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The effects of alcohol and nicotine pretreatment during adolescence on adulthood responsivity to alcohol

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The Effects of Alcohol and Nicotine Pretreatment During Adolescence on Adulthood
Responsivity to Alcohol

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Masters of Arts
Department of Psychology
College of Arts and Sciences
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Dedication

To my mom, Sylvia Ann Valdez. Mom, you are my very best friend and I do not know what I would have done without all of the support you gave me through every single step of this journey. You make such a difference in my life and I cannot even begin to describe how grateful I am to have you still here with me. I thank God everyday for letting me keep you here with me to enjoy it all each and every day.

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Table of Contents

List of Figures	iii
Abstract	iv
Chapter One: Background	1
Human drug use: Emphasis on Adolescent Alcohol and Cigarette Use	1
Animal Model of Adolescence	3
Effects of Alcohol on Behavior	4
Adults	4
Adolescents	5
Effects of Ethanol in the Brain	5
Adults	5
Adolescents	7
Long-term Neurobehavioral Effects of Ethanol Exposure During Adolescence	8
Effects of Nicotine on Behavior	9
Adults	9
Adolescents	10
Effects of Nicotine in the Brain	10
Adults	10
Adolescents	11
Combined Effects of Alcohol and Nicotine on Behavior	12
Adults	12
Adolescents	13
Combined Effects of Alcohol and Nicotine in the Brain	14
Overview of the Present Study	15
Hypotheses	16
Chapter Two: Effects of Alcohol and/or Nicotine Pretreatment During Adolescence On Adulthood Alcohol-Related Behaviors	
Materials and Methods	
Subjects	18
Apparatus	18
Open Field	18
Conditioned Place Preference Apparatus	19
Procedure	19
Pretreatment	19
Novelty Preference	20

Conditioned Place Preference	20
Design and Analyses	21
Chapter Three: Results	23
Effects of Ethanol and/or Nicotine Pretreatment on Novelty-Related Behaviors in Adulthood	23
Novelty-Induced Exploration	23
Novelty Preference	24
Novel Environment Induced Exploration	25
Examining the Rewarding Effects to Ethanol After Adolescent Treatment of Alcohol and/or Nicotine	26
Chapter Five: Discussion	30
Comorbid Use of Alcohol and Nicotine in Humans	30
Conditioned Place Preference Paradigm and Ethanol History	30
Combined Ethanol and Nicotine Treatment	33
Conclusions	36
References	38

List of Figures

Figure 1A:	Ethanol and/or Nicotine During Adolescence Did Not Alter Novelty-Induced Exploration in Adulthood: Chronic Ethanol and/or Nicotine	24
Figure 1B:	Ethanol and/or Nicotine During Adolescence Did Not Alter Novelty-Induced Exploration in Adulthood: Repeated Intermittent Ethanol and/or Nicotine	24
Figure 2A:	Ethanol and/or Nicotine During Adolescence Did Not Alter Novelty Preference in Adulthood: Chronic Ethanol and/or Nicotine	25
Figure 2B:	Ethanol and/or Nicotine During Adolescence Did Not Alter Novelty Preference in Adulthood: Repeated Intermittent Ethanol and/or Nicotine	25
Figure 3A:	Ethanol and/or Nicotine During Adolescence Did Not Alter Novel Environment Induced Exploration in Adulthood: Chronic Ethanol and/or Nicotine	26
Figure 3B:	Ethanol and/or Nicotine During Adolescence Did Not Alter Novel Environment Induced Exploration in Adulthood: Repeated Intermittent Ethanol and/or Nicotine	26
Figure 4:	Chronic Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Conditioned Place Preference in Adulthood	28
Figure 5:	Repeated Intermittent Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Conditioned Place Preference in Adulthood	29

The Effects of Alcohol and Nicotine Pretreatment During Adolescence on Adulthood

Responsivity to Alcohol

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ABSTRACT

Adolescence is a period of development that is associated with increased risk taking behaviors and experimenting with drugs of abuse, including alcohol and nicotine. Early onset of use of these agents may be associated with long-term changes in behavior and enhanced sensitivity to the subsequent effects of alcohol in adulthood. The present experiment was designed to assess the long-term behavioral alterations that occur due to adolescent exposure to ethanol and nicotine, either alone or in combination, on adulthood responsivity to the rewarding properties of environmental cues paired with ethanol. It was hypothesized that adolescent rats exposed to the combination of ethanol and nicotine would exhibit enhanced novelty seeking behaviors in adulthood. When assessing the rewarding properties of environmental cues paired with ethanol in adulthood using the CPP paradigm, it was hypothesized that adolescent rats exposed to the combination of a moderate dose of alcohol (0.75 g/kg) and nicotine (0.4 mg/kg) would more readily acquire a CPP in adulthood as compared to animals exposed to either drug alone. However, no changes in novelty seeking behaviors or conditioned place preference in adulthood were observed due to exposure to ethanol and/or nicotine during adolescence.

Methodological considerations are discussed. Currently, other experiments are being conducted to assess the effects of nicotine on voluntary ethanol treatment in adolescent and adult male rats.

Chapter One: Background

Human Drug Use: Emphasis on Adolescent Alcohol and Cigarette Use

Alcohol and cigarettes are two of the most widely used drugs of abuse (SAMSHA, 2003), and the co-use and abuse of alcohol and nicotine is well documented in humans (Bien & Burge, 1990; Istvan & Matarazzo, 1984; Miller & Gold, 1998). Interestingly, individuals dependent on cigarettes consume approximately twice as much alcohol as nonsmokers (Carmody et al., 1985) and it has been estimated that up to 90% of alcoholics are regular smokers (Batel et al., 1995; Bien & Burge, 1990; DiFranza & Guerrera, 1990; Grant, 1998; Miller & Gold, 1998,). Considerable evidence indicates that use of either of these substances increases associated risk for disease development, and indeed, the combined health risks of alcohol and smoking are estimated to be as much as 50% higher than the use of either substance alone (Bien & Burge, 1990). It is important to note that adolescence is a common developmental period in which initiation of use of these substances occurs.

Substantial evidence supports the notion that adolescence is a unique developmental period in which individuals are more likely engage in risk-taking behavior, such as experimenting with drugs of abuse including alcohol and cigarettes. Alcohol is not only one of the most commonly abused psychoactive substances, but also the use of alcohol is quite prevalent in adolescents (Bates & Labouvie, 1997; Windle, 1990). During the adolescent period, there is a dramatic increase in the use of alcohol

with as many as 43% of 8th grade, 65% of 10th grade, and 73% of 12th grade students reported using alcohol in the past year, and 8%, 24% and 32%, respectively, reported being drunk in the past month (Johnston et al., 2001). Additionally, the time course from casual use to dependence on alcohol during adolescence is accelerated relative to adults who initiate use after the age of 21 (Clark et al., 1998). Importantly, it has been suggested that use of alcohol during the adolescent developmental period may render individuals at more risk for developing dependence on alcohol (Andersen et al., 2003; Dewit et al., 2000; Hawkins et al., 1997; Rose et al., 2001) and to abuse alcohol as adults (Duncan et al., 1997). These data suggest that level of consumption of alcohol is high in adolescents and that initiation of use during this period can produce long-term changes in alcohol-related behaviors.

Additionally, the rates of smoking initiation during adolescence are also extremely high, with as many as 50% of high school students reported having tried cigarettes at least once in their lifetime and 25% of those individuals progressing to sustained cigarette use in adulthood (CDC, 2001). Approximately 80% of individuals who smoke started before the age of 18 (CDC, 2001) and it has been suggested that the level of addiction to nicotine is higher among individuals who initiate use of this substance at an early age (Kandel & Chen, 2000; Taouli & Wynder, 1991). Importantly, there appears to be a strong correlation between age of onset of smoking and level of dependence on alcohol and the propensity to develop addiction to these substances later in life (Abelson et al., 1977; DiFranza & Guerrera, 1990; Grant, 1998). All of these data demonstrate an increased vulnerability of adolescents to alcohol and cigarette use and long-term behavioral effects that may arise from use of one or both of these substances

during this developmental period.

Although it is important to note that studying the effects of adolescent alcohol and nicotine exposure is critical, it is unethical to systematically examine the effects of these substances in human adolescents. Therefore, it is important to develop an adolescent animal model to enable systematic investigation of the short-term and long-term effects of alcohol and nicotine exposure during adolescence on subsequent adulthood responsiveness to these agents.

Animal Model of Adolescence

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

been raised (Witt, 1994).

Effects of Ethanol on Behavior

Adults

Ethanol has been shown to produce different effects on behavior in adult animals that may be related to the rewarding and reinforcing or aversive properties of ethanol. Alcohol has biphasic effects on behavior (Lewis & June, 1990), and some studies have yielded mixed results using low and high doses of ethanol. In adult rats, high doses of ethanol produce sedative/hypnotic effects on behaviors, such as motor coordination (White et al., 2002) and locomotor activity (e.g., Little et al., 1996) and appear to be aversive in a conditioned place preference (CPP) paradigm (van der Kooy et al., 1983). In contrast, low doses of ethanol have been shown to produce stimulatory effects of locomotor activity (Correa et al., 2003) when animals were separated into high and low responders to novelty (Hoshaw & Lewis, 2001). Adult animals have been shown to demonstrate ethanol-induced CPP (Bozarth, 1990; Bienkowski et al., 1995; Gauvin & Holloway, 1992), however, others have had difficulty demonstrating ethanol-induced CPP in adult animals (Asin et al., 1985). It is important to note that the development of an ethanol-induced CPP is dependent on previous alcohol treatment (Bozarth, 1990; Bienkowski et al., 1995; Gauvin & Holloway, 1992). Thus it appears that in adult animals, prior exposure to ethanol is necessary for the development of an ethanol-induced CPP. All of these data demonstrate the complexity of the effects of alcohol on behavior in adult animals. High and low doses of ethanol have different effects on behavior, and prior exposure to alcohol can alter CPP. Novelty-related behaviors also appear to be related to alcohol's effects on behavior. Given that adolescents appear to be differentially

sensitive to the effects of alcohol relative to adults, it is important to examine the long-term behavioral effects of alcohol during this developmental period.

Adolescents

Adolescent rats are especially sensitive to the effects of alcohol on a number of behavioral measures (for a review see Spear & Varlinskaya, 2005). Adolescents have been reported to be less sensitive to the sedative/hypnotic and motor incoordinating effects of alcohol (Little et al., 1996; Silveri & Spear, 1998; White et al., 2002), to develop an ethanol-induced CPP more readily (Philpot, Badanich & Kirstein, 2003), and to voluntarily consume more ethanol than adults (Doremus et al., 2005). Additionally, adolescent rats reach peak blood ethanol concentrations (Little et al., 1996) and develop tolerance to alcohol more rapidly than adults (Silveri & Spear, 1999). Together, these data suggest that adolescents experience more of the rewarding properties of ethanol than adults, rendering them especially sensitive to the effects of ethanol. Importantly, the effects of ethanol pretreatment during adolescence have been shown to produce long-term behavioral alterations in novelty preference (Stansfield & Kirstein, accepted pending revisions) and locomotor activity (Maldonado & Kirstein, manuscript in prep) in adulthood. All of these data demonstrate that adolescents and adults are differentially sensitive to the behavioral effects of ethanol and that ethanol can produce long-term changes in novelty preference and ethanol-related behaviors in adulthood, which may be mediated by ethanol's effects on the developing brain.

Effects of Ethanol in the Brain

Adults

Ethanol has been shown to produce a number of neurochemical alterations in the

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

Adolescents

Reward mechanisms in the brain, including alterations of the mesolimbic DA system, continue to undergo significant developmental changes during adolescence (Lidow et al., 1991; Nakano et al., 1996; Seeman et. al., 1987; Spear, 2000; Teicher et. al., 1995). However, relatively little information is available related to changes induced by ethanol in the developing adolescent brain and how these changes may be associated with the differential sensitivity of adolescents to ethanol. Following repeated treatment with ethanol, periadolescent animals (postnatal day (PND) 25) have been shown to exhibit a shift to the left in the temporal peak of stimulated DA relative to the effects of acutely administered ethanol (Philpot & Kirstein, 1998). Additionally, adolescent (PND 45) rats have been shown to have greater basal DA levels and lack of change in DOPAC/DA turnover ratio relative to younger and older animals (Philpot & Kirstein, 2004). This unique neurochemical profile in adolescent animals may be indicative of a lack of tolerance to the rewarding effects of ethanol. These specific age-related neurochemical patterns related to mesolimbic DA may be implicated in the rewarding effects of ethanol that is unique to adolescents.

Although adolescents are less sensitive behaviorally to many of the effects of ethanol, when focusing on brain alterations, adolescents appear more sensitive to cortical and hippocampal neurotoxic alterations induced by ethanol. Swartzwelder and colleagues observed that adolescents suffered from more ethanol-induced disruptions of hippocampal plasticity and memory (Swartzwelder et al., 1995a, b). In a hippocampal-dependent task, adolescents also appear to be more impaired in the Morris water maze to 1.0 or 2.0 g/kg ethanol (Markweise et al., 1998) and larger impairments in working

memory were observed in adolescent animals exposed to repeated 5.0 g/kg ethanol every 48 hours (White et al., 2000). Crews and colleagues have also seen greater ethanol-induced neurotoxicity in adolescent animals (Crews et al., 2000, 2006). Specifically, adolescents demonstrated more frontal damage following a binge model of ethanol administration over a period of four days to 9-10 g/kg/day (Crews et al., 2000) and inhibition of neurogenesis in hippocampal and forebrain regions following acute ethanol administration over a range of ethanol doses ranging from 1.0-5.0 g/kg (Crews et al., 2006). All of these data indicate that adolescent animals are uniquely sensitive to the effects of ethanol in the brain, with increased DA-related activity and greater hippocampal and cortical damage induced by ethanol. These alterations occurred during adolescence and resulted in long-term neuroadaptations, which appears to cause long-term changes in ethanol-associated behaviors.

Long-term Neurobehavioral Effects of Ethanol Exposure During Adolescence

Adolescents have been shown to be uniquely sensitive to the effects of ethanol, with less sensitivity expressed behaviorally, but greater neurotoxic effects observed in the brain. When animals were exposed to ethanol during preweaning (Hayashi & Tadokoro, 1985), or postweaning (Ho et al., 1989), later increases in preference for ethanol were observed. However others have reported no change in preference for ethanol later in life when preexposure occurred during adolescence (Kakihana & McClean, 1963; Parisella & Pritham, 1964; Tolliver & Samson, 1991). Exposure to ethanol during adolescence has been shown to induce impairments in attention and memory (Slawecki et al., 2004) and fear conditioning (Bergstrom et al., 2006) in adulthood. Additionally, adolescent ethanol exposure produced enhanced anxiety- and depressive-like behaviors (Slawecki et al.,

2004) and long-term tolerance in adulthood (Silvers et al., 2003). Exposure to ethanol during adolescence impaired spatial memory (Sircar & Sircar, 2005) and altered hippocampal-mediated neurophysiological function (Slawecki et al., 2001) in adulthood. Furthermore, adolescent ethanol drinking has been shown to alter stimulated ethanol-induced DA efflux in adulthood in alcohol preferring (P) rats (Sahr et al., 2004). All of these data suggest that, indeed, adolescent ethanol exposure produces long-term behavioral and neurochemical alterations in anxiety and depressive-like behaviors and adaptations of hippocampal and DA systems. However, long-term alterations to the rewarding effects of ethanol and importantly, the long-term effects of other drugs, such as nicotine, on ethanol -related behaviors have not been systematically investigated.

Nicotine Effects on Behavior

Adults

Nicotine is believed to be the major psychoactive substance in cigarettes that drives addiction. Animal models using nicotine have shown that adult animals developed different behavioral responses to nicotine. Repeated exposure to nicotine in adult rats has been shown to result in behavioral sensitization (Benwell & Balfour, 1992; Clarke & Kumar, 1983; Janhunen et al., 2005; Walter & Kuschinsky, 1989), which has been suggested to be cue-dependent (Schroeder et al., 2001). Additionally, adult rats have also been shown to self-administer nicotine (Corrigall, 1992; Donney et al., 1995, Shoaib et al., 1997). Nicotine has been shown to be rewarding in adult animals using a CPP paradigm, with several reports supporting the ability of nicotine to establish a CPP at doses ranging from 0.1 – 2.0 mg/kg (sc) with maximal conditioning at the modest doses ranging from 0.1 to 1.4 mg/kg/sc (Fudala et al., 1985; Janhunen et al., 2005; Le

Foll & Goldberg, 2005). All of these data demonstrate that adult animals respond to nicotine in a manner that leads to increased reward associated with repeated drug exposure. Given that adolescents and adults are differentially responsive to nicotine, it is important to focus on this critical developmental period when use of cigarettes in humans is high.

Adolescents

To date, only a limited number of studies have investigated behavioral differences of nicotine between adolescent and adult animals. Adolescent animals have been shown to self-administer higher levels of nicotine (Levin et al., 2003) and exhibit fewer somatic signs of withdrawal than adults (O'Dell et al., 2006). Age-related differences in the anxiolytic and rewarding effects of nicotine in adolescents relative to adults have been investigated, with adolescents exhibiting greater anxiolytic effects (Torella et al., 2004; Vastola et al. 2002) and reward (Shram et al., 2006; Vastola et al. 2002) to a moderate dose of nicotine as compared to adults. However, an absence of drug-cue conditioning has also been demonstrated in periadolescent animals (Schochet et al., 2004). Repeated administration of nicotine during adolescence has been shown to increase self-administration in adulthood (Adriani et al., 2003). These data suggest that adolescents are more sensitive to the rewarding effects and less sensitive to the aversive effects of nicotine as compared to adult animals, which may be mediated by age-related neurochemical differences in responsivity to nicotine in the brain.

Nicotine Effects in the Brain

Adults

Behaviorally, nicotine has been shown to alter responsivity in adult animals, and

these differences are likely mediated by neurochemical systems, including the DA system (Singer et al., 1982). The central effects of nicotine are mediated via nicotinic acetylcholine receptors (nAChRs) (Stolerman, 1991), which are located within the mesolimbic DA system in both the VTA and NAcc (Clarke et al., 1985; Schwartz et al., 1984; Soderpalm et al., 2000). However, the DA-enhancing effects of nicotine appear to be mediated primarily by activation of these receptors in the VTA (Corrigall et al., 1994; Nisell et al., 1994). When nicotine is administered into the VTA (Ferrari et al., 2002; Imperato et al., 1986), specifically, the posterior portion of the VTA (Ikemoto et al., 2006), increases in DA are observed in the NAcc (Benwell & Balfour, 1992; Ericson et al., 2003, Nisell et al., 1994). Many studies have demonstrated that nicotine will increase accumbal DA, however, it has also been shown that repeated administration of nicotine reduces subsequent nicotine-induced increases of DA in adult animals (Vezina et al., 1992, Benwell & Balfour, 1992, Imperato et al., 1986, Benwell et al., 1993). Together these studies demonstrate that nicotine is able to induce DA release in the NAcc, which appears to be mediated, at least partially, by nicotinic receptors in the VTA.

Adolescents

In adults, it is well documented that nicotine is able to induce DA release in the nucleus accumbens via activation of nAChRs in the VTA. However, these effects have not been well documented in adolescent animals. A recent study has demonstrated that adolescent and adult rats are differentially affected by acute and repeated nicotine in terms of nicotine-stimulated accumbal DA release. Specifically, it was observed that adult animals exhibit an elevation in DA when acutely administered nicotine, but this pattern was not evident in adolescent animals. However, after repeated nicotine

treatment, the increase in DA disappeared (i.e., tolerance) in adult animals (Badanich & Kirstein, 2004). Similarly, it has been shown that after repeated nicotine treatment for seven days, adult rats demonstrated an increase in nAChR binding, but this effect was absent in adolescent rats (Collins et al., 2004). These data suggest that adolescent and adult animals are differentially responsive to acute and repeated nicotine treatment when examining DA-related activation. Overall, adolescence appears to be a period of vulnerability to the different behavioral and neurochemical effects of ethanol and nicotine, although, data focusing on the combined effects of these two substances is sparse. Therefore, investigations focusing on the interactive effects of ethanol and nicotine are needed to elucidate the level of vulnerability of adolescents to the commonly combined use of ethanol and nicotine.

Combined Effects of Ethanol and Nicotine on Behavior

Adults

Nicotine has been shown to have interactive effects with ethanol in adult animals. These effects have been observed when both drugs are co-administered or when animals are pretreated with one drug and challenged with another. Nicotine treatment has been shown to increase ethanol intake and preference (Blomqvist et al., 1996, Clark et al., 2001, Le et al., 2000, Lopez-Moreno et al., 2004; Pothoff et al., 1983, Smith et al., 1999) and increase ethanol reinstatement (Le et al., 2003) in adult animals. However, these effects appear to be dose dependent with lower doses of nicotine increasing ethanol consumption and higher doses suppressing consumption after acute nicotine treatment (Gauvin et al., 1993). These data suggest a complex interaction of nicotine on ethanol - intake.

Nicotine has been shown to produce effects on other ethanol-related behaviors as well. Pretreatment of nicotine blocked an ethanol-induced conditioned taste aversion (Kunin et al., 1999). Nicotine has been shown to enhance ethanol discrimination (Signs & Schechter, 1986), but to impair performance on working memory and attention task performance (Bizarro et al., 2003; Rezvani & Levin, 2002). However, ethanol has been shown to have no effect on nicotine discrimination (Le Foll & Goldberg, 2006). Furthermore, nicotine has been shown to increase the rate of tolerance to ethanol (Hjeresen, 1989) and cross-tolerance has been observed between these two substances (Collins et al., 1988); an effect that has also been observed in adolescent animals (Lopez et al., 2001). Low doses of nicotine have been shown to enhance the motor stimulatory effects of ethanol in mice (Blomqvist et al., 1992) and rats (Schaefer & Michael, 1992) and an additive increase in intracranial self-stimulation was observed relative to administration of either drug alone (Schaefer & Michael, 1992). All of these data suggest that there are interactive effects between ethanol and nicotine on behavior in adult animals, and this drug combination should be systematically examined in adolescent animals.

Adolescents

To date, there is a limited amount of data on the behavioral and neurochemical effects of the co-administration of ethanol and nicotine. Neurochemical alterations due to the co-administration of these substances appear to be complex. Of particular importance is that nicotine administration during adolescence, via subcutaneous injections, produced long-term increases of ethanol intake into adulthood (Tsui et al., 2001, Le, 2002). However, it has also been suggested that chronic continuous nicotine infusion, via

subcutaneous implantation of nicotine pellets, during adolescence does not increase ethanol intake in adulthood (Smith et al., 2002). The results from these studies demonstrate that the delivery method of adolescent nicotine (sc or pellet) can differentially affect ethanol -related behaviors in adulthood. These data are consistent with the adult data suggesting that continuous administration of nicotine does not produce alterations in ethanol consumption, whereas repeated subcutaneous administration, does indeed increase voluntary ethanol consumption. When animals were given a choice to consume ethanol, nicotine and water in a limited access paradigm there were no interactive effects on intake when both ethanol and nicotine were offered. That is to say that there was not an additive effect on ethanol intake when nicotine was also offered in young and older rats (Marshall et al., 2002). When nicotine and alcohol were systemically administered in combination, but not either drug alone, unique age-related behavioral outcomes were observed, with adolescents exposed to the combination of these two drugs showing greater hyperthermia relative to their adult counterparts (Rezvani & Levin, 2004). These data imply unique, and possibly additive, effects of nicotine and ethanol in adolescent rats in behavior and brain mechanisms may mediate these effects.

Combined Effects of Ethanol and Nicotine in the Brain

It is well documented that nicotine (via nAChRs in the VTA), as well as ethanol, causes activation of the DA system. Ethanol has been shown to produce stimulatory effects on different nAChR subtypes in the VTA (Jerlhag et al., 2006; Solderpalm et al., 2000). Additionally, ethanol (Gessa, 1985) and nicotine (Calabresi et al., 1989) facilitates DA release in the NAcc (Le et al., 2001) via activation of the VTA in adult rats

(Blomqvist et al., 1996). An additive effect of locally administered nicotine into the VTA on ethanol-induced DA release in the NAcc was observed with a moderate dose of nicotine (Tizabi et al., 2002). Furthermore, a synergistic effect of ethanol and nicotine on spontaneous firing of the VTA has been observed (Clark & Little, 2004). Mecamylamine, a noncompetitive nAChR antagonist, administered in the VTA, but not NAcc, blocked ethanol-induced accumbal DA increases and reduced ethanol preference and intake (Blomqvist et al., 1997; Ericson et al., 1998). All of this evidence supports the notion that ethanol's actions are at least partially mediated by its action on nicotinic receptors, especially those in the VTA (Soderpalm et al., 2000), and provides evidence that these two substances work together at the neurochemical level to modulate ethanol-induced DA release in the NAcc.

Overview of the Present Study

Blomqvist and colleagues have speculated that nicotine abuse, especially during adolescence, may render individuals more sensitive to developing alcohol dependence in adulthood (Blomqvist et al., 1996). Furthermore, adolescents not only appear to be more sensitive to the effects of alcohol or nicotine, but also especially sensitive to the interactive effects of this drug combination. Therefore, the goal of the present experiment was to assess the long-term effects of ethanol, nicotine, or the combination of ethanol and nicotine during adolescence on adulthood novelty preference and ethanol-related behaviors. Additionally, given that human adolescents do not usually consume alcohol everyday, a repeated-intermittent ethanol exposure-dosing regimen was also used to mimic adolescent human alcohol consumption and assess if this produced different results from chronic exposure to ethanol. Furthermore, given that a CPP has been

demonstrated at moderate, but not lower or higher doses of nicotine, a moderate dose of 0.4 mg/kg nicotine was used in the present experiment. Specifically, adolescent animals were chronically or repeated-intermittently administered either drug alone (ethanol or nicotine or saline) or in combination (ethanol and nicotine) during adolescence (PND 30-47). For the repeated-intermittent exposure, adolescent animals were administered nicotine or saline everyday and exposed to ethanol on PND 30-33, PND 37-40, and PND 44-47. Subsequently, in adulthood, following a washout period, novelty preference (PND 64-67) and conditioned place preference to ethanol (PND 68-73) was assessed. Preliminary data from our laboratory indicate that chronic exposure to a moderate dose of ethanol during adolescence alters novelty-related behaviors in adulthood (Figure 1; Stansfield and Kirstein, 2007).

Hypotheses

The overall goal of the proposed experiments was to assess the long-term behavioral alterations that occur due to adolescent exposure of ethanol and nicotine, either alone or in combination, on adulthood responsivity to the rewarding properties of environmental cues paired with ethanol. Given that in humans, adolescent exposure to this drug combination appears to facilitate the development of alcohol dependence in adulthood, it was hypothesized that adolescent animals exposed to the combination of ethanol and nicotine would be more vulnerable to developing alterations in ethanol-related behaviors in adulthood. It has already been established that adolescent ethanol exposure enhanced novelty-related behaviors in adulthood (Stansfield & Kirstein, 2007). Given that adolescent animals are more sensitive to the anxiolytic effects of nicotine, it is hypothesized that adolescent rats exposed to nicotine alone during adolescence would

exhibit greater novel environment induced behavioral activation. Furthermore, adolescent animals exposed to the combination of ethanol and nicotine would exhibit greater novelty-seeking behaviors as compared to animals exposed to either drug alone. Overall, it was expected that animals exposed to the combination of ethanol and nicotine would exhibit the highest behavioral responsivity to the rewarding properties of environmental cues paired with ethanol in adulthood. When assessing the rewarding properties of ethanol in adulthood using the CPP paradigm, it was hypothesized that adolescents animals exposed to the combination of a moderate dose of alcohol (0.75 g/kg) and nicotine (0.4 mg/kg) would more readily acquire a CPP in adulthood as compared to animals exposed to either drug alone. Thus, animals exposed to both ethanol and nicotine during adolescence would exhibit the greatest level of responsivity to alcohol in adulthood.

Chapter Two: Effects of Ethanol and/or Nicotine Pretreatment During Adolescence on
Adulthood Ethanol -Related Behaviors
Materials and Methods

Subjects

Two hundred and forty two male Sprague-Dawley rats, derived from established breeding pairs at the University of South Florida, Tampa (Harlan laboratories, IN), were used in the present study. Litters were sexed and culled to 10 pups per litter on postnatal day (PND) 1, with the date of birth designated as PND 0. Pups were housed with their respective dams until PND 21 when they were weaned and housed in groups of three/four with same-sex littermates. The colony room was maintained in a humidity- and temperature-controlled vivarium on a 12:12 hour light/dark cycle, with lights on from 0700 hours to 1900 hours. Animals were allowed ad libitum access to food and water in the home cage. Each animal was tested across development beginning on PND 30 and ending on PND 75. No more than one male pup per litter was used in any given condition. In all respects, maintenance and treatment of the animals was within the guidelines for animal care by the National Institutes of Health.

Apparatus

Open field. The apparatus consisted of an open circular field with a black plastic floor (D = 96.5 cm) and an opaque plastic circular barrier (H = 45.7 cm) in which animals were allowed free access to move about. A camera was suspended above the open field and movement (cm) of the animal was digitally recorded. This signal was tracked,

quantified, and analyzed using an Ethovision video tracking system (Noldus Information Technology, Utrecht, Netherlands). All behavioral testing occurred under dimly-lit conditions and occurred between 1000 and 1400 hr.

Conditioned Place Preference Apparatus. The conditioning apparatus consisted of a single black Plexiglas (Rohm and Haas Company, Philadelphia, Pennsylvania) runway that could be separated by a removable Plexiglas wall into two equal sized compartments (21 x 24.5 x 20.5 cm), containing distinct visual and tactile cues. One compartment consisted of vertically striped walls (1 inch thick) with a wire-mesh floor. The other compartment consisted of horizontally striped walls (1 inch thick) with a grey sandpaper floor (100 grit).

Procedure

Pretreatment. Animals were randomly assigned to one of four pretreatment conditions based on drug combinations of ethanol (EtOH), nicotine (NIC), and saline (SAL) administered. The four pretreatment conditions used were: SAL/SAL, NIC/SAL, SAL/EtOH and NIC/EtOH. All animals were pretreated with SAL (0.9 % NaCl; subcutaneous (sc) or intraperitoneal (ip)), NIC (0.0 or 0.4 mg/kg/sc; expressed as the salt), or EtOH (17% v/v; 0.0 or 0.75 g/kg/ip) from PND 30 to 47. Rats that were chronically administered ethanol were exposed to both a sc (nicotine or saline) and ip (ethanol or saline) everyday from PND 30-47. Rats that were repeated-intermittently administered ethanol were exposed to a sc (nicotine or saline) everyday from PND 30-47 and were administered an ip injection (ethanol or saline) on PND 30-33, PND 37-40, and PND 44-47. During Pretreatment (PND 30-47), animals were transported to the lab, weighed and administered their respective injections. All animals received two injections

in the home-cage (or one sc injection on PND 34-36 and PND 41-43 if they underwent repeated-intermittent exposure to ethanol). The first injection was a sc injection (saline or 0.4 mg/kg nicotine). Immediately following the first injection, animals received an ip injection (saline or 0.75 g/kg/ip EtOH). Following drug administration animals were immediately returned to the colony. From PND 48-63 animals remained undisturbed in the colony, except for regular cage maintenance.

Novelty Preference. From PND 64-66, all animals were subjected to a novelty preference probe (Stansfield et al., 2004; Stansfield & Kirstein, 2006). Beginning on the morning of PND 64, animals were transported to the lab (0900-1000) and placed on the novel open field as described above, and behavior recorded for 5 min. Immediately following the five-minute habituation trial, animals were returned to the colony. On the afternoon on PND 64 (1400-1500), animals were again transported to the lab and placed on the open field for a five-minute habituation trial, and then immediately returned to the colony. This process was repeated on PND 65-67, for a total of 8 habituation trials. Immediately following the eighth habituation trial, animals were returned to their home-cage for one minute, and then returned to the open field where a novel object (7 cm in height) was placed in the center of the open field. Time spent near the novel object (sec), frequency of approaches to the novel object, latency to approach (sec) the novel object were recorded for the ninth trial. Total Distance Moved (cm) was recorded for all trials.

Conditioned Place Preference (CPP). The CPP procedure is a biased procedure, which occurred over six days, in three phases. The first phase is pre-conditioning baseline (PND 68), the second phase is conditioning (PND 69-72), and the final phase is post-conditioning test (PND 73). During baseline, animals were presented in the center

of the CPP apparatus with the center wall removed to allow free access to both chambers for 15 minutes. Time (sec) spent in each chamber was recorded by the EthoVision video tracking system. The chamber that the animal spent the least amount of time in (least preferred) was assigned as the drug-paired chamber, and for phase two animals were conditioned with alcohol to this chamber. Control animals received saline injections on both sides of the apparatus. Phase two (conditioning) occurred over four days, PND 69-72. Each morning (1000-1100 hr) animals were transported to the lab, weighed, administered saline, and immediately confined to the initially preferred chamber for 5 minutes. Immediately following the 5-minute conditioning session, animals were returned to their home-cage and returned to the colony. Approximately four hours later, (1400-1500 hr), animals were again transported to the lab, weighed, administered their respective saline or ethanol injection, and immediately confined to the initially least preferred chamber for 5 minutes. Immediately following the 5-minute conditioning session, animals were returned to the home-cage and returned to the colony. This procedure was repeated over a period of four days. The apparatus was cleaned with Quatricide (Pharmal Research Laboratories Incorporated) and EtOH (70%) prior to each trial to remove lingering odors. During phase three, PND 73, animals were transported to the lab, weighed, and introduced to the CPP apparatus with the center wall removed to allow free access to both chambers for 15 minutes and Time (sec) spent in each chamber was digitally recorded and quantified via the EthoVision video tracking system. This procedure is identical to that of phase one, with animals in a drug-free state.

Design and Analyses

The present experimental design is a two-way between subjects design ANOVA

for Pretreatment (4; SAL/SAL, NIC/SAL, SAL/EtOH, NIC/EtOH) and Post-treatment (2; SAL, EtOH). Data were analyzed separately for each dosing regimen (chronic vs repeated-Intermittent exposure to ethanol). Therefore, animals that were assigned to a SAL Pretreatment and SAL Post-treatment served as controls because these animals never received EtOH. Furthermore, for any Pretreatment condition (SAL/SAL, NIC/SAL, SAL/EtOH, NIC/EtOH), after the washout period in adulthood, half of the animals were subsequently be administered SAL and the other half was administered EtOH in the conditioned place preference paradigm. The level for significance was set at 0.05 for all analyses.

Data for novelty preference were analyzed using a one-way between subjects ANOVA for and Pretreatment (SAL, NIC, EtOH, NIC/EtOH). Frequency to approach the novel object, time spent with the novel object, and total distance moved on trial one were used as dependent measures to assess the effects of pretreatment during adolescence on adulthood-novelty behaviors.

Data for CPP were analyzed using a two-factor between subject design ANOVA with Pretreatment (SAL/SAL, NIC/SAL, SAL/EtOH, NIC/EtOH) and Post-Treatment (SAL, EtOH) as factors. Difference scores (test – baseline) of time (sec) spent in the least preferred chamber were used as the dependent measure. A CPP was defined as an EtOH post-treated animal spending significantly more time on the initially least preferred side at test relative SAL post-treated animals.

Chapter Three: Results

Effects of Ethanol and/or Nicotine Pretreatment on Novelty-Related Behaviors in Adulthood

Novelty-Induced Exploration

Adult animals that were previously treated with either saline, ethanol alone, nicotine alone or ethanol combined with nicotine were assessed on a number of behavioral measures related to novelty seeking. One of the measures that are assessed in this paradigm is novelty-induced exploration. With this measure, the number of times an animal approaches the novel object on the final trial was assessed. As indicated in Figure 1A, there were no significant differences among any of the groups in the number of times adult animals that were chronically exposed to ethanol alone, nicotine alone, or the combination of ethanol and nicotine during adolescence approached the novel object ($F(3, 113) = .96, p > 0.05$). Similarly, as depicted in Figure 1B, in animals that were repeated-intermittently exposed to saline, ethanol alone, nicotine alone, or ethanol combined with nicotine during adolescence; there were no significant differences among any of the groups ($F(3, 110) = 1.14, p > 0.05$). Therefore, these data do not replicate recent findings that indicate that treatment with a moderate dose of ethanol during adolescence increases novelty induced exploration in adult rats (Stansfield & Kirstein, 2007).

Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Novelty-Induced Exploration in Adulthood

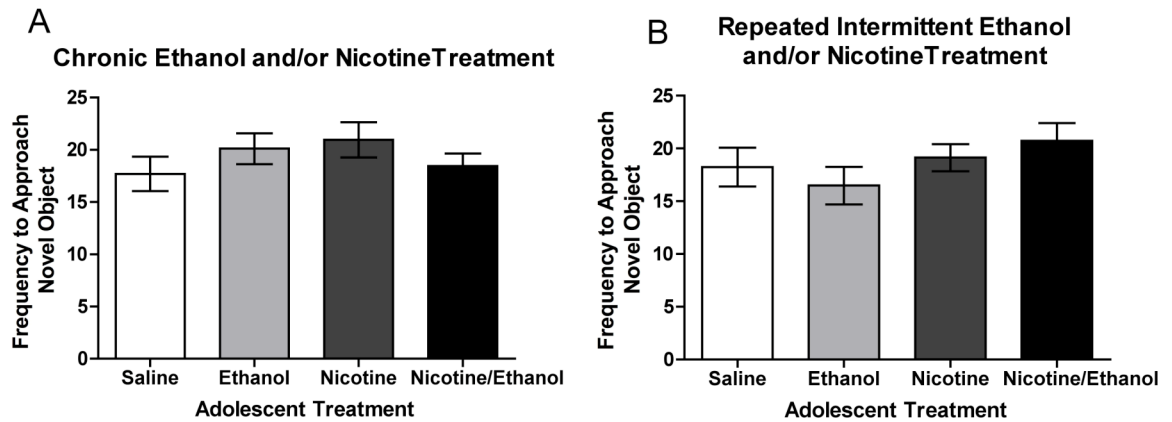


Figure 1: Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Novelty Induced Exploration in Adulthood. There were no long-term changes in novelty induced exploration due to either chronic (Panel A) or repeated intermittent (Panel B) exposure to ethanol and/or nicotine during adolescence. Panel A: Saline n= 30; Ethanol n= 27; Nicotine n= 24; Ethanol/Nicotine n= 33. Panel B: Saline n= 26; Ethanol n= 27; Nicotine n= 32; Ethanol/Nicotine n= 27.

Novelty Preference

Novelty preference is another behavior that was assessed in the behavioral paradigm indicated above. Novelty preference is defined as time (seconds) spent near and around the novel object on the final trial. As indicated in Figure 2A, there were no significant differences among any of the groups that were chronically treated with saline, ethanol alone, nicotine alone, or ethanol combined with nicotine during adolescence in the amount of time spent with the novel object on the final trial ($F(3, 113) = 1.87, p > 0.05$). Similarly, as depicted in Figure 2B, there were no significant differences among

any of the groups that were repeated-intermittently exposed to saline, ethanol alone, nicotine alone, or ethanol combined with nicotine during adolescence ($F(3, 110) = .69, p > 0.05$).

Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Novelty Preference in Adulthood

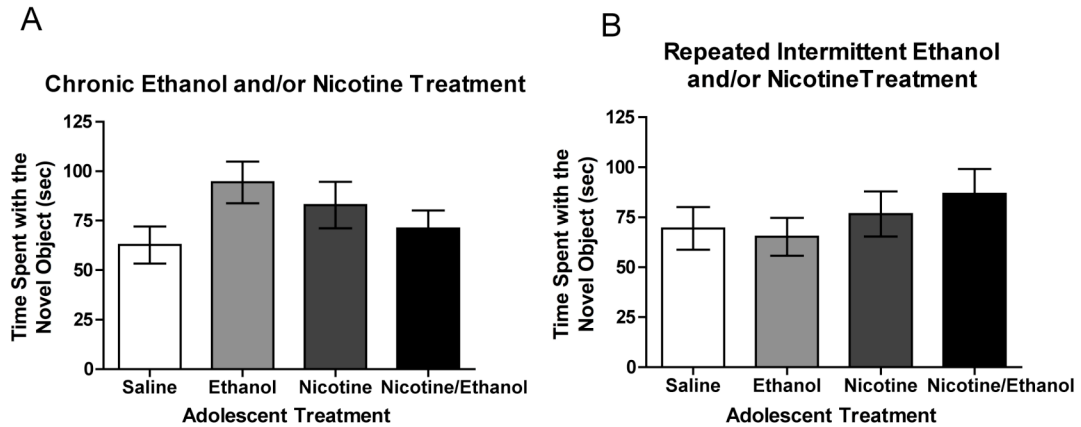


Figure 2: Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Novelty Preference in Adulthood. There were no long-term changes in novelty preference due to either chronic (Panel A) or repeated intermittent (Panel B) exposure to ethanol and/or nicotine during adolescence. Panel A: Saline $n = 30$; Ethanol $n = 27$; Nicotine $n = 24$; Ethanol/Nicotine $n = 33$. Panel B: Saline $n = 26$; Ethanol $n = 27$; Nicotine $n = 32$; Ethanol/Nicotine $n = 27$.

Novel Environment Induced Exploration

Novel environment induced exploration is a measure that is commonly used to assess novelty-related behaviors as measured by the total amount of distance traveled on the first trial when animals were exposed to a novel environment. As depicted in Figure 3A, there were no significant differences in the distance traveled on the first trial among

among animals that were repeated-intermittently treated with saline, ethanol alone, of the groups that were exposed to saline, ethanol alone, nicotine alone, or ethanol combined with nicotine during adolescence ($F(3, 121) = 1.74, p > 0.05$). Similarly nicotine alone, or ethanol combined with nicotine, there were no significant differences in the distance traveled upon exposure to a novel environment ($F(3, 114) = 1.41, p > 0.05$).

Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Novel Environment Induced Exploration in Adulthood

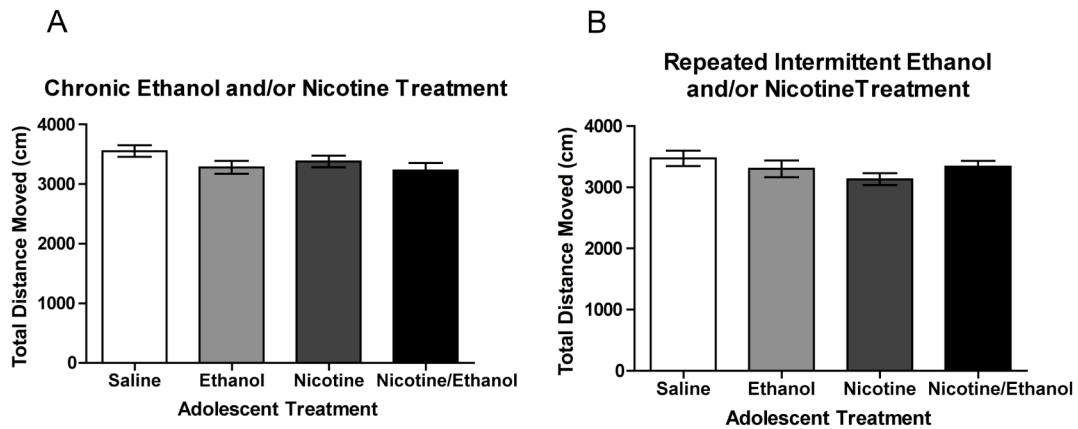


Figure 3: Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Novel Environment Induced Exploration. There were no long-term changes in novel environment induced exploration due to either chronic (Panel A) or repeated intermittent (Panel B) exposure to ethanol and/or nicotine during adolescence. Panel A: Saline $n = 30$; Ethanol $n = 27$; Nicotine $n = 24$; Ethanol/Nicotine $n = 33$. Panel B: Saline $n = 26$; Ethanol $n = 27$; Nicotine $n = 32$; Ethanol/Nicotine $n = 27$.

Examining the Rewarding Effects to Ethanol After Adolescent Treatment of Alcohol and/or Nicotine

Using the CPP paradigm, changes in the rewarding properties of environmental

cues associated with ethanol were assessed after adolescent treatment with saline, ethanol alone, nicotine alone or the combination of ethanol and nicotine during adolescence. As depicted in Figure 4, among animals that were chronically treated during adolescence with saline, ethanol alone, nicotine alone or ethanol and nicotine, there was an overall pattern of decreased time spent in the chamber paired with ethanol relative to animals that were treated with saline in both chambers, regardless of pretreatment as supported by a significant main effect for Posttreatment ($F(1, 112) = 6.89, p < 0.01$). The main effect for Pretreatment ($F(3, 112) = .38, p > 0.05$) and the Pretreatment by Posttreatment interaction ($F(3, 112) = .89, p > 0.05$) failed to reach significance. As illustrated in Figure 5, among animals that were repeated-intermittently treated with saline, ethanol alone, nicotine alone, or ethanol combined with nicotine, there were no significant differences among any of the grouped in the amount of time spent in the chamber paired with ethanol as compared to animals that were administered saline in both chambers as indicated by a nonsignificant main effect for Pretreatment ($F(3, 109) = .96, p > 0.05$), Posttreatment ($F(1, 109) = .98, p > 0.05$) or Pretreatment by Posttreatment interaction ($F(3, 109) = .38, p > 0.05$).

Chronic Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Conditioned Place Preference in Adulthood

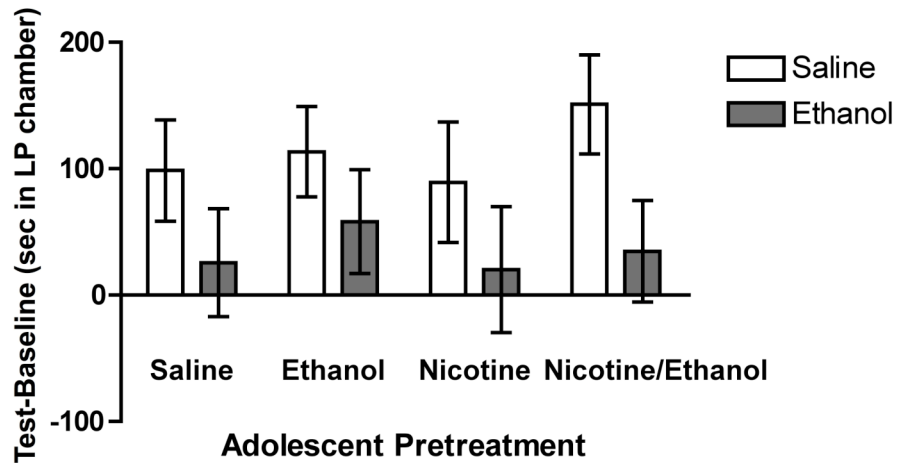


Figure 4: Chronic Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Conditioned Place Preference in Adulthood. There were no long-term changes in conditioned place preference to ethanol in adulthood following chronic exposure to ethanol and/or nicotine during adolescence. Pretreatment-Posttreatment: Saline-Saline n=15; Saline-Ethanol n= 14; Ethanol-Saline n= 15; Ethanol-Ethanol n= 14; Nicotine-Saline n= 14; Nicotine-Ethanol n= 15; Ethanol/Nicotine-Saline n= 17; Ethanol/Nicotine-Ethanol n= 16.

Repeated Intermittent Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Conditioned Place Preference in Adulthood

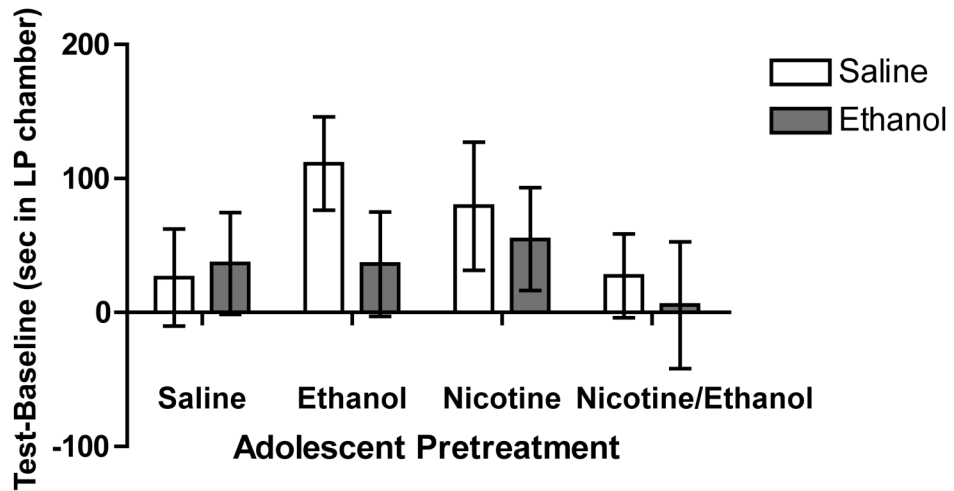


Figure 5: Repeated Intermittent Ethanol and/or Nicotine Treatment During Adolescence Did Not Alter Conditioned Place Preference in Adulthood. There were no long-term changes in conditioned place preference to ethanol in adulthood following repeated intermittent exposure to ethanol and/or nicotine during adolescence. Pretreatment-Posttreatment: Saline-Saline n=15; Saline-Ethanol n= 14; Ethanol-Saline n= 16; Ethanol-Ethanol n= 14; Nicotine-Saline n= 16; Nicotine-Ethanol n= 16; Ethanol/Nicotine-Saline n= 15; Ethanol/Nicotine-Ethanol n= 13.

Chapter Four: Discussion

Comorbid use of Alcohol and Nicotine in Humans

In humans, there appears to be a dose-dependent increase in the level of tobacco use and alcohol consumption (Falk et al., 2006). Additionally, co-use of alcohol and tobacco is highest among young people aged 18-24 and the rates of co-use decline with age (Falk et al., 2006). Human males that smoked nicotine-containing cigarettes consumed more alcohol than their non-nicotine-containing cigarette counterparts (Barrett et al., 2006). Additionally, humans that consumed ethanol reported increased satisfaction ratings of nicotine-containing cigarettes relative to denicotinized cigarettes (Rose et al., 2002). Together these data support the notion that alcohol and tobacco are commonly used in humans and that there is greater positive effects associated with the combined use of these drugs.

Conditioned Place Preference Paradigm and Ethanol History

The CPP paradigm is a commonly used behavioral model designed to assess the rewarding effects associated with environmental cues paired with drug administration. It is believed that environmental cues paired with drug administration are deemed more rewarding if animals spent more time in the environment paired with drug administration following a number of conditioning trials. If animals spend less time in the environment paired with drug administration then the animals are believed to develop an aversion to those drug-associated cues. The evidence to establish a CPP with ethanol is mixed.

Some have reported establishment of a conditioned place preference Bienkowski et al., 1995 (Bienkowski et al., 1995; Bozarth, 1990; Gauvin & Holloway, 1991), whereas others have reported conditioned place aversions with ethanol (Asin et al., 1985; Cunningham et al., 1993; Schechter, 1992), and others have reported no change in preference for the environment paired with ethanol (Ciccocioppo et al., 1999; Davies and Parker, 1990; Schechter, 1992; Stewart et al., 1996) across a range of doses. However, it appears that usually a dose of approximately 1.0 g/kg ethanol is needed to establish a CPP with ethanol (Bozarth, 1990). However, many others have not been able to establish a conditioned place preference with ethanol. The discrepancy in the results appears to be due to a number of factors, including dose, route of administration, length of conditioning trial, number of conditioning trials, and previous history with ethanol.

It appears that animals that have a history with ethanol more easily establish a conditioned place preference for ethanol (Bienkowski et al., 1995; Bozarth 1990; Gauvin & Holloway, 1991). However, others have failed to observe a CPP in animals that had a history of ethanol exposure (Davies & Parker, 1990). Bozarth (1990) was able to establish a conditioned place preference with a moderate 1.0 g/kg/ip ethanol dose after 15 conditioning trials that lasted 30 minutes each. Bienkowski and colleagues observed an ethanol-induced CPP in animals that were chronically pretreated with 0.5 g/kg ethanol for 20 days prior to conditioning (Bienkowski et al., 1995). Rats in the Bozarth (1990) experiment had more and longer drug conditioning trials and were not conditioned to the alternate chamber with saline. The present experiment conditioned rats with four conditioning trials paired with ethanol and four conditioning trials paired with saline that lasted five minutes each. Animals in the Bienkowski et al. (1995) experiment

immediately underwent conditioning following preexposure. Animals used in the present experiment underwent a washout period from ethanol until they matured into young adulthood. Therefore, different results may have been observed for animals in the present experiment if animals had undergone conditioning immediately following pretreatment rather than waiting for the two-week washout period to allow animals to mature to adulthood. It is possible, that the present experiment did not include a sufficient number of conditioning trials, and that if animals in the present experiment were conditioned with longer and a greater number of drug-paired conditioning trials that a CPP for ethanol may have been observed.

Among animals that did not have a previous history with ethanol, route of administration appears to be an important factor in the ability to establish a conditioned place preference with ethanol. When rats were administered ethanol intraperitoneally (ip), there was either an aversion (Cunningham et al., 1993; Schechter, 1992) or no change in preference for the environment paired with ethanol (Asin et al., 1985). Using the CPP paradigm, there was no significant difference in preference for the chamber paired with ethanol that was administered intraperitoneally (ip) using animals that were selectively bred to prefer alcohol (P rats) or not to prefer alcohol (NP rats; Schechter, 1992). Indeed, both P and NP rats found the environment paired with a moderate dose of ethanol (1.0 g/kg/ ip) aversive (Schechter, 1992). However, NP rats showed a depression in locomotor activity, an effect that was not observed in P rats (Schechter, 1992). Additionally, in another line of genetically selected alcohol preferring rats, the Marchigian Sardinian alcohol preferring (msP) rats, when a moderate dose of ethanol (0.70 g/kg/ip) was administered ip, similar to that used in the present study (0.75 g/kg/ip),

no change in preference was observed for the chamber paired with ethanol (Ciccocioppo et al., 1999). The absence of a CPP was observed in msP rats administered ethanol ip, regardless of a previous history of alcohol consumption or greater number of pairings of ethanol with the environment (Ciccocioppo et al., 1999). Additionally, there was no evidence of ethanol-induced place preference in P or NP rats administered 0.5 g/kg/ ip ethanol and conditioned place aversions were observed at higher 1.0 or 1.5 g/kg/ip ethanol doses (Stewart et al., 1996). These data suggest that in out bred strains of rats or in rats that are selectively bred to prefer alcohol, there was no establishment of CPP when ethanol was administered ip. However, in animals with a long history of voluntary oral consumption, a conditioned place preference was observed when animals were confined to one compartment and allowed voluntary access to ethanol (Gauvin & Holloway, 1991). Therefore, it appears that when ethanol is administered ip, there is no establishment of CPP in animals that do not have prior experience with ethanol. In the present experiment, ethanol was administered ip and no change in preference for the environment paired with ethanol was observed, regardless of dosing regimen or pretreatment history. It is possible that a CPP may have been established in the animals of the present if a different route of administration had been used in the present experiment because they had a long prior history with ethanol and/or nicotine during adolescence.

Combined Ethanol and Nicotine Treatment

Recent work indicates that humans that are exposed to both alcohol and tobacco during adolescence exhibit characteristics that are associated with enhanced risk-taking behaviors (Schmid et al., 2007). These risk-taking behavioral characteristics may be

associated with enhanced substance abuse problems during adolescence and later in life. Concurrent adolescent use of alcohol and tobacco was associated with an earlier age of onset of drinking and greater and heavier drinking episodes relative to consumption of alcohol alone (Schmid et al., 2007). Additionally, concurrent alcohol and tobacco using adolescents expressed greater positive effect expectancies for alcohol using the Alcohol Expectancy Questionnaire (Brown et al., 1987; Schmid et al., 2007). Previously it has been demonstrated in rats that adolescent treatment with a moderate dose of ethanol (1.0 g/kg/ip) enhanced novelty induced exploration in adulthood (Stansfield & Kirstein, 2007). In the present experiment, it was hypothesized that adolescent exposure to ethanol alone or ethanol in combination with nicotine would replicate these findings. However, there was no change in any novelty-related behaviors in adulthood (See Figures 1-3) observed due to adolescent pretreatment with ethanol and/or nicotine. This could have been due to the fact that all adolescents were administered two injections, one administered ip (ethanol or saline) and one administered subcutaneously (sc; nicotine or saline). The added stress of the second injection may have dampened the effect previously observed of higher novelty induced exploration due to adolescent exposure to a moderate dose of alcohol. Alternatively, a slightly lower ethanol dose of 0.75 g/kg/ip was used in the present experiment as compared to the 1.0 g/kg/ip used in the previous study (Stansfield & Kirstein, 2007). This slightly lower dose may not have been sufficient to alter adulthood novelty-related behaviors.

Using mice, there was no additive effect of ethanol to enhance CPP for nicotine as compared to animals that were administered nicotine alone (Korkosz et al., 2006). Similarly, in animals that had a long exposure to alcohol consumption and later tested for

elevations in locomotor activity to nicotine, no enhancement of prior ethanol history on nicotine-induced locomotor activity was observed (Darbra et al., 2004). Adolescent naïve alcohol-preferring (P) rats show enhanced nicotine self-administration and nicotine-reinstatement relative to their alcohol-nonpreferring (NP) counterparts (Le et al., 2006). Previous work suggests that withdrawal from ethanol and nicotine produces greater aversion to the open arms of an elevated plus maze relative to withdrawal from either drug alone (Onaivi et al., 1989). Tolerance from ethanol and cross-tolerance from nicotine alone, or nicotine combined with ethanol were observed in response to ethanol as measured by ethanol-induced hypothermia and locomotor activity (Collins et al., 1996). Together, these data indicate that there is an interactive effect of ethanol and nicotine on behavior. However, these studies indicate that ethanol has effects on subsequent nicotine-induced behaviors. The present experiment assessed changes induced by prior ethanol and/or nicotine exposure on subsequent ethanol-induced conditioned place preference. When similar effects were examined cross-tolerance was observed due to exposure to alcohol and/or nicotine on subsequent ethanol-induced hypothermia and locomotor activity (Collins et al., 1996). Funk and colleagues suggest that reduced sensitivity to alcohol may result from chronic exposure to nicotine or vice versa (Funk et al., 2006). Therefore, if reduced sensitivity to alcohol was established due to adolescent exposure to nicotine alone or nicotine combined with alcohol, then a CPP would not be expected for alcohol in adulthood. This speculation is quite plausible given that we did not observe a CPP or conditioned place aversion to ethanol in adulthood, in any group.

When ethanol and nicotine were administered either alone or in combination during adolescence in mice, there were no long-lasting effects on cognitive performance

in male rats (Abreu-Villaca et al., 2007). However, there was an improvement in cognitive performance in females in adulthood after adolescent co-exposure to ethanol and nicotine (Abreu-Villaca et al., 2007). Therefore, the results obtained in the present experiment in male rats of no enhancement of alcohol-induced CPP in adulthood after adolescent exposure to ethanol and nicotine administration alone or in combination are consistent with the results obtained by Abreu-Villaca and colleagues (2007).

Conclusions

Although enhanced CPP in adulthood was not observed due to adolescent exposure to ethanol and/or nicotine in the present experiment, nicotine was able to increase ethanol consumption in rodents (Clark et al., 2001; Larsson & Engel, 2004; Le et al., 2000; Smith et al., 1999). In animals that were chronically exposed to nicotine and later tested for voluntary ethanol consumption, nicotine enhanced subsequent voluntary ethanol intake after a washout period (Blomqvist et al., 1996). Therefore, prior nicotine administration is able to increase voluntary ethanol consumption after a washout period. Blomqvist and colleagues (1996) did not examine if nicotine combined with ethanol pretreatment would later enhance voluntary ethanol intake relative to administration of ethanol alone. Therefore, if we had examined voluntary ethanol intake in adulthood to measure the reinforcing properties of ethanol, we may have observed enhanced intake in adulthood, as many experiments have reported enhanced ethanol intake in animals administered nicotine (Blomqvist et al., 1996; Clark et al., 2001; Larsson & Engel, 2004; Le et al., 2000; Smith et al., 1999).

Nicotine exposure during adolescence was able to increase the reinforcing properties of cocaine in adulthood as measured using operant responding (McQuown et

al., 2007). A similar effect was expected for ethanol, in that adolescent animals that were exposed to nicotine were expected to show a CPP in adulthood to ethanol. Additionally, it was expected that animals that were exposed to the combination of ethanol and nicotine during adolescence were expected to show an enhanced CPP relative to all other groups. Given that the evidence for establishment of an ethanol-induced conditioned place preference is mixed and that ip administration of ethanol appears to produce either an aversion or no change in preference for the environment paired with ethanol, this paradigm and route of ethanol administration may not have been the most appropriate paradigm to use to assess the rewarding properties of ethanol in adulthood. Data from another set of experiments conducted to assess the effects of nicotine on voluntary ethanol intake in adolescent and adult male rats indicate that adolescents that were exposed to nicotine showed enhanced voluntary ethanol intake relative to their saline counterparts. This effect was not observed for similarly treated adult males (Maldonado & Kirstein, manuscript in prep). However, the long-term effects of nicotine on voluntary ethanol intake were not examined in that set of experiments. Together these experiments indicate that nicotine does increase voluntary ethanol intake in adolescent, but not adult male rats. Therefore, the adolescent period is one where there are enhanced interactive effects of nicotine and alcohol. However given the mixed literature on ethanol-induced CPP, perhaps other behavioral paradigms should be utilized to assess the long-term interactive effects of this drug combination when administered during adolescence. Currently, experiments are being conducted to assess if nicotine exposure during adolescence or adulthood increases subsequent voluntary ethanol intake in male rats after a washout period.

References

- Abelson H, Fishburne RM & Cisin I. 1977. National Survey of Drug Abuses 1977. National Institute on Drug Abuse, Rockville, MD.
- Abreu-Villaca Y, Medeiros AH, Lima CS, Faria FP, Filgueiras CC & Manhaes AC. 2007. Combined exposure to nicotine and ethanol in adolescent mice differentially affects memory and learning during exposure and withdrawal. *Behav Brain Res*, 181, 136-146.
- Adriani W, Spijker S, Deroche-Gamonet V, Laviola G, Le Moal M, Smit AB & Piazza PV. 2003. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. *J Neurosci*, 23, 4712-4716.
- Andersen A, Due O, Holstein BE & Iversen L. 2002. Tracking drinking behaviour from age 15-19 years. *Addiction*, 98, 1505-1511.
- Anthony JC & Petronis KR. 1995. Early-onset drug use and risk of later drug problems. *Drug Alcohol Depend* 40(1): 9-15.
- Appel SB, McBride WJ, Diana M, Diamond I, Bonci A, & Brodie MS. 2004. Ethanol effects on dopaminergic "reward" neurons in the ventral tegmental area and the mesolimbic pathway. *Alcoholism-Clinical and Experimental Research* 28:1768-1778.
- Asin KE, Wirtshafter D & Tabakoff, B. 1985. Failure to establish a conditioned place preference with ethanol in rats. *Pharmacology Biochemistry and Behavior*, 22(2):169-173.
- Badanich KA & Kirstein CL. 2004. Nicotine administration alters accumbal dopamine

- in the adult but not in the adolescent rat. *Ann NY Acad Sci*, 1021, 410-417.
- Barrett SP, Tichauer M, Leyon M & Pihl, RO. 2006. Nicotine increases alcohol self-administration in non-dependent male smokers. *Drug Alcohol Depend*, 81, 197-204.
- Batel P, Pessione F, Maitre C & Rueff B. 1995. Relationship between alcohol and tobacco dependencies among alcoholics who smoke. *Addiction*, 90, 977-980.
- Bates ME & Labouvie EW. 1997. Adolescent risk factors and the prediction of persistent alcohol and drug use into adulthood. *Alcohol Clin. Exp. Res*, 21: 944-950.
- Benwell ME & Balfour DJ. 1992. The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. *Br J Pharmacol*, 105, 849-856.
- Benwell ME, Balfour DJ & Lucchi HM. 1993. Influence of tetrodotoxin and calcium on changes in extracellular dopamine levels evoked by systemic nicotine. *Psychopharmacology*, 112, 467-474.
- Bergstrom HC, McDonald CG & Smith RF. 2006. Alcohol exposure during adolescence impairs auditory fear conditioning in adult Long-Evans rats. *Physiol Behav*, 88, 466-472.
- Bienkowski, P, Kuca P & Kostowski W. 1995. Conditioned place preference after prolonged pre-exposure to ethanol. *Pol. J. Pharmacol.*, 47(2): 189-191.
- Bizarro L, Patel S & Stolerman IP. 2003. Comprehensive deficits in performance of an attentional task produced by co-administering alcohol and nicotine to rats. *Drug and Alcohol Depend*, 72, 287-295.
- Blomqvist O, Engel JA, Nissbrandt H & Soderpalm B. 1993. The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. *Eur J*

- Pharmacol, 249, 207-213.
- Blomqvist O, Ericson M, Engel JA & Soderpalm B. 1997. Accumbal dopamine overflow of ethanol: Localization of the antagonizing effect of mecamylamine. *Eur J Pharmacol*, 334, 149-156.
- Blomqvist O, Ericson M, Johnson DH, Engel JA & Soderpalm B. 1996. Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. *Eur J Pharmacol*, 314, 257-267.
- Blomqvist O, Soderpalm B & Engel JA. 1992. Ethanol-induced locomotor activity: involvement of central nicotinic acetylcholine receptors? *Brain Res Bull*, 29, 173-178.
- Bozarth, MA. 1990. Evidence for the rewarding effects of ethanol using the conditioned place preference method. *Pharmacol Biochem Behav*, 35(2):485-487.
- Brown SA, Christiansen BA & Goldman MS. 1987. The alcohol expectancy questionnaire: an instrument for the assessment of adolescent and adult alcohol expectancies. *J Stud Alcohol*, 48, 483-491.
- Calabresi P, Lacey MG & North RA. 1989. Nicotine excitation of rat ventral tegmental neurons in vitro studied by intracellular recording. *British Journal of Pharmacology*, 98, 135-140.
- Carmondy TP, Brischetto CS, Matarazzo JD, O'Donnell RP & Conner WE. 1985. Concurrent use of cigarettes, alcohol, and coffee in healthy, community-living men and women. *Health Psychol*, 4, 323-335.
- CDC. 2002. Tobacco Information and Prevention Sources (TIPS). National Center for Chronic Disease Prevention and Health Promotion.

- Center for Disease Control and Prevention. 2001. The youth risk behavior survey: Trends in cigarette smoking among high school students. Washington, D.C.
- Chambers RA, Taylor JR, Potenza MN. 2003. Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *Am J Psychiatry*. 160, 1041-52.
- Ciccocioppo R, Panocka I, Frolidi R, Quiradamo E & Massi M. 1999. Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. *Psychopharmacol*, 141, 235-241.
- Clark DB, Kirisci L & Tarter RE. 1998. Adolescent versus adult onset and the development of substance use disorders in males. *Drug Alcohol Depend*, 49, 115-121.
- Clark A, Lingren S, Brooks SP, Watson WP & Little HJ. 2001. Chronic infusion of nicotine can increase operant self-administration of alcohol. *Neuropharmacol*, 41, 108-117.
- Clark A & Little HJ. 2004. Interactions between low concentrations of ethanol and nicotine on firing rate of ventral tegmental dopamine neurons. *Drug Alcohol Depend*, 75, 199-206.
- Clarke PB & Kumar R. 1983. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol*, 78, 329-337.
- Clarke PB, Schwartz RD, Paul SM, Pert CB & Pert A. 1985. Nicotinic binding in rat brain: autoradiographic comparison of ³H-acetylcholine, ³H-nicotine and ¹²⁵I-alpha-bungarotoxin. *J Neurosci*, 5, 1307-1315.
- Collins AC, Burch JB, de Fiebre CM & Marks MJ. 1988. Tolerance to and cross

- tolerance between ethanol and nicotine, *Pharmacol Biochem Behav*, 29, 365-373.
- Collins AC, Wilkins LH, Slobe BS, Cao JZ & Bullock AE. 1993. Long-term ethanol and nicotine treatment elicit tolerance to ethanol. *Alcohol Clin Exp Res*, 20, 990-999.
- Collins SL, Wade D, Ledon J & Izenwasser S. 2004. Neurochemical alterations produced by daily nicotine exposure in periadolescent vs. adult male rats. *Eur J Pharmacol*, 502, 75-85.
- Correa M, Arizzi MN, Betz A, Mingote S, Salamone JD. 2003. Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res Bull* 62:197-202.
- Corrigall WA. 1992. A rodent model for nicotine self-administration. In: Boulton AA, Baker GB, Wu PH (eds) *Animal models of drug addiction (neuromethods)*. Humana Press, Totowa, NJ, pp 315-344.
- Corrigall WA, Coen KM & Adamson KL. 1994. Self-administered nicotine activated the mesolimbic dopamine system through the ventral tegmental area. *Brain Res*, 653, 278-284.
- Crews FT, Braun CJ, Hoplight B, Switzer 3rd RC & Knapp DJ. 2000. Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. *Alcohol Clin Exp Res*, 24, 1712-1723.
- Crews FT, Mdzinarishvili D, Kim D, Je J & Nixon K. 2006. Neurogenesis in adolescent brain is potently inhibited by ethanol. *Neuroscience*, 137, 437-445.
- Cunningham CL, Niehus JS & Noble D. 1993. Species differences in sensitivity to ethanol's hedonic effects. *Alcohol*, 10, 97-102.
- Cunningham MG, Bhattacharyya S & Benes FM. 2002. Amygdalo-cortical sprouting

- continues into early adulthood: implications for the developmental of normal and abnormal function during adolescence. *J Comp Neur*, 453, 116-130.
- Darbra S, Pallares M & Ferre N. 2004. Effects of voluntary alcohol intake on nicotine-induced behavioral sensitisation in rats. *Pharmacol, Biochem and Behav*, 77, 815-822.
- Davies BT & Parker LA. 1990. Novel versus familiar ethanol: a comparison of aversive and rewarding properties. *Alcohol*, 7, 523-529.
- Dewit DJ, Adlaf EM, Offord DR & Ogborne AC. 2000. Age at first alcohol use: a risk factor for the development of alcohol disorders. *Am J Psychiatr*, 157, 745-750.
- Darbra S, Prat G, Pallares M, Ferre N. 2002. Tolerance and sensitization to the hypnotic effects of alcohol induced by chronic voluntary alcohol intake in rats. *J Psychopharmacol*. 1, 79-83.
- DiFranza JR & Guerrera MP. 1990. Alcoholism and smoking. *J Stud Alcohol*, 51, 130-135.
- Donny EC, Caggiula AR, Knopf S & Brown C. 1995. Nicotine self-administration in rats. *Psychopharmacol*, 122, 390-394.
- Doremus TL, Brunell SC, Rajendran P & Spear LP. 2005. Factors influencing ethanol consumption in adolescent relative to adult rats. *Alcohol Clin Exp Res*, 29(10), 1796-1808.
- Duncan SC, Alpert A, Duncan TE & Hops H. 1997. Adolescent alcohol use development and young adult outcomes. *Drug Alcohol Depend*, 49, 39-48.
- Engel JA, Fahlke C, Hulthe P, Hard E, Johannessen K, Snape B & Svensson L. 1988. Biochemical and behavioral evidence for an interaction between ethanol and calcium

- channel antagonists. *J Neural Trans*, 74, 181-193.
- Ericson M, Blomqvist O, Engel JA & Soderpalm B. 1998. Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *Eur J Pharmacol*, 358, 189-196.
- Ericson M, Molander A, Lof E, Engel JA & Soderpalm B. 2003. Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. *Eur J Pharmacol*, 467, 85-93.
- Falk DE, Yi H & Hiller-Sturmhofel S. 2006. An epidemiologic analysis of co-occurring alcohol and tobacco use and disorders: Findings from the national epidemiologic survey on alcohol and related conditions. *Alcohol Res Health*, 29, 162-171.
- Ferrari R, Le Novere N, Picciotto MR, Changeux JP & Zoli Z. 2002. Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. *Eur J Neurosci*, 15, 1810-1818.
- Fudala PJ, Teoh KW, Iwamoto ET. 1985. Pharmacologic characterization of nicotine-induced conditioned place preference. *Pharmacol Biochem Behav*. 22, 237-41.
- Funk D, Marinelli PW & Le AD. 2006. Biological processes underlying co-use of alcohol and nicotine: neuronal mechanisms, cross-tolerance, and genetic factors.
- Gatto GJ, McBride WJ, Murphy JM, Lumeng L & Li TK. 1994. Ethanol self-infusion into the ventral tegmental area by alcohol-preferring (P) rats. *Alcohol*, 11: 557-564.
- Gauvin DV & Holloway FA. 1992. Historical factors in the development of EtOH-conditioned place preference. *Alcohol*, 9, 1-7.
- Gauvin DV, Moore KR, Holloway FA. 1993. Do rat strain differences in ethanol consumption reflect differences in ethanol sensitivity or preparedness to learn?

- Alcohol, 10, 37-43.
- George SR, Fan T, Ng GY, Jung SY, O'Dowd BF & Naranjo CA. 1995. Low endogenous dopamine function in brain predisposes to high alcohol preference and consumption: reversal by increasing synaptic dopamine. *J. Pharm. Exp. Ther.* 273: 373-379.
- Gessa GL, Muntoni F, Collu M, Vargiu L & Mereu G. 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Research*, 348, 201-203.
- Gonzales RA, Job MO & Doyon WM. 2004. The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. *Pharmacol and Ther*, 103, 121-146.
- Grant BF. 1998. Age at smoking onset and its association with alcohol consumption and DSM-IV alcohol abuse and dependence: results from the national longitudinal alcohol epidemiologic survey. *Journal of Substance Abuse*, 10, 59-73.
- Hawkins JD, Graham JW, Maugin E, Abbott R, Hill KG & Catalona RF. 1997. Exploring the effects of age of alcohol use initiation and psychosocial risk factors on subsequent alcohol misuse. *J Stud Alcohol*, 58, 280-290.
- Hayashi T & Tadokoro S. 1985. Learning retardation and enhanced ethanol preference produced by postnatal pretreatments with ethanol in adult rats. *J Pharmacol*, 37, 269-276.
- Hjeresen DL. 1989. Nicotine interactions with ethanol tolerance. *Pharmacol Biochem Behav*, 31, 617-622.
- Ho A, Chin AJ & Dole VP. 1989. Early experience and the consumption of alcohol by

- adult C57BL/6J mice. *Alcohol*, 6, 511-515.
- Hoshaw BA, Lewis MJ 2001. Behavioral sensitization to ethanol in rats: evidence from the Sprague-Dawley strain. *Pharmacol Biochem Behav* 68:685-690.
- Ikemoto S, Qin M & Lui ZH. 2006. Primary reinforcing effects of nicotine are triggered from multiples regions inside and outside the ventral tegmental area. *J Neurosci*, 26, 723-730.
- Imperato A & Di Chiara G. 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther*, 239, 219-228.
- Imperato A, Mulas A & Di Chiara G. 1986. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur J Pharmacol*, 132, 337-338.
- Istvan J & Matarazzo JD. 1984. Tobacco, alcohol and caffeine use: a review of their interrelationships. *Psychol Bull*, 95, 301-326.
- Janhunen S, Linnervuo A, Svensk M & Ahtee L. 2005. Effects of nicotine and epibatidine on locomotor activity and conditioned place preference in rats. *Pharmacol Biochem Behav*, 82, 758-765.
- Jerlhag E, Grotli M, Luthman K, Svensson L & Engel JA. 2006. Role of the subunit composition of central nicotinic acetylcholine receptors for the stimulatory and dopamine-enhancing effects of ethanol. *Alcohol and Alcoholism*, 486-493.
- Johnston LD, O'Malley PM & Bachman JG. 2001. National survey results on drug use from the Monitoring the Future Study, 1975-2000. Vol 1, NIH Publication No. 01-4924, Bethesda, MD: Department of Health and Human Services.
- Kakihana R & McClearn GE. 1963. Development of alcohol preference in BALB/c

- mice. *Nature*, 199, 511-512.
- Kalsbeek A, Voorn P, Buds RM, Pool CW & Uylings HB. 1988. Development of the dopaminergic innervation in the prefrontal cortex of the rat. *J Comp Neurol*, 269, 58-72.
- Kandel DB. 1992. Epidemiological trends and implications for understanding the nature of addiction. *Res Publ Assoc Res Nerv Ment Dis* 70: 23-40.
- Kandel DB & Chen K. 2000. Extent of smoking and nicotine dependence in the United States: 1991-1993. *Nicotine Tob Res*, 2, 263-274.
- Katner SN, McBride WJ, Lumeng L, Li TK, Murphy JM. 1997. Involvement of CNS cholinergic systems in alcohol drinking in P rats. *Addict Biol*, 2, 215-223.
- Kohl RR, Katner JS, Chernet E & McBride WJ. 1998. Ethanol and negative feedback regulation of mesolimbic dopamine release in rats. *Psychopharmacology*, 139: 79-85.
- Korkosz A, Zatorski P, Taracha E, Plaznik A, Kostowski W & Bienkowski P. 2006. Effects of ethanol on nicotine-induced conditioned place preference in C57BL/6J mice. *Prog in Neuro-Psychopharmacol & Biol Psychiat*, 30, 1283-1290.
- Kunin D, Smith BR & Amit Z. 1999. Nicotine and ethanol interaction on conditioned taste aversions induced by both drugs. *Pharmacol Biochem Behav*, 62, 1999.
- Koob GF. 1992. Drug of abuse: Anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci*, 13: 177-184.
- Larsson A & Engel JA. 2004. Neurochemical and behavioral studies on ethanol and nicotine interactions. *Neurosci Biobehav Rev*, 27, 713-720.
- Larsson A, Jerlhag E, Svensson L, Soderpalm B & Engel JA. 2004. Is an α -conotoxin MII-sensitive mechanism involved in the neurochemical, stimulatory, and rewarding

- effects of ethanol? *Alcohol*, 24, 239-250.
- Le AD. 2002. Effects of Nicotine on Alcohol Consumption. *Alcohol Clin Exp Res*, 26, 1915-1916.
- Le AD, Corrigan WA, Harding S, Juzysch W, Li TK. 2000. Involvement of nicotine receptors in alcohol self-administration. *Alcohol: Clin and Exper Res*, 24, 155-163.
- Le AS, Li Z, Funk D, Shram M, Li TK & Shaham Y. 2006. Increased vulnerability to nicotine self-administration and relapse in alcohol-naïve offspring of rats selectively bred for high alcohol intake. *J Neurosci*, 26, 1872-1879.
- Le AD, Wang A, Harding S, Juzysch W & Shaham Y. 2003. Nicotine increases alcohol self-administration and reinstates alcohol seeking in rats. *Psychopharmacology*, 168, 216-221.
- Le Foll D & Goldberg SR. 2005. Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacol*, 178, 481-492.
- Le Foll D & Goldberg SR. 2006. Ethanol does not affect discriminative-stimulus effects of nicotine in rats. *Eur J Pharmacol*, 519, 96-102.
- Levin ED, Rezvani AH, Montoya D, Rose HE, Swartzwelder HS. 2003. Adolescent-onset nicotine self-administration modeled in female rats. *Psychopharmacology*, 169, 141-149.
- Little PJ, Kuhn CM, Wilson WA & Swartzwelder HS. 1996. Differential effects of ethanol in adolescent and adult rats. *Alcohol Clin Exp Res*, 20, 1346-1351.
- Lopez MF, Simpson DD, White NM & Randall CL. 2003. Age- and sex-related differences in alcohol and nicotine effects in CBL/6J mice. *Addiction Biology*, 8, 419-427.

- Lopez MF, White NM & Randall CL. 2001. Alcohol tolerance and nicotine cross tolerance in adolescent mice. *Addict Biol*, 6, 119-127.
- Lopez-Moreno JA, Trigo-Diaz JM, Rodriguez de Fonseca F, Gonzalez Cuevas G, Gomez de Heras R, Crespo Galan I, Navarro M. 2004. Nicotine in alcohol deprivation increases alcohol operant self-administration during reinstatement. *Neuropharmacol*, 47, 1036-1044.
- Maldonado AM & Kirstein CL. Differential dose effects on ethanol-induced locomotor activity in animals treated in adolescence and challenged as adults. *Alcohol*, manuscript in prep.
- Markweise BJ, Acheson SK, Levin ED, Wilson WA & Swartzwelder HS. 1998. Differential effects of ethanol on memory in adolescent and adult rats. *Alcohol Clin Exp Res*, 22, 416-421.
- Marshall CE, Dadmarz M, Hofford JM, Gottheil E & Vogel WM. 2002. Self-administration of both ethanol and nicotine. *Pharmacology*, 67, 143-149.
- McQuown AC, Belluzzi LD & Leslie FM. 2007. Low dose nicotine treatment during early adolescence increases subsequent cocaine reward. *Neurotoxicol Teratol*, 29, 66-73.
- Mereu G, Fadda F & Gessa GL. 1984. Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. *Brain Res.*, 292: 63-69.
- Miller NS & Gold MS. 1998. Comorbid cigarette and alcohol addiction: epidemiology and treatment. *J Addict Dis*, 17, 55-66.
- Moghaddam B & Bunney BS. 1989. Differential effect of cocaine on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens: Comparison

- to amphetamine. *Synapse* 4:156-161.
- Nadal R, Chappell AM & Samson HH. 1998. Effects of nicotine and mecamylamine microinjections into the nucleus accumbens on ethanol and sucrose self-administration. *Alcohol Clin Exper Res*, 22, 1190-1198.
- Nakano K, Mochizuki K, Sato M (1996). Self-control and the Type A behavior pattern. *J Behav Ther Exp Psychiatry* 27:169-174,
- Nakahara D, Ozaki N, Miura Y & Nagatsu T. 1989. Increased dopamine and serotonin metabolism in rat nucleus accumbens produced by intracranial self-stimulation of medial forebrain bundle as measured by in vivo microdialysis. *Brain Research*, 495, 178-181.
- Nisell M, Nomikos GG & Svensson TH. 1994. Infusion of nicotine in the ventral tegmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. *Pharmacol Toxicol*, 75, 348-352.
- Nisell M, Nokimos GG & Svernnsson TH. 1994. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse*, 16, 36-44.
- O'Dell LE, Bruijnzeel AW, Smith RT, Parsons LH, Merves ML, Goldberger BA, Richardson HN, Koob GF & Markou A. 2006. Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. *Psychopharmacology*, 186, 612-619.
- Ojeda SR & Urbanski HF. Puberty in the rat. In: Knobil E & Neill JD (Eds.). *The Physiology of Reproduction*, 2nd Edition, New York, Raven Press, 1994, 363-409.
- Onaivi ES, Todd S & martin BR. 1989. Behavioral effects in the mouse during and

- following withdrawal from ethanol ingestion and/or nicotine administration. *Drug Alcohol Depend*, 24, 205-211.
- Panocka I, Ciccocioppo R, Mosca C & Massi M. 1995. Effects of the dopamine D1 receptor antagonist SCH 39166 on the ingestive behaviour of alcohol-preferring rats. *Psychopharmacology*, 120: 227-235.
- Parisella RM & Pritham GH. 1964. Effect of age on alcohol preference in rats. *Q J Stud Alcohol*, 25, 248-252.
- Phillips AG, Coury A, Fiorino D, LePiane FG, Brown E & Fibiger HC. 1992. Self-stimulation of the ventral tegmental area enhances dopamine release in the nucleus accumbens: a microdialysis study. *Ann N Y Acad Sci.*, 654: 199-206.
- Philpot RM, Badanich KA & Kirstein CL. 2003. Place conditioning: age-related changes in the rewarding and aversive effects of alcohol. *Alcoholism, Clinical and Experimental Research*, 27, 593-599.
- Philpot RM & Kirstein CL. 2004. Developmental differences in the accumbal dopaminergic response to repeated ethanol exposure. *Ann NY Acad Sci*, 1031, 422-426.
- Potthoff AD, Ellison G & Nelson L. 1983. Ethanol intake increases during continuous administration of amphetamine and nicotine, but not several other drugs. *Pharmacol Biochem Behav*, 18, 489-493.
- Primus RJ & Kellogg CK. 1989. Pubertal-related changes influence the development of environment-related social interaction in the male rat. *Dev Psychobiol*, 22, 633-643.
- Rezvani AH & Levin ED. 2002. Nicotine-alcohol interactions and cognitive functions in rats. *Pharmacol Biochem Behav*, 72, 865-872.

- Rezvani AM & Levin ED. 2004. Adolescent and adult rats respond differently to nicotine and alcohol: motor activity and body temperature. *International Journal on Developmental Neuroscience*, 22, 349-354.
- Rose JE, Brauer LH, Behm FM, Cramblett M, Calkins K & Lawhon D. 2002. Potentiation of nicotine reward by alcohol. *Alcohol Clin Exp Res*, 26, 1930-1931.
- Rose RJ, Dick DM, Viken RJ, Pulkkinen L & Kaprio L. 2001. Drinking or abstaining at age 14? A genetic epidemiological study. *Alcohol Clin Exp Res*, 25, 1594-1604.
- Rosenberg DR & Lewis DA. 1994. Changes in the dopaminergic innervation of monkey prefrontal cortex during late postnatal development: A tyrosine hydroxylase study. *Biol Psychiat*, 36, 272-277.
- Sahr AE, Thielen RJ, Lumeng L, Li TK & McBride WJ. 2004. Long-lasting alterations of the mesolimbic dopamine system after periadolescent ethanol drinking by alcohol-preferring rats. *Alcohol Clin Exp Res*, 28, 702-711.
- SAMSHA. 2003. Results from the 2002 National Survey on Drug Use and Health: National Findings. U.S. Office of Applied Studies.
- Samson HH, Hodge CW, Tolliver GA & Haraguchi M. 1993. Effect of dopamine agonists and antagonists on ethanol reinforced behavior: the involvement of the nucleus accumbens. *Brain Res. Bull.*, 30, 133-141.
- Schaefer GJ & Michael RP. 1992. Interactions between alcohol and nicotine on intracranial self-stimulation and locomotor activity in rats. *Drug & Alcohol Depend*, 30, 37-47.
- Schechter MD. 1992. Locomotor activity but not conditioned place preference is differentially affected by a moderate dose of ethanol administered to P and NP rats.

- Alcohol, 9, 185-188.
- Schmid B, Hohm E, Blomeyer D, Zimmermann US, Schmidt MH, Esser G & Laucht M. 2007. Concurrent alcohol and tobacco use during early adolescence characterizes a group at risk. *Alcohol Alcohol*, 42, 219-225.
- Sharpe AL & Samson HH. 2002. Repeated nicotine injections decrease operant self-administration. *Alcohol*, 28, 1-7.
- Shram MJ, Funk D, Li Z & Le AD. 2006. Periadolescent and adult rats respond differently in tests measure the rewarding and aversive effects of nicotine. *Psychopharmacology*, 186, 201-208.
- Schroeder BE, Binzak JM & Kelley AE. 2001. A common profile of prefrontal cortical activation following exposure to nicotine- or chocolate-associated contextual cues. *Neuroscience*, 105, 535-545.
- Schwartz RD, Lehmann J & Kellar KJ. 1984. Presynaptic nicotinic cholinergic receptors labeled by [3H]acetylcholine on catecholamine and serotonin axons in brain. *J Neurochem*, 42, 1495-1498.
- Shoaib M, Schindler CW, & Goldberg SR. 1997. Nicotine self-administration in rats: strain and nicotine-pre-exposure effects on acquisition. *Psychopharmacol*, 129, 35-43.
- Signs SA & Schechter MD. 1986. Nicotine-induced potentiation of ethanol discrimination. *Pharmacol Biochem Behav*, 18, 484-493.
- Silveri MM & Spear LP. 1998. Decreased hypnotic effects of ethanol early in ontogeny. *Alcohol Clin Exp Res*, 22, 670-676.
- Silveri MM & Spear LP. 1999. Ontogeny of rapid tolerance to the hypnotic effects of

- ethanol. *Alcohol Clin Exp Res*, 23, 1180-1184.
- Silvers JM, Tokunaga S, Mittleman G & Matthews DB. 2003. Chronic intermittent injections of high-dose ethanol during adolescence produce metabolic, hypnotic, and cognitive tolerance in rats. *Alcohol Clin Exp Res*, 27, 1606-1612.
- Singer G, Wallace M & Hall R. 1982. Effects of dopaminergic nucleus accumbens lesions on the acquisition of schedule-induced self injection of nicotine in the rat. *Pharmacol, Biochem Behav*, 17, 579-581.
- Sircar R & Sircar D. 2005. Adolescent rats exposed to repeated ethanol treatment show lingering behavioral impairments. *Alcohol Clin Exp Res*, 29, 1402-1410.
- Slawecki CJ, Betancourt M, Cole M & Ehlers CL. 2001. Periadolescent alcohol exposure has lasting effects on adult neurophysiological function in rats.
- Slawecki CJ & Ehlers CL. Enhanced prepulse inhibition following adolescents ethanol exposure in Sprague-Dawley rats. *Alcohol Clin Exp Res*, 29, 1829-1836.
- Slawecki CJ, Thorsell A & Ehlers CL. 2004. Long-term neurobehavioral effects of alcohol or nicotine exposure in adolescent animal models. *Ann NY Acad Sci*, 1021, 448-452.
- Smith AM, Kelley RB & Chen WJ. 2002. Chronic continuous nicotine exposure during periadolescence does not increase ethanol intake during adulthood in rats. *Alcohol Clin Exp Res*, 26, 976-979.
- Smith BR, Horan JT, Gaskin S & Amit Z. 1999. Exposure to nicotine enhances acquisition of ethanol drinking in laboratory rats in a limited access paradigm. *Psychopharmacology*, 142, 408-412.
- Smith RF. 2003. Animal models of periadolescent substance abuse. *Neurotoxicol*

- Teratol 25(3): 291-301.
- Schochet TL, Kelley AE & Landry CF. 2004. Differential behavioral effects of nicotine exposure in adolescent and adult rats. *Psychopharmacology*, 175, 265-273.
- Solderpalm B, Ericson M, Olausson P, Blomqvist O & Engel JA. 2000. Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behav Brain Res*, 113, 85-96.
- Spear LP. 2000. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev*, 24, 417-463.
- Spear LP & Brake SC. 1983. Periadolescence: Age-dependent behavior and psychopharmacological responsivity in rats. *Dev Psychobiol*, 16, 83-109.
- Spear LP & Varlinskaya EI. 2005. Adolescence. Alcohol sensitivity, tolerance, and intake. *Recent Dev Alcohol*, 17, 143-159.
- Stansfield KH, Philpot RM & Kirstein CL. 2004. An animal model of sensation seeking: the adolescent rat. *Ann N Y Acad Sci*, 1021, 453-458.
- Stansfield KH & Kirstein CL. 2006. Effects of novelty on behavior in the adolescent and adult rat. *Dev Psychobiol* 48, 10-15.
- Stansfield KH & Kirstein CL. Chronic drug exposure during adolescence alters novelty-related behaviors in adulthood. *Pharmacol, Biochem & Behav*, manuscript accepted pending revisions.
- Stewart RB, Murphy JM, McBride WJ, Lumeng L & Li TK. 1996. Place conditioning with alcohol in alcohol-preferring and –nonpreferring rats. *Pharmacol, Biochem Behav*, 53, 487-491.
- Stolerman IP. 1991. Behavioural pharmacology of nicotine: multiple mechanisms. *Br J*

- Addict, 86, 533-536.
- Swartzwelder HS, Wilson WA & Tayyeb MI. 1995a. Differential sensitivity of NMDA receptor-mediated synaptic potentials to ethanol in immature versus mature hippocampus. *Alcohol Clin Exp Res*, 19, 320-323.
- Swartzwelder HS, Wilson WA & Tayyeb MI. 199b. Age-dependent inhibition of long-term potentiation by ethanol in immature versus mature hippocampus. *Alcohol Clin Exp Res*, 19, 1480-1485.
- Taioli E, Wynder EL. 1991. Effect of the age at which smoking begins on frequency of smoking in adulthood. *N Engl J Med* 325:968-969
- Tizabi Y, Copeland RL, Louis VA & Taylor RE. 2002. Effects of combined systemic alcohol and central nicotine administration into the ventral tegmental area on dopamine release in the nucleus accumbens. *Alcohol Clin Exp Res*, 26, 394-399.
- Tolliver GA & Samson HH. 1991. The influence of early postweaning ethanol exposure on oral self-administered behavior in the rat. *Pharmacol Biochem Behav*, 38, 575-580.
- Torella T, Philpot RM, Wecker L & Kirstein CL. 2004. Developmental differences in nicotine place conditioning. *Annals of the New York Academy of Sciences*, 1021; 399-403.
- Tsui T, Le AD, Wang A, Juztsch W & Harding S. 2001. Exposure to nicotine but not alcohol during adolescence enhances acquisition of alcohol consumption in adulthood. *Alcohol Clin Exp Res*, 25, Suppl: #73.
- van der Kooy D, O'Shaughnessy M, Mucha RF & Kalant H. 1983. Motivational properties of ethanol in naïve rats as studied by place conditioning. *Pharmacol*,

- Biochem & Behav, 19, 441-445.
- Vastola BJ, Douglas LA, Varlinskaya EI, Spear LP. 2002. Nicotine-induced conditioned place preference in adolescent and adult rats. *Physiol Behav.* 77, 107-114.
- Vezina P, Blanc G, Glowinski J & Tassin JP. 1992. Nicotine and morphine differentially activate brain dopamine in prefrontocortical and subcortical terminal fields: effects of acute and repeated injections. *J Pharmacol Exp Ther*, 261, 484-490.
- Walter S & Kuschinsky K. 1989. Conditioning of nicotine effects on motility and behaviour in rats. *Naunyn Schmiedebergs Arch Pharmacol*, 339, 208-213.
- Weiss F, Lorang MT, Bloom FE & Koob GF. 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *Journal of Pharmacology and Experimental Therapeutics*, 267, 250-258.
- Weiss F, Mitchiner M, Bloom FE & Koob GF. 1990. Free choice responding for ethanol versus water in alcohol preferring (P) and unselected Wistar rats is differentially modified by naloxone, bromocriptine, and methysergide. *Psychopharmacology*, 101: 178-186.
- White AM, Ghis AJ, Levin ED & Swartzwelder HS. 2000. Binge pattern ethanol exposure in adolescent and adult rats: Differential impact on subsequent responsiveness to ethanol.
- White AM, Truesdale MC, Bae JG, Ahmad S, Wilson WA, Best PJ & Swartzwelder HS. 2002. Differential effects of ethanol on motor incoordination in adolescent and adult rats. *Pharmacol Biochem Behav*, 73, 673-677.
- Williams-Hemby L & Porrino LJ. 1994. Low and moderate doses of ethanol produce

- distinct patterns of cerebral metabolic changes in rats. *Alcohol Clin Exp Res*, 18: 982-988.
- Windle M. 1990. Alcohol use and abuse: some findings from the National Adolescent Student Health Survey. *Alcohol Health and Research World*, 15, 5-10.
- Wise RA & Rompre PP. 1989. Brain dopamine and reward. *Annu. Rev. Psychol.* 40: 191-255.
- Witt ED. 1994. Mechanisms of alcohol abuse and alcoholism in adolescents: a case for developing animal models. *Behav Neural Biol*, 62, 168-177.