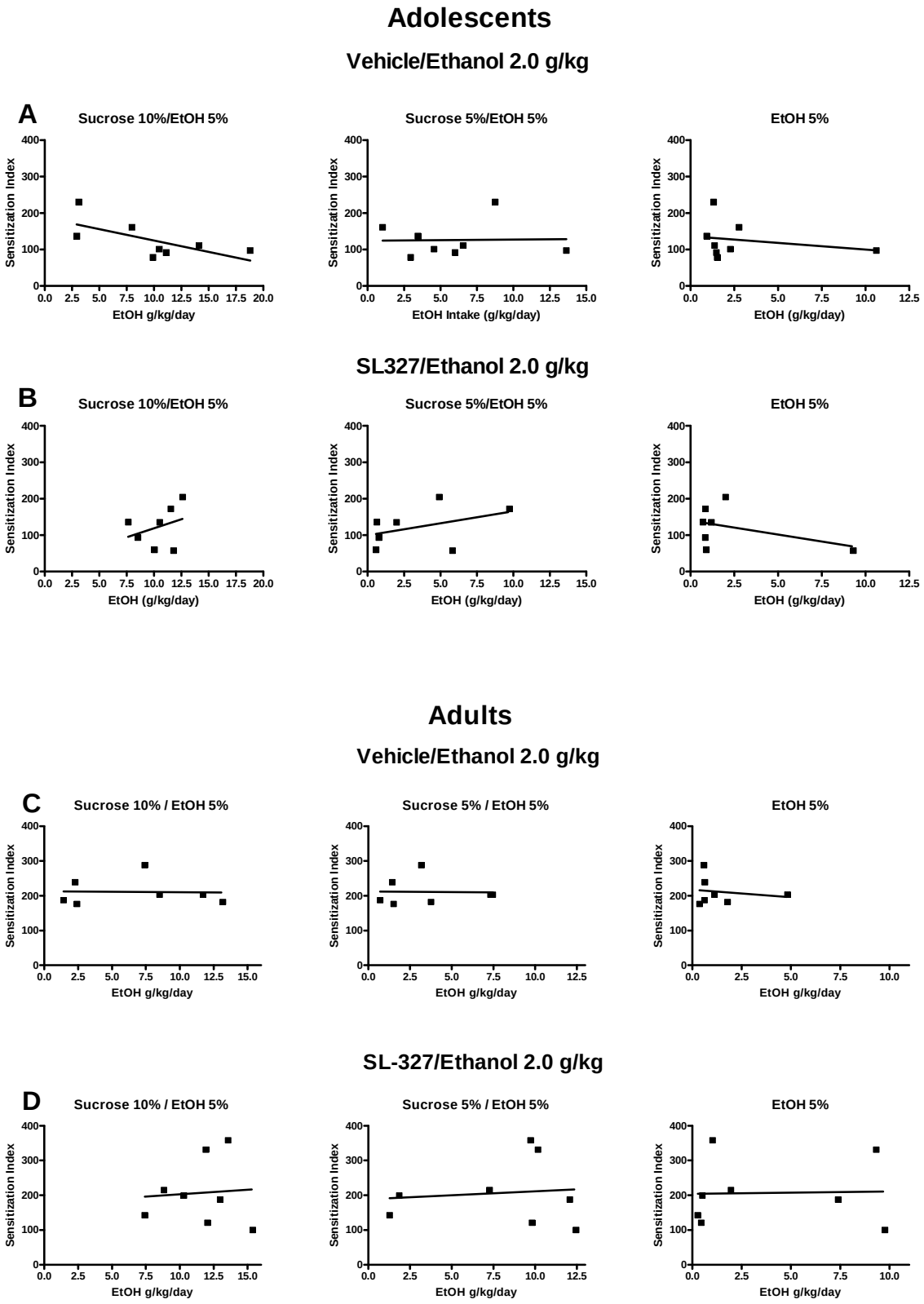

Figure 19. No correlation between ethanol intake and level of ethanol sensitization in adolescent or adult DBA/2J mice. **A.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.0 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adolescent mice pretreated with vehicle (SL327 0 mg/kg). **B.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.0 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adolescent mice pretreated with SL327 (30 mg/kg). **C.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.0 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adult mice pretreated with vehicle (SL327 0 mg/kg). **D.** Linear regression of ethanol intake (g/kg) as a function of level of ethanol 2.0 g/kg sensitization (distance traveled on day 11/distance traveled on day 1 X 100, %) in adult mice pretreated with SL327 (30 mg/kg).

DISCUSSION

Previous studies have shown that ethanol sensitized adult DBA/2J mice self-administer more ethanol than non-sensitized mice (Camarini and Hodge 2004). Since it has been proposed that adolescents are particularly vulnerable to ethanol-induced neuroadaptations that may lead to future alcoholism, this study examined ethanol intake during adulthood in mice that had been treated for ethanol sensitization as adolescents. The results of this study are surprising, as it was discovered that mice exposed to ethanol during adolescence did not show increased ethanol intake in adulthood, whereas mice that were treated during adulthood did show subsequent increases in ethanol intake. The results also suggest that inhibition of $ERK_{1/2}$ during ethanol sensitization can result in subsequently greater ethanol intake and preference in the adult mice.

In the first experiment, neither the adolescent nor adult mice showed an increase in ethanol intake or preference following ethanol (2.0 or 2.5 g/kg) sensitization treatment. This finding is likely due to a difference in the sucrose fading procedure employed in the present study as compared to previous work (Camarini and Hodge 2004). In the present study, mice were first given one bottle containing sucrose (10%) and one containing water. All the mice showed greater than 98% preference for the sucrose solution over the water solution (data not shown). This indicates that the DBA/2J mice in the present study have a high preference for a sweetened solution compared to water. As the sucrose was faded out of the solution, all the groups showed high intake levels of the sweetened solution compared to water. A comparison of ethanol preference in the saline groups from Figure 15 and Figure 18 showed that the mice preferred sweetened ethanol much more versus water (Figure 15) than versus sucrose alone (Figure 18). Together, this data pattern suggests that sucrose fading versus water is not an accurate measurement of ethanol intake because the DBA/2J mice will drink high levels of

sweetened ethanol for the sucrose component (Figure 15). In the second experiment, when both solutions were sweetened (Figure 18; Camarini and Hodge 2004), the effects of previous ethanol treatment on ethanol intake can be revealed because intake and preference for ethanol can be dissociated from intake and preference of sucrose.

Evidence of a link between locomotor sensitization and drug self-administration is mixed. For ethanol, previous research has indicated an increase in self-administration following repeated ethanol treatment in DBA/2J and C57BL/6, but the repeated ethanol treatment did not elicit locomotor sensitization (Camarini and Hodge 2004). Another study found that voluntary ethanol consumption in C57BL/6 mice could elicit subsequent ethanol sensitization (Lessov et al. 2001). The present study found an increase in ethanol self-administration in DBA/2J adult mice following ethanol sensitization, while the adolescent mice, which did not show sensitization, did not show an increase in self-administration. One conclusion to be drawn from the present data is that locomotor sensitization to ethanol is necessary for subsequent ethanol self-administration. However, the correlation data indicate no relationship between locomotor activity and ethanol intake in any group. This would indicate that repeated ethanol treatment in adults could lead to an increase in ethanol intake that is not related to locomotor activity. In fact, many studies show that drug exposure leads to subsequent increases in drug self-administration or reward, unrelated to sensitized locomotor activity (Horger et al. 1992; Lorrain et al. 2000; Valadez and Schenk 1994). Further, pretreatment with amphetamine, morphine, and cocaine leads to enhanced conditioned place preference of these drugs (Lett 1989). Overall, there appears to be a link between drug pre-exposure and drug self-administration in adult animals but locomotor response to ethanol appears to be dissociated from this effect.

Dopamine neurotransmission between the ventral tegmental area and the nucleus accumbens is thought to underlie both the acute effects of drugs, including

locomotor activity, and drug self-administration (Wise and Bozarth 1987). For the psychomotor stimulant drugs, locomotor sensitization leads to a sensitized dopamine release in the nucleus accumbens, which is though to underlie locomotor sensitization and subsequent self-administration (Vezina 2004). However, ethanol may differ from other drugs of abuse as it has been shown that ethanol sensitization does not cause a sensitized dopamine response in the nucleus accumbens (Zapata et al. 2006). These data indicate that the link between ethanol sensitization and ethanol self-administration may involve other neurotransmitters.

Glutamate has been shown to be a major player in psychostimulant sensitization, and previous evidence from our lab and others has shown that antagonists of glutamate receptors can inhibit both ethanol sensitization and ethanol self-administration (Broadbent et al. 2003; Hodge et al. 2006; McMillen et al. 2004; Vanderschuren and Kalivas 2000). Therefore, it is possible that long-lasting upregulation of glutamatergic neurotransmission underlies the ability of ethanol sensitization to increase ethanol self-administration in the present study. It has been shown that glutamate neurotransmission, and not dopamine neurotransmission, in the nucleus accumbens mediates relapse to cocaine seeking (Cornish and Kalivas 2000). Furthermore, it has been shown that glutamatergic neurotransmission is being pruned throughout adolescence. Perhaps these ongoing changes in glutamate transmission prohibit an increase in ethanol self-administration in the adolescent mice.

The data from the present study implicate long-lasting changes in $ERK_{1/2}$ signaling as a mediator of increased ethanol intake. It has been shown that both chronic ethanol exposure and SL327 treatment inhibit $ERK_{1/2}$ phosphorylation, and that chronic ethanol exposure followed by withdrawal leads to long-lasting changes in $ERK_{1/2}$ phosphorylation (Sanna et al. 2002). The present study suggests that inhibition of $ERK_{1/2}$ phosphorylation with SL327, while simultaneously effecting $ERK_{1/2}$

phosphorylation with ethanol (2.0 g/kg), leads to an increase in ethanol intake 17 days later in adult mice. It is known that the $ERK_{1/2}$ signaling cascade is downstream from G-protein coupled receptors and Ca2+ signaling, which can be mediated by glutamate and dopamine receptors (Haddad 2005; Roberson et al. 1999). We hypothesize that ethanol and SL327 during ethanol sensitization lead to a decrease in $ERK_{1/2}$ phosphorylation, and this is followed by a subsequent increase in $ERK_{1/2}$ phosphorylation during 17 days with no treatment. Increases in $ERK_{1/2}$ phosphorylation might cause changes in gene expression and synaptic plasticity that could lead to the increase in ethanol intake observed in the present study (Di Cristo et al. 2001; Grewal et al. 1999; Qi and Elion 2005; Sweatt 2004; Thomas and Huganir 2004; Wang et al. 2007). In fact, it has recently been shown that cocaine sensitization in adult rats followed by 14-days of withdrawal leads to an increase in phosphorylated $ERK_{1/2.}$ This increase in $pERK_{1/2}$ was associated with an increase in AMPA receptor expression in the nucleus accumbens of previously sensitized rats (Boudreau et al. 2007). As it has been shown repeatedly that AMPA receptors are involved in drug-seeking in adult rodents, the authors hypothesized that the increase in AMPA receptors might cause the sensitized animals to be at a greater vulnerability for drug-seeking (Boudreau and Wolf 2005; Fasano and Brambilla 2002; Gerdeman et al. 2003; Sutton et al. 2003; Winder et al. 2002). Therefore, a similar cascade of events might underlie the propensity of the sensitized and/or SL327 treated adult mice in the current study to drink more ethanol. Interestingly, a study comparing the long-lasting effects of nicotine exposure during adolescence or adulthood on AMPA receptor expression showed that, two-months after nicotine treatment, AMPA receptor expression in the striatum was decreased in the animals exposed during adolescence and increased in the animals exposed during adulthood (Adriani et al. 2004). Thus, it is possible that the adolescent mice in the present study did not increase

ethanol intake because of developmental differences in ethanol-induced AMPA receptor expression in the nucleus accumbens.

The present study showed that ethanol sensitization treatment during adulthood, but not adolescence, leads to a subsequent increase in ethanol intake and preference. The data revealed that this effect is enhanced by inhibition of $ERK_{1/2}$ phosphorylation, possibly downstream from glutamate receptors, and that the differences in adolescent and adult mice might be due to differences in glutamate signaling during development. As it has been proposed that adolescents are more vulnerable to drug-induced neuroadaptations which lead to a propensity to develop alcoholism in adulthood (Spear 2000a), we hypothesized that ethanol sensitization in adolescent mice would cause a significant increase in ethanol intake during adulthood. The results of this study, however, suggest that ethanol sensitization is not a model of the adolescent vulnerability to alcoholism. Instead, ethanol sensitization appears to be a model of adult neurobiological changes that occur which can lead to the propensity to increase ethanol intake.

CHAPTER V: GENERAL CONCLUSIONS

DISCUSSION

The results from the three sets of experiments show that adolescent DBA/2J mice are less sensitive to ethanol sensitization, to the modulation of ethanol sensitization by the mGluR5 antagonist MPEP, and to ethanol intake following ethanol sensitization. These data add to a growing body of literature indicating that adolescent rodents are differentially sensitive to drugs of abuse. The age-dependent differences in sensitivity have been proposed to be due to the ongoing developmental neurobiological changes in the adolescent brain, and the present studies support this theory.

Pierce and Kalivas (1997) proposed that the neurobiological changes which underlie locomotor sensitization include increased glutamatergic output from the prefrontal cortex to the nucleus accumbens and VTA along with increased dopaminergic output from the VTA to the nucleus accumbens, as shown in Figure 20. We propose that adolescent mice are less sensitive to ethanol sensitization based on the neurobiological changes taking place during the developmental period, as illustrated in Figure 21. These changes include decreased glutamatergic output from the prefrontal cortex to the nucleus accumbens and VTA, which are opposite to the critical increases in glutamatergic output which underlie adult sensitization.

The possibility that differences in glutamatergic system development are important for age-dependent differences in sensitization is supported by the second set of experiments. That is, the metabotropic glutamate receptor subtype 5 antagonist MPEP was able to significantly blunt ethanol sensitization in the adult mice, but was ineffective in altering sensitization in the adolescent mice (Figure 22). Along with

studies from other laboratories, it seems clear that glutamate neurotransmission is critical for ethanol sensitization in adult mice. In adolescent mice, however, MPEP did not blunt ethanol (2.5 g/kg) sensitization which indicates that mGluR5 may not be involved in adolescent ethanol sensitization. It is possible that in adolescent mice, dopaminergic or GABAergic neurotransmission is more important for ethanol sensitization than glutamatergic neurotransmission. It has been shown that ethanol induces greater GABA receptor-mediated inhibitory postsynaptic currents in adult rats compared to adolescent rats (Li et al. 2006). We can speculate that the high ethanol dose (2.5 g/kg) in adolescent mice is able to elicit strong enough GABA-mediated inhibition from the nucleus accumbens to the ventral pallidum, thus allowing ethanol sensitization to develop independent of glutamatergic input to the nucleus accumbens. Since glutamatergic signaling is critical for adult ethanol sensitization, it is unclear why GABA might be more important in adolescent sensitization.

It has been proposed that age-dependent differences in sensitivity to ethanol might underlie the propensity for ethanol intake during adolescence to lead to alcoholism later in life (Spear and Varlinskaya 2005). The finding that adolescents are less sensitive to ethanol sensitization is significant because it has been shown in humans that sons of alcoholics, a group at high risk for developing alcoholism, are differentially sensitive to the physiological effects of ethanol when given repeated ethanol treatments (Newlin and Thomson 1991). It is possible, therefore, that blunted sensitivity to the neuroadaptations that occur during the induction of ethanol sensitization in adolescents may be one factor that contributes to the epidemiological observation that adolescent alcohol use is associated with increased risk of abuse in adulthood (Grant and Dawson 1998)

The final set of experiments was designed to directly assess the hypothesis that adolescent mice are more vulnerable than adult mice to the long-term neurobiological

changes induced by ethanol sensitization. In this experiment, all mice were treated for ethanol sensitization, followed by two-bottle sweetened ethanol intake 17 days later. The results indicate that adolescent mice are less vulnerable than adult mice to the effects of ethanol sensitization on subsequent ethanol intake. That is, ethanol sensitization in the adult mice caused a subsequent increase in ethanol intake and preference, whereas ethanol sensitization in adolescent mice caused no changes in subsequent ethanol intake or preference. Although the results could be due to several factors, as presented in the experiment 3 discussion section, it is possible that ethanol sensitization is not an appropriate measure of the adolescent vulnerability to alcoholism. In both the adolescent and adult mice, ethanol sensitization was not correlated with ethanol intake. This would indicate that locomotor activity is dissociated from drug intake, and therefore may not be the best available method for assessing the neuroadaptations that lead to drug intake.

This conclusion is further supported by the results from the second part of experiment 3. In this study, adult mice that received the drug SL327 during ethanol sensitization did not show any difference in sensitization compared to vehicle treated mice. However, treatment with SL327 during ethanol sensitization caused a significant increase in subsequent ethanol intake in the adult mice. These results indicate that a drug which does not alter ethanol sensitization is able to affect ethanol intake, again dissociating ethanol sensitization from intake.

Despite the lack of a correlation between locomotor sensitization and ethanol intake, the results suggest that changes in glutamatergic signaling induced by repeated ethanol treatment in the adult mice can affect subsequent ethanol intake. The models in Figures 23 and 24 show the proposed changes that may occur following ethanol sensitization treatment in the adult mice, which could underlie the later increase in ethanol intake and preference. That is, chronic ethanol exposure leads to a

downregulation of $ERK_{1/2}$ phosphorylation. When ethanol is no longer present, this downregulation is followed by a compensatory upregulation in $ERK_{1/2}$ phosphorylation. When ethanol is re-introduced, there is even greater upregulation of $ERK_{1/2}$ phosphorylation, and this upregulation may underlie a progressive increase in ethanol intake (Sanna et al. 2002). We hypothesize that the upregulation in ERK1/2 phosphorylation leads to an increase in glutamate signaling from the prefrontal cortex to the nucleus accumbens, and that this increase in glutamatergic neurotransmission is responsible for the greater levels of ethanol intake observed in the present study (Figure 24).

Overall, the clinical data clearly show that human adolescents are vulnerable to drug-induced neuroadaptations that lead to an enhanced susceptibility to alcoholism. The present studies add to the rodent literature showing that adolescents respond differently than adults to drugs of abuse, including ethanol. This differential responding is likely due to the ongoing neurobiological changes that take place during the adolescent period, such as the pruning of glutamatergic synapses. These experiments raise the concern that ethanol sensitization may not be the best method for modeling the adolescent vulnerability to future alcoholism.

FUTURE DIRECTIONS

Future studies will examine the brain regional involvement in ethanol sensitization in both adolescent and adult mice. In the present experiments, c-Fos and ΔFosB immunohistochemistry did not show any sensitization-related changes in expression (data not shown). Future experiments will use immunohistochemistry to the transcription factor CREB, which has been linked to numerous addiction related behaviors (Pandey 2004). This examination will elucidate gene-expression changes in brain regions that might underlie the behavioral differences observed in the two age-

groups. In order to further probe the lack of an effect of MPEP in adolescents, immunohistochemical analysis examining mGluR5 expression in adolescent and adult mice should be conducted. Although previous studies have shown that mRNA expression of mGluR5 remains the same from P21 through adulthood, the immunohistochemical study would determine if the mGluR5 is expressed in the same number and location in adolescent and adult DBA/2J mice (Catania et al. 1994).

As the present study was the first to use MPEP in adolescents, the effects of MPEP in adolescent mice remain unknown. In adults, MPEP is known to increase the ethanol-induced loss of righting reflex and to increase time spent in the open arm of the elevated plus maze (Sharko and Hodge 2008; Spooren et al. 2000). Therefore, a study of the effectiveness of MPEP in adolescent mice could examine the effect of MPEP on ethanol-induced loss of righting reflex or the effect of MPEP in the elevated plus maze.

Another future study to be conducted is the analysis of phosphorylated $ERK_{1/2}$ in the adult mice following the sucrose-fading/ethanol intake experiment. We could examine directly the hypothesis that long-term changes in $pERK_{1/2}$ expression underlie the effect of SL327 and ethanol during ethanol sensitization to lead to an increase in subsequent ethanol intake.

Figure 20. Neurocircuitry of Ethanol Sensitization in Adults.

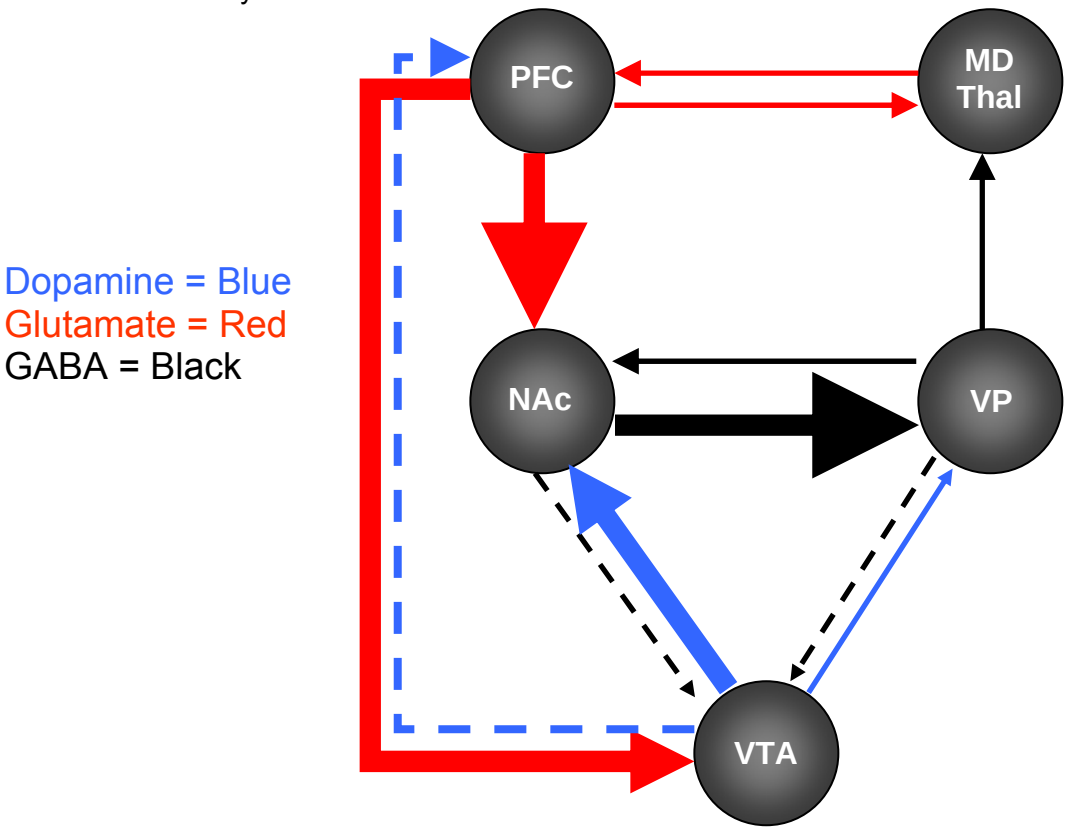

Figure 20. Neurocircuitry following ethanol sensitization in adult DBA/2J mice. Proposed changes that occur due to locomotor sensitization in adult mice, adapted from Pierce and Kalivas 1993. Locomotor sensitization occurs following an increase in dopaminergic neurotransmission from the VTA to the NAc, along with increases in glutamatergic neurotransmission from the PFC to the NAc. Dashed lines indicate decreases in neurotransmission, while bold lines indicate increases. VTA=ventral tegmental area; NAc=Nucleus Accumbens; PFC=Prefrontal Cortex; VP=Ventral Pallidum; MD Thal=Medial dorsal Thalamus

Figure 21. Neurocircuitry During the Adolescent Period.

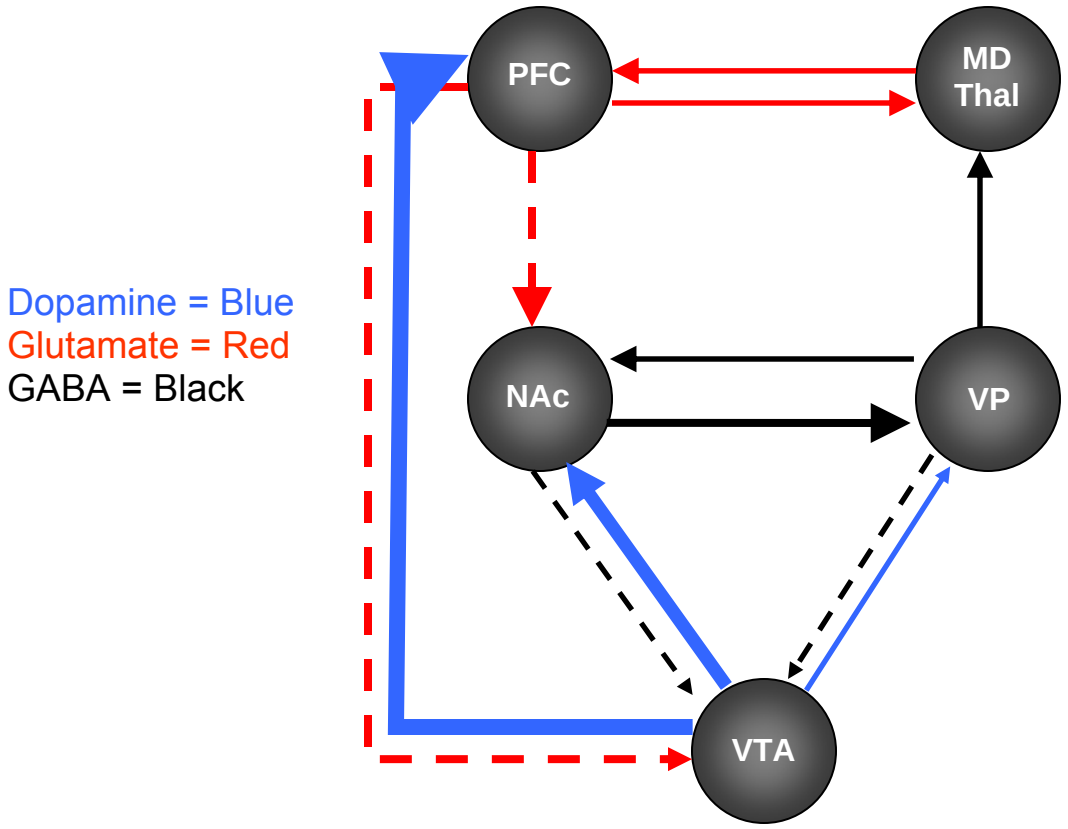

Figure 21. Neurocircuitry adaptations occurring during the adolescent period of development. Proposed changes occurring in the adolescent include decreases in glutamatergic output from the PFC to the VTA and increases in dopaminergic output from the VTA to the PFC and NAc. Dashed lines indicate decreases in neurotransmission, while bold lines indicate increases. VTA=ventral tegmental area; NAc=Nucleus Accumbens; PFC=Prefrontal Cortex; VP=Ventral Pallidum; MD Thal=Medial dorsal Thalamus

Figure 22. Proposed Effect of MPEP on Ethanol Sensitization.

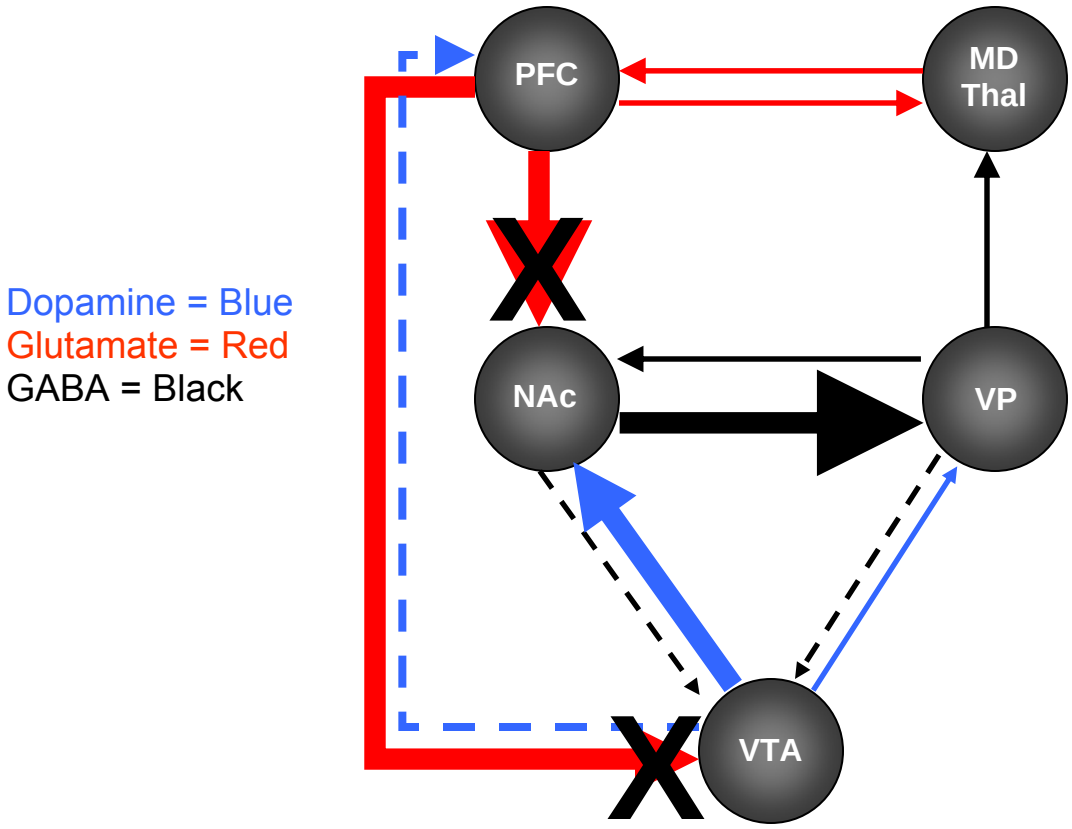

Figure 22: Effect of MPEP on ethanol sensitization. In adults, MPEP blocks the critical glutamatergic transmission from the PFC to the NAc and the VTA, which inhibits ethanol sensitization (shown with the X). Dashed lines indicate decreases in neurotransmission, while bold lines indicate increases.

Figure 23. Proposed Regulation of $ERK_{1/2}$.

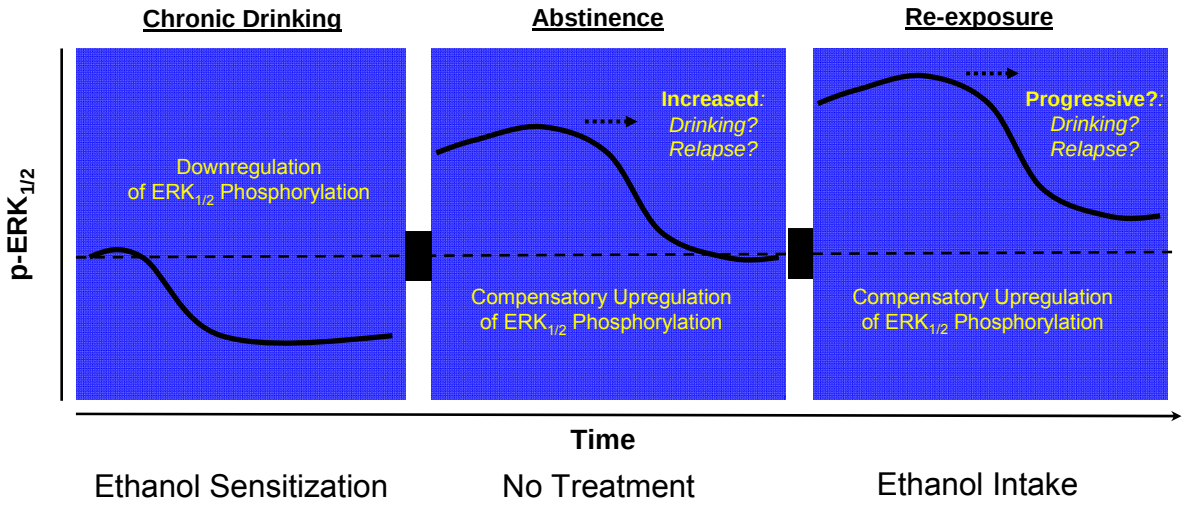

Figure 23. Proposed regulation of $ERK_{1/2}$ phophorylation following ethanol exposure, abstinence, and re-exposure. Chronic ethanol exposure has been shown to downregulate the phosphorylation of $ERK_{1/2}$. This downregulation is followed by an upregulation in $ERK_{1/2}$ phosphorylation during abstinence from ethanol. When ethanol is re-introduced, there is even greater upregulation of $ERK_{1/2}$ phosphorylation which may underlie a progressive increase in ethanol intake. Based on Sanna et al 2002.

Figure 24. Proposed Long-Term Changes Produced By Ethanol Sensitization.

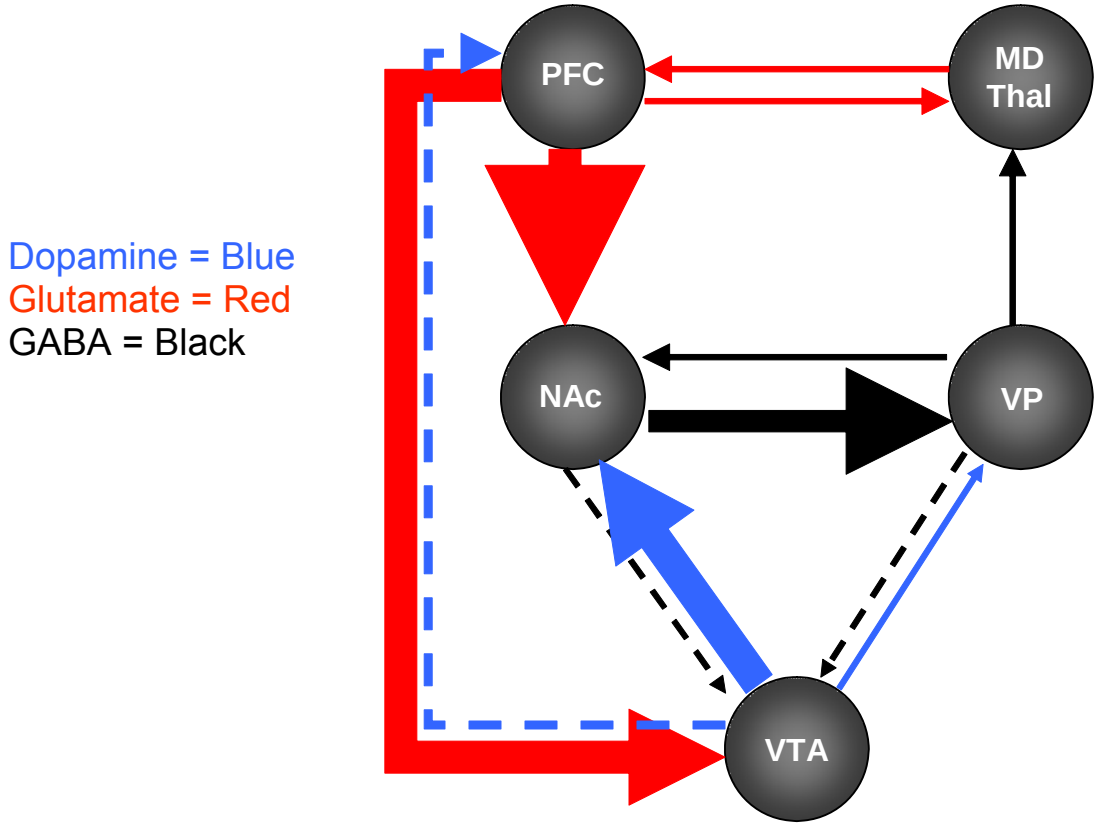

Figure 24. Proposed long-term changes induced by ethanol or SL327 treatment during ethanol sensitization in adult DBA/2J mice. We propose that treatment with SL327 or ethanol during ethanol sensitization significantly inhibits $pERK_{1/2}$, but that a compensatory long-term increase in $pERK_{1/2}$ leads to increased glutamatergic signaling, which might underlie the propensity of the adult mice to self-administer more ethanol. Dashed lines indicate decreases in neurotransmission, while bold lines indicate increases.

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