

EFFECTS OF NICOTINE EXPOSURE IN ADOLESCENT RATS ON
ACQUISITION OF ALCOHOL DRINKING AND RESPONSE TO NICOTINE
IN ADULTHOOD

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ABSTRACT

Amy L. Bracken

EFFECTS OF NICOTINE EXPOSURE IN ADOLESCENT RATS ON ACQUISITION OF ALCOHOL DRINKING AND RESPONSE TO NICOTINE IN ADULTHOOD

Nicotine is one of the most widely abused drugs in the world, and most smokers begin smoking during their adolescent years. Adolescence is a unique developmental period during which vulnerability to the effects of drug exposure is especially high. This dissertation uses rodent models to investigate the persistent effects of adolescent nicotine exposure on both neurobiological and behavioral measures of drug sensitivity in adulthood. The aims of this dissertation were to 1) determine whether nicotine would be self-administered into the posterior ventral tegmental area (pVTA), a neuroanatomical component of the mesolimbic dopamine (DA) system, which is known to be involved in reward and reinforcement; 2) investigate whether adolescent nicotine exposure would alter the sensitivity of the mesolimbic DA system as measured by DA release in the nucleus accumbens (NAc) in response to nicotine microinjections into the pVTA; 3) examine the effects of adolescent nicotine exposure on behavioral sensitization to nicotine in adulthood; and 4) investigate whether adulthood alcohol drinking behavior, in both Wistar and alcohol-preferring (P) rats, would be augmented by nicotine exposure during adolescence. Results of this dissertation demonstrated that 1) the pVTA is a neuroanatomical site that

supports nicotine self-administration; and that adolescent nicotine exposure results in 2) increased nicotine-stimulated DA release in the NAc during adulthood; 3) augmented behavioral sensitization to nicotine in adult animals; and 4) enhanced acquisition of alcohol drinking behavior in adult Wistar and P rats. Overall, this dissertation provides insight into the diverse and persistent changes, in both neurobiology and behavior, caused by exposure to nicotine during the critical developmental period of adolescence.

William J. McBride, PhD., Chair

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LIST OF ABBREVIATIONS

6-OHDA	6-hydroxydopamine
AA rats	Alko Alcohol rats
ACh	acetylcholine
aCSF	artificial cerebrospinal fluid
ADE	alcohol deprivation effect
ANA rats	Alko Non-Alcohol rats
ANOVA	analysis of variance
aVTA	anterior ventral tegmental area
CNS	central nervous system
DA	dopamine
DHBE	dihydro- β -erythroidine
EMIT	electrolytic microinfusion transducer
EtOH	ethanol
ETS	environmental tobacco smoke
HPLC	high-performance liquid chromatography
ICSA	intracranial self-administration
i.v.	intravenous
LMA	locomotor activity
LTP	long-term potentiation
MLA	methyllycaconitine
nAChR	nicotinic acetylcholine receptor
NAc	nucleus accumbens
NP rats	alcohol-non-preferring rats
P rats	alcohol-preferring rats
PD	post-natal day
PFC	prefrontal cortex
PPTg	pedunculopontine tegmental nucleus
pVTA	posterior ventral tegmental area
RN	red nucleus
s.c.	subcutaneous
SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticulata
VTA	ventral tegmental area

I. INTRODUCTION

A. Nicotine and nicotinic receptors

Drug addiction is a worldwide problem that carries high monetary and societal costs. Tobacco is one of the world's most widely-abused drugs. Here in the United States, cigarette smoking is the leading cause of preventable disease, disability, and death (NIDA 2006). It is estimated that 30% of the U.S. population over age 12 are regular smokers (SAMHSA 2003). Smoking use increases risk of cancer, cardiovascular disease, and stroke. Worldwide, only malaria is responsible for more deaths than smoking (Mansvelder and McGehee 2002).

Tobacco contains thousands of chemicals. Of these, nicotine is the primary psychoactive ingredient. It occurs naturally in the tobacco plant, the leaves of which have been used for medicinal and recreational purposes for thousands of years. Tobacco is most commonly smoked, but can also be absorbed through the membranes inside the mouth in the form of smokeless tobacco. More recently, nicotine chewing gum and transdermal nicotine patches have been marketed primarily to aid in smoking cessation.

Once in the bloodstream, nicotine flows throughout the body and readily crosses the blood-brain barrier. It reaches the brain within 60 seconds, at an initial concentration of 100-500 nM in the blood and brain (Karan et al. 2003). Its primary site of action is the nicotinic acetylcholine receptor (nAChR). nAChRs are membrane-bound ligand-gated ion channels distributed widely both

peripherally, at the neuromuscular junctions of somatic muscles, and in the central nervous system (CNS), in the autonomic ganglia and throughout the brain. Nicotine activates nAChRs in the CNS to elicit its psychoactive effects, which include increased arousal, muscle relaxation, decreased anxiety, and mood elevation.

Because of their prevalence throughout the body, nAChRs have been widely studied (reviews by Vidal 1996; Changeux et al. 1998; Leonard and Bertrand 2001; Mansvelder and McGehee 2002). The nAChR is made up of five subunits, arranged symmetrically around a central pore. Genes for ten different α subunits and four different β subunits have been identified, although the most prevalent receptors in the mammalian CNS contain either a combination of $\alpha 4$ and $\beta 2$ subunits, or they are $\alpha 7$ homomers (Jones et al. 1999). When acetylcholine (ACh), the endogenous ligand and neurotransmitter, or nicotine, an exogenous nAChR agonist, binds to the receptor on an α subunit near the N terminus, a conformation change increases the probability that cations will pass through the central pore. Typically, sodium (Na^+) ions enter the cell and potassium (K^+) ions exit, with the net result being an inward flow of positively charged ions. This excites the cell and can lead to action potential firing. In some neuronal nAChRs, calcium (Ca^{2+}) ions can also flow into the cell, which means that nAChR activity could influence Ca^{2+} -dependent processes such as ion pumps and neurotransmitter release (Wonnacott 1997).

nAChRs are distributed widely throughout the brain, including in the brainstem and cerebellum, the midbrain, the hippocampus and other limbic

areas, the olfactory bulbs, the prefrontal cortex, and throughout the different cell layers of the cortex (Tribollet et al. 2004). They can be found on cell bodies and dendrites, where they may modulate postsynaptic effects, or on axon terminals, where they can modulate synaptic transmission. The $\alpha 4\beta 2$ -containing heteromeric and $\alpha 7$ homomeric receptors were identified early on in the study of nAChRs and differentiated by their different affinities for nicotine binding (Changeux et al. 1998). The $\alpha 4\beta 2$ receptors have a high affinity for nicotine, and the $\alpha 7$ receptors are low-affinity nicotine binding sites. Equally important are the desensitization properties of these two receptor subtypes; at nicotine concentrations similar to those experienced by smokers, both the $\alpha 4\beta 2$ and $\alpha 7$ nAChRs are activated, but the $\alpha 4\beta 2$ nAChRs are desensitized within minutes (Mansvelder and McGehee 2002). The $\alpha 7$ nAChRs require higher nicotine concentrations to become desensitized, and they are slower to do it. Therefore, when studying nicotinic modulation of neuronal pathways, both the activation and desensitization patterns must be considered.

B. Nicotine and the mesolimbic dopamine system

Because nAChRs are widely distributed throughout the brain, it is possible that nicotine acts on many different cellular processes that are either directly or indirectly related to the process of reward and the development of drug addiction. However, a wide breadth of research has led to the conclusion that dopamine (DA) is the neurotransmitter that plays the major role in the behavioral stimulus

properties of nicotine (reviewed by Stolerman and Shoaib 1991; Dani and Heinemann 1996; Di Chiara 2000; Balfour 2004).

The intracranial self-administration (ICSA) technique has been used to identify discrete brain regions involved in the initiation of response-contingent behaviors for the delivery of a reinforcer (Bozarth and Wise 1980; Goeders and Smith 1987; McBride et al. 1999). Previous research using the ICSA procedure in rodents has demonstrated central nervous system sites where drugs of abuse such as ethanol (Gatto et al. 1994; Rodd-Henricks et al. 2000a), amphetamine (Hoebel et al. 1983), and cocaine (Goeders and Smith 1983, 1986; McKinzie et al. 1999) are self-infused. The rewarding properties of nicotine have been linked to the drug's ability to stimulate the mesolimbic DA system (Di Chiara 2000), similar to many other drugs of abuse (Wise and Bozarth 1987; Robinson and Berridge 1993). The major neuronal pathway in this system originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc) as well as limbic structures and the prefrontal cortex.

There is much evidence to support a role for this pathway in the reinforcing effects of nicotine, which could be related to the development of nicotine addiction. First, receptor binding studies have shown that nAChRs are highly localized in the VTA compared to surrounding areas (Klink et al. 2001; Wooltorton et al. 2003). Behavioral studies have also lent support. Clarke and colleagues (1988) knew that other psychomotor stimulants such as cocaine and amphetamine activated the mesolimbic DA system to exert both their behavioral stimulating and reinforcing actions. By using local microinjections of 6-

hydroxydopamine (6-OHDA) into the NAc to selectively deplete DA terminals in rats and observing a subsequent decrease in locomotor response to nicotine injections, Clarke and colleagues were able to determine that nicotine's locomotor stimulant effects were also predicated on activation of the mesolimbic DA system. Nicotine injections directly into the VTA have been shown to increase locomotor activity in rats (Panagis et al. 1996). Repeated intra-VTA microinjections of the nicotinic receptor agonist cytisine also resulted in locomotor sensitization, further indicating that nAChRs within the VTA can produce locomotor effects (Museo and Wise 1994).

Corrigall and colleagues used DA receptor antagonists and site-specific 6-OHDA lesion techniques to investigate the role of DA in nicotine self-administration behavior (Corrigall and Coen 1991; Corrigall et al. 1992). They observed that systemic injections of both DA D1 and D2 receptor antagonists reduced intravenous (i.v.) nicotine self-administration, and that this reduction was not due to overall motor impairment. Corrigall and colleagues also concluded that DA release in the NAc was essential for nicotine self-administration, as the bilateral lesions of DA terminals in this area significantly reduced responding for i.v. nicotine. Later, Corrigall's group shed even more light on the specific site of action of nicotine when they demonstrated that infusions of the $\alpha 4\beta 2$ -selective nAChR antagonist dihydro- β -erythroidine (DHBE) directly into the VTA attenuated i.v. nicotine self-administration (Corrigall et al. 1994). DHBE did not, however, cause similar reductions in operant responding for food or cocaine, or in overall locomotor activity. Intra-VTA nicotine was also shown to induce

conditioned place preference, which is yet another indication that nicotine is rewarding within the VTA (Laviolette and van der Kooy 2003b). Finally, intra-VTA nicotine perfusion increased DA levels in the NAc, and this increase was potentiated when the rats were pretreated with systemic nicotine for 5 days preceding the microdialysis study (Rahman et al. 2004). Taken together, all of these studies indicate that the VTA is a specific site of action within the mesolimbic DA system at which nicotine exerts its motivational stimulus effects.

While these studies and many more have concluded that nicotine acts directly on DA neurons in the VTA to exert its excitatory effects, there are other cell types within the VTA that also express nAChRs (Klink et al. 2001). Specifically, GABA interneurons and glutamatergic presynaptic terminals both synapse onto DA neurons in the VTA and express nAChRs. Studies on the subunit compositions of the different VTA nAChRs have revealed that the DA and GABA neurons express mostly non- $\alpha 7$, and therefore high-affinity and fast-desensitizing, nAChRs (Pidoplichko et al. 1997; Klink et al. 2001). Conversely, glutamatergic neurons originating in the prefrontal cortex express presynaptic nAChRs in the VTA which are mostly $\alpha 7$ -containing, and therefore low-affinity and slower to desensitize (Kalivas et al. 1989). Mansvelder and colleagues proposed two synaptic mechanisms by which all three neuron types in the VTA may contribute to long-lasting changes in mesolimbic DA activity and thus development of nicotine addiction (Mansvelder and McGehee 2000; Mansvelder et al. 2002).

Since nAChRs are present both on DA neurons and glutamatergic terminals in the VTA, nicotine activates the DA neurons both directly at nAChRs and through the release of glutamate into the synapse and its activation of postsynaptic NMDA receptors (Bonci and Malenka 1999). The depolarization induced by nAChR activation would remove the magnesium block on the NMDA receptor, and allow glutamate to bind to the receptor and Ca^{2+} ions to enter the cell. Together, this pre- and post-synaptic activation of the cell can induce long-term potentiation (LTP). And while the nAChRs on the DA neurons are mostly the non- $\alpha 7$ type and therefore subject to quick desensitization, the $\alpha 7$ nAChRs on the glutamatergic terminals resist desensitization and therefore allow for continued activation of the DA neurons via NMDA receptors (Mansvelder and McGehee 2002). Thus, LTP of VTA DA neurons could be involved in elevated DA release in the NAc. Notably, Mansvelder and McGehee (2000) demonstrated that such LTP could be induced by nicotine concentrations comparable to that of human cigarette smokers after just one cigarette.

GABA interneurons in the VTA also play a role in nicotine's actions on the mesolimbic DA system. Nicotine binds to the nAChRs on these interneurons, resulting in a transient increase in inhibitory GABA input to the DA neurons. Since most of the nAChRs on the GABA interneurons are of the non- $\alpha 7$ variety, they desensitize rapidly and reduce the short-lived inhibitory control on the DA neurons. This was also shown to take place in the presence of low nicotine concentrations, similar to those achieved by smokers (Mansvelder and McGehee 2002). Thus, the DA neuron excitement and the glutamate-driven LTP

mentioned above are facilitated by a reduction in inhibitory GABAergic tone. Together, these three types of nAChR-expressing neurons in the VTA promote nicotine-induced DA release in the NAc and, over time, can change the way the mesolimbic pathway responds to nicotine which can lead to nicotine addiction.

In addition to the VTA, other brain areas have been identified as possible sites of action of nicotine in relation to rewarding effects. Accumbal DA levels are elevated when nicotine is infused directly into the NAc itself, but this effect is transient compared to nicotine infusions into the VTA (Nisell et al. 1994b). The pedunclopontine tegmental nucleus (PPTg) has also been identified as a site of action for nicotine; specifically, cholinergic neurons projecting from the PPTg to the VTA DA neurons are activated during systemic nicotine self-administration, and lesions of these neurons reduce self-administration behavior (Lanca et al. 2000). However, both of these sites of action seem to result in input to VTA DA neurons, so this seems to be the consensus primary action site for the reinforcing effects of nicotine.

C. Nicotine and ethanol interactions

Often, drug addicts will co-abuse two or more different drugs. Nicotine and ethanol (EtOH) are two of the most highly co-abused drugs in the world. In the United States, it is estimated that 90% of alcoholics are smokers, compared with just 30% of the general population (Daepfen et al. 2000). Similarly, alcoholism is estimated to be 10-14 times more common among smokers than

non-smokers (DiFranza and Guerrera 1990). The amount of tobacco smoked is positively correlated with alcohol intake and the severity of alcohol dependence (Grant et al. 2004).

Due to the high rate of nicotine and EtOH co-abuse in humans, significant research has been directed at elucidating the interactions of these two drugs in laboratory animals. Nicotine's locomotor-activating effects were enhanced when EtOH was co-administered, even though EtOH alone decreased locomotor activity (Schaefer and Michael 1992). Home-cage EtOH drinking was increased in rats during chronic nicotine treatment, and persisted even one week after cessation of nicotine exposure (Pothoff et al. 1983; Blomqvist et al. 1996). Conflicting results have emerged from studies investigating the effects of chronic nicotine treatment on operant responding for EtOH in rats, with some data demonstrating an increase in EtOH self-administration (Le et al. 2000; Clark et al. 2001) whereas one study demonstrated a decrease (Sharpe and Samson 2002). Nicotine has been shown to reinstate EtOH seeking in rats following extinction of operant responding (Le et al. 2003; Lopez-Moreno et al. 2004). Although any divergent results could be due to differences in nicotine administration (repeated injections vs. continuous infusion) and/or rat strain, it is clear that the underpinnings of nicotine/EtOH interactions are complex.

In addition to investigation of systemic effects, research has also been aimed at elucidating the neuroanatomical sites regulating nicotine and EtOH interactions. Several studies have indicated a role for VTA nAChRs in the actions of EtOH, including EtOH-stimulated NAc DA release and EtOH-

stimulated locomotor activation (Larsson et al. 2002). In vivo microdialysis has been used to demonstrate that the noncompetitive nAChR antagonist mecamylamine blocked the EtOH-stimulated rise in NAc DA (Blomqvist et al. 1993). However, all drugs in this experiment were administered systemically, which makes the effects on specific brain regions difficult to differentiate. Later, this same group followed up on these results using site-specific techniques, and determined that local infusion of mecamylamine into the VTA blocked the rise in NAc DA induced by EtOH reverse microdialysis into the NAc (Ericson et al. 2008). However, the specific regions mediating EtOH-stimulated NAc DA release implicated by this group differ from the regions implicated by our laboratory. Ericson's group argues that EtOH infusion into neither the aVTA nor the pVTA results in NAc DA release; rather, the NAc itself is the primary site of action of EtOH in the mesolimbic DA system, whereas the VTA is a secondary site in a NAc-VTA-NAc signaling loop. Additionally, their 2008 study indicated that nAChRs in the anterior VTA (aVTA), but not posterior VTA (pVTA), were involved in mediating NAc DA release. Our laboratory has shown that rats self-administer EtOH into the pVTA but not the aVTA (Rodd-Henricks et al. 2000a) and that EtOH microinjections into the pVTA stimulate DA release in the NAc (Ding et al. 2009; Rodd et al. 2007). So, while there is disagreement about the primary site of action of EtOH within this system, one can conclude that nAChRs are likely involved in the actions of EtOH in some capacity.

The nAChR subtypes mediating certain effects of EtOH have been investigated. Systemic pretreatment of either DHBE, which is selective for $\alpha 4\beta 2$

nAChRs, or methyllycaconitine (MLA), which is selective for $\alpha 7$ nAChRs, failed to reduce both EtOH-stimulated locomotor activity and EtOH-induced DA overflow in the NAc in mice (Larsson et al. 2002). However, the less selective negative allosteric nAChR modulator mecamylamine did block both of these EtOH-stimulated effects, so it appears that EtOH may not act on these nAChR subtypes.

nAChRs have also been implicated in the self-administration of EtOH. Daily nicotine injections (0.8 mg/kg) were shown to increase EtOH consumption after repeated treatment, following an initial suppression of EtOH drinking on the first day (Le et al. 2000). Systemic injections of the nAChR antagonist mecamylamine reduced EtOH intake. Together, these results suggest that activation of nAChRs are involved in EtOH consumption behavior. Furthermore, systemic injections of the competitive nAChR antagonist DHBE, which is selective for $\alpha 4\beta 2$ nAChRs, did not reduce EtOH intake (Le et al. 2000), which indicates that this receptor subtype is not involved in EtOH consumption behavior. A related study was conducted by administering mecamylamine directly into the VTA using reverse microdialysis, and assessing its effects of EtOH drinking as well as NAc DA release (Ericson et al. 1998). Their findings, consistent with the study from Le and colleagues, were that a single mecamylamine infusion in the VTA reduced EtOH intake and preference for EtOH, and blocked the EtOH-induced increase in NAc DA levels.

D. The alcohol-preferring P rat

A useful genetic tool for studying EtOH-related behaviors in the laboratory is the alcohol-preferring P rat. This selectively-bred line of rats voluntarily consumes an average of 5-8 g/kg body weight/day of EtOH and meets the criteria proposed for an animal model of alcoholism (Lumeng et al. 1977; Cicero 1979; McMillin 1997; Murphy et al. 2002). However, there is evidence that they may show differential sensitivity to other drugs of abuse as well, as compared to alcohol-non-preferring NP rats. Adult P rats are less sensitive to the locomotor activating effects of amphetamine (McKinzie et al. 2002), but more sensitive to locomotor activation by nicotine (Gordon et al. 1993). Importantly for this dissertation, P rats also self-administer higher levels of intravenous nicotine than NP rats, and were more susceptible to relapse of nicotine self-administration behavior (Le et al. 2006). Studies on different lines of rats selectively bred for high and low EtOH intake, the Alko Alcohol (AA) and Alko Non-Alcohol (ANA) rats, did not reveal any differential effects of nicotine on locomotor sensitization or DA release in the NAc (Kiiianmaa et al. 2000). However, P rats have been shown to abuse nicotine, as measured by self-administration and relapse following a period of deprivation (Le et al. 2006), in addition to being considered an animal model of alcohol abuse. Thus, the P and NP rats are likely a more appropriate genetic model of alcoholism than the AA and ANA rats in which to test the effects of nicotine.

E. Adolescence: a critical neurobiological time period

Adolescence is a unique developmental period that is characterized by landmarks such as physical growth, behavioral maturation, and neuronal development. Behaviorally, adolescence is also the time when novelty- and sensation-seeking emerge, which, in both humans and laboratory animals, has been associated with drug use (Martin et al. 2002; Cain et al. 2005).

The National Institute of Drug Abuse 2005 Monitoring the Future Study indicates that among 12th graders surveyed, 74% had used alcohol, 50% had smoked cigarettes, and 45% had smoked marijuana (NIDA 2006). While some experimentation with drugs appears to be the norm during adolescence, it has been shown that early onset of alcohol and drug use is a predictor of later alcohol and drug abuse and dependence (Yamaguchi and Kandel 1984; Anthony and Petronis 1995; Chen et al. 2009).

Adolescence is the period during which most human smokers initiate drug use. The deleterious effects on the individual and the high healthcare costs of smoking are well known. Of particular concern is initiation of tobacco use during the teenage years, which can result in long-term consequences including an increased risk for progression to use of other illegal drugs (Kandel et al. 1992), as well as a higher risk of development of alcohol dependence (John et al. 2003), and a decreased probability of smoking cessation (Chen and Millar 1998). Data indicate that the vast majority of smokers subject themselves to these additional risks, because approximately 90% of cigarette smokers begin smoking before the

age of 18 (CDC 2007). Children of smokers may be involuntarily exposed to environmental tobacco smoke (ETS) in their homes. This is also a public health concern, because in addition to the acute health effects of ETS, childhood ETS exposure has been correlated with significantly higher rates of adulthood smoking (Larsson et al. 2001).

Research has demonstrated that adolescents are more sensitive to some drug effects and less sensitive to others, but a consistent theme in the literature is that adolescent brains respond differently to drugs of abuse than those of adults (reviewed by Spear, 2000). An underlying cause of differential drug sensitivity during adolescence could be the extensive neuronal development and maturation that take place in the forebrain during this time. A vast amount of synaptic pruning takes place during adolescence, following the overproduction of synaptic connections during prenatal and neonatal time periods. The presumed purpose of this developmental plasticity is to allow neural networks to form in a way as to best accommodate environmental needs. Thus, it is plausible that drugs used during this time could result in neurobiological changes, and that those changes might persist well into adulthood.

Studies in animals have identified specific changes that occur during adolescence in brain regions important for reward and behavioral reinforcement, including the mesolimbic DA system. Specifically, the density of dopaminergic fibers projecting to the prefrontal cortex (PFC) and striatum increase until 60 days of age in rats, at which time adult levels are reached (Kalsbeek et al. 1988). Additionally, D1 and D2 receptor levels in the PFC and striatum peak during

adolescence before declining into adulthood (Tarazi and Baldessarini 2000). D1 and D2 receptor binding also peaks in the NAc during adolescence, before declining by about one-third by 60 days of age. Activation of these receptors and neurons could also have downstream consequences which may undergo changes during adolescence. For example, stimulatory (D1 receptor) and inhibitory (D2 receptor) effects on adenylyl cyclase (AC) in the NAc were attenuated in adolescence, as compared to adulthood, in rats. Together, these findings indicate that adolescence is likely a peak time for dopaminergic activity in brain regions important for reward, and these regions might thus be susceptible to drug-induced alterations.

F. Adolescence and nicotine

Overall, studies in rodents have indicated that adolescent rats are more sensitive to the rewarding effects of nicotine than adults (reviewed by Slotkin, 2002). Low doses of nicotine induced place preference conditioning in adolescent rats but not adult rats (Vastola et al. 2002; Belluzzi et al. 2004). Cross-sensitization has also been reported; adolescent rats treated with nicotine showed an increased locomotor elevation in response to amphetamine administration, whereas adult rats did not under similar study conditions (Collins et al. 2004a). Additionally, adolescent rats have been shown to intravenously self-administer a greater number of infusions of nicotine than their adult counterparts over a 4-week self-administration paradigm (Levin et al. 2003).

In animal studies, adolescent exposure to nicotine has been shown to produce behavioral effects that last into adulthood, such as anxiety-like behavior and changes in fear conditioning in rats (Slawecki et al. 2003; Smith et al. 2006). Adolescent nicotine treatment has also resulted in an increased propensity for rats to self-administer nicotine during adulthood. Adult rats intravenously self-administered more nicotine following peri-adolescent pretreatment with nicotine than with no pretreatment. These lasting effects of nicotine seem to be specific to exposure during the adolescent time period, as equivalent exposure during adulthood did not result in similar behavioral changes (Adriani et al. 2003).

Faraday et al. (2003) demonstrated that chronic nicotine treatment had both acute and long-lasting motor activity increasing effects in adolescent rats, which were distinct from the effects observed when the rats were pretreated with nicotine during adulthood. However, shortcomings of this study included the lack of a cross-wise study design, which would have allowed for examination of within-subject effects of different adolescent and adult treatments. Additionally, the adolescent nicotine exposure occurred in the behavioral testing apparatus, which did not allow for analysis of effects of adolescent nicotine treatment on subsequent novel environment responding to be studied. However, together with the knowledge that the locomotor activating properties of nicotine are predicated on mesolimbic DA release (Clarke et al. 1988), the results of the Faraday et al. (2003) study indicate that the same neurobiological substrates that underlie nicotine-induced locomotor activation may also underlie nicotine reward sensitivity.

Previous work demonstrated that nAChR levels are up-regulated in midbrain areas implicated in nicotine reward and dependence, including the VTA, in adult rats following adolescent treatment with nicotine (Trauth et al. 1999; Abreu-Villaca et al. 2003; Adriani et al. 2003). The VTA is an essential neural substrate for EtOH self-administration, as evidenced by Rodd-Henricks and colleagues' work (2000a) demonstrating intracranial self-administration of EtOH into the pVTA but not areas surrounding the pVTA in Wistar rats. Given that nAChRs have been implicated for their involvement in EtOH self-administration by Le and colleagues (2000), it is possible that adolescent-nicotine-induced changes in nAChRs may influence EtOH drinking both acutely and later in life.

Mechanisms underlying nicotine and EtOH co-abuse have also been investigated in adolescent animal models. Only two previous studies have focused on EtOH drinking following adolescent nicotine exposure. One, by Smith and colleagues (2002), concluded that adulthood EtOH drinking was not affected by peri-adolescent nicotine exposure in Sprague-Dawley rats, a non-selected line. A more recent published study used alcohol-preferring AA rats to study the effects of adolescent nicotine exposure on adulthood EtOH intake (Kempainen et al. 2009). That study also concluded that adulthood EtOH intake was unaffected by adolescent nicotine exposure. However, AA rats are not well-characterized in terms of EtOH intake or seeking, or relapse-like behavior following deprivation of EtOH, in contrast to the P rats used in our laboratory. Even though these two studies concluded that adolescent nicotine exposure did not affect EtOH drinking in adulthood, the fact that teenage smoking is

associated with a greater risk of EtOH and other drug abuse in adulthood points toward a common underlying mechanism, and calls for further study into the topic.

One additional consideration is the number of other components of tobacco smoke. There are thousands of chemicals in cigarette smoke, and one or more of them may act on biological systems. For example, acetaldehyde, which is a major metabolite of EtOH within the brain, is a component of cigarette smoke. So, exposure to the additional chemicals in smoke, in addition to the nicotine itself, may play a role in the link between adolescent smoking and adulthood EtOH drinking.

G. Hypotheses and specific aims

Previous research has indicated that activation of the mesolimbic dopamine system plays a vital role in mediating the reinforcing effects of nicotine. nAChRs have been shown to be highly localized in the VTA, a major point of origin of mesolimbic DA projections. Therefore, the first aim of this dissertation was to investigate whether nicotine would be self-administered directly into the VTA of Wistar rats. The intracranial self-administration (ICSA) technique has been used to identify specific brain regions involved in mediating the reinforcing effects of drugs of abuse (Bozarth and Wise 1980; Goeders and Smith 1987). This procedure has been used to demonstrate site-specific self-administration of several drugs including EtOH, morphine, and amphetamine (Gatto et al. 1994;

Bozarth and Wise 1981; Chevrette et al. 2002). Functional heterogeneity between the anterior and posterior portions of the VTA has also been reported (Ikemoto et al. 1998), and drugs such as EtOH have been shown to be self-administered into the pVTA but not into the aVTA (Rodd-Henricks et al. 2000a). Therefore, it was hypothesized that nicotine would be self-administered directly into the pVTA of Wistar rats in a dose-dependent manner.

Given that adolescence is a time period when the brain is especially susceptible to the effects of drugs of abuse, it is no surprise that the vast majority of smokers begin using tobacco during their teenage years. For the next three aims of this dissertation, the long-lasting effects of nicotine exposure during adolescence were examined. Because nicotine exerts its reinforcing properties by stimulating the mesolimbic DA system and causing DA release in the NAc, the second aim of this dissertation was to investigate whether exposure to nicotine during adolescence would result in changes in nicotine-induced DA release in the NAc during adulthood in Wistar rats. Using the information gathered in the first aim of this dissertation about the reward-mediating actions of nicotine within the VTA, it was hypothesized that DA release in the NAcc as a result of nicotine injections directly into the VTA would be enhanced in rats that had been exposed to nicotine during adolescence.

Following investigation of nicotine-mediated DA release in the NAc, the next logical step was to study the effects of adolescent nicotine treatment on a behavioral correlate of mesolimbic dopaminergic activation. Previous research has established that the locomotor stimulating effect of nicotine is mediated by

mesolimbic DA activity (Clarke et al. 1988). Therefore, the third aim of this dissertation was to investigate the effects of adolescent nicotine exposure on behavioral sensitivity to nicotine during adulthood in Wistar rats. A locomotor activity study was employed to investigate the initial sensitivity of adolescent-exposed rats to nicotine as well as the development of behavioral sensitization following repeated daily injections of the drug. In the locomotor sensitization paradigm, repeated doses of a drug incrementally increase behavioral responsiveness to the drug, and this effect persists over time (Robinson and Berridge 1993). It was hypothesized that nicotine exposure during adolescence would yield an increase in sensitivity to the locomotor-activating effects of nicotine during adulthood.

Finally, the high rate of co-abuse of nicotine and EtOH in human populations prompts the question of whether exposure to one drug can cross-sensitize a system to the effects of the other. Since persistent neurobiological and behavioral effects of adolescent nicotine exposure had been explored in the second and third aims of this dissertation, the fourth aim was to investigate the effects of adolescent nicotine exposure on EtOH consumption during adulthood. Adolescent nicotine exposure results in a number of long-lasting behavioral effects, and several previous studies have shown that EtOH consumption was increased when preceded by nicotine treatment (Pothoff et al. 1983; Blomqvist et al. 1996). Therefore, it was hypothesized that adolescent nicotine exposure would result in increased EtOH consumption during adulthood. This experiment was conducted not only in Wistar rats but also in alcohol-preferring P rats, since

earlier studies have shown that P rats may be more sensitive to certain effects of nicotine (Gordon et al. 1993; Le et al. 2006). It was also hypothesized that the effects of adolescent nicotine exposure on adulthood EtOH consumption would be enhanced in P rats as compared to Wistar rats, because there may be an underlying genetic influence on the co-abuse of these two drugs.

II. MATERIALS AND METHODS

A. Aim 1: Nicotine intracranial self-administration

1. Animals

Female Wistar rats (Harlan, Indianapolis, IN) arrived in the laboratory at approximately post-natal day (PD) 60 and were pair-housed until after cannula implantation surgery 2-4 weeks later. Female rats were used because they have been used in our previous ICSA studies, and because females maintain their body weight and head size better than male rats, which allows for more accurate stereotaxic placements (Ikemoto et al. 1998). Estrous cycles were not monitored in the current study, although previous studies have indicated that ICSA behavior is not affected by estrous cycle (Gatto et al. 1994; Rodd-Henricks et al. 2000a). The vivarium conditions were constant for this and all subsequent experiments in this dissertation. It was maintained on a 12-hour reverse light/dark cycle (lights off at 0900 hours) with food and water available *ad libitum*, and temperature (21°C) and humidity (50%) were controlled.

At the time of surgery, animals weighed approx. 250-300 g. With the subjects under isoflurane anesthesia, a 22 gauge guide cannula were stereotaxically implanted into the right hemisphere; the guide cannulae was aimed 1.0 mm above the target region. Coordinates (Paxinos and Watson 1998) for placements into the pVTA were 5.7 mm posterior to bregma, 2.1 mm lateral to the midline, and 8.5 mm ventral from the surface of the skull at a 10 degree angle

to the vertical. In between experimental sessions, a 28 gauge stylet was placed into the guide cannula and extended 0.5 mm beyond the tip of the guide. After surgery, rats were housed individually and allowed to recover for 7 days. Animals were handled for at least 5 min daily after the third recovery day. Animals were not acclimated to the experimental chambers before the commencement of data collection.

2. Drugs and solutions

Appropriate amounts of nicotine ((-)-1-Methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt, Sigma-Aldrich) were dissolved into artificial cerebrospinal fluid (aCSF), which consisted of 120.0 mM NaCl, 4.8 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 2.5 mM CaCl₂, and 10.0 mM D-glucose (pH of 7.3 ± 0.1). Doses used in this study were 10, 50, 100, 200, 400, and 800 µM nicotine. Blood and brain nicotine concentrations in human smokers are roughly 100-500 nM (Karan et al. 2003), but previous animal studies involving intra-VTA microinjections of nicotine have used doses in the mM range, from 0.4 to 25 mM (David et al. 2006; Ikemoto et al. 2006), so the range of doses used here were chosen because they fell in between these two extremes.

3. Apparatus

The test chambers (30 x 30 x 26 cm) were situated in sound-attenuating cubicles (64 x 60 x 50 cm; Colbourn Instruments, Allentown, PA) and illuminated by a dim house light during testing. Two identical levers (3.5 x 1.8 cm) were

mounted on a single wall of the test chamber, 15 cm above a grid floor, and were separated by 12 cm. Directly above each lever was a row of three different colored cue lights. The light (red) to the far right over the active bar was illuminated during resting conditions. An electrolytic microinfusion transducer (EMIT) system (Bozarth and Wise, 1980) was used to control microinfusions of drug or vehicle. Two platinum electrodes were placed in an infusate-filled gas-tight cylinder (20 mm in length by 6 mm in diameter) equipped with a 28 gauge injection cannula (Plastics One, Roanoke VA). The electrodes were connected by a spring-coated cable (Plastics One) and a swivel to a constant current generator (MNC, Shreveport LA) that delivered 6 μ A of quiescent current and 200 μ A of infusion current between the electrodes. Depression of the active lever delivered the infusion current for 5 sec, which lead to the rapid generation of H₂ gas (raising the pressure inside the gas-tight cylinder), and, in turn, forced 100 nl of the infusate through the injection cannula. During the 5-sec infusion and additional 5-sec timeout period, the house light and red cue light turned off, and the green cue light over the active lever flashed on and off at 0.5 sec intervals.

4. General test condition

Subjects were brought into the testing room, the stylet removed, and the injection cannula was screwed into place. Injection cannulae extended 1.0 mm beyond the tip of the guide. The test chambers were equipped with two levers. Depression of the “active lever” (FR1 schedule of reinforcement) caused the

delivery of a 100 nl bolus of infusate over 5 sec, followed by a 5-sec timeout period. During that 10-sec total time, additional responses on the active lever were recorded but did not result in additional infusions. Responses on the “inactive lever” were recorded but did not result in infusions. The assignment of active and inactive levers with respect to right and left was counterbalanced among subjects, with the active and inactive levers remaining the same for each rat throughout the experiment.

5. Dose response study

Wistar rats were randomly assigned to one of five groups (n = 6-10 per group). Each rat was tested during seven sessions in the self-administration chamber for 4 hours each session, with sessions occurring every other day. One group received infusions of vehicle (aCSF) for all seven sessions. The other groups received infusions of either 10, 50, 100, 200, 400, or 800 μ M nicotine for the first four sessions. During the 5th and 6th sessions, all groups received infusions of aCSF. During the 7th session, rats were allowed to respond for their original infusate. This seven-session paradigm is the standard for ICSCA experiments in our laboratory, as a previous study on self-administration of ethanol indicated that stable lever responding was attained by sessions 3 and 4, extinction was reached within two sessions, and active lever responding was reinstated within one session when original infusate was restored (Rodd-Henricks et al. 2000a). A desktop computer equipped with an operant control

system recorded the data and controlled delivery of the infusate in relation to lever responding.

At the end of the experimental day, rats were euthanized in a CO₂ chamber, and 1% bromophenol blue (0.5 µl) was injected into the infusion site. The animals were decapitated and brains removed, and immediately frozen at -70°C. Frozen brains were sliced in a cryostat microtome into 40 µM sections. Sections were stained with cresyl violet and examined under a light microscope for verification of the injection site using the rat brain atlas of Paxinos and Watson (1998).

6. Statistical analysis

Data analysis consisted of a one-way analysis of variance (ANOVA) performed on the number of infusions across the four acquisition sessions. Additionally, for each individual group, lever discrimination was determined by a Lever (active or inactive) x Session mixed ANOVA with a repeated measure of Session. Lever discrimination is a key factor when a stimulant is self-administered (e.g., nicotine, cocaine, amphetamine) to distinguish between reinforcement-contingent behavior and drug-stimulated locomotor activity. To study extinction (5th and 6th sessions) and reinstatement (7th session) in each infusate group, first a one-way ANOVA was performed on the active lever presses in sessions 4-7. Then, paired t-tests (two-tailed) with a 95% confidence interval were used to determine whether the lever responding on aCSF days was significantly different than on days when nicotine was available.

B. Aim 2: Effects of adolescent nicotine exposure on adulthood nicotine-stimulated DA release in the NAc

1. Animals

Male Wistar rats (Harlan, Indianapolis, IN) arrived in the laboratory at PD 23-25 and were housed 4 to a cage, then later adjusted to pair-housed when body weights reached approximately 300 g. Following cannulae implantation surgery at approx PD 75-80, they were single housed.

With the subjects under isoflurane anesthesia, two guide cannulae were implanted at a 10 degree angle to the vertical. For the microinjection site (pVTA), a 22-gauge cannula was used, and for the microdialysis probe site (NAc), an 18-gauge cannula was used. Coordinates (Paxinos and Watson 1998) for placements into the posterior VTA were 5.7 mm posterior to bregma, 2.1 mm lateral to the midline, and 8.5 mm ventral from the surface of the skull. Cannulae were placed at a 10 degree angle to the vertical. Coordinates for the NAc shell were 1.7 mm anterior to bregma, 2.3 mm lateral to the midline, and 5.4 mm ventral from the surface of the skull. In between experimental sessions, stylets were placed into the guide cannulae which extended 0.5 mm beyond the tip of the guides. After surgery, rats were housed individually and allowed to recover for at least 5 days before the insertion of microdialysis probes. Rats were habituated to the Plexiglas microdialysis chambers (22.5 x 44.5 x 38 cm, width x length x height) for approximately 4-5 hours daily, and handled for at least 10 minutes on the 3 days immediately preceding the microdialysis experiment.

On the third habituation day, after removal from the chambers, animals were briefly anesthetized, and loop-style microdialysis probes were inserted into the guide cannulae aimed at the NAc and cemented in place. Probes were made as previously described (Perry and Fuller 1992; Kohl et al. 1998) and inserted into the guides so that the loop was oriented along the anterior/posterior axis. The probes extended past the tip of the guide cannulae a total of 3.0 mm: 2.0 mm of this was active microdialysis membrane.

2. Drugs and solutions

For adolescent exposure, 0.5 mg/kg nicotine ((-)-1-Methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt, Sigma-Aldrich) or saline was administered via a once-daily subcutaneous (s.c.) injection on PD 30-41. Animals were weighed, injected, and placed directly back in the home cage. Nicotine dose was based on several previous studies (including Adriani et al. 2006; Belluzzi et al. 2004; Berg and Chambers 2008). The dose was calculated as the weight of the base, and was dissolved in a sterile 0.9% saline solution (pH adjusted to 7.1 ± 0.1) and delivered at an injection volume of 1 ml/kg.

For the intra-VTA microinjections, nicotine solutions were prepared as described above for the ICOSA experiments in section II.A.2. The doses used in this experiment were 100 and 200 μ M, because they were determined to be the optimal doses in the ICOSA dose-response experiment in the first part of this dissertation.

For microdialysis in the NAc, a standard aCSF that did not include D-glucose was used. Microdialysis aCSF consisted of 145.0 mM NaCl, 2.7 mM KCl, 1.0 mM MgCl₂, 2.5 mM CaCl₂, and 2.0 mM Na₂HPO₄ (pH of 7.4 ± 0.05).

3. Microinjection–microdialysis test procedure

On the experimental day, animals were transferred to Plexiglas testing chambers. The inputs of the probes were connected to a microinfusion syringe pump (Harvard Apparatus, South Natick, MA) through a length of clear polyethylene (PE) tubing. The outlets were connected to opaque black PE tubing to reduce light-induced degradation of DA, and the samples were collected into 0.5-ml PE tubes containing 5.0 µl of 0.1 N perchloric acid.

Microdialysis aCSF was perfused through the probes at a flow rate of 1.0 µl/min for 2 hr for washout. After the 2-hr equilibration, samples were collected every 20 min. The first 3 samples collected were considered baseline samples.

After collection of the baseline samples, the injection cannula was inserted into the VTA guide cannula. The EMIT system was used for injections in this experiment, as described in section II.A.3. above. This time, injection delivery was controlled by the experimenter rather than a two-lever operant system. Immediately after insertion of the injection cannula, injections of either aCSF, 100, or 200 µM nicotine were delivered for a duration of 5 sec each, 3 times per min, for 10 min. This was done to mimic a temporal pattern of microinjections that a rat might self-administer in the ICSPA paradigm (Rodd-Henricks et al. 2000a). The injector cannula was gently removed approximately 1 min following

the last microinjection. Samples continued to be collected every 20 min until a total of 7 samples had been collected after the microinjections.

All samples were immediately frozen on dry ice and stored at -70°C until assayed. At the end of the experimental day, rats were euthanized in a CO_2 chamber, and 1% bromophenol blue ($0.5\ \mu\text{l}$) was injected into the infusion site and perfused through the microdialysis probe. The animals were decapitated and brains removed, and immediately frozen at -70 degrees C. Frozen brains were sliced in a cryostat microtome into $40\ \mu\text{M}$ sections. Sections were stained with cresyl violet and examined under a light microscope for verification of the injection and probe sites using the rat brain atlas of Paxinos and Watson (1998).

4. High-Performance Liquid Chromatography

The microdialysis samples were analyzed for DA content with a reverse-phase high-performance liquid chromatography coupled with an electrochemical detection (HPLC-EC) system. Samples were loaded into a $5\text{-}\mu\text{l}$ loop and injected onto a small-bore analytical column (BDS Hypersil C18, $150\ \text{mm} \times 2.1\ \text{mm}$, Thermo). The mobile phase ($50\ \text{mM}$ phosphoric acid, $100\ \text{mg/l}$ OSA, $0.1\ \text{mM}$ EDTA, $8\ \text{mM}$ KCl, and 6.0% acetonitrile, pH 6.0) was delivered by a Shimadzu LC-20AD solvent delivery system (Shimadzu Corporation, Tokyo Japan). DA was detected by a VT-03 glassy carbon electrode (Antec Leyden, The Netherlands) and an Antec DECADE II amperometric detector (Antec Leyden, The Netherlands) with the potential set at $320\ \text{mV}$ and sensitivity setting of $50\ \text{pA/V}$. The outputs from the detector were sent to a ChromPerfect (Version 5.5.5,

Justice Innovations, Inc., Palo Alto, CA) chromatography data analysis system. The lower detection limit for dopamine was approximately 0.1 nM. Calibration was performed using standards of known DA concentration.

5. Statistical analysis

DA levels in the NAc were normalized and expressed as percent of baseline. Timecourse data for DA release in the NAc in response to pVTA microinjections were analyzed using a two-way ANOVA (Adolescent Treatment x Time) with a repeated measure of Time. Where appropriate, individual means were compared using paired t-tests with a 95% confidence interval.

C. Aim 3: Effects of adolescent nicotine exposure on adulthood behavioral sensitivity to nicotine

1. Animals

Male Wistar rats (Harlan, Indianapolis, IN) arrived in the laboratory at PD 23-25 and were housed 4 to a cage, then later adjusted to pair-housed when body weights reached approximately 300 g.

2. Drugs and solutions

Drugs, solutions, and adolescent nicotine exposure regimen were as described above in section II.B.2. For this experiment, there were two different

adolescent nicotine exposure groups. One group received s.c. injections of 0.25 mg/kg nicotine, another received 0.5 mg/kg nicotine, and a control group received an equivalent volume of saline.

3. Apparatus

LMA sessions took place in Plexiglas recording chambers (43.2 x 43.2 x 30.5 cm), which were each equipped with 16 infrared beam transmitters spanning the x and y axes of the field (Med Associates, Inc., St. Albans, VT). Transmitter and detector arrays for translational motion were located 5 cm from the chamber floor and spaced 2.5 cm apart. Data were collected using the Activity Monitor 5.0 software (Med Associates, Inc., St. Albans, VT), which was configured to separate small, quick movements (stereotypic counts) from translational locomotion (ambulatory beam break counts, distance traveled).

4. General test condition

Starting on PD 80, locomotor behavior was assessed over ten 2-hour sessions (1 session per day) occurring Monday through Friday of two consecutive weeks. Each 2-hour locomotor recording session consisted of a 1-hour baseline phase and a 1-hour post-injection phase. Activity data were collected in 10-min blocks. After each 1-hour baseline period ended, animals received a subcutaneous injection of saline or 0.25 mg/kg or 0.5 mg/kg nicotine, according to randomly assigned drug treatment groups. Each animal received the same dose of nicotine (or saline) across all ten days of the LMA study.

5. Statistical analysis

Analysis for the LMA data consisted of a repeated measures ANOVA with between-subject variables of adolescent nicotine group or adult nicotine group and repeated measures of session. Roy's Largest Root was used to correct the violations of the assumptions of the repeated measures ANOVA test, namely sphericity and heterogeneity of variance. Additionally, LMA was decomposed into 10-min blocks and analyzed through a repeated-measures ANOVA with between-subject variables of adolescent nicotine group or adult nicotine group and repeated measures of block. Post hoc analyses (Student-Newman-Keuls and Tukey's b) were used to determine which groups were significantly ($p < 0.05$) different from each other.

D. Aim 4: Effects of adolescent nicotine exposure on adulthood EtOH drinking behavior

1. Animals

Male Wistar (Harlan, Indianapolis, IN) or alcohol-preferring P (Indiana University, Indianapolis, IN) arrived in the laboratory at PD 23-25 and were housed 4 to a cage, then later adjusted to pair-housed when body weights reached approximately 300 g. A control group of Wistar rats, which received nicotine exposure first during adulthood rather than adolescence, arrived at approximately PD 60 and were pair-housed.

2. Drugs and solutions

Drugs, solutions, and adolescent nicotine exposure regimen were as described above in section II.B.2. For the adult control group of Wistar rats, nicotine injections were given on PD 60-71.

For EtOH drinking, 95% (190 proof) EtOH was mixed with deionized water to the appropriate volume/volume concentrations (5, 10, 15, or 30%). During drinking studies, EtOH solutions were stored in 8-liter plastic carboys (Nalgene Labware) in the vivarium for convenience, and fresh solutions were made every 2 weeks.

3. Experimental procedure

At PD 70 days of age (or PD 110, for the Wistar age control group), the animals were transferred to hanging wire mesh cages (1 rat per cage) with water and food freely available throughout the experiment. Following 5 days of habituation to the hanging wire mesh cages, all rats were given 24-hour concurrent access to multiple concentrations of ethanol (EtOH). Multiple concentrations were used so that any shift in preference between lower and higher concentrations of EtOH could be observed. For Wistar rats, EtOH was available at concentrations of 5% and 10% v/v. Initially, those same EtOH concentrations were used for the P rats, to allow for direct comparisons to be drawn between the Wistar and P rats. In addition, a separate cohort of P rats underwent the same adolescent nicotine exposure treatment as the other cohorts, and was given access to higher concentrations of EtOH (15% and 30%,

v/v) during adulthood. This was done to counteract the possibility of a ceiling effect in the cohort of P rats drinking the lower EtOH concentrations, as it has been shown that P rats will voluntarily drink EtOH at concentrations of up to 30%.

Access to EtOH solutions was continuous for the first 8 weeks of the experiment. The first 4 weeks are considered the acquisition phase of EtOH drinking behavior, and the second 4 weeks are considered the maintenance phase. Following those 8 weeks, rats underwent three cycles of 2 weeks of EtOH deprivation and 2 weeks of EtOH reinstatement. This was done to look for possible adolescent-nicotine-induced changes in the expression of an Alcohol Deprivation Effect (ADE), defined as a temporary increase in EtOH intake compared to baseline drinking conditions, following a period of EtOH deprivation.

Rats had continuous access to their drinking solutions except during measurement of body and bottle weights. Beginning on the first EtOH access day, body and bottle weights were obtained, at least 6 days per week, using a Sartorius Balance BP 1600 and Sartorius Interface V24/V28-RS232C(-S)/423 (Sartorius Instruments, McGaw Park, IL) and recorded by a personal computer program (Software-Wedge, Professional Edition v 5.0 for DOS; Sartorius Instruments). Weights were rounded to the nearest 0.1 g. Weights for the 7th day of the week, when not recorded, were determined to be the average of the weights obtained on the preceding and following days.

Bottles for water and EtOH were standard glass bottles with a capacity of approximately 300 ml of fluid, with a stopper (no. 10) holding an angled (~135°) stainless steel sipper tube with ball-bearing tip. Spillage was calculated by using

a set of “spill bottles,” hanging on an empty cage, which were removed and weighed along with the other bottles. The fluids in the spill bottles were the same as the fluids rats had access to, i.e. water and two concentrations of EtOH. An average of approximately 0.5 ml of fluid was spilled with each weighing, so this amount was subtracted from all daily bottle weights. All bottles were refilled at least twice per week, and were replaced every 2 weeks.

4. Statistical analysis

Analysis for the EtOH intake data and preference data consisted of a repeated measures ANOVA with a between-subject variable of Adolescent Treatment and repeated measures of Week (or Day, in the case of analysis of initial 7 days of EtOH drinking). Roy’s Largest Root was used to correct the violations of the assumptions of the repeated measures ANOVA test, namely sphericity and heterogeneity of variance. For the EtOH intake during each relapse EtOH drinking session following periods of deprivation, repeated measures ANOVAs with a between-subject variable of Adolescent Treatment and repeated measures of Day were used. Then, paired t-tests (two-tailed) with a 95% confidence interval were used to compare EtOH intake on individual re-exposure days to the pre-deprivation baseline EtOH intake amount.

III. RESULTS

A. Nicotine intracranial self-administration

The posterior VTA (pVTA) was defined neuroanatomically as the VTA region at the level of the interpeduncular nucleus, coronal sections from -5.6 to -6.3 mm bregma (Fig. 1). Cannula placements outside the pVTA included injection sites located in the substantia nigra (both in the pars compacta, SNc; and pars reticulata, SNr), and red nucleus (RN).

In our seven-session intracranial self-administration (ICSA) paradigm, the first four sessions were considered “acquisition” of the lever-pressing behavior. The 5th and 6th sessions, during which responses on the active lever resulted in infusions of aCSF rather than nicotine, were considered the “extinction” sessions. The 7th session, during which the original nicotine infusate was returned, was considered the “reinstatement” session. A range of nicotine concentrations infused into the pVTA supported response-contingent behaviors (Fig. 2). Since rats typically establish stable responding by the third 4-hour ICSA session, a one-way ANOVA was used to compare the average number of nicotine infusions received during sessions 3 and 4, and it revealed a significant main effect of Group ($F_{6,47} = 4.459$, $p < .0001$). Post hoc comparisons (Tukey's b) indicated that the 50, 100, and 800 μM groups received significantly more infusions than the aCSF group, and that the rats self-administering 200 or 400 μM nicotine

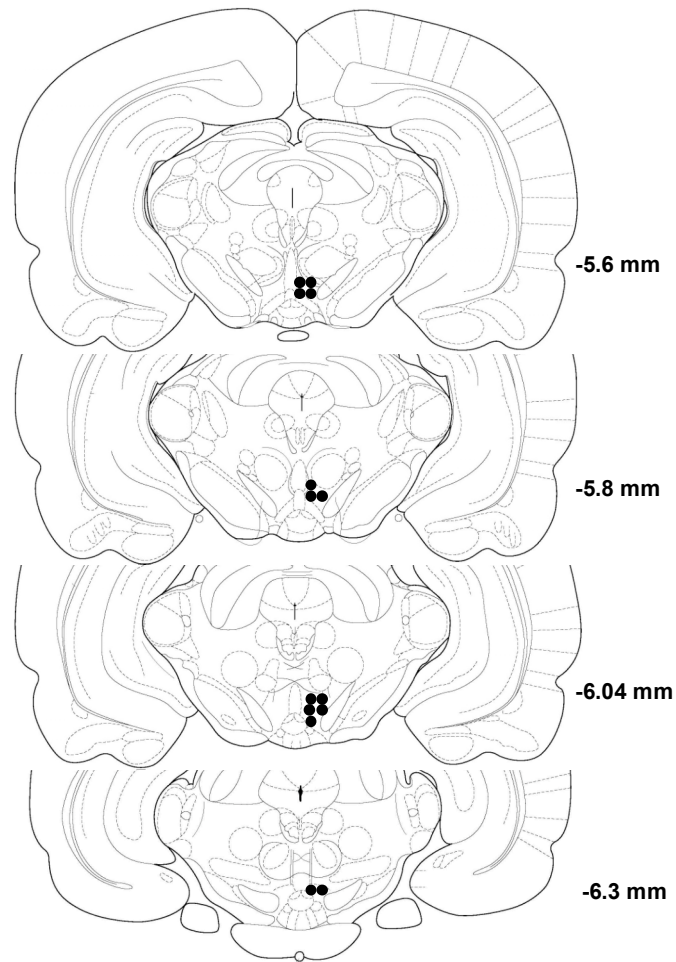


Figure 1. Representative placements of the microinjector cannulae within the pVTA. Distances (in millimeters) from bregma are shown to the right of the diagrams. This figure was adapted from Paxinos and Watson (1998).

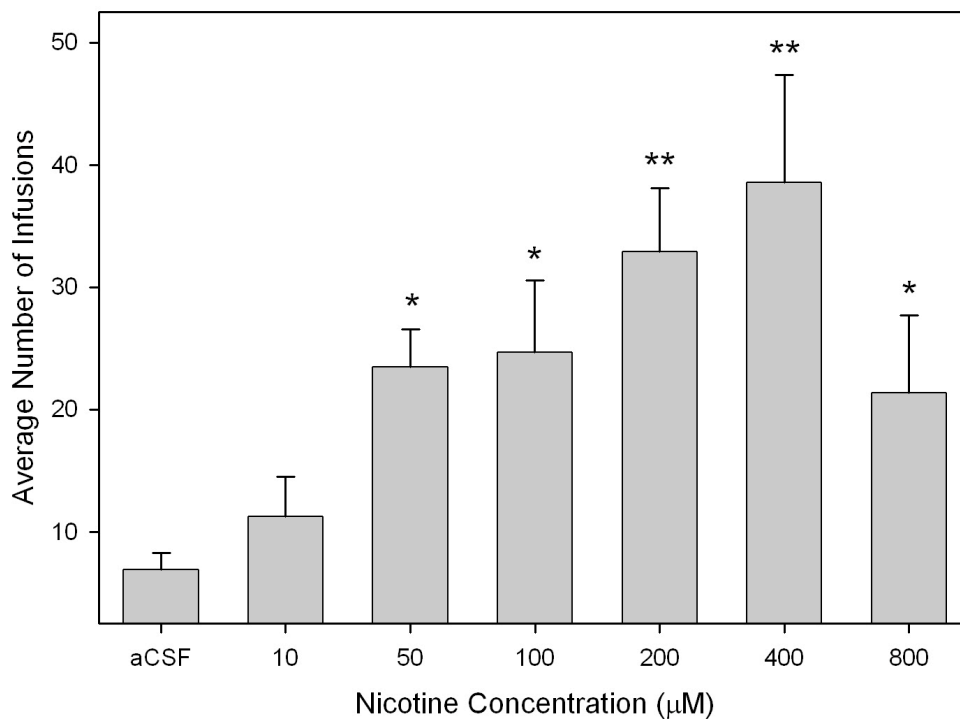


Figure 2. The mean (\pm SEM) number of infusions during intracranial self-administration sessions 3 and 4 by Wistar rats self-infusing 10-800 μM of nicotine, or aCSF ($n = 6-10$ per group). * Indicates that the average number of infusions is significantly higher than the aCSF group ($p < 0.05$). ** Indicates that the average number of infusions is significantly greater than both the aCSF and 10 μM groups ($p < 0.05$).

received significantly more infusions than both the aCSF and 10 μ M groups (Fig. 2).

To determine whether the Wistar rats showed discrimination between the active and inactive levers, first a 3-way ANOVA was performed with repeated measures of Lever (active or inactive) and Session and a between-subjects factor of Group using all nicotine dose groups. The ANOVA indicated a significant Session x Lever x Group interaction ($F_{36,282} = 1.563$, $p = 0.026$). Next, each nicotine dose Group was held constant and 2-way ANOVAs were performed with repeated measures of Lever and Session across all 7 sessions. Active and inactive lever presses for each infusate group are represented in Fig. 3 and described below.

To assess whether extinction (in sessions 5 and 6) and reinstatement (in session 7) of response-contingent behavior took place, each Group was held constant and repeated measures ANOVAs were performed with a repeated measure of Session, using sessions 4 thru 6 for extinction, and sessions 5 thru 7 for reinstatement. Finally, paired t-tests were performed to compare active lever responding between sessions.

For the Wistar rats self-infusing aCSF during all 7 sessions, there was no significant main effect of Lever ($F_{1,8} = 0.016$, $p = 0.902$) or Session x Lever interaction ($F_{6,3} = 1.877$, $p = 0.323$) during sessions 4-6 or 5-7. The average number of responses in all sessions was generally low on both levers (< 25 responses per session).

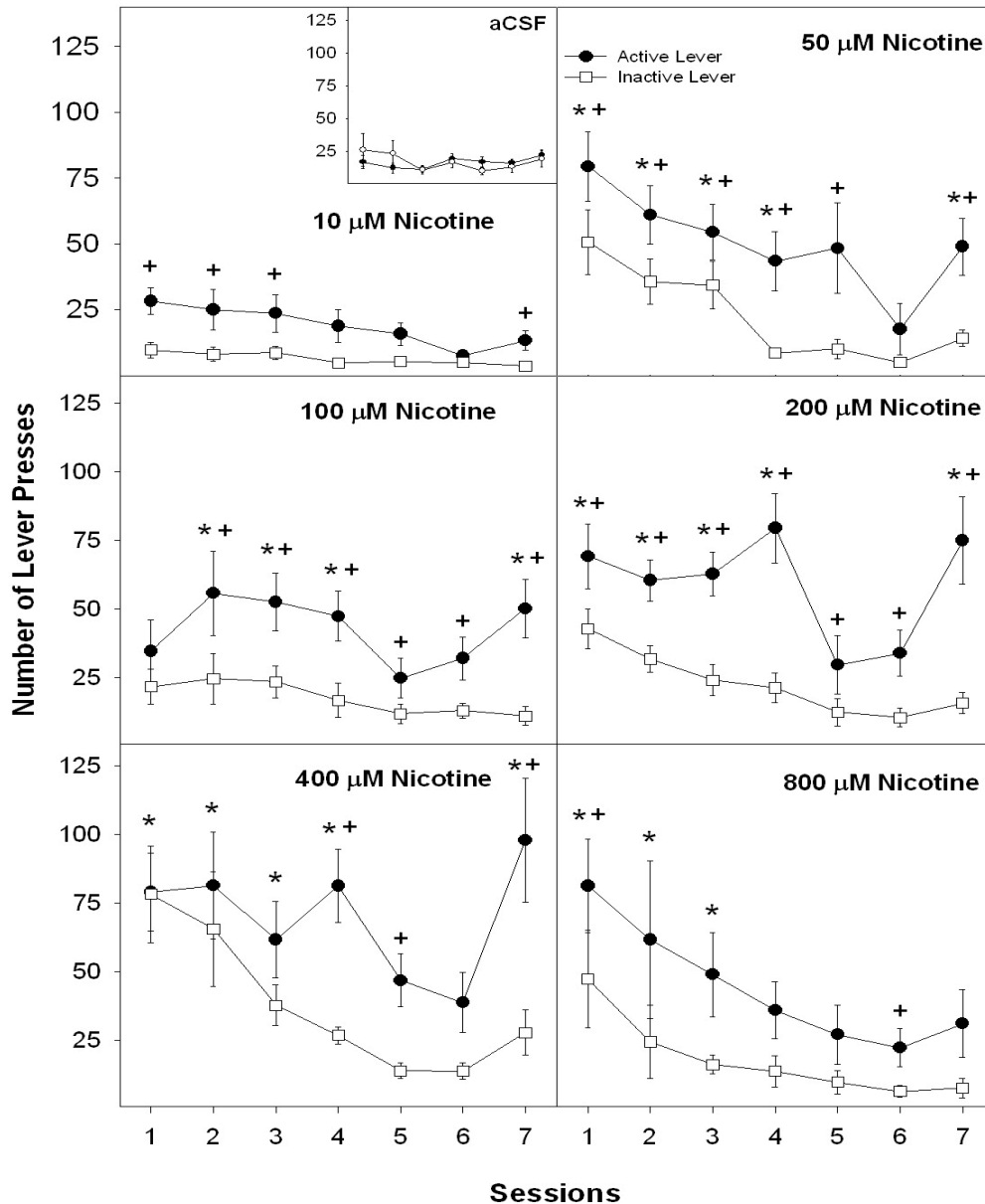


Figure 3. The mean (\pm SEM) number of active (closed circles) and inactive (open squares) lever presses for Wistar rats self-administering aCSF (inset) or 10-800 μ M nicotine into the pVTA during sessions 1-4 and session 7 ($n = 6-10$ per group). Nicotine was replaced by aCSF for sessions 5 and 6. * Indicates significantly ($p < 0.05$; Tukey's b) higher active lever responding compared to active lever responding by rats self-administering aCSF. + Indicates lever discrimination ($p < 0.05$) for that particular session within a given infusate group.

For the 10 μ M nicotine group, the 2-way ANOVA indicated a significant main effect of Lever ($F_{1,5} = 13.479$, $p = 0.014$), but no Session x Lever interaction ($F_{3,3} = 1.042$, $p = 0.487$). Paired t-tests indicated that these rats responded significantly more on the active than inactive lever during the first three acquisition sessions ($df = 5$, p values < 0.045). When aCSF was substituted for nicotine during sessions 5 and 6, a repeated measures ANOVA on sessions 4 thru 6 revealed a significant main effect of Session ($F_{2,4} = 7.042$, $p = 0.049$) which indicated extinction of the response-contingent behavior, and lever discrimination was no longer apparent (p values > 0.073). When 10 μ M nicotine was returned in session 7, an ANOVA on sessions 5 thru 7 showed a significant main effect of Session ($F_{2,4} = 7.152$, $p = 0.048$), which indicated reinstatement of active lever pressing, and rats responded significantly more on the active than the inactive lever ($p = 0.043$). However, across all sessions, the average number of active lever responses was relatively low (< 25 responses per session) and did not differ from active lever responding by the aCSF group.

A 2-way ANOVA on the lever presses in the 50 μ M nicotine group revealed a significant main effect of Lever ($F_{1,6} = 17.473$, $p = 0.006$) but no Session x Lever interaction ($F_{6,1} = 6.787$, $p = 0.286$). Paired t-tests indicated that this group of rats demonstrated significantly greater responding on the active lever than the inactive lever during all 4 acquisition sessions ($df = 6$, p values < 0.022). When aCSF replaced nicotine for the two extinction sessions, lever discrimination was apparent during the 5th session ($p = 0.037$) but no longer present in the 6th session ($p = 0.245$). When 50 μ M nicotine was returned in the

reinstatement session, active lever responding was once again significantly greater than inactive lever responding ($p = 0.012$). A repeated measures ANOVA on the number of active lever presses during sessions 4 thru 6 indicated no significant main effect of Session ($F_{2,5} = 3.791$, $p = 0.10$), and a similar ANOVA on the active lever presses during sessions 5 thru 7 also approached significance ($F_{2,5} = 2.069$, $p = 0.06$).

For the 100 μM nicotine group, a 2-way ANOVA indicated a significant main effect of Lever ($F_{1,9} = 17.094$, $p = 0.003$) but no Session x Lever interaction ($F_{6,4} = 2.572$, $p = 0.190$). Paired t-tests showed lever discrimination during all but the 1st session ($df = 9$, p values < 0.024), including the sessions in which aCSF replaced the nicotine infusate (sessions 5 and 6). A repeated measures ANOVA on the active lever presses in sessions 4-6 revealed a significant main effect of Session ($F_{2,8} = 6.023$, $p = 0.025$), and paired t-tests indicated that active lever presses in sessions 5 and 6 were significantly lower than in session 4 (p values < 0.014), indicating extinction of the response-contingent behavior. When comparing sessions 5-7, a repeated measures ANOVA indicated no significant main effect of Session ($F_{2,8} = 3.202$, $p = 0.095$).

For the 200 μM nicotine group, a 2-way ANOVA revealed a significant main effect of Lever ($F_{1,9} = 134.3$, $p < 0.001$) but no Session x Lever interaction ($F_{6,4} = 2.584$, $p = 0.189$). Paired t-tests indicated discrimination between the active and inactive levers during all 7 sessions of the experiment ($df = 9$, p values < 0.026). To assess extinction, a repeated measures ANOVA performed on the number of active lever presses during sessions 4-6 revealed a significant main

effect of Session ($F_{2,8} = 6.001$, $p = 0.026$), and paired t-tests indicated that active lever responses were significantly lower in sessions 5 and 6 than session 4 (p values < 0.021). An ANOVA on sessions 5-7 showed no main effect of Session ($F_{2,8} = 1.854$, $p = 0.218$).

A 2-way ANOVA on the rats self-infusing 400 μ M nicotine revealed a significant main effect of Lever ($F_{1,5} = 15.810$, $p = 0.011$) but no Session x Lever interaction ($F_{3,3} = 2.288$, $p = 0.257$). Paired t-tests indicated significant lever discrimination during sessions 4, 5, and 7 ($df = 5$, p values < 0.02), but not session 6. Repeated measures ANOVAs performed on sessions 4-6 and sessions 5-7 revealed no significant main effect of Session ($F_{2,4} < 2.661$, p values > 0.184).

Finally, for the 800 μ M nicotine group, a 2-way ANOVA indicated a significant main effect of Lever ($F_{1,5} = 11.546$, $p = 0.019$) but no Session x Lever interaction ($F_{3,3} = 0.297$, $p = 0.828$). Paired t-tests indicated that lever discrimination was observed only during the sessions 1 and 6 ($df = 5$, p values < 0.046). Repeated measures ANOVAs revealed that neither extinction (sessions 4-6) nor reinstatement (sessions 5-7) of active lever pressing were significant ($F_{2,4} < 3.717$, p values > 0.122).

A one-way ANOVA was performed on the average number of active lever presses during all 7 sessions in all groups, and this indicated that, in at least three of the five nicotine sessions, rats in the 50-800 μ M nicotine groups responded significantly (p values < 0.05 ; Tukey's b) more on the active lever for nicotine infusions than did the rats responding for aCSF (Fig. 3).

Wistar rats self-infusing the same concentrations of nicotine into areas surrounding the pVTA, including the SNc, SNr, and RN, showed an overall low level of infusions and active lever responding throughout all sessions. These rats displayed comparable levels of infusions and active lever responding with the rats self-infusing aCSF into the pVTA. The average number of infusions by each injector placement group in sessions 3 and 4 are depicted in Fig. 4. A one-way ANOVA indicated no significant differences among the three non-pVTA groups ($F_{2,41} = 2.248$, $p = 0.118$).

The number of active lever presses by the rats self-infusing nicotine into areas outside the pVTA were generally low compared to the pVTA groups: approximately 20-30 lever presses/session for rats self-infusing nicotine into the subregions of the SN, and generally 10 or fewer lever presses/session for the rats self-infusing into the RN. However, some lever discrimination within injector placement groups was observed (Fig. 5). A 3-way ANOVA performed with repeated measures of Lever (active or inactive) and Session and a between-subjects factor of Injector Placement revealed a significant main effect of Lever ($F_{1,41} = 8.879$, $p = 0.005$). Next, Injector Placement was held constant and ANOVAs were performed with repeated measures of Lever and Session. For the rats infusing concentrations of nicotine into the SNr, a significant main effect of Lever was observed ($F_{1,13} = 10.275$, $p = 0.007$), but there was no Session x Lever interaction ($F_{6,8} = 0.892$, $p = 0.543$). Paired t-tests indicated that these rats responded significantly more on the active lever than the inactive lever in all but the 1st session ($df = 13$, p values < 0.036 ; Fig. 5, top panel). For the rats infusing

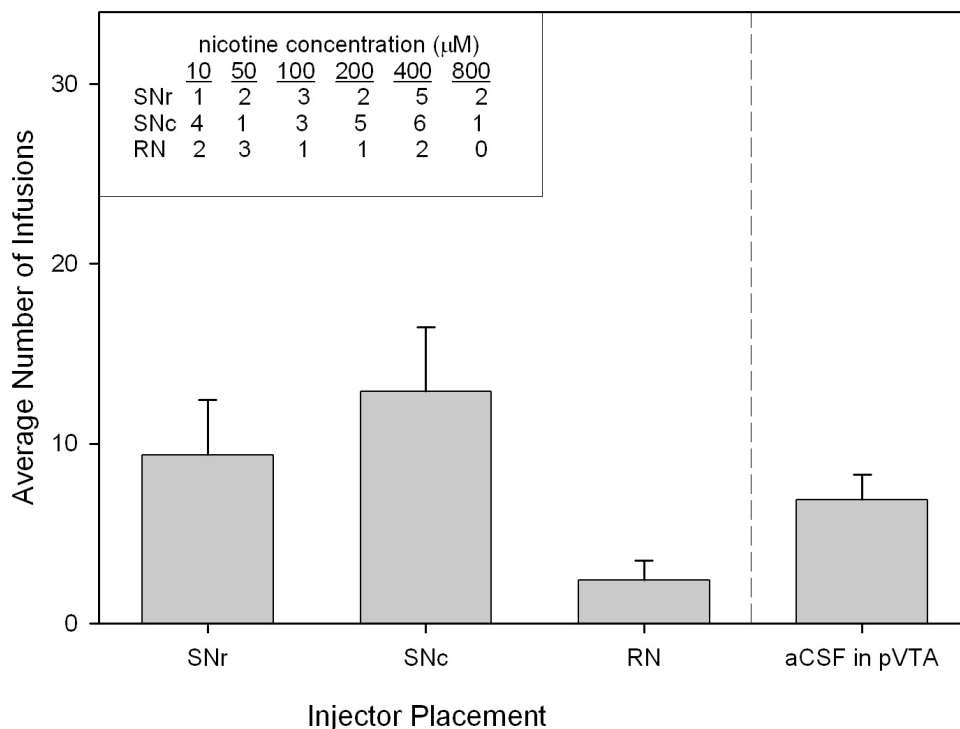


Figure 4. The mean (\pm SEM) number of infusions during intracranial self-administration sessions 3 and 4 by rats self-administering various concentrations of nicotine (10 – 800 μM) into the SNr ($n = 14$), SNc ($n = 20$), or RN ($n = 10$), as well as for rats self-administering aCSF into the pVTA (right of dotted line). No significant differences between injector placement groups were observed. Number of rats in each group self-infusing each nicotine concentration is indicated in the inset.

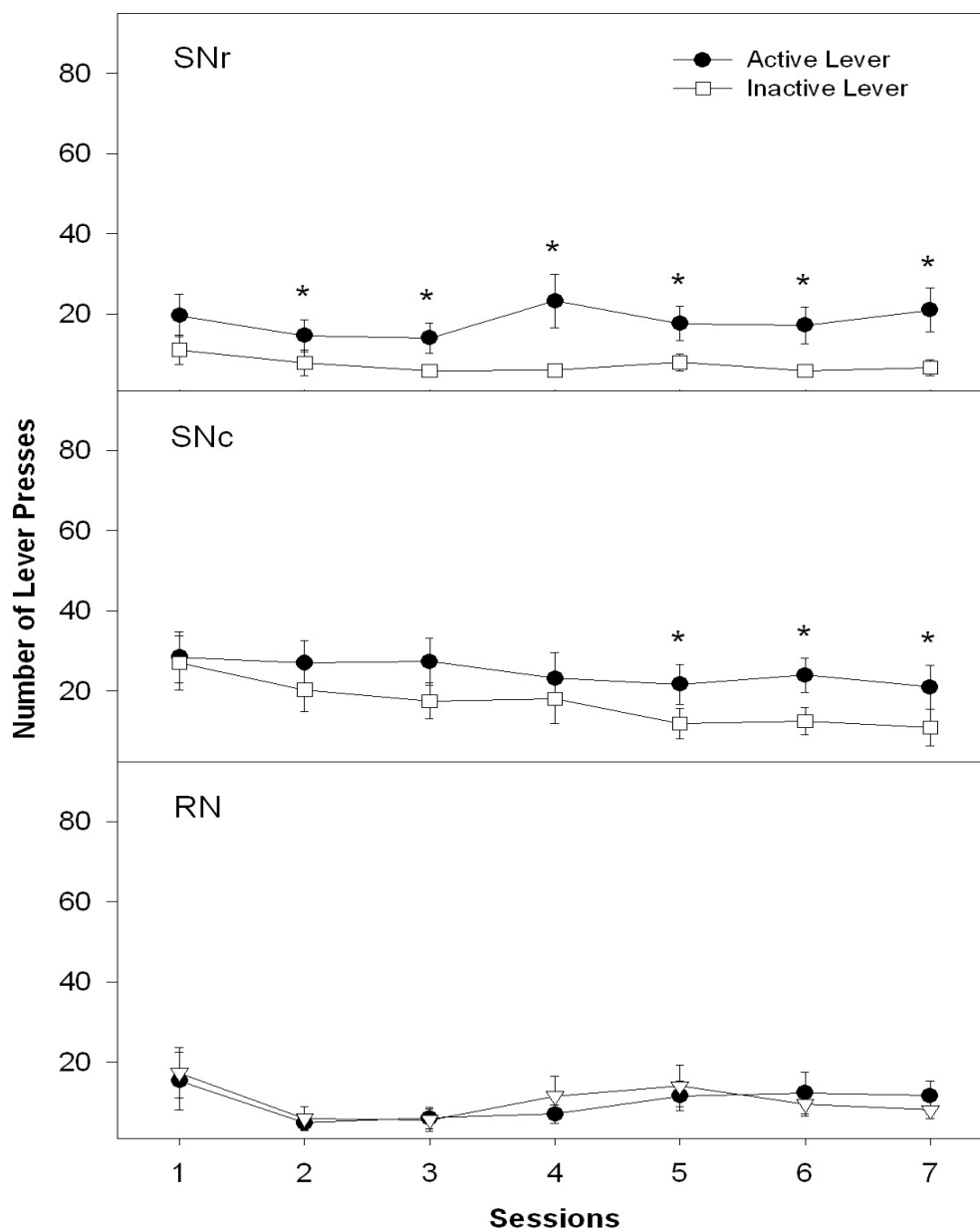


Figure 5. The Mean (\pm SEM) number of active (closed circles) and inactive (open circles) lever presses for rats self-administering various concentrations of nicotine (10 – 800 μ M) into the SNr (top panel, n = 14), SNc (middle panel, n = 20), or RN (bottom panel, n = 10) during sessions 1-4, aCSF in sessions 5 and 6, and the original nicotine infusate in session 7. * Indicates significant ($p < 0.05$) lever discrimination within a given injector placement group.

concentrations of nicotine into the SNc, a significant main effect of Lever was observed ($F_{1,19} = 5.005$, $p = 0.037$), but there was no Session x Lever interaction ($F_{6,14} = 0.798$, $p = 0.587$). Paired t-tests indicated significant lever discrimination during sessions 5-7 ($df = 19$, p values < 0.006 ; Fig. 5, middle panel). For the rats infusing nicotine into the RN, a repeated measures ANOVA indicated neither a significant main effect of Lever ($F_{1,9} = 0.094$, $p = 0.767$) nor a Session x Lever interaction ($F_{6,4} = 2.511$, $p = 0.196$). No lever discrimination was observed in this group (Fig. 5, bottom panel).

B. Effects of adolescent nicotine exposure on adulthood nicotine-stimulated DA release in NAc

Only data from animals with the microinjector cannula correctly implanted in the pVTA and the microdialysis probe correctly implanted in the shell of the NAc were included in the analysis. Representative anatomical placements of the NAc probes and the pVTA injector cannulae are depicted in Fig. 6A and 6B, respectively. The pVTA was defined neuroanatomically as the VTA region located at -5.6 to -6.3 mm relative to bregma. Because of the diameter and length of the probes, a portion of the active probe membrane was often located within the NAc core. Therefore, only data from rats with the probes placed at least two-thirds in the shell vs. the core were used.

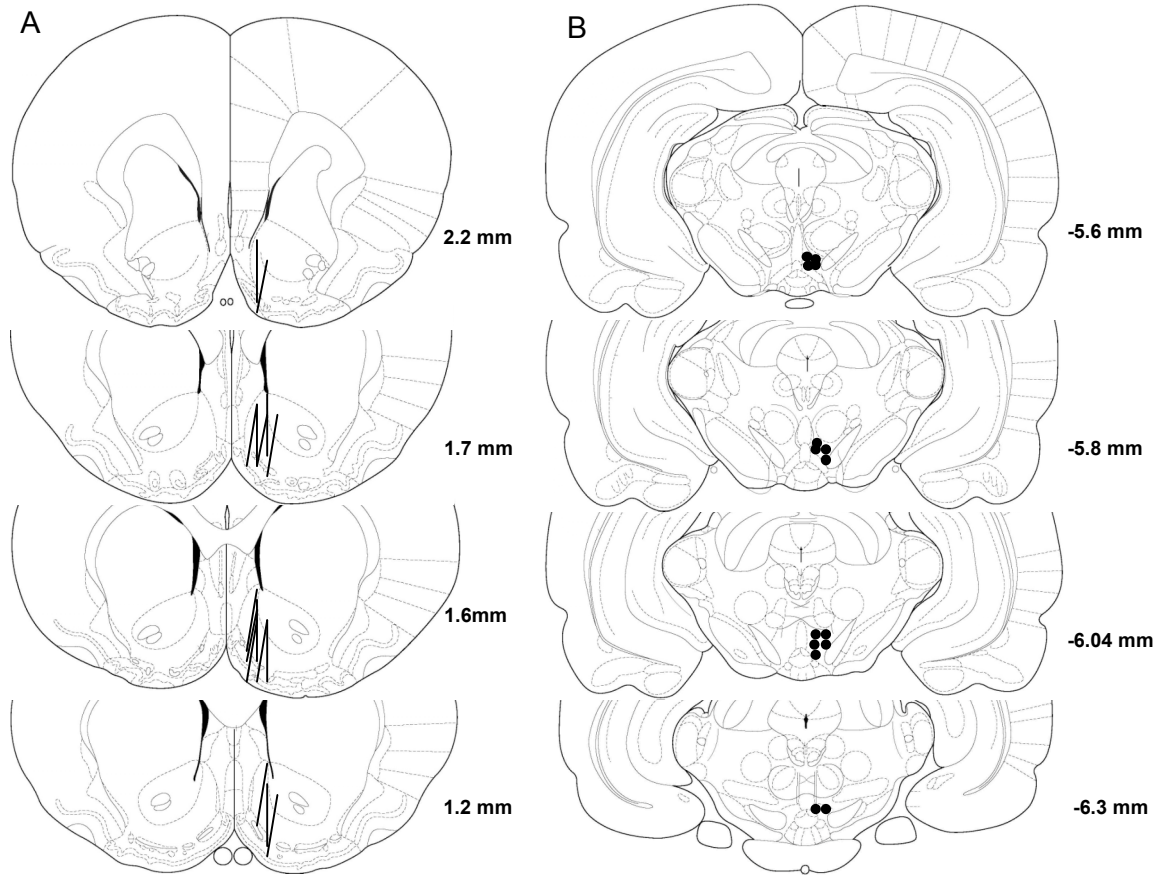


Figure 6. Representative placements of (A) the microdialysis probes within the NAc and (B) the microinjector cannulae within the pVTA. Distances (in millimeters) from bregma are shown to the right of the diagrams. This figure was adapted from Paxinos and Watson (1998).

Numerous unanticipated challenges were faced with the HPLC machine during the data analysis portion of this study. Some of the HPLC chromatograms obtained during this study showed puzzling inconsistencies in DA peak size and peak time, even within individual animals. Inconsistent amounts of storage time for the microdialysis samples between collection and HPLC analysis may have contributed to this issue, but other unknown factors were likely also at play. After consulting with HPLC specialists in the laboratory, data from some animals had to be excluded based on these inconsistencies. This resulted in a low n for some groups, particularly the groups that received nicotine microinjections of 100 μM during microdialysis. Time constraints prevented the experiment, with its roughly 2.5-month time course, from being repeated in additional animals.

Examination of basal extracellular DA levels (average of the three 20-minute baseline samples) in the NAc using an unpaired t-test revealed no differences between adolescent treatment groups ($t_{21} = 0.594$, $p = 0.117$). Average baseline DA levels were approximately 4.8 ± 1 .

Microinjection of 100 or 200 μM nicotine into the pVTA increased extracellular DA in the NAc to approximately 160% of baseline in Wistar rats treated with saline during adolescence (Fig. 7). However, in rats treated with 0.5 mg/kg nicotine during adolescence, microinjection of 100 or 200 μM nicotine into the pVTA resulted in extracellular DA increases of 300-330% above baseline in the NAc (Fig. 7). aCSF microinjections in rats with either one of the adolescent treatments (saline or 0.5 mg/kg nicotine) did not result in any significant increase in extracellular DA levels in the NAc. A repeated measures ANOVA was

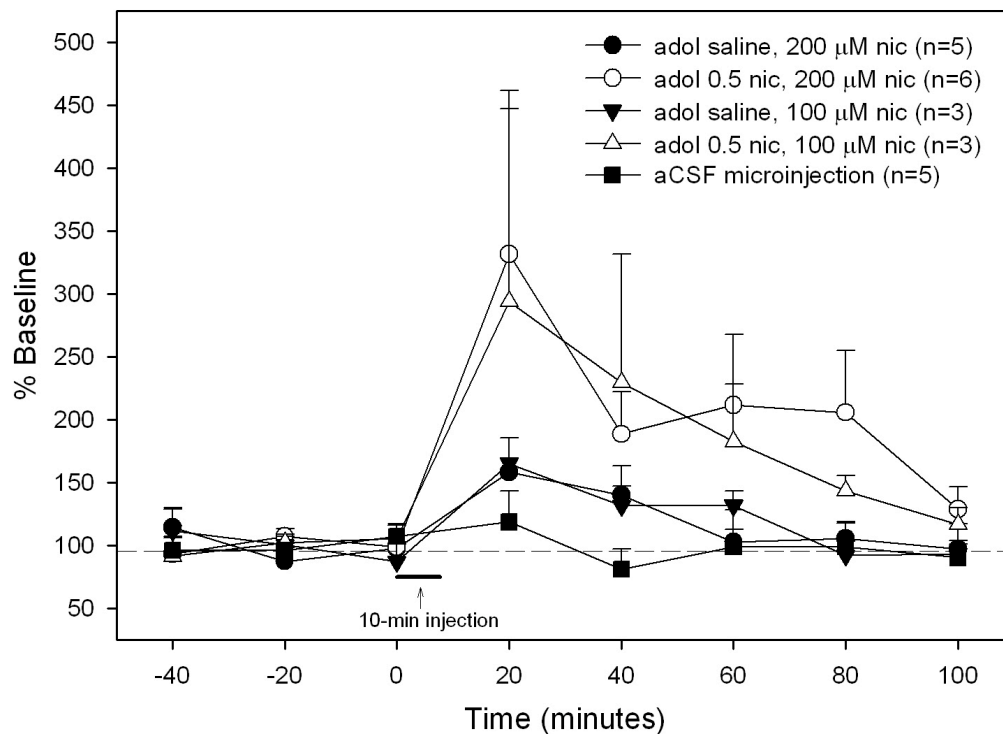


Figure 7. Time-course effects of microinjections (over 10 minutes) of 100 μ M (triangles), 200 μ M (circles), or aCSF (squares) on extracellular DA levels in the NAc of adult Wistar rats treated with 0.5 mg/kg nicotine (closed symbols) or saline (open symbols) during adolescence. The closed squares represent all rats that received aCSF microinjections, regardless of adolescent treatment. Data are expressed as percentages of baseline (mean \pm SEM). No significant differences were observed.

performed on the percent increases in DA levels from baseline, with a repeated measure of Time and between-subjects factors of Adolescent Treatment and Microinjection Concentration. Neither the 3-way interaction term ($F_{8,30} = 0.659$, $p = 0.722$) nor the individual main effects of Time ($F_{4,14} = 1.518$, $p = 0.25$), Adolescent Treatment ($F_{1,17} = 3.714$, $p = 0.71$), or Microinjection Concentration ($F_{2,17} = 1.017$, $p = 0.383$) were significant.

Due to the relatively low n in each group, especially the 100 μM microinjection group, and the similar percent increases in NAc DA levels resulting from 100 and 200 μM nicotine microinjections within each adolescent treatment group, data were collapsed across nicotine microinjection concentrations and analyzed by repeated measures ANOVA with a between-subject factor of Adolescent Treatment. While the interaction term was not significant ($F_{4,13} = 0.978$, $p = 0.453$), there was a significant main effect of Adolescent Treatment ($F_{1,16} = 4.475$, $p = 0.050$) (Fig. 8). Paired t-tests indicated that for the first two post-injection time points, both of the Adolescent Treatment groups had extracellular DA levels in the NAc that were significantly increased above baseline ($df = 8$, p values < 0.05). For the remaining post-injection time points, the NAc DA levels for the adolescent saline group were no longer significantly different from baseline (p values > 0.073). NAc DA levels in the adolescent 0.5 mg/kg nicotine group remained significantly elevated above baseline for the third and fourth time points (p values < 0.046), and were no longer significantly different from baseline by the final time point ($p = 0.101$).

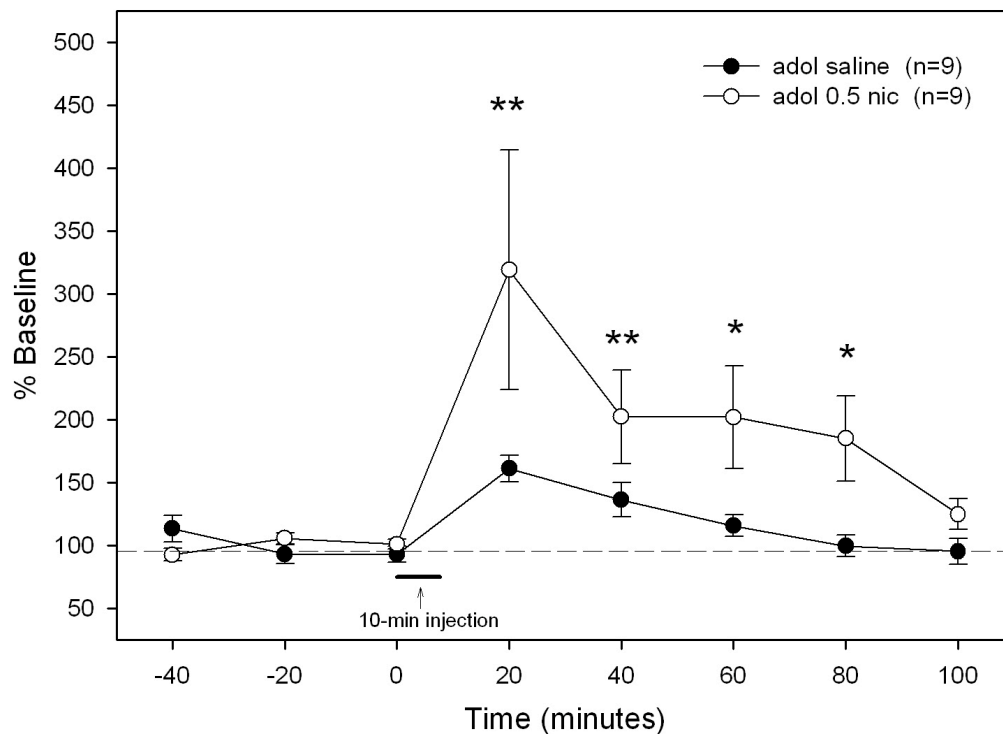


Figure 8. Time-course effects of microinjections (over 10 minutes) of nicotine (100 or 200 μ M) on extracellular DA levels in the NAc of adult Wistar rats treated with saline (closed circles) or 0.5 mg/kg nicotine (open circles) during adolescence. Data are expressed as percentages of baseline (mean \pm SEM). * Indicates that that DA levels in the adolescent nicotine group were significantly higher than pre-injection baseline ($p < 0.05$). ** Indicates that DA levels in both adolescent treatment groups were significantly higher than pre-injection baseline ($p < 0.05$).

C. Effects of adolescent nicotine exposure on adulthood behavioral sensitivity
to nicotine

1. Effects on subsequent baseline locomotor activity

The possibility that adolescent exposure to nicotine would influence baseline locomotor activity (LMA) during the initial period of exposure to the activity chamber was examined (Fig. 9). A repeated measures ANOVA (within subject factor time, between subject factor adolescent pre-exposure) revealed a significant interaction of Adolescent Treatment x Time ($F_{10,114} = 2.0, p = 0.04$). Decomposing the interaction term by performing ANOVAs on activity segmented into 10-min bins revealed significant group effects during the first 40 min of the pre-injection hour ($F_{2,60}$ values > 6.85 ; p values < 0.002). Wistar rats treated with 0.5 mg/kg nicotine during adolescence had significantly higher LMA than rats treated with saline during all 4 time periods (Post-hoc comparisons, Tukey's b); while rats treated with 0.25 mg/kg nicotine during adolescence had significantly higher LMA than control rats during the 2nd and 4th 10-min bin. Rats that were treated with nicotine (0.25 or 0.5 mg/kg) during adolescence did not differ from each other during any time point. The differences in baseline activity among the 3 groups were not observed during the last 20 min of the baseline session (p values > 0.29).

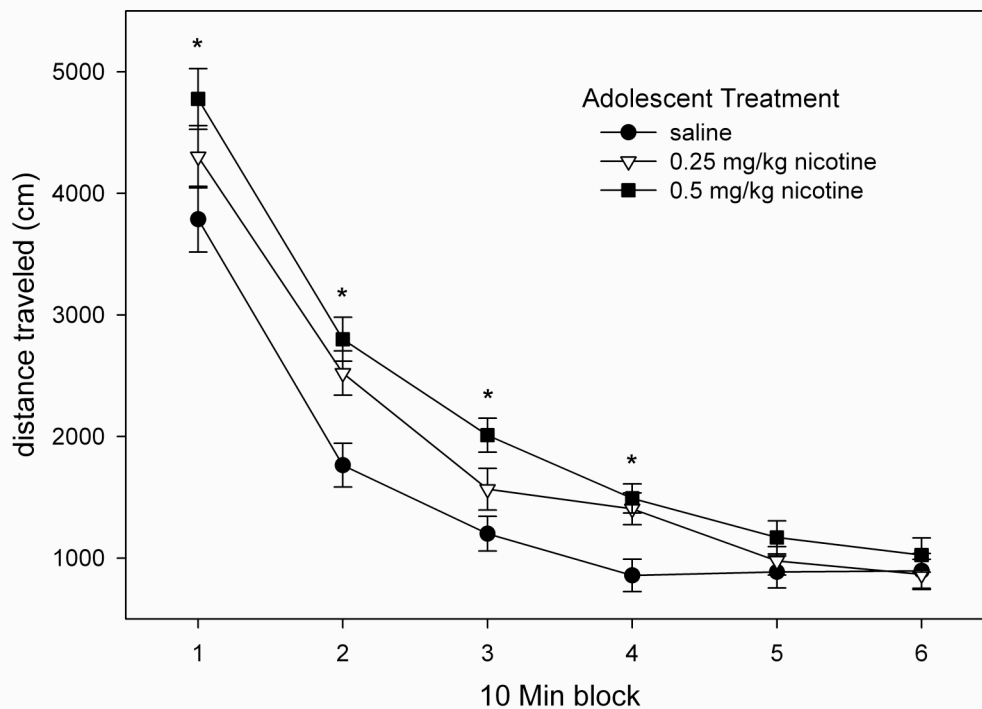


Figure 9. The mean (\pm SEM) distances traveled in 10-min blocks during the initial 60 minutes, before any injections, in the locomotor activity chambers by Wistar rats that were treated with saline (closed circles, n=19), 0.25 mg/kg nicotine (open triangles, n=22), or 0.5 mg/kg nicotine (closed squares, n=22) during adolescence. * Indicates that the groups that received 0.25 mg/kg or 0.5 mg/kg nicotine were significantly ($p < 0.05$) different than the saline group.

2. Effects on locomotor sensitization

A repeated measures ANOVA on all rats revealed a significant Day x Adolescent Treatment x Adult Treatment interaction ($F_{9,49} = 2.495$, $p = 0.02$) across the 10 days of the LMA study. The significant 3-way interaction was initially decomposed by holding adolescent treatment constant.

The effects of novel environment and initial (Day 1) adulthood nicotine treatment were examined (Fig. 10). A one-way ANOVA indicated that novelty-induced LMA in adulthood was affected by adolescent nicotine treatment ($F_{2,60} = 7.684$, $p = 0.001$), with 25-40% higher LMA observed for the 2 groups exposed to nicotine during adolescence compared to the adolescent saline-treated group. For the initial adulthood injection of 0.25 or 0.5 mg/kg nicotine, significant differences in LMA were observed based on differing adolescent treatments ($F_{2,19} = 6.479$ and 4.333 , $p = 0.007$ and 0.028 , respectively), with adult nicotine treatment producing a 2-fold greater stimulation in the groups exposed to nicotine during adolescence compared to the adolescent saline-treated rats. Adolescent nicotine treatment did not have an effect on the LMA observed following an adulthood saline injection ($F_{2,15} = 1.182$, $p = 0.334$).

A repeated measures ANOVA on the adult LMA data collected from rats treated with saline during adolescence did not reach significance, but showed a trend toward the expected overall effect of adult nicotine treatment (Fig. 11, top panel). A priori hypothesis asserted that nicotine should increase LMA; therefore, individual ANOVAs were performed. However, examining the overall activity revealed no significant differences between the 3 injections conditions,

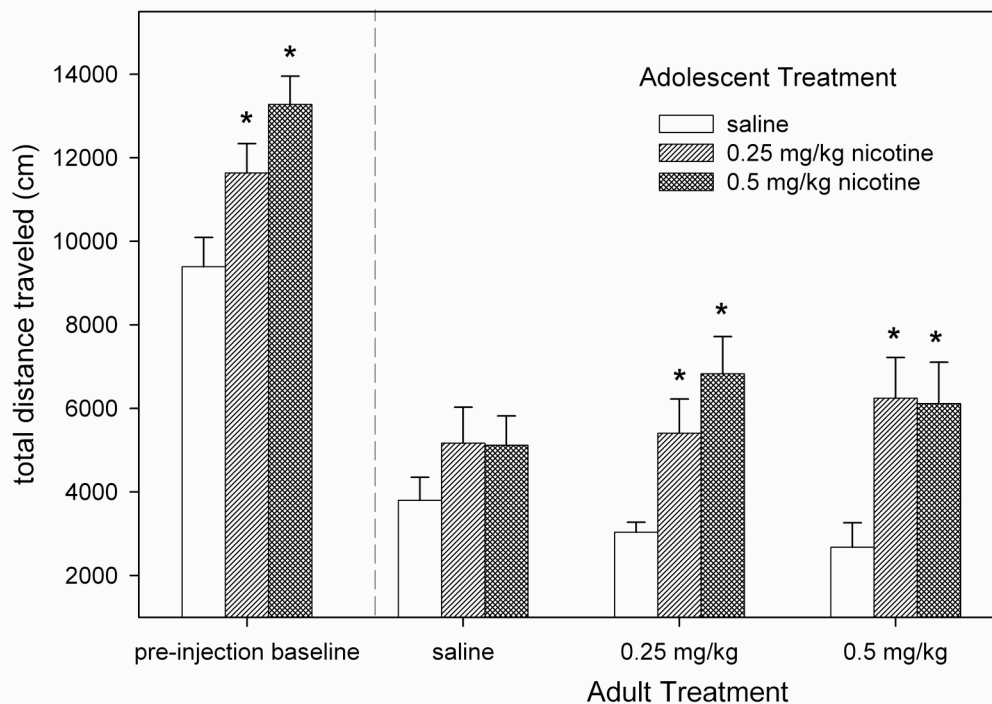


Figure 10. The mean (\pm SEM) total distances traveled by Wistar rats during the 60 minutes immediately preceding the first adulthood injection (left of dotted line, $n = 19-22$ /group) and the 60 minutes following the first adulthood injection (right of dotted line, $n = 6-8$ /group). * Indicates that, in each respective grouping, the 0.25 mg/kg and 0.5 mg/kg nicotine adolescent-treated groups were significantly ($p < 0.05$) different than the saline adolescent-treated group.

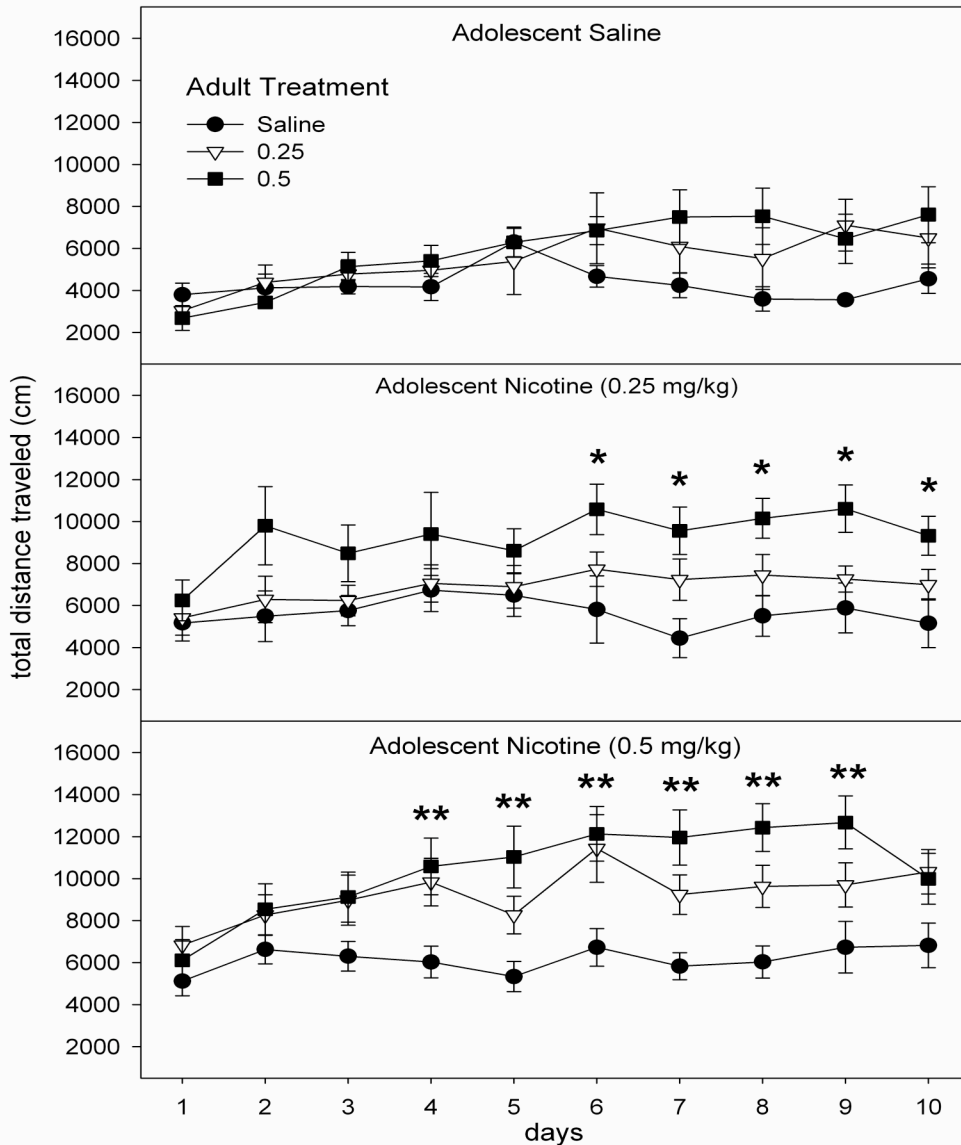


Figure 11. The mean (\pm SEM) total distances traveled across the 10 post-injection locomotor activity sessions by adult Wistar rats that received (a) top panel, saline during adolescence (saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine in adulthood, $n = 6-7/\text{group}$); (b) middle panel, 0.25 mg/kg nicotine during adolescence (saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine in adulthood, $n = 6-8/\text{group}$), or (c) bottom panel, 0.5 mg/kg nicotine during adolescence (saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine in adulthood, $n = 6-8/\text{group}$). Symbols and legend indicate drug treatment administered during the adulthood 10-day locomotor activity study. * Indicates that the 0.5 mg/kg nicotine adult-treated group was significantly ($p < 0.05$) different than saline. ** Indicates that the 0.25 mg/kg and 0.5 mg/kg nicotine adult-treated groups were significantly different than saline.

but between-group differences on Day 9 approached significance ($F_{2,16} = 3.388$, $p = 0.059$).

For the adolescent saline treated group, although significant differences in the total daily distances traveled were expected but not observed, differences did emerge when activity was reduced to 10-min blocks (Time x Adult Treatment: $F_{5,13} = 10.472$; $p < 0.001$). Beginning on Test Day 3 (Fig. 12, top panel), rats treated with nicotine displayed an increase in LMA compared to saline treated rats during individual 10-min blocks; a significant effect of Adult Treatment ($p < 0.026$) was observed during the 3rd and 4th 10-min blocks, with post hoc analyses revealing that the 0.25 and 0.5 mg/kg nicotine groups were different from saline controls. On Day 6 (Fig. 13, top panel), for the adolescent saline treated group, there was a significant Time x Adult Treatment interaction ($p = 0.046$); individual ANOVA indicated a significant group difference during the 3rd bin with post-hoc comparisons indicating that the 0.25 and 0.5 mg/kg nicotine groups were different from saline controls. During the 9th test session (Fig. 14, top panel), for the adolescent saline-treated rats, there was a significant effect of Adult Treatment ($p < 0.023$) during the 2nd and 3rd time period with post-hoc comparisons indicating that the 0.25 and 0.5 mg/kg nicotine groups were different from saline controls.

A repeated measures ANOVA on the adult LMA data collected from rats treated with 0.25 mg/kg nicotine during adolescence (Fig. 11, middle panel) revealed a significant effect of Day ($F_{9,11} = 4.482$, $p = 0.011$) and a Day x Adult Treatment interaction that approached significance ($F_{9,12} = 2.67$, $p = 0.058$).

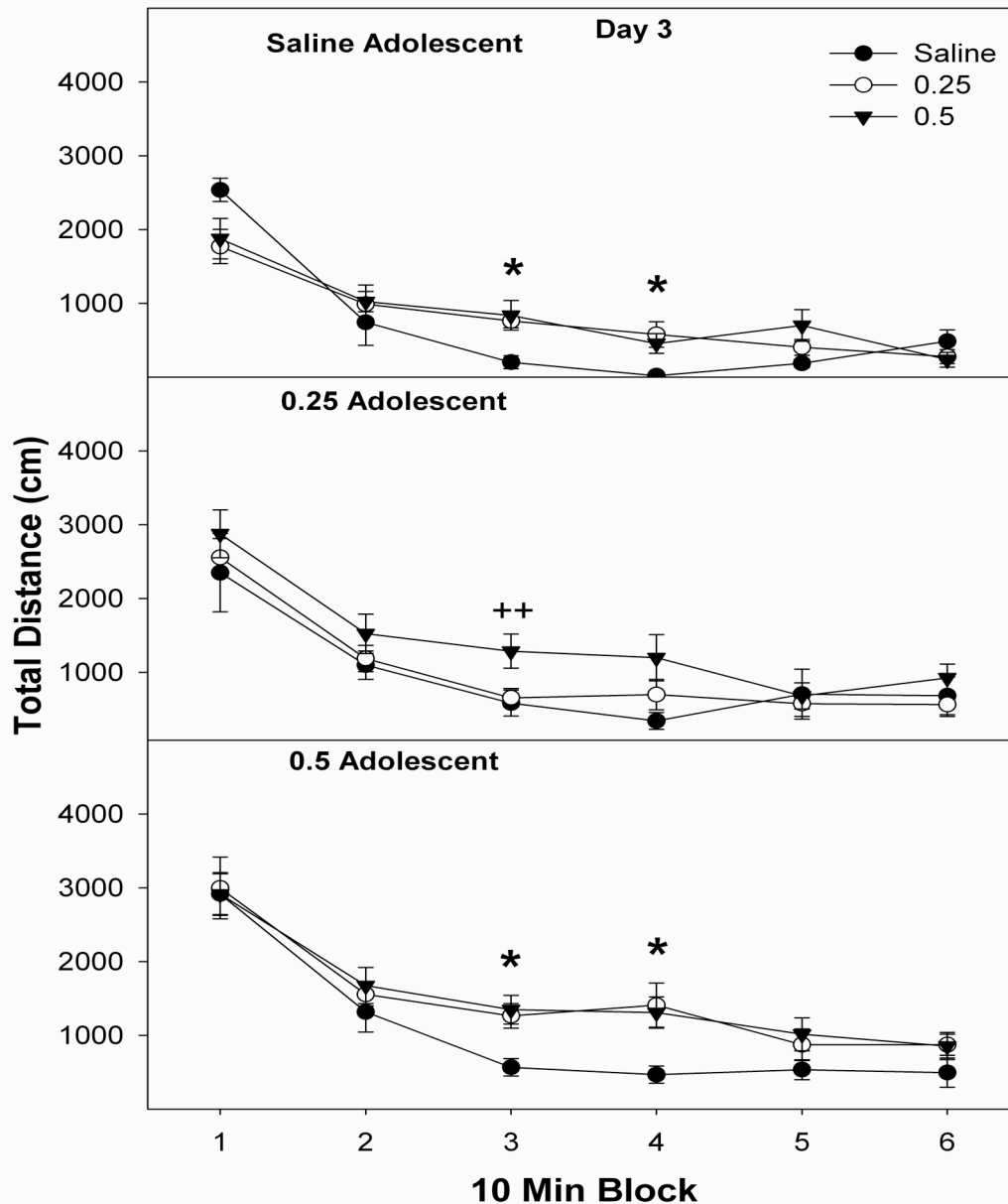


Figure 12. The mean (\pm SEM) distances traveled across the six 10-minute time blocks following injection on Day 3 by adult Wistar rats that received saline (top panel), 0.25 mg/kg nicotine (middle panel), and 0.5 mg/kg nicotine (bottom panel) during adolescence. Symbols and legend indicate drug treatment administered during the adult portion of the study. * Indicates that the 0.25 mg/kg and 0.5 mg/kg nicotine adult-treated groups were significantly ($p < 0.05$) different than saline. ++ Indicates that the 0.5 mg/kg nicotine adult-treated group was significantly different than both other groups.

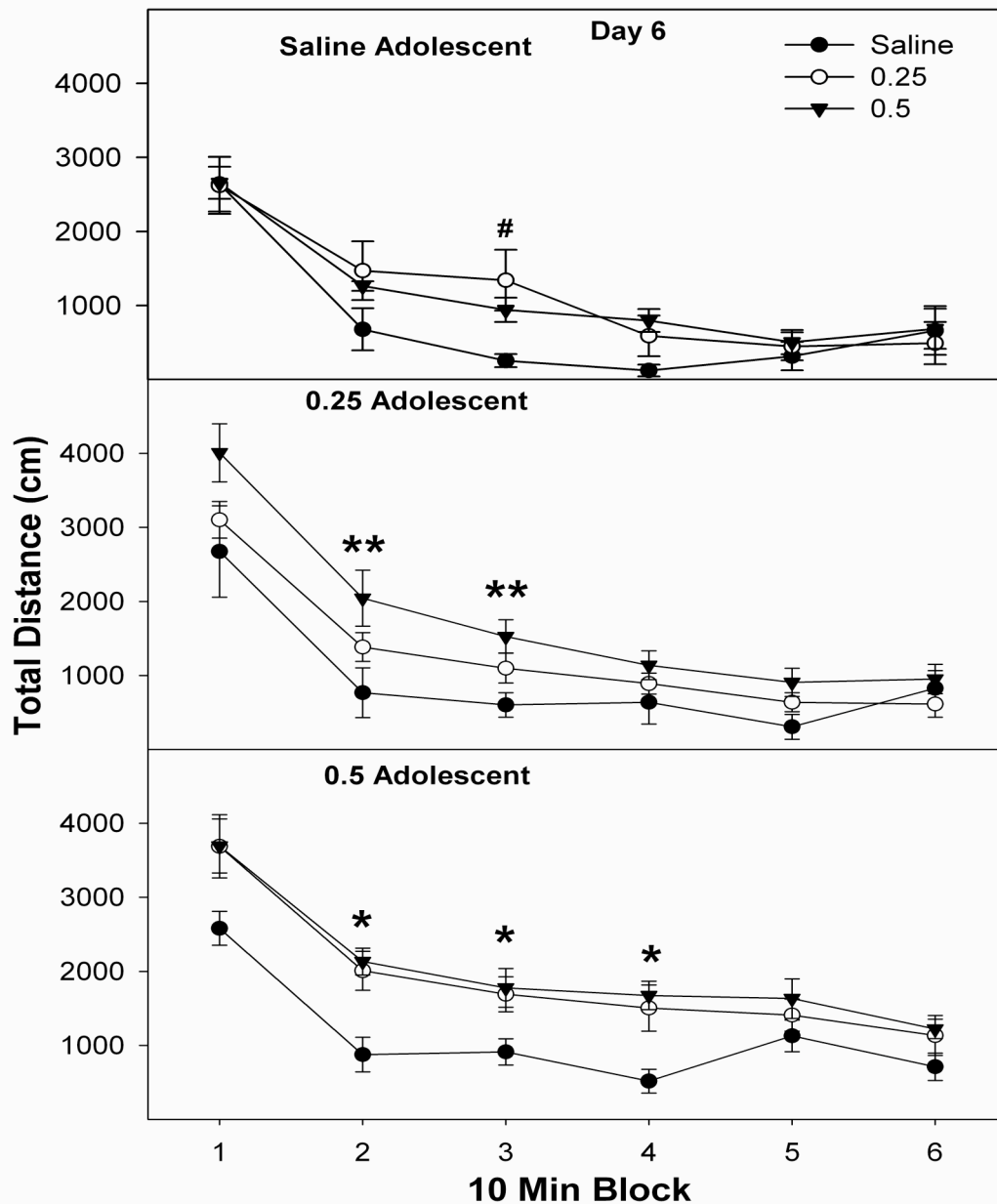


Figure 13. The mean (\pm SEM) distances traveled across the six 10-minute time blocks following injection on Day 6 by rats that received saline (top panel), 0.25 mg/kg nicotine (middle panel), and 0.5 mg/kg nicotine (bottom panel) during adolescence. Symbols and legend indicate drug treatment administered during the adult portion of the study. # Indicates that the 0.25 mg/kg nicotine adult-treated group was significantly ($p < 0.05$) different than saline. ** Indicates that the 0.5 mg/kg nicotine adult-treated group was significantly different than saline. * Indicates that the 0.25 mg/kg and 0.5 mg/kg nicotine adult-treated groups were significantly different than saline.

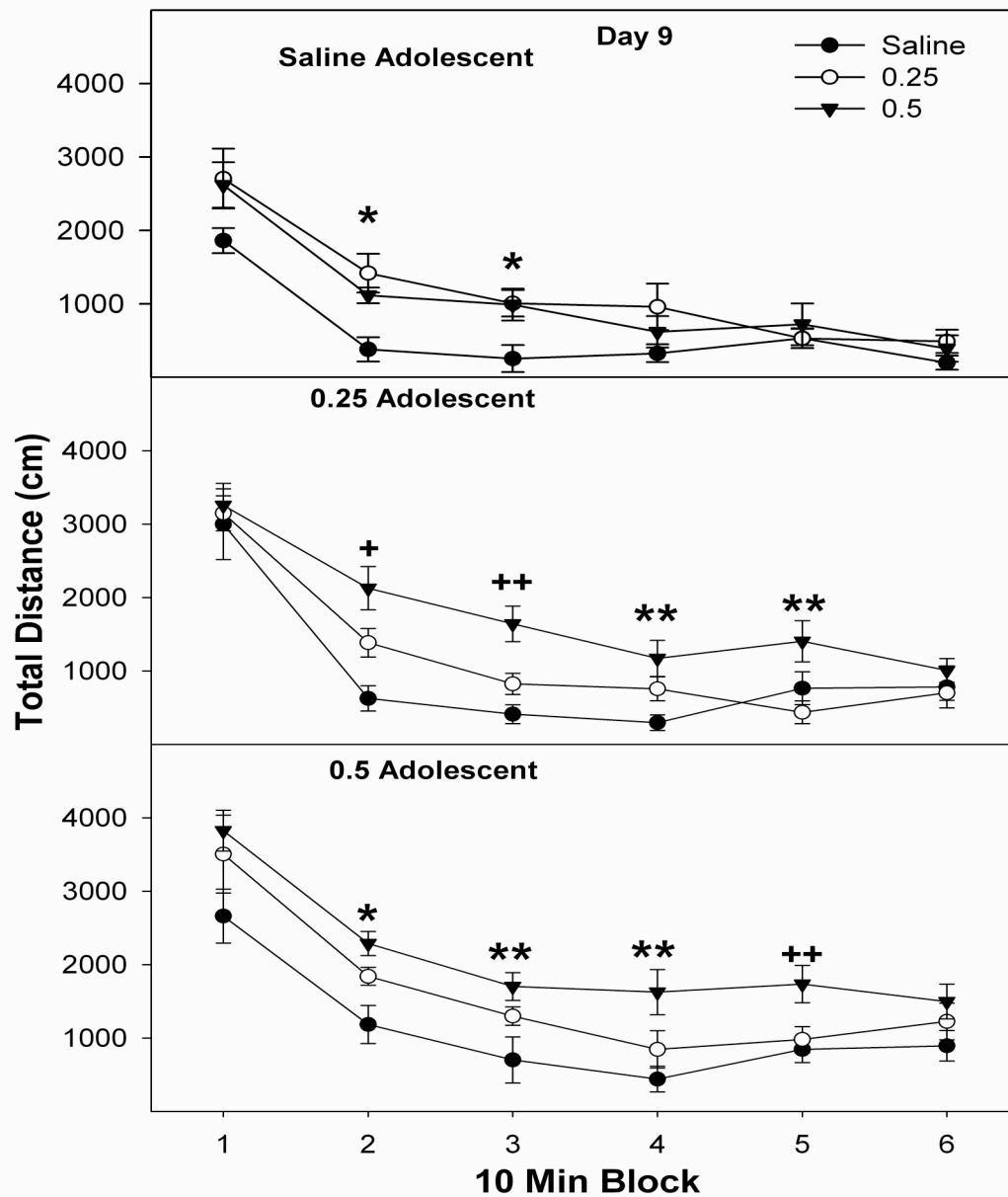


Figure 14. The mean (\pm SEM) distances traveled across the six 10-minute time blocks following injection on Day 9 by rats that received saline (top panel), 0.25 mg/kg nicotine (middle panel), and 0.5 mg/kg nicotine (bottom panel) during adolescence. Symbols and legend indicate drug treatment administered during the adult portion of the study. * Indicates that the 0.25 mg/kg and 0.5 mg/kg nicotine adult-treated groups were significantly ($p < 0.05$) different than saline. + Indicates that all three adult-treated groups were significantly different from each other. ++ Indicates that the 0.5 mg/kg nicotine adult-treated group was significantly different than both other groups. ** Indicates that the 0.5 mg/kg nicotine adult-treated group was significantly different than saline.

Post hoc analyses (Tukey's b) revealed that the adults receiving 0.5 mg/kg nicotine showed significantly greater LMA than saline controls and 0.25 mg/kg nicotine on days 6 through 10.

Analysis of the individual 10-minute blocks within each 60-min activity session revealed more striking differences. On Day 3 (Fig. 12, middle panel), for the group exposed to 0.25 mg/kg nicotine during adolescence, there was a significant effect of Adult Treatment ($p = 0.033$) during the 3rd time period with post hoc analyses indicating that the 0.5 mg/kg nicotine group was different from the saline and 0.25 mg/kg nicotine groups. On the 6th test day (Fig. 13, middle panel), a significant effect of Adult Treatment ($p < 0.036$) was observed during the 2nd and 3rd time periods with post hoc analyses indicating that the 0.5 mg/kg nicotine group was higher than saline controls. On Day 9 (Fig. 14, middle panel), a significant overall Time x Adult Treatment interaction was observed ($F_{5,16} = 3.109$, $p = 0.038$) for the adolescent 0.25 mg/kg nicotine-treated group, with post-hoc comparisons revealing differences during all but the first and last 10-min blocks ($p < 0.018$). Adult rats receiving 0.5 mg/kg nicotine showed significantly more locomotion than saline controls during the 4th and 5th blocks, and more than both the saline controls and 0.25 mg/kg nicotine group during the 3rd block. During the 2nd time block, all three groups were significantly different from each other.

A repeated measures ANOVA on the adult LMA data collected from rats treated with 0.5 mg/kg nicotine during adolescence (Fig. 11, bottom panel) revealed a significant effect of Day ($F_{9,11} = 4.901$, $p = 0.008$). Post hoc analyses

(Tukey's b) revealed that the adults receiving 0.25 mg/kg or 0.5 mg/kg nicotine showed significantly greater LMA than saline controls on days 4 through 9.

Analysis of the individual 10-min blocks within each 60-min activity session revealed that the group that received the 0.5 mg/kg adolescent dose of nicotine resulted in LMA differences. On Day 3 (Fig. 12, bottom panel), a significant Adult Treatment ($p < 0.027$) was observed during the 3rd and 4th 10-min blocks, with both the 0.25 and 0.5 mg/kg nicotine groups differing from saline controls. On Day 6 (Fig. 13, bottom panel), for the adolescent 0.50 mg/kg nicotine-treated group, a significant overall Time x Adult Treatment interaction was observed ($F_{5,16} = 3.305$, $p = 0.031$), and post hoc analyses revealed that for the 2nd, 3rd and 4th time blocks, both nicotine groups were significantly higher than saline controls ($p < 0.049$). On Day 9 (Fig. 14, bottom panel), a significant effect of Adult Treatment ($p < 0.018$) was observed for the adolescent 0.50 mg/kg nicotine exposed group, wherein the 0.5 mg/kg adult treatment was significantly higher than saline controls during the 3rd and 4th time periods, and higher than both the saline and 0.25 mg/kg groups during the 5th time period. In the 2nd time period, both nicotine groups were significantly different from saline controls.

D. Effects of adolescent nicotine exposure on adulthood EtOH drinking

1. Initial EtOH intake

In order to study the effects of adolescent nicotine exposure, which concluded 35 days earlier, on initial EtOH drinking in adulthood, the EtOH intakes during the first 7 days of access were analyzed. Differences in EtOH intake between groups can be noted even on the first day of EtOH access. Wistar rats consumed 2-4 g/kg EtOH on the first day, whereas P rats, with access to 5% and 10% EtOH, consumed 6-7 g/kg EtOH and P rats, with access to 15% and 30% EtOH, consumed 8-10 g/kg EtOH on the first day.

A repeated measures ANOVA on the daily EtOH intake averages by Wistar rats revealed a significant main effect of Day ($F_{6,32} = 3.7$, $p = 0.007$), but no significant main effect of Adolescent Treatment ($F_{2,37} = 2.37$, $p = 0.108$) or Day x Adolescent Treatment interaction term ($F_{6,33} = 1.916$, $p = 0.107$) (Fig. 15, top panel).

A repeated measures ANOVA on the daily EtOH intake averages by P rats, with access to 5% and 10% EtOH, revealed a significant main effect of Day ($F_{6,12} = 3.7$, $p = 0.002$), as well as a significant Day x Adolescent Treatment interaction ($F_{6,13} = 4.406$, $p = 0.012$), but no significant main effect of Adolescent Treatment ($F_{2,17} = 0.477$, $p = 0.628$) (Fig. 15, middle panel). However, post hoc analyses (Tukey's b) did not indicate significant group differences on any individual day ($F_{2,17} < 2.285$, 0.052 , p values > 0.196).

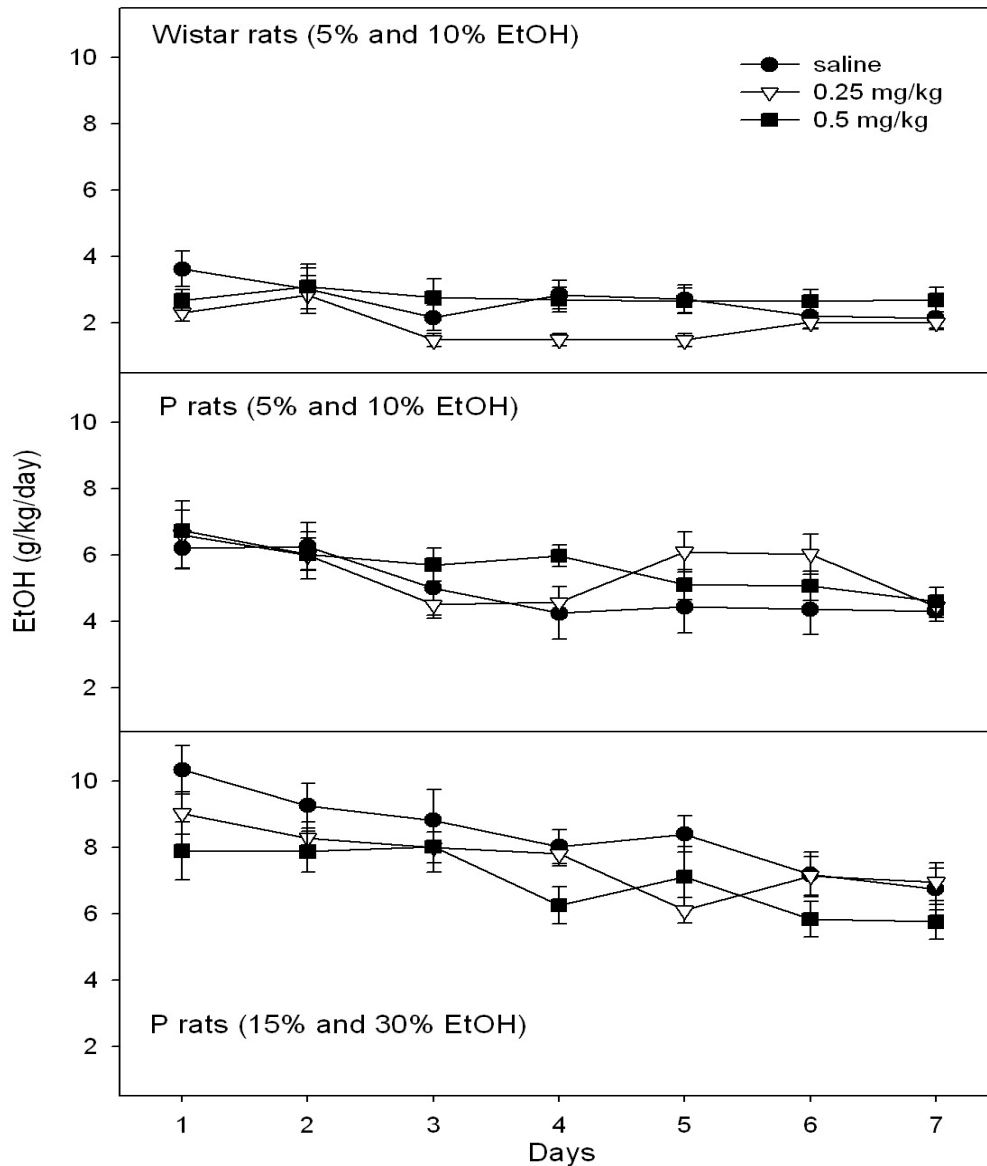


Figure 15. The mean (\pm SEM) daily average EtOH intakes (g/kg/day) during the initial 7 days of continuous EtOH access by Wistar rats (top panel), P rats with access to 5% and 10% EtOH (middle panel), and P rats with access to 15% and 30% EtOH (bottom panel). Symbols indicate adolescent nicotine treatment with saline (closed circles), 0.25 mg/kg nicotine (open triangles), and 0.5 mg/kg nicotine (closed squares). $n = 7-10$ per group. No significant differences were observed.

In P rats drinking higher (15% and 30%) EtOH concentrations, a repeated measures ANOVA on the daily EtOH intake averages of the first 7 days of EtOH access revealed a significant main effect of Day ($F_{6,9} = 6.606$, $p = 0.007$), as well as a significant Day x Adolescent Treatment interaction ($F_{6,10} = 46.011$, $p = 0.007$), but no significant main effect of Adolescent Treatment ($F_{2,14} = 6.011$, $p = 0.122$) (Fig. 15, bottom panel). Post hoc analyses did not indicate significant group differences on any individual day ($F_{2,27} < 2.936$, p values > 0.086).

2. Acquisition and maintenance of EtOH drinking behavior

a. Wistar rats

The effects of adolescent nicotine exposure on EtOH intake by Wistar rats during the 8 weeks of continuous access were examined. Of the 8 weeks of continuous EtOH access, the first 4 weeks are considered the acquisition phase of EtOH drinking behavior, and the second 4 weeks are considered the maintenance phase. A repeated measures ANOVA on the weekly averages of the initial 4 weeks of EtOH drinking by Wistar rats revealed a significant Week x Adolescent Treatment interaction ($F_{3,38} = 2.876$, $p = 0.049$) (Fig. 16). There were no significant main effects of Week ($F_{3,37} = 1.944$, $p = 0.139$) or Adolescent Treatment ($F_{2,39} = 2.608$, $p = 0.086$). Post hoc analyses (Tukey's b) revealed that for the 2nd and 3rd weeks of EtOH drinking, Wistar rats treated with 0.5 mg/kg nicotine during adolescence consumed significantly more EtOH than rats treated with 0.25 mg/kg nicotine or saline during adolescence (p values < 0.038).

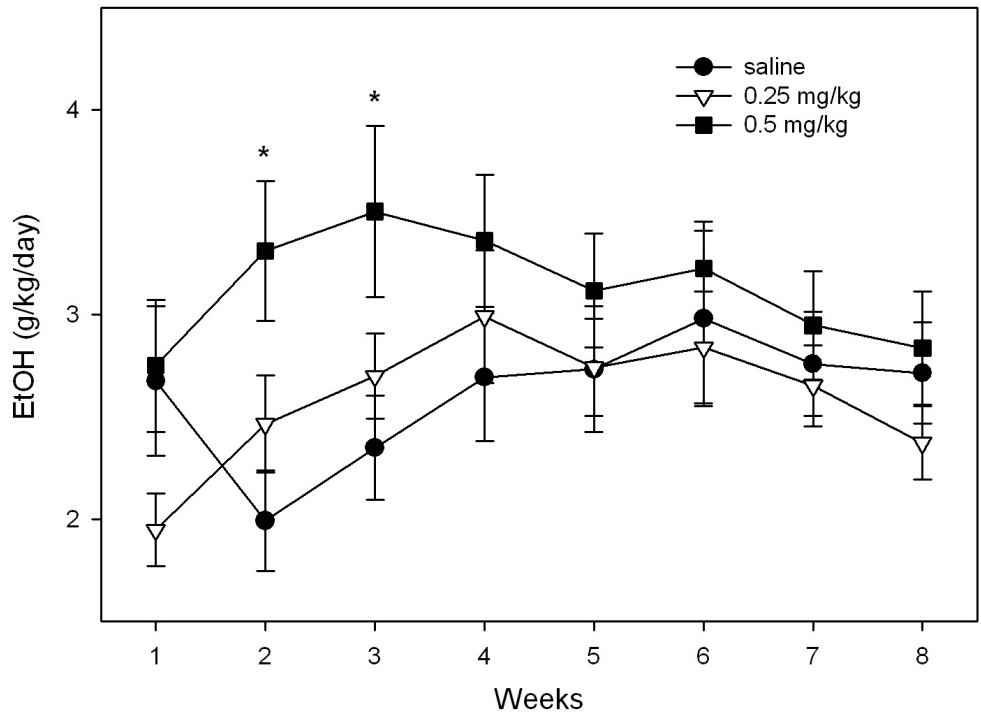


Figure 16. The mean (\pm SEM) average weekly EtOH intake amounts (g/kg/day) during the 8 weeks of continuous EtOH access by Wistar rats that were treated with saline (closed circles, $n = 7$), 0.25 mg/kg nicotine (open triangles, $n = 8$), or 0.5 mg/kg nicotine (closed squares, $n = 7$) during adolescence. * Indicates that rats in the 0.5 mg/kg group had higher intake than the saline group.

In addition to analyzing amount (g/kg of body weight) of EtOH consumed throughout the study, it is also important to investigate whether adolescent nicotine exposure had effects on preference for EtOH as a percentage of total fluid intake, as well as any differences in preference between the multiple concentrations of EtOH to which the rats had access. In Wistar rats, repeated measures ANOVAs on the average weekly preference of 5% or 10% EtOH as a percentage of total fluid intake during either the 4 week acquisition period or the entire 8 weeks of continuous EtOH access indicated no significant main effects of Week ($F_{3,16} < 2.071$, p values > 0.128) or Adolescent Treatment ($F_{3,16} < 3.434$, p values > 0.104) or Adolescent Treatment x Week interactions ($F_{3,17} < 3.434$, p values > 0.186) (Fig. 17, top and middle panels). However, there was a significant main effect of Week ($F_{7,12} = 12.277$, $p < 0.001$) on the preference for total EtOH (Fig. 17, bottom panel). This indicates that, while there were no differences in preference for one of the two available EtOH concentrations over the 8 weeks of continuous EtOH access, there was an increase in EtOH intake as a percentage of total fluid intake for all groups of Wistar rats.

b. P rats – 5% and 10% EtOH

Next, the effects of adolescent nicotine exposure on EtOH intake by P rats, with access to 5% and 10% EtOH, during the 8 weeks of continuous access were examined. A repeated measures ANOVA on the 8 weeks of EtOH drinking revealed a significant main effect of Week ($F_{7,11} = 6.967$, $p = 0.003$) and a Week x Adolescent Treatment interaction ($F_{7,12} = 3.614$, $p = 0.025$) (Fig. 18). There

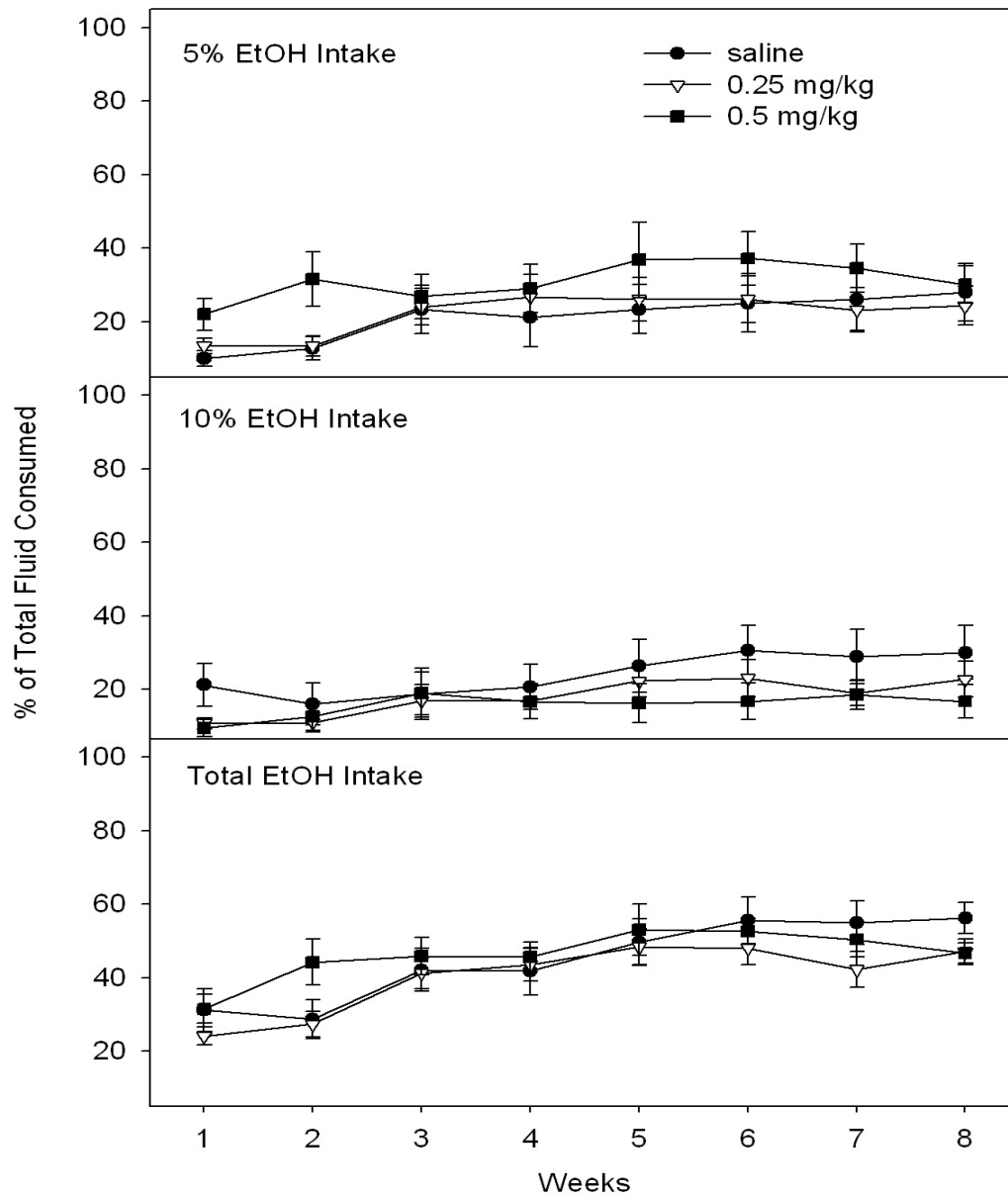


Figure 17. The mean (\pm SEM) average weekly intake of 5%, 10%, and total EtOH, expressed as a percentage of total fluid intake, by Wistar rats that were treated with saline (closed circles, $n = 7$), 0.25 mg/kg nicotine (open triangles, $n = 8$), or 0.5 mg/kg nicotine (closed squares, $n = 7$) during adolescence. No significant ($p < 0.05$) differences were observed.

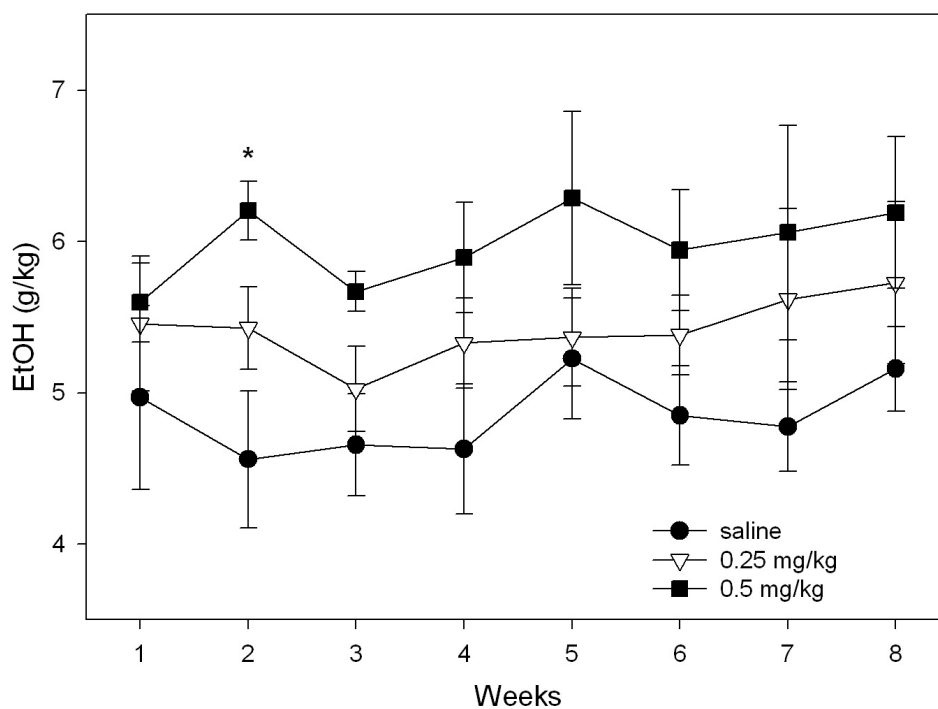


Figure 18. The mean (\pm SEM) average weekly EtOH intakes (g/kg/day) during the 8 weeks of continuous EtOH access by P rats, with access to 5% and 10% EtOH, that were treated with saline (closed circles, $n = 8$), 0.25 mg/kg nicotine (open triangles, $n = 8$), or 0.5 mg/kg nicotine (closed squares, $n = 7$) during adolescence. * Indicates that the group that received 0.5 mg/kg nicotine is significantly ($p < 0.05$) different from the other groups.

was almost a significant main effect of Adolescent Treatment ($F_{2,17} = 3.36$, $p = 0.059$). The P rats treated with 0.5 mg/kg nicotine during adolescence had a tendency to have higher EtOH intakes than the saline group. Post hoc analyses (Tukey's b) revealed that for the second week of EtOH access, rats that were treated with 0.5 mg/kg nicotine during adolescence consumed significantly more EtOH than rats treated with 0.25 mg/kg nicotine or saline during adolescence ($p = 0.025$).

Next, the EtOH preference data were analyzed using repeated measures ANOVAs on the 8 weeks of access to 5% and 10% EtOH. For both 5% EtOH preference and total EtOH preference, a significant main effect of Adolescent Treatment was observed ($F_{2,17} > 4.43$, p values < 0.028) (Fig. 19, top and bottom panels). Post hoc analyses (Tukey's b) indicated that the rats treated with 0.5 mg/kg nicotine during adolescence showed a significantly greater preference for 5% EtOH during week 5 of EtOH access ($p = 0.009$), as well as a greater preference for total EtOH during the 3rd, 4th, and 5th weeks of EtOH access (p values < 0.045), compared to all other groups. During the 7th week of EtOH access, both the 0.5 and 0.25 mg/kg nicotine treated groups showed a significantly greater preference for 5% EtOH than rats treated with saline ($F_{2,17} = 4.997$, $p = 0.02$). There was no significant effect of Adolescent Treatment on preference for 10% EtOH ($F_{2,17} = 0.394$, $p = 0.680$) (Fig. 19, middle panel).

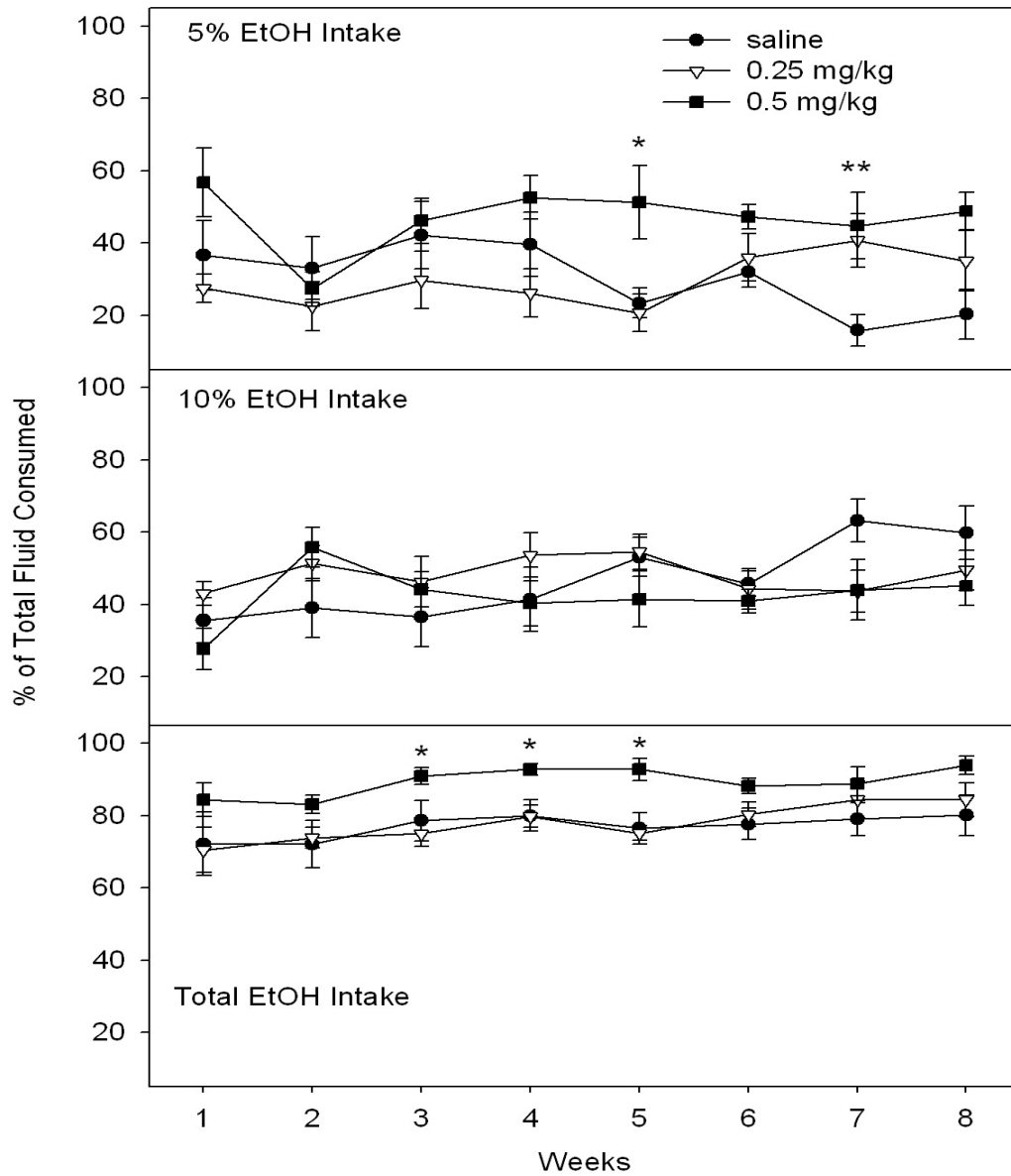


Figure 19. The mean (\pm SEM) average weekly intakes of 5%, 10%, and total EtOH, expressed as a percentage of total fluid intake, by P rats that were treated with saline (closed circles, $n = 8$), 0.25 mg/kg nicotine (open triangles, $n = 8$), or 0.5 mg/kg nicotine (closed squares, $n = 7$) during adolescence. * Indicates that the group that received 0.5 mg/kg nicotine is significantly ($p < 0.05$) different from the other groups. ** Indicates that the saline group is significantly ($p < 0.05$) different from the other groups.

c. P rats – 15% and 30% EtOH

The effects of adolescent nicotine exposure on EtOH intake by P rats, with access to 15% and 30% EtOH, during the 8 weeks of continuous access were examined. A repeated measures ANOVA on the 8 weeks of EtOH drinking revealed a significant main effect of Week ($F_{7,8} = 4.682$, $p = 0.023$), but no main effect of Adolescent Treatment and no significant interaction term, both on the initial 4 weeks and on the entire 8 weeks of continuous EtOH access ($F_{14,18} < 0.784$, p values > 0.264) (Fig. 20). Although the EtOH intake levels changed across the 8 weeks, there were no effects of adolescent nicotine treatment on EtOH intake.

Repeated measures ANOVAs on the preference for 15%, 30%, or total EtOH as a percentage of total fluid intake across the 8 weeks of acquisition and maintenance of EtOH drinking revealed significant main effects of Week for both 15% and 30% preference ($F_{7,8} > 4.558$, p values < 0.025) (Fig. 21, top and middle panels). However, there was no significant main effect of Week on total EtOH preference ($F_{7,8} = 2.298$, $p = 0.133$) (Fig. 21, bottom panel), and no significant main effects of Adolescent Treatment on preference for 15% or 30% EtOH or total EtOH ($F_{2,14} < 0.494$, p values > 0.621).

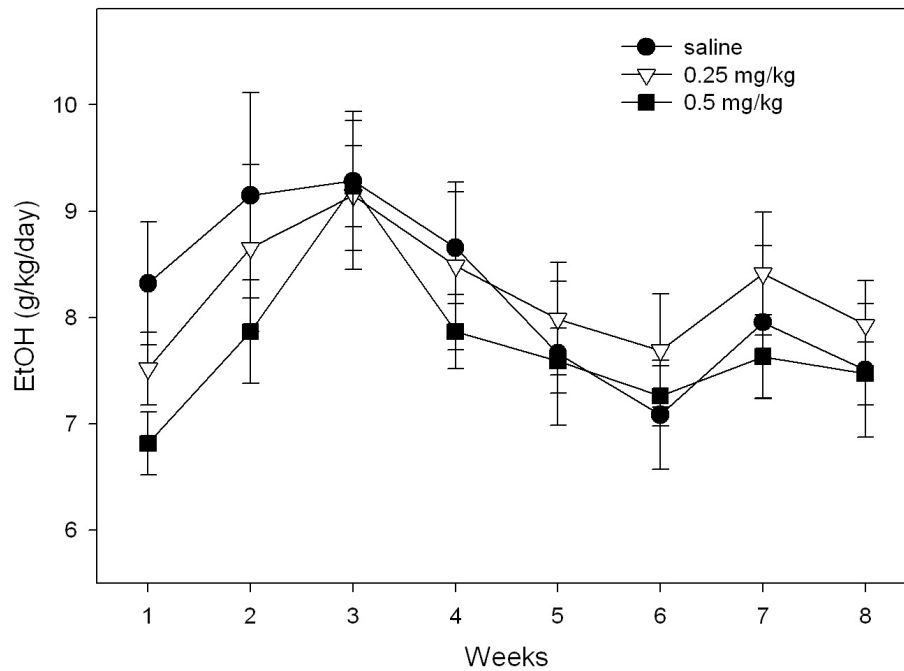


Figure 20. The mean (\pm SEM) average weekly EtOH intakes (g/kg/day) during the 8 weeks of continuous EtOH access by P rats, with access to 15% and 30% EtOH, that were treated with saline (closed circles, $n = 10$), 0.25 mg/kg nicotine (open triangles, $n = 10$), or 0.5 mg/kg nicotine (closed squares, $n = 10$) during adolescence. There were no significant differences between groups at any of the time points.

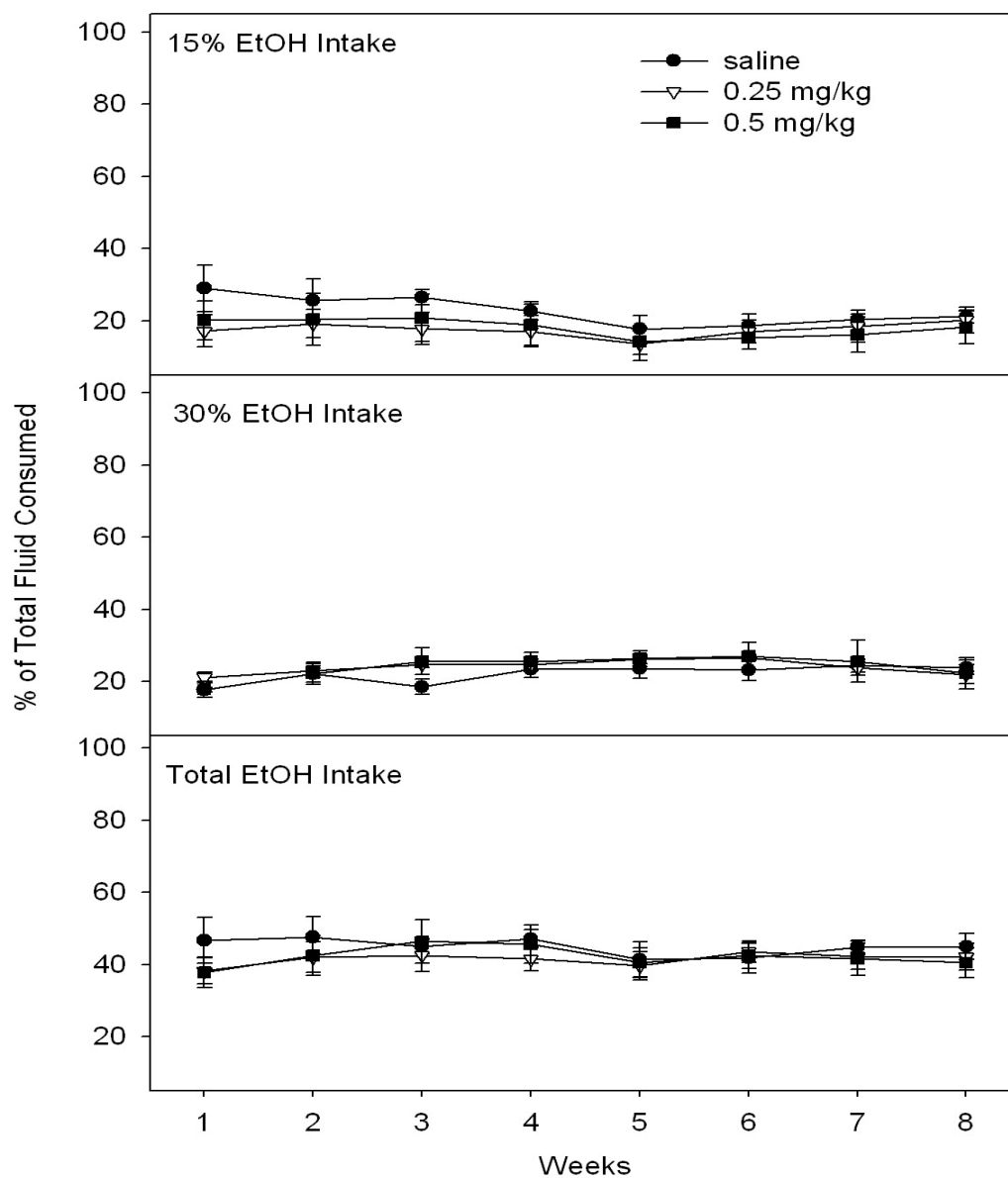


Figure 21. The mean (\pm SEM) average weekly intakes of 15%, 30%, and total EtOH, expressed as a percentage of total fluid intake, by P rats that were treated with saline (closed circles, $n = 10$), 0.25 mg/kg nicotine (open triangles, $n = 10$), or 0.5 mg/kg nicotine (closed squares, $n = 10$) during adolescence. There were no significant differences.

3. Relapse EtOH drinking

a. Wistar rats

The possibility that adolescent nicotine exposure may have altered relapse EtOH drinking in Wistar rats was examined. To assess whether adolescent nicotine exposure altered EtOH relapse drinking following the first EtOH deprivation, a repeated measures ANOVA was performed on the pre-deprivation baseline and first 4 EtOH re-exposure days, with repeated measures of Day and a between-subject factor of Adolescent Treatment. This ANOVA indicated a significant main effect of Day ($F_{5,14} = 6.438$, $p = 0.003$), but no significant main effect of Adolescent Treatment ($F_{2,18} = 0.277$, $p = 0.761$) or interaction term ($F_{10,30} = 0.746$, $p = 0.677$). Next, paired t-tests indicated that all groups of rats showed significantly greater EtOH intake on the first three re-exposure days compared to the pre-deprivation baseline ($df = 14$, p values < 0.015) (Fig. 22). By the 4th re-exposure day, EtOH intake was not significantly different from the pre-deprivation baseline ($df = 14$, p values > 0.11). However, a one-way ANOVA indicated no significant differences between adolescent nicotine groups on either the pre-deprivation baseline or any of the re-exposure days ($F_{2,39} < 0.784$, p values > 0.46).

Two additional cycles of 2-week EtOH deprivations and 2-week EtOH reinstatements were performed. All Wistar rats consumed significantly more EtOH in the days following the deprivations as compared to the respective pre-deprivation baselines, similar to the first EtOH deprivation and reinstatement

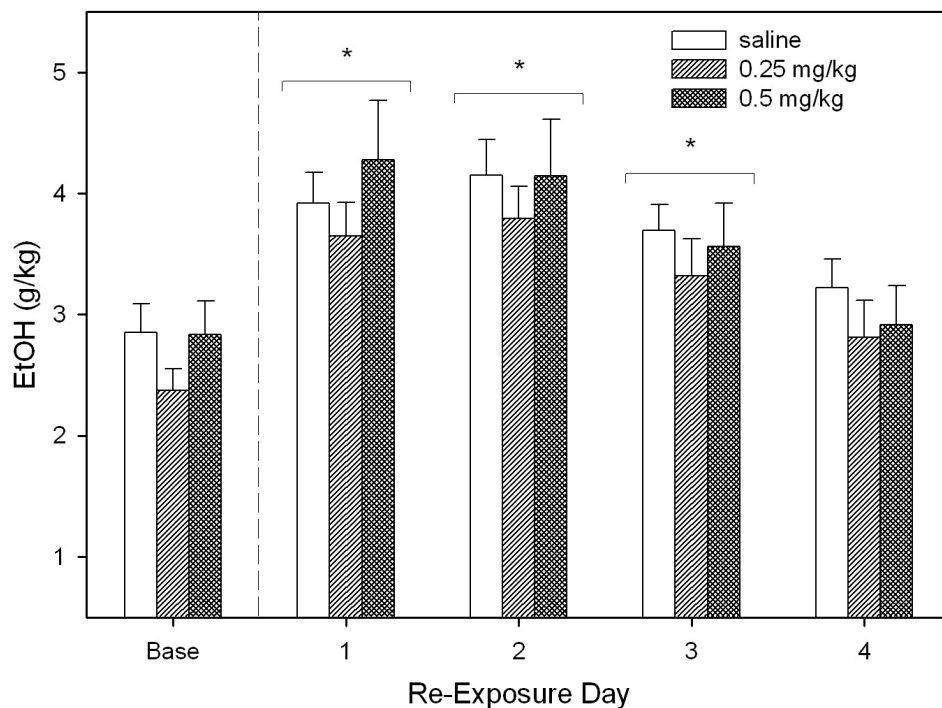


Figure 22. The mean (\pm SEM) average daily EtOH intakes (g/kg/day) preceding and following a 2-week EtOH deprivation by Wistar rats treated with saline (open bars, $n = 7$), 0.25 mg/kg nicotine (gray bars, $n = 8$), and 0.5 mg/kg nicotine (black bars, $n = 7$) during adolescence. Baseline EtOH intake levels (left of dotted line) were taken as the average daily EtOH intake during the week immediately preceding the deprivation. * Indicates all nicotine treatment groups are significantly ($p < 0.05$) different than baseline.

cycle, however there were no effects of adolescent nicotine exposure on the relapse EtOH drinking in either of these cycles (data not shown).

Next, preference for 5%, 10%, and total EtOH during the cycles of EtOH deprivation and reinstatement were analyzed using repeated measures ANOVAs. Following the first EtOH deprivation, there were no significant main effects of Adolescent Treatment on the preferences for 5% or 10% EtOH or for EtOH as a percentage of total fluid intake ($F_{5,14} < 2.284$, p values > 0.099) (Fig. 23). Following the second EtOH deprivation, however, analysis of the preference for both 5% and 10% EtOH revealed a significant Day x Adolescent Treatment interaction ($F_{5,15} > 2.781$, p values < 0.047) (Fig. 24, top and middle panels), and a significant main effect of adolescent treatment was observed on preference for 10% EtOH ($F_{2,18} = 6.715$, $p = 0.007$) (Fig. 24, middle panel). Interestingly, post-hoc analyses (Tukey's b) indicated that the rats treated with saline showed a significantly greater preference for 10% EtOH than nicotine-treated rats on all but the 4th reinstatement day ($F_{2,18} > 5.165$, p values < 0.017). No significant effects of adolescent nicotine treatment on total EtOH preference were observed (Fig. 24, bottom panel).

Finally, following the third EtOH deprivation, a significant main effect of Adolescent Treatment was observed in the preference for 10% EtOH ($F_{2,18} = 3.995$, $p = 0.037$) (Fig. 25, middle panel), but no significant effects of Adolescent Treatment were observed in preference for 5% EtOH or total EtOH ($F_{4,16} < 1.63$, p values > 0.078) (Fig. 25, top and bottom panels). Again, it was the adolescent

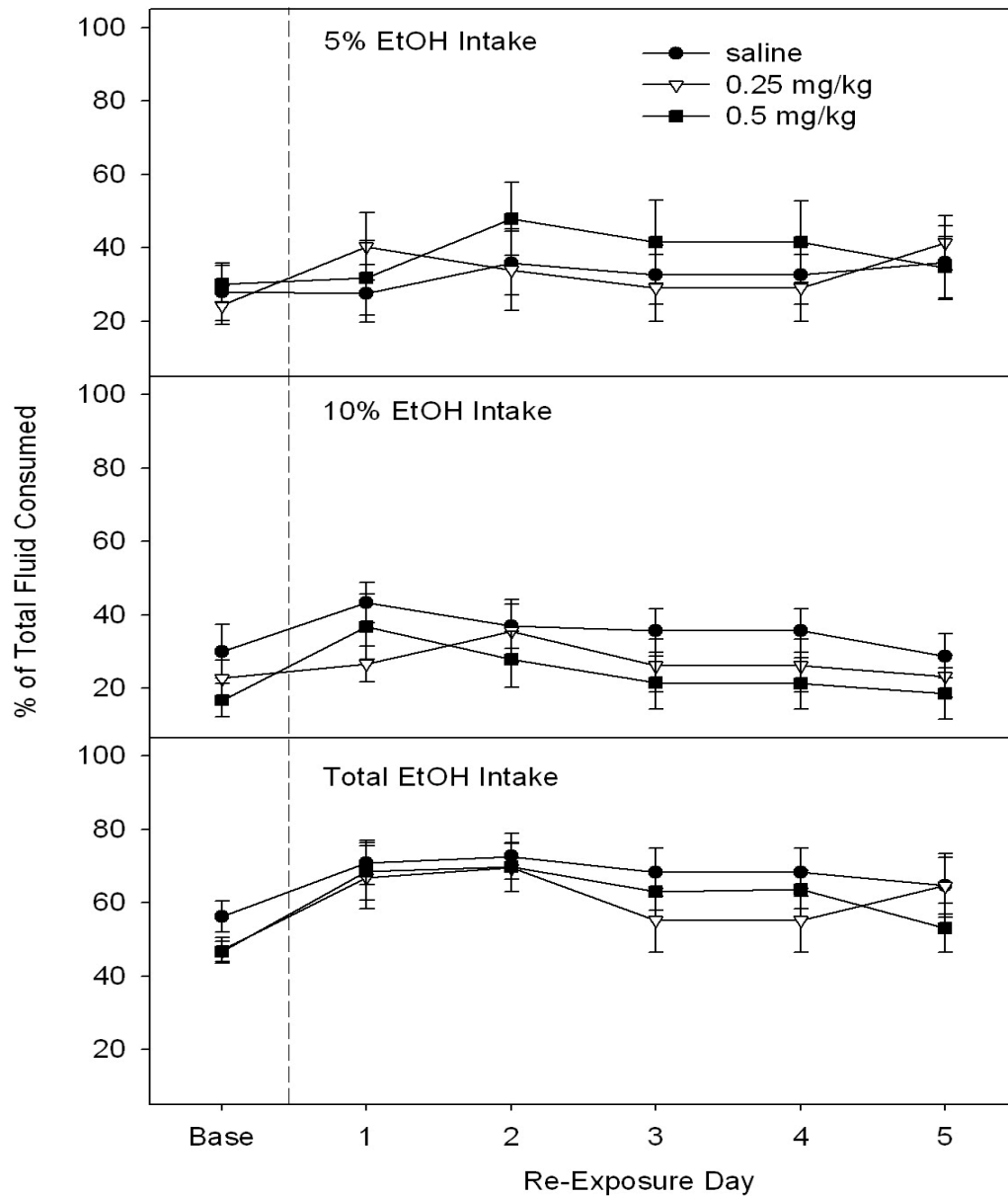


Figure 23. The mean (\pm SEM) average daily intake of 5%, 10%, and total EtOH, expressed as a percentage of total fluid in take, preceding and following the first 2-week EtOH deprivation period by Wistar rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. No significant differences were observed.

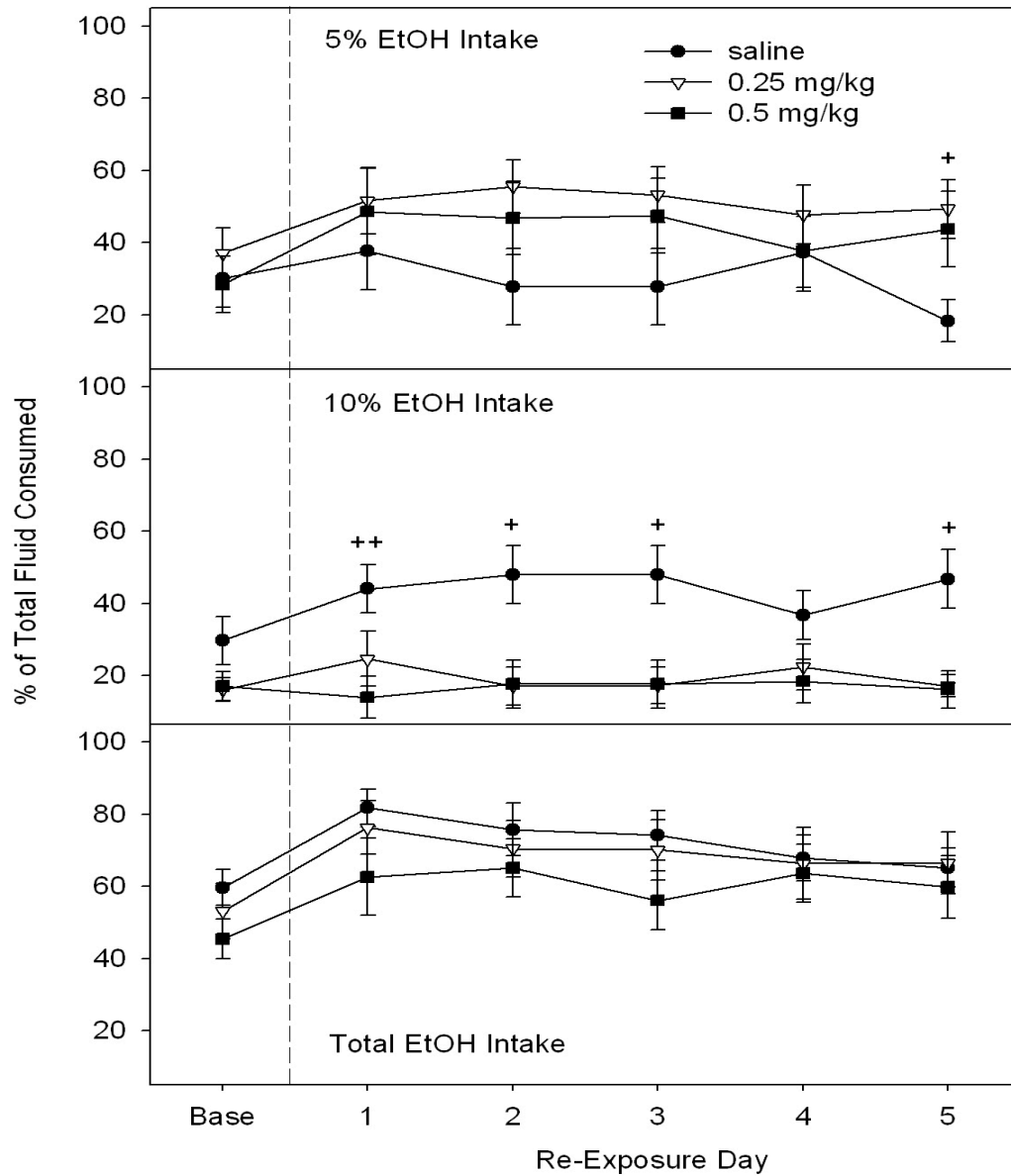


Figure 24. The mean (\pm SEM) average daily intake of 5%, 10%, and total EtOH, expressed as a percentage of total fluid in take, preceding and following the second 2-week EtOH deprivation period by Wistar rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. + Indicates that the adolescent saline group is significantly ($p < 0.05$) different from the other groups. ++ Indicates that the adolescent saline group is significantly ($p < 0.05$) different from the 0.5 mg/kg nicotine group.

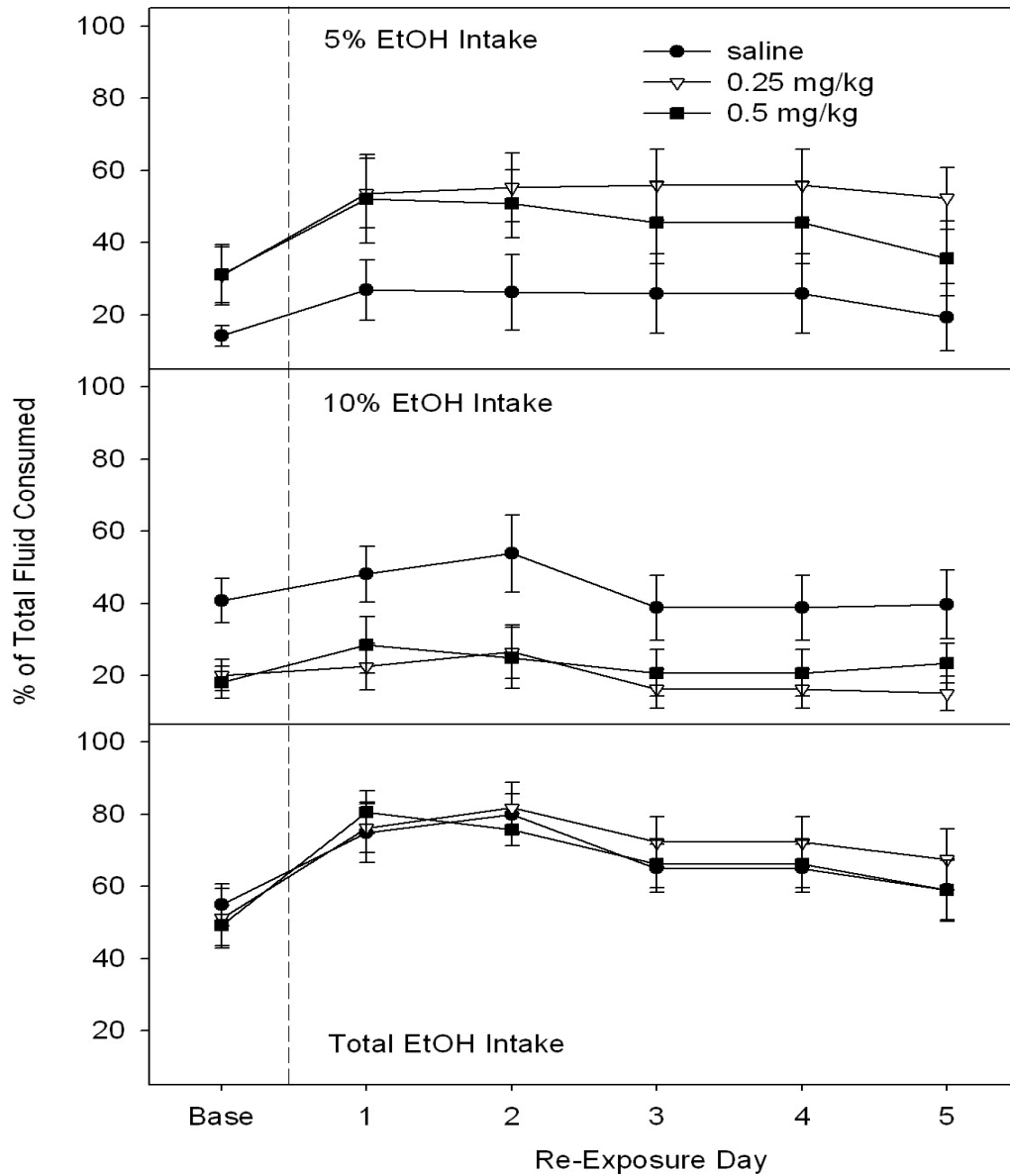


Figure 25. The mean (\pm SEM) average daily intake of 5%, 10%, and total EtOH, expressed as a percentage of total fluid in take, preceding and following the third 2-week EtOH deprivation period by Wistar rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. No significant differences between nicotine treatment groups were observed.

saline-treated rats that showed a greater preference for 10% EtOH than the other two groups following the third EtOH deprivation.

b. P rats – 5% and 10% EtOH

The possibility that adolescent nicotine exposure may have altered relapse EtOH drinking in P rats, with access to 5% and 10% EtOH, was examined. To assess whether adolescent nicotine exposure altered EtOH relapse drinking following the first EtOH deprivation, a repeated measures ANOVA was performed on the pre-deprivation baseline and first 4 EtOH re-exposure days, with repeated measures of Day and a between-subject factor of Adolescent Treatment. This ANOVA indicated a significant main effect of Day ($F_{4,14} = 23.047$, $p < 0.001$), but no significant main effect of Adolescent Treatment ($F_{2,17} = 0.835$, $p = 0.731$) or interaction term ($F_{8,30} = 0.792$, $p = 0.614$). Paired t-tests indicated that all groups of rats showed a significant increase in EtOH intake on the first re-exposure day compared to the pre-deprivation baseline ($df = 19$, $p = 0.002$) (Fig. 26). By the second re-exposure day, EtOH intake was not significantly different from the pre-deprivation baseline ($p = 0.499$). However, a one-way ANOVA indicated no significant differences between adolescent nicotine groups on either the pre-deprivation baseline or any of the re-exposure days ($F_{2,17} > 0.039$, p values > 0.301).

Two additional cycles of 2-week EtOH deprivations and 2-week EtOH reinstatements were performed. All P rats consumed significantly more EtOH in the days following the deprivations as compared to the respective pre-deprivation

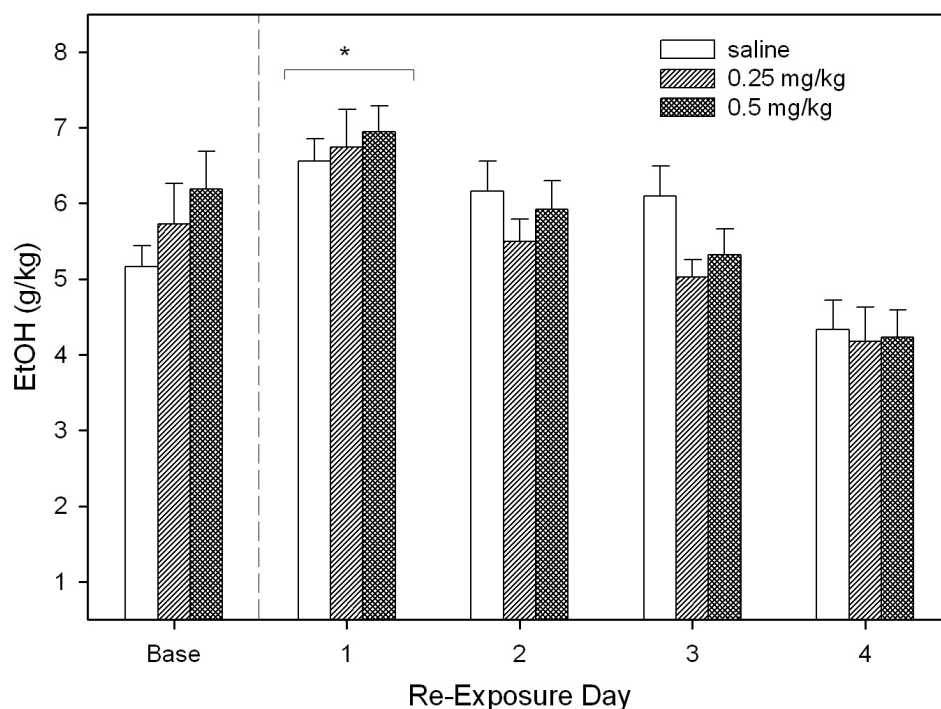


Figure 26. The mean (\pm SEM) average daily EtOH intakes (g/kg/day), preceding and following a 2-week EtOH deprivation by P rats with access to 5% and 10% EtOH, treated with saline (open bars, $n = 8$), 0.25 mg/kg nicotine (gray bars, $n = 8$), and 0.5 mg/kg nicotine (black bars, $n = 7$) during adolescence. Baseline EtOH intake levels (left of dotted line) were taken as the average daily EtOH intake during the week immediately preceding the 2-week EtOH deprivation period. * Indicates that EtOH intakes in all three groups are significantly ($p < 0.05$) different than baseline.

baselines, however there were no effects of adolescent nicotine exposure on the relapse EtOH drinking in either of these cycles (data not shown).

Next, the effects of adolescent nicotine treatment on EtOH preference were analyzed using repeated measures ANOVAs. Following the first EtOH deprivation period, a significant main effect of Adolescent Treatment was observed on both 5% and 10% EtOH preference ($F_{1,18} > 4.307$, p values < 0.031) (Fig. 27, top and middle panels). When looking at total EtOH preference, no differences were observed ($F_{2,17} = 2.639$, $p = 0.10$), although it should be noted that all groups of rats were drinking almost exclusively EtOH during the first few days of reinstatement of EtOH access (Fig. 27, bottom panel). Following the second and third EtOH deprivations, no significant main effects of Day ($F_{5,14} < 2.532$, p values > 0.082) or Adolescent Treatment ($F_{2,17} < 2.653$, p values > 0.121) on preference for 5%, 10%, or total EtOH were observed (Figs. 28 and 29).

c. P rats – 15% and 30% EtOH

Finally, the effects of adolescent exposure on relapse EtOH drinking were examined in P rats with access to higher concentrations (15% and 30%) of EtOH. A repeated measures ANOVA performed on the pre-deprivation baseline and first 4 EtOH re-exposure days indicated a significant main effect of Day ($F_{4,24} = 23.917$, $p < 0.001$), but no significant main effect of Adolescent Treatment ($F_{2,27} = 0.285$, $p = 0.754$) or interaction term ($F_{8,50} = 0.322$, $p = 0.954$). Paired t-tests indicated that all groups of rats showed significantly elevated EtOH intake on the

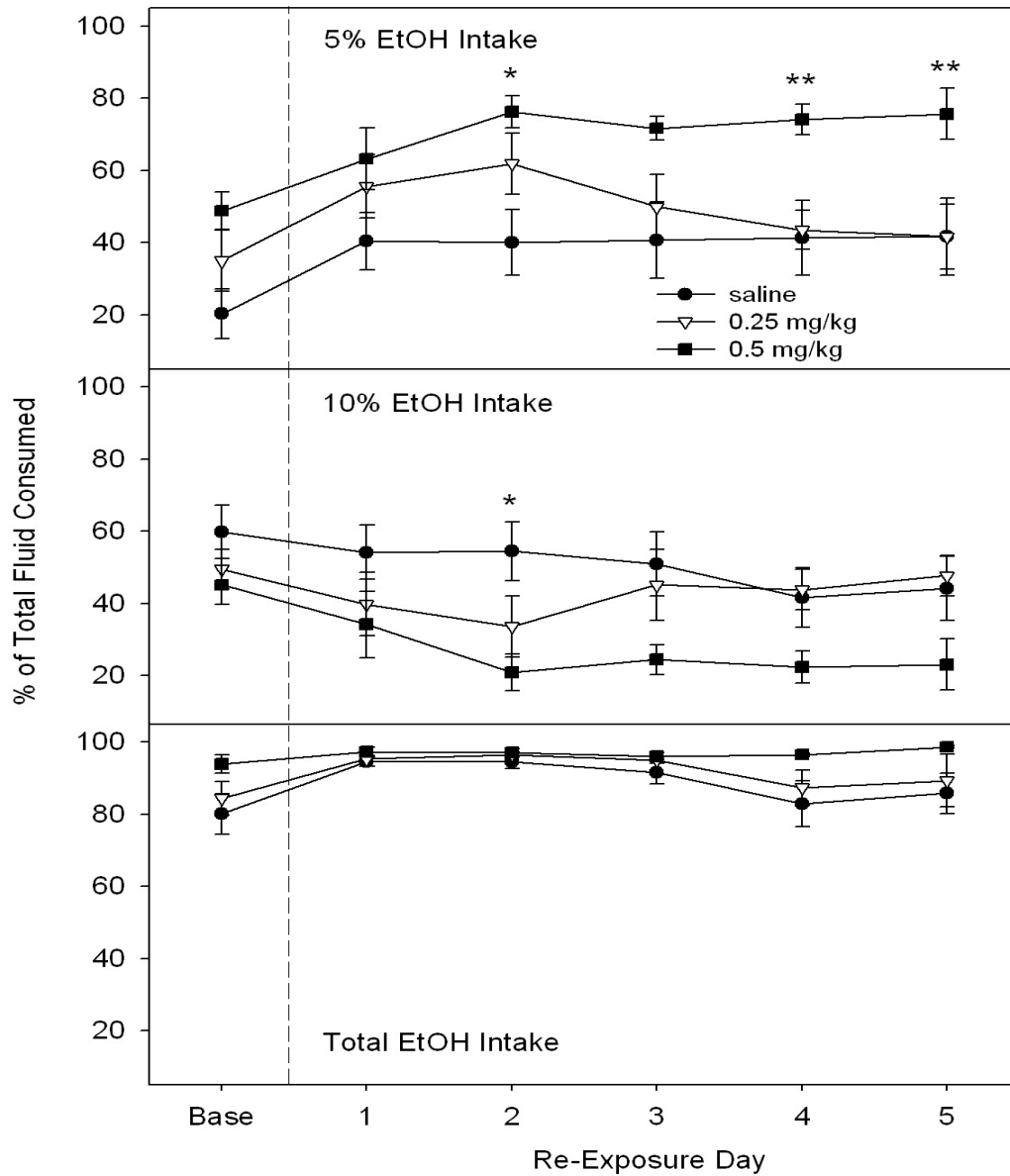


Figure 27. The mean (\pm SEM) average daily intakes of 5%, 10%, and total EtOH, expressed as a percentage of total fluid intake, preceding and following the first 2-week EtOH deprivation period by P rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. * Indicates that the 0.5 mg/kg nicotine group is significantly ($p < 0.05$) different from the saline group. ** Indicates that the 0.5 mg/kg nicotine group is significantly ($p < 0.05$) different from the other two groups.

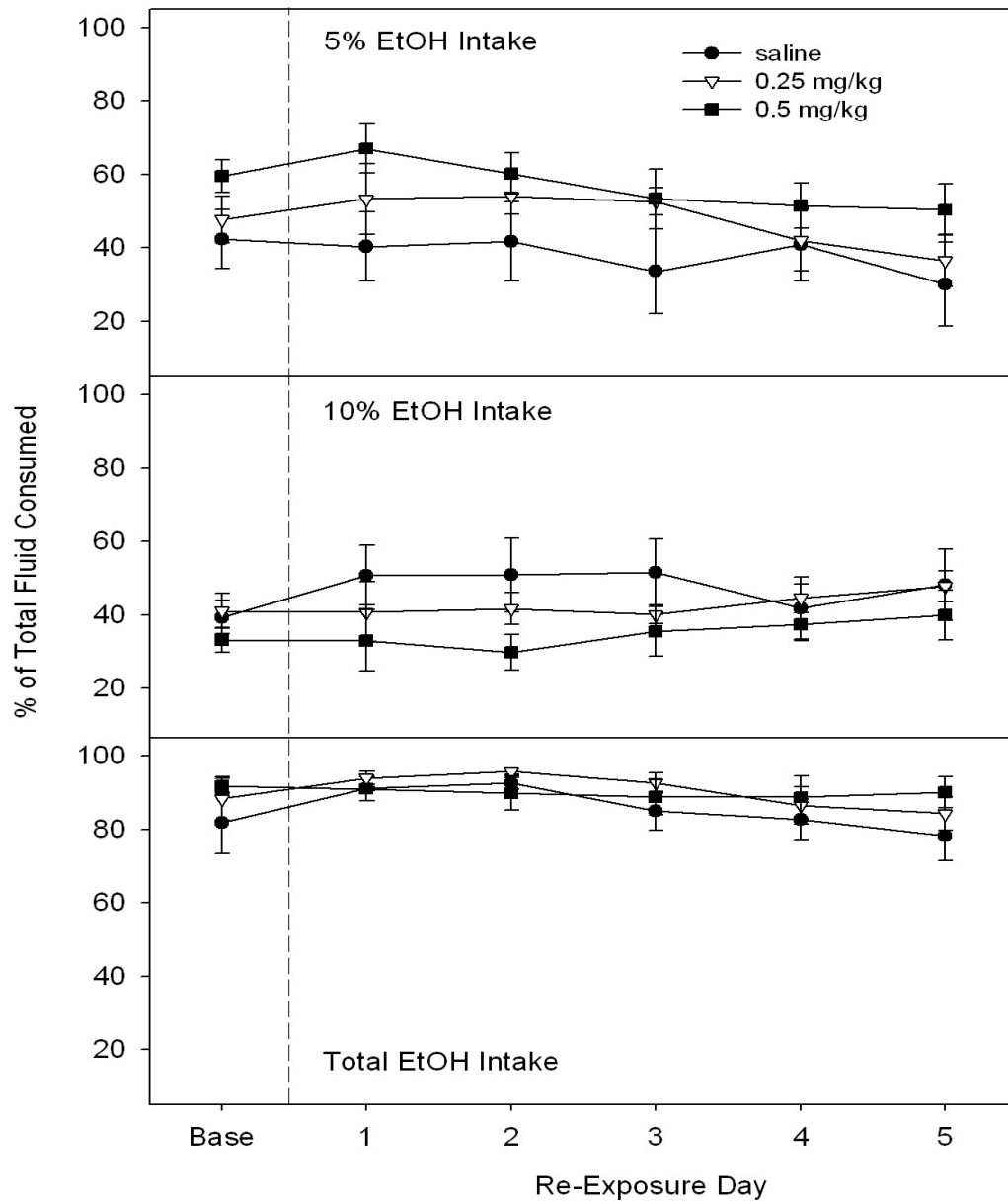


Figure 28. The mean (\pm SEM) average daily intake of 5%, 10%, and total EtOH, expressed as a percentage of total fluid intake, preceding and following the second 2-week EtOH deprivation period by P rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. No significant differences were observed.

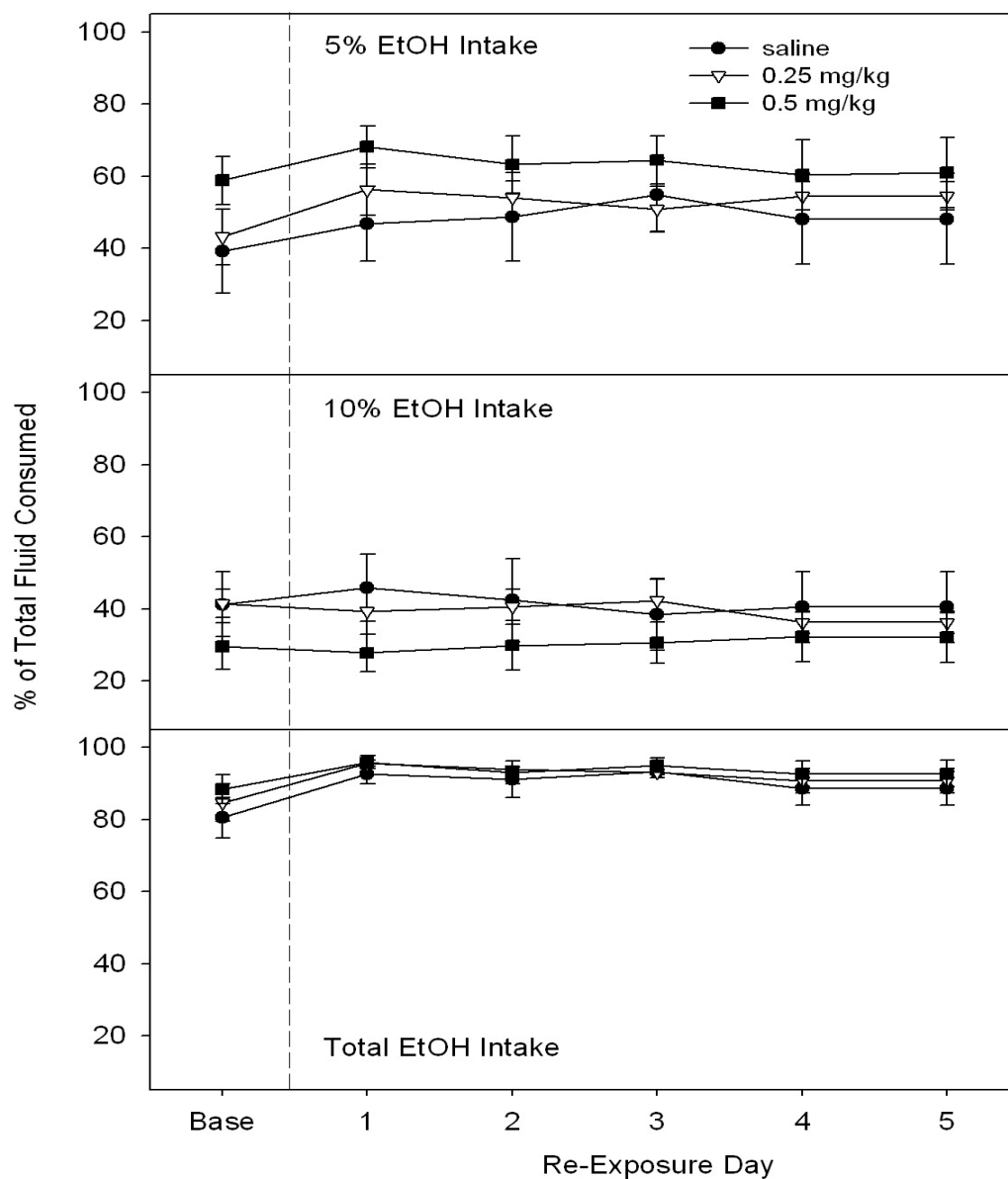


Figure 29. The mean (\pm SEM) average daily intakes of 5%, 10%, and total EtOH, expressed as a percentage of total fluid intake, preceding and following the third 2-week EtOH deprivation period by P rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. No significant differences were observed.

first re-exposure day ($df = 29$, $p < 0.001$) (Fig. 30). By the second re-exposure day, EtOH intake levels did not differ from the pre-deprivation baseline ($p = 0.130$). A one-way ANOVA indicated no significant differences between adolescent nicotine groups on either the pre-deprivation baseline or any of the re-exposure days ($F_{2,27} < 0.789$, p values > 0.465).

Two additional cycles of 2-week EtOH deprivations and 2-week EtOH reinstatements were performed with these rats. All P rats consumed significantly more EtOH in the days following the deprivations as compared to the respective pre-deprivation baselines, however there were no effects of adolescent nicotine exposure on the relapse EtOH drinking in either of these cycles (data not shown).

The effects of adolescent nicotine treatment on EtOH preference were analyzed using repeated measures ANOVAs for each of the 3 deprivations (Figs. 31-33). Following all 3 EtOH deprivations, significant main effects of Day ($F_{5,10} < 3.681$, p values > 0.038) were observed on preference for each 15%, 30%, and total EtOH, but there were no significant main effects of Adolescent Treatment ($F_{2,14} < 1.278$, p values > 0.309) on preference for 15%, 30%, or total EtOH.

4. Effects of nicotine exposure during adulthood

In order to assess whether any observed effects of adulthood EtOH drinking were correlated with nicotine exposure specifically during adolescence, a group of adult Wistar rats was exposed to either saline or 0.5 mg/kg nicotine

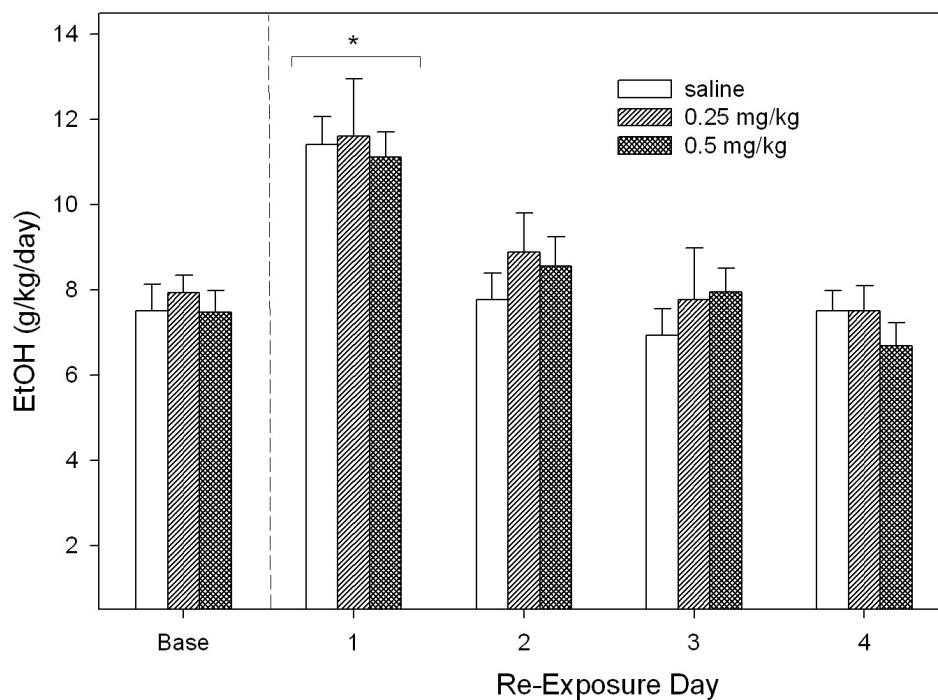


Figure 30. The mean (\pm SEM) average daily EtOH intakes (g/kg/day) preceding and following a 2-week EtOH deprivation by P rats with access to 15% and 30% EtOH, treated with saline (open bars, $n = 10$), 0.25 mg/kg nicotine (gray bars, $n = 10$), and 0.5 mg/kg nicotine (black bars, $n = 10$) during adolescence. Baseline EtOH intake levels (left of dotted line) were taken as the average daily EtOH intake during the week immediately preceding each 2-week EtOH deprivation period. * Indicates that EtOH intakes in all three groups are significantly ($p < 0.05$) different than baseline.

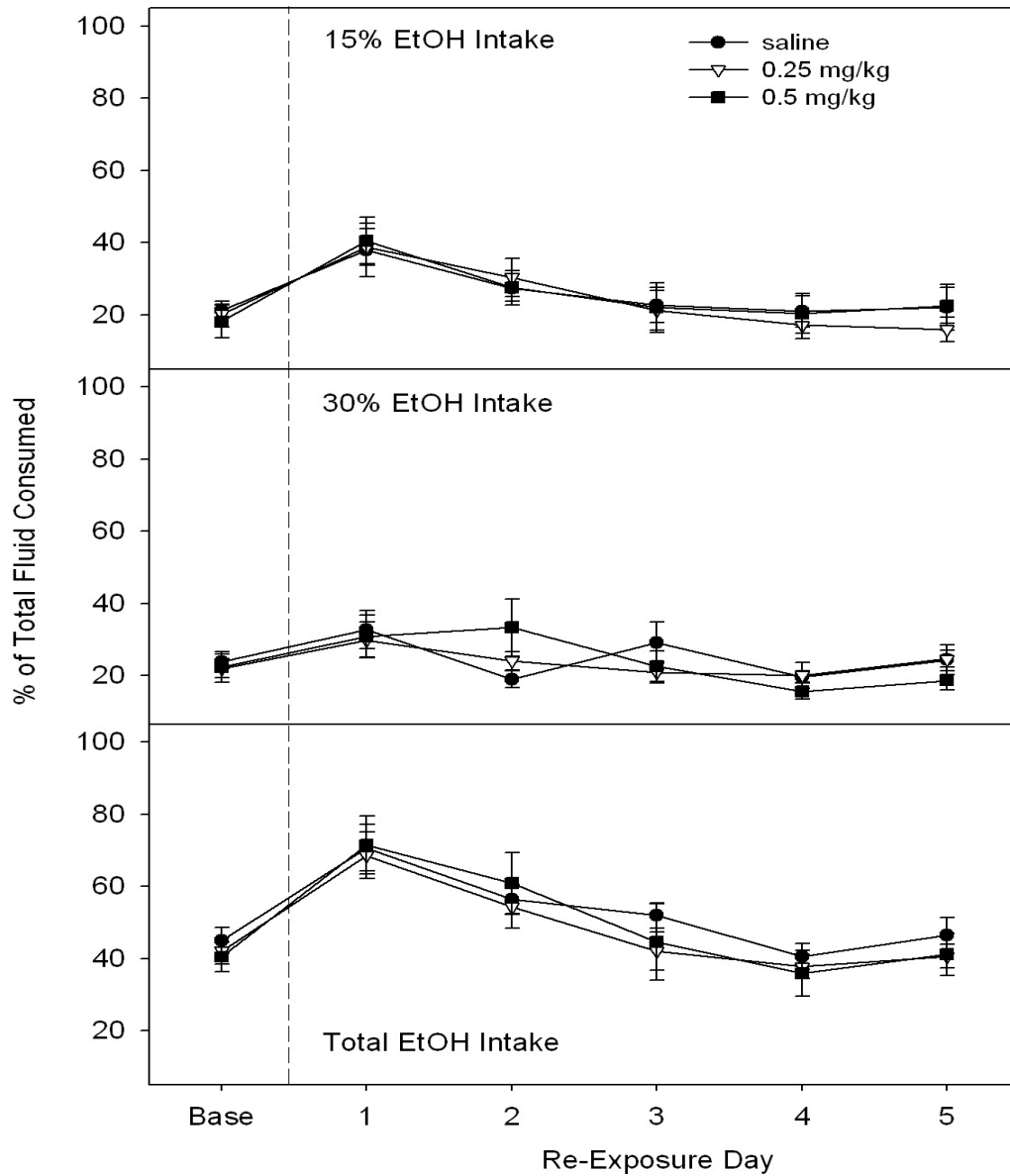


Figure 31. The mean (\pm SEM) average daily intakes of 15%, 30%, and total EtOH, expressed as a percentage of total fluid intake, preceding and following the first 2-week EtOH deprivation period by P rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. No significant differences were observed.

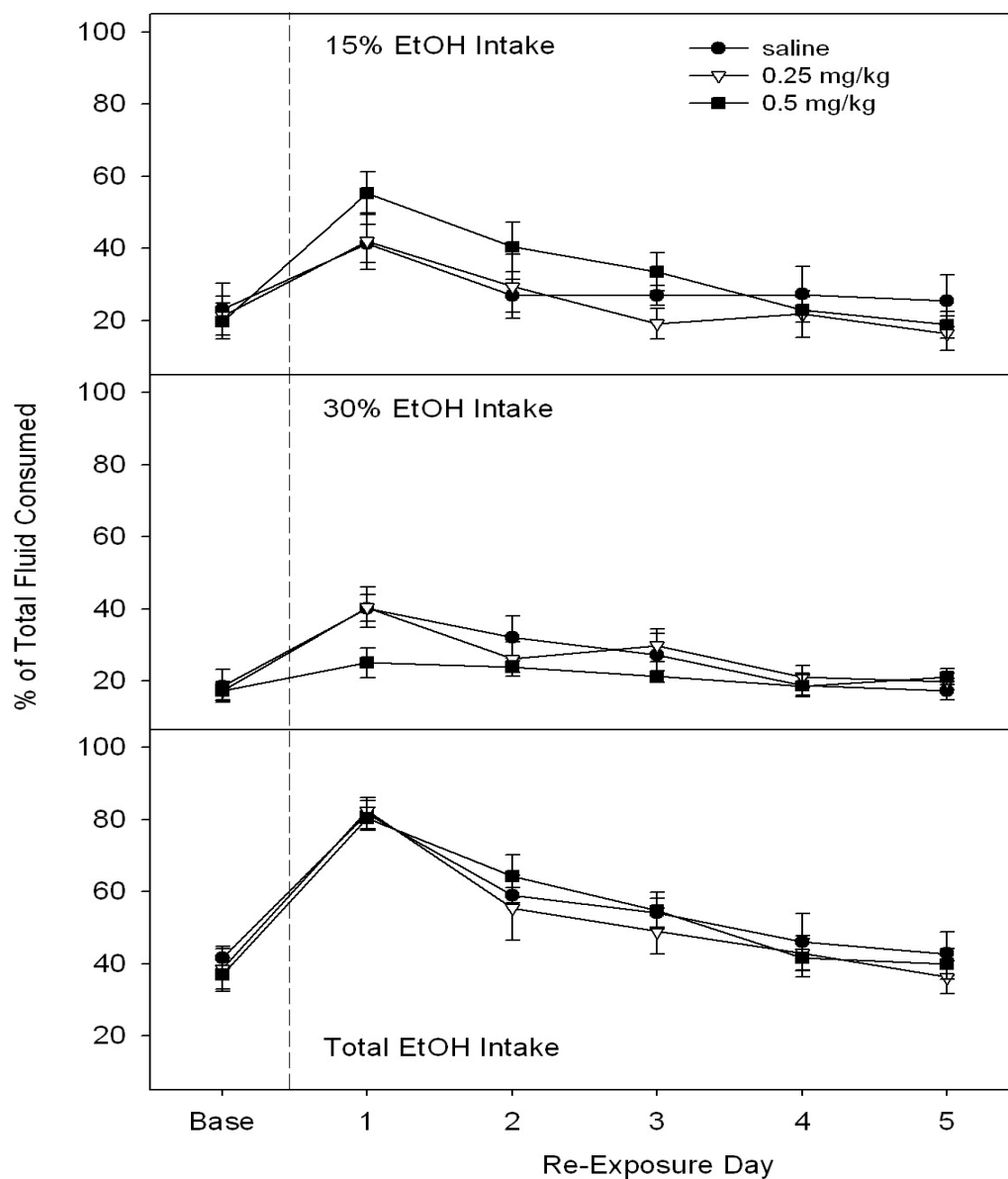


Figure 32. The mean (\pm SEM) average daily intakes of 15%, 30%, and total EtOH, expressed as a percentage of total fluid intake, preceding and following the second 2-week EtOH deprivation period by P rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. No significant differences were observed.

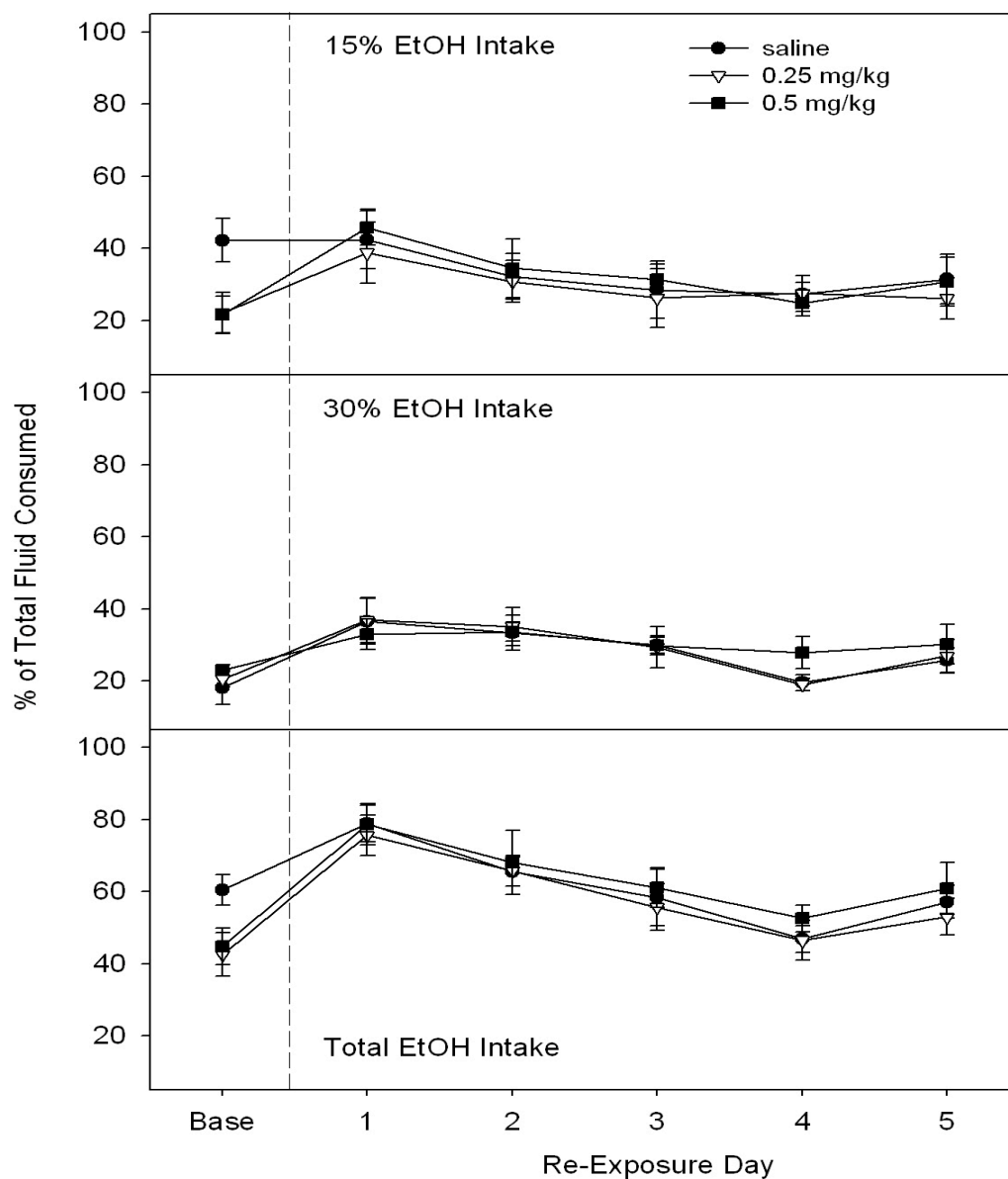


Figure 33. The mean (\pm SEM) average daily intakes of 15%, 30%, and total EtOH, expressed as a percentage of total fluid intake, preceding and following the third 2-week EtOH deprivation period by P rats treated with saline, 0.25 mg/kg nicotine, and 0.5 mg/kg nicotine during adolescence. No significant differences were observed.

from PD 60-71 and then given subsequent 24-hour access to 5% and 10% EtOH beginning on PD 110. A repeated measures ANOVA on the EtOH intake during the initial 7 days of access revealed no significant main effects of Day ($F_{6,7} = 0.947$, $p = 0.518$) or Treatment ($F_{1,12} = 0.253$, $p = 0.624$). A repeated measures ANOVA on the EtOH intake of this control group of Wistar rats showed a significant main effect of Week ($F_{7,6} = 5.517$, $p = 0.027$) but no significant effect of Treatment ($F_{1,12} = 0.09$, $p = 0.77$) or significant interaction term ($F_{7,6} = 0.548$, $p = 0.775$) during the 8-week period of continuous EtOH access (Fig. 34).

5. Water intake

The possibility that adolescent nicotine exposure may have influenced water intake during adulthood was investigated. A repeated measures ANOVA on the water intake of Wistar rats during the initial 7 days of EtOH access revealed no significant main effects of Day ($F_{6,7} = 0.947$, $p = 0.518$) or Adolescent Treatment ($F_{1,12} = 0.253$, $p = 0.624$). A repeated measures ANOVA on the water intake during the 8-week period of continuous EtOH access revealed a significant main effect of Week ($F_{4,36} = 2.749$, $p = 0.043$) but no significant effects of Adolescent Treatment ($F_{2,39} = 2.548$, $p = 0.091$). During the 1st week of EtOH access, all groups consumed approximately 30-35 g/day of water, and by the 4th week, average water consumption was reduced to approximately 25-27 g/day. The decrease in water intake over time (data not

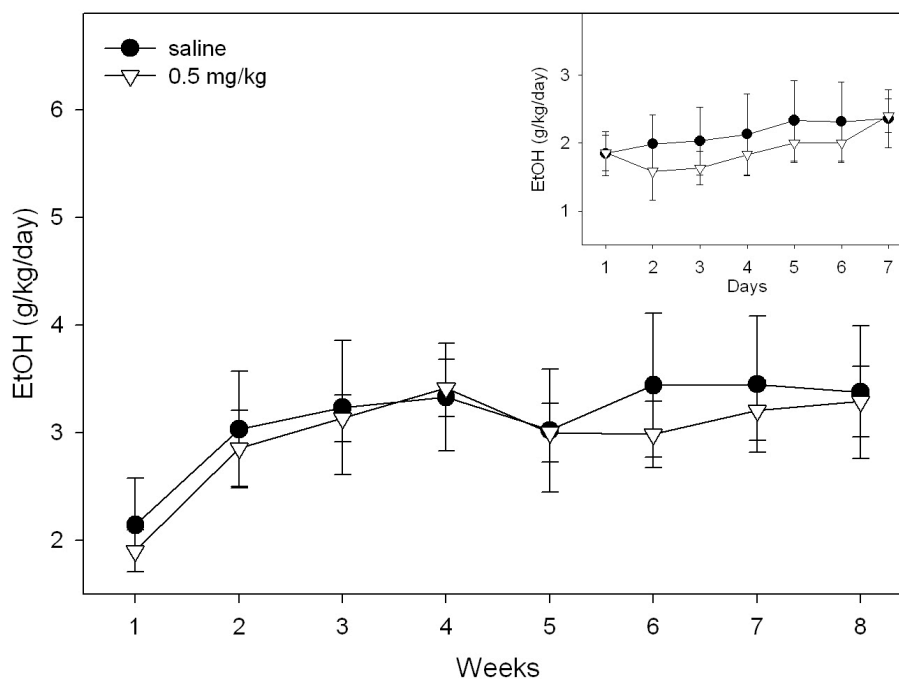


Figure 34. The mean (\pm SEM) average weekly EtOH intakes (g/kg/day) during the 8 weeks of continuous EtOH access by a group of Wistar rats that were previously treated with saline (closed circles, $n = 7$), or 0.5 mg/kg nicotine (open triangles, $n = 7$) during adulthood. The mean (\pm SEM) average daily EtOH intake amounts for the initial 7 days of EtOH access are depicted in the inset. No significant differences were observed.

shown) was not unexpected, due to the increase of EtOH intake by all rats over time, especially during the initial 4 weeks of EtOH access.

Next, the effects of adolescent nicotine exposure on adulthood water intake in P rats with access to 5% and 10% EtOH were examined. A repeated measures ANOVA on the water intake during the initial 7 days of EtOH access revealed no significant main effects of Day ($F_{6,12} = 2.681$, $p = 0.069$) or Adolescent Treatment ($F_{2,17} = 1.127$, $p = 0.347$). A repeated measures ANOVA on the water intake during the 8-week period of continuous EtOH access revealed a significant main effect of Week ($F_{4,14} = 3.573$, $p = 0.033$) but no significant effects of Adolescent Treatment ($F_{2,17} = 3.428$, $p = 0.056$). During the 1st week of EtOH access, all groups consumed approximately 9-12 g/day of water, and by the 4th week, average water consumption was reduced to approximately 4-9 g/day.

Finally, the effects of adolescent nicotine exposure on adulthood water intake in P rats with access to 15% and 30% EtOH were examined. A repeated measures ANOVA on the water intake during the initial 7 days of EtOH access revealed a significant main effect of Day ($F_{6,9} = 13.09$, $p = 0.001$), but no significant main effect of Adolescent Treatment ($F_{2,14} = 0.256$, $p = 0.778$). A repeated measures ANOVA on the water intake during the 8-week period of continuous EtOH access revealed no significant main effects of Week ($F_{7,8} = 1.679$, $p = 0.241$) or Adolescent Treatment ($F_{2,14} = 0.667$, $p = 0.529$). During the 1st week of EtOH access, all groups consumed approx. 22-24 g/day of water,

and by the 4th week, average water consumption remained relatively unchanged at approximately 23-25 g/day.

IV. DISCUSSION

A. The pVTA is a neuroanatomical site that supports nicotine reinforcement

In the first part of this dissertation, it was investigated whether nicotine would be self-administered directly into the pVTA by Wistar rats, since this is a neuroanatomical site known to support self-infusion of other drugs of abuse, and also a region where nAChRs are highly localized. The primary finding of the first aim of this dissertation is that that Wistar rats initiated and maintained intracranial self-administration (ICSA) of nicotine into the pVTA in a dose-dependant manner (Figs. 2 and 3). The average numbers of self-infusions into areas surrounding the pVTA, including the SNr, SNc, and RN, were low (< 15 infusions per session) (Fig. 4), which indicates that the reinforcing effects of nicotine in the pVTA are not likely due to diffusion of the drug into adjacent areas. Although the aVTA was not specifically tested in this study, previous studies have demonstrated that it is not a site mediating the reinforcing properties of nicotine (Ikemoto et al. 2006) or EtOH (Rodd-Henricks et al. 2000a). Taken together, these results indicate that the pVTA is a neuroanatomical site responsible for mediating the rewarding properties of nicotine in the central nervous system. The study of specific sites involved in nicotine reinforcement should be expanded in the future. Such further studies should include the aVTA, but based on previous research, it would be hypothesized that the aVTA would not be a site that mediates nicotine reinforcement.

With any self-administration study, there is the concern that observed increases in lever responding may be due to elevations in locomotor activity, rather than motivational stimulus effects of the drug. Here, the self-administration of nicotine into the pVTA did not appear to be a result of a general increase in locomotor activity, because the rats readily discriminated between the active and inactive levers for the self-infusion of 10 - 400 μ M nicotine. Additionally, across all nicotine doses, when aCSF was substituted for nicotine during the extinction sessions (sessions 5 and 6), responses on the active lever decreased toward the levels of those on the inactive lever. Finally, greater responding on the active lever was reinstated when nicotine was restored during session 7.

Looking closer at the between sessions lever pressing patterns (Fig. 3) reveals some interesting observations. In rats responding for 10 μ M nicotine, the number of active lever presses was similar to that of the rats responding for aCSF throughout the experiment; however, the rats responding 10 μ M for nicotine showed significant lever discrimination in acquisition sessions 1-3 and in the reinstatement (7th) session. This indicates that while the low dose of nicotine may not have been highly reinforcing (compared to the higher doses of nicotine), it has reinforcement salience; that is, the rats were able to detect and differentiate between infusions of 10 μ M nicotine and aCSF in the pVTA.

With some nicotine doses, inactive lever presses were initially high. For example, the rats self-infusing 50 μ M nicotine responded 30-50 times on the inactive lever during each of the first 3 sessions. However, by the 4th session,

inactive lever pressing was low (< 15 responses per session), and remained low throughout the remainder of the sessions. A similar pattern of responding on the inactive lever was observed in the rats self-infusing 200 and 400 μM nicotine. It is possible that this relatively high level of inactive lever pressing was due to locomotor activation by nicotine, which has been widely reported as an effect of systemic (Clarke and Kumar 1983a and 1983b; Clarke et al. 1988) and local intra-VTA (Leikola-Pelho and Jackson, 1992) nicotine treatment. However, even when inactive lever responding was high, the rats self-infusing 50, 200, and 400 μM discriminated between the active and inactive levers. Thus, nicotine seems to have reinforcement saliency in rats even when they are behaviorally activated by the drug's locomotor effects.

Interestingly, the rats self-administering the 100 μM dose of nicotine did not demonstrate the relatively high level of inactive lever responding in the early sessions that was seen in the groups self-administering 50, 200, or 400 μM nicotine. In this group, the number of inactive lever presses was below 25 per session across all 7 sessions. It is unknown why this group did not demonstrate the initially higher inactive lever responding like the other groups. In the first ICSEA session, the 100 μM nicotine group also showed a relatively low number of active lever responses (approx. 30-35, not significantly different from active lever responses by rats self-infusing aCSF) and did not discriminate between levers, and the reason for this is also unknown. However, following that first session, rats in this group discriminated between the active and inactive levers, so it seems as though the initial session was the only aberrant one.

In the group of rats self-infusing 800 μM nicotine, lever discrimination was observed in the first session and then was not present during the rest of the sessions in which nicotine was available. Additionally, active lever responding declined across the sessions, and did not differ from active lever responding in the aCSF group in sessions 4-7. This indicates that the 800 μM dose of nicotine was not as rewarding as the 50-400 μM doses, and may have even had aversive properties. Thus, one can conclude that the dose-response pattern of nicotine in the pVTA is an inverted U-shaped curve, as depicted in Fig. 2.

Analyses on the extinction (sessions 4-6) and reinstatement (sessions 5-7) of active lever responding revealed that the response-contingent behavior was significantly extinguished only in the 10, 100, and 200 μM nicotine groups, and significantly reinstated only in the 10 μM nicotine group. It is likely that between-subject variability in each nicotine dose group was the reason why statistically significant extinction and reinstatement were not seen in other groups. However, looking at Fig. 3, one can see the tendency for active lever responding to decline during the extinction sessions and increase in the reinstatement session, especially in the 50-400 μM nicotine groups.

When injector cannulae were aimed at areas adjacent to the pVTA, the numbers of nicotine infusions were low (< 15 infusions per session, Fig. 4) and active lever responses were also low (< 30 lever presses per session, Fig. 5), compared to the data from rats self-infusing nicotine into the pVTA. While there were no significant differences among injector placement groups during sessions 3 and 4 of the ICSEA experiment, rats self-infusing nicotine into the RN appeared

to show a lower level of infusions than rats self-infusing nicotine into the SNc (Fig. 4). In fact, the number of infusions approached zero on these days. The RN is involved in motor coordination and has no connectivity to the mesolimbic DA pathway or any other pathways known to be involved in reward, so the low number of infusions may have been due to motor effects.

The finding that nicotine is reinforcing within the pVTA is consistent with previous studies demonstrating that nicotine's reinforcing effects are mediated by the mesolimbic DA neurons projecting from the pVTA to the shell of the nucleus accumbens (Corrigall et al. 1992; Ikemoto and Wise 2002). Additionally, other drugs of abuse such as ethanol and morphine have been shown to be reliably self-infused into the pVTA of rats (Rodd-Henricks et al. 2000a; Devine and Wise 1994). One previous study by Ikemoto et al. (2006) demonstrated dose-dependent self-infusion of nicotine into ventral midbrain areas including the pVTA as well as the supramammillary nucleus and the central linear nucleus, although the doses used were in the mM range (12.5-50 mM) and therefore approximately 1000-fold higher than the doses examined in the current study. Considering that pharmacologically relevant brain concentrations of nicotine in human cigarette smokers is in the 100-500 nM range (Karan et al. 2003), the doses from the aforementioned previous study are much higher than what would be physiologically relevant in humans, and therefore difficult to compare with the results of the current study. Higher concentrations of nicotine would be more likely to diffuse from the microinjection site to other nearby brain regions, which complicates the interpretation of the results.

Differences in study design also exist. In our study, each rat was given the opportunity to self-infuse only one concentration of nicotine during the four acquisition sessions and the final reinstatement session. In the Ikemoto study, each rat self-administered a variety of nicotine concentrations, starting with high ones (12.5-50 mM) and concluding with some in a lower range (0.1-10 mM). Therefore, order effects have possibly complicated the expression of response-contingent behavior. This is further complicated by the use of only one extinction session (during which only vehicle was infused upon active lever pressing) was used between the high-dose and lower-dose phases. Results from the current study show that, for some doses, extinction of active lever pressing is not fully extinguished until the second drug-free session. However, while some methodological differences exist, the finding that nicotine is self-administered into the pVTA and not areas adjacent to the VTA is supported by Ikemoto's previous study.

The lower levels of infusions and active lever responses by rats with injector cannulae in the SN subregions and RN as compared to the pVTA may be due to a lesser ability of nicotine to activate neurons originating in these areas. Although nicotine was shown to activate neurons in the SN in electrophysiological studies (Clarke et al. 1985), a later study showed that nicotine-elicited currents in DA neurons originating in the VTA were significantly larger than those in SNc dopaminergic projections (Klink et al. 2001). Unfortunately, that particular study did not differentiate between currents originating in the SNc or SNr. Ikemoto's (2006) study also concluded that

nicotine was not self-administered into the SN. So, while nAChRs are present in the SN, it appears that nicotine activation of these nAChRs is not reinforcing.

The inverted U-shaped dose response curve could be explained by nicotinic modulation of both GABAergic and glutamatergic input to DA neurons within the VTA, which was mentioned in the Introduction section of this dissertation. Nicotine infused locally into the VTA could bind to nAChRs on any of three different types of neurons: directly on VTA DA neurons via nAChRs, most of which are of the high-affinity non- $\alpha 7$ variety; GABA interneurons within the VTA, which also express primarily non- $\alpha 7$ nAChRs; and presynaptic nAChRs on glutamatergic terminals, most of which are the low-affinity $\alpha 7$ -containing variety (Pidoplichko et al. 1997; Klink et al. 2001). At the lowest concentrations tested here, nicotine was not self-administered at a rate greater than that of aCSF. Therefore, it is possible that the nAChRs activated by these lowest concentrations were primarily the high-affinity non- $\alpha 7$ -containing ones on the DA and GABA neurons, and that the net result of DA neuron activation and GABAergic inhibition was not sufficient to result in reinforcement for the rats. At the doses that supported response-contingent behavior and lever discrimination (100-400 μM), it is more likely that the low-affinity $\alpha 7$ -containing nAChRs on the presynaptic glutamatergic terminals would have also been activated, thereby providing enough additional excitatory input for the summation of signaling from all three neuron types to potentiate activation of the mesoaccumbens DA neurons, even in the wake of desensitization of the high-affinity non- $\alpha 7$ -containing nAChRs on the DA and GABA neurons. At these concentrations,

desensitization of the non- $\alpha 7$ nAChRs on the DA and GABA neurons may come into play, which would make the excitatory glutamatergic input onto the pathway all the more important. Finally, at the highest nicotine concentrations tested, even the low-affinity $\alpha 7$ nAChRs on the glutamate terminals may have become desensitized, shifting the mesoaccumbens pathway back to a state which was not reinforcing to the rats.

A study of nicotine ICSA has also been performed in mice (David et al. 2006). The paradigm was different from the current study in that the self-infusions were triggered by successful completion of a Y-maze, which makes it a preference-based paradigm that was not dependent on rate of responses. As such, the results are not directly comparable to those of the current ICSA study. However, the results from this previous study are important because they confirm that the VTA is a region that supports nicotine reinforcement. Additionally, they show that the competitive nAChR antagonist DHBE, when co-infused with nicotine, rapidly and significantly decreased the ICSA response. Since DHBE is selective for the $\alpha 4\beta 2$ nAChRs, this study supports the idea that this nAChR subtype is crucial for nicotine reinforcement. This finding was in agreement with an earlier study showing that mutant mice lacking the $\beta 2$ subunit of the nAChR showed impaired i.v. nicotine self-administration and nicotine-stimulated NAc DA release (Picciotto et al. 1998).

Building on this information about the $\beta 2$ subunit, a recent study used mutant mice to ascertain the role of different α subunits in the VTA on systemic nicotine self-administration (Pons et al. 2008). $\beta 2$ subunits are known to

combine most commonly in the VTA with $\alpha 4$ subunits, although they are found to a lesser extent in combination with $\alpha 6$ subunits. Genetic knockout (KO) mice missing each of these individual subunits, plus an $\alpha 7$ KO and the wild-type mouse, were tested for acquisition of i.v. nicotine self-administration. Pons and colleagues reported that self-administration was promptly acquired by the WT and $\alpha 7$ KO mice, but not the mice missing the $\alpha 4$ or $\alpha 6$ subunits. Additionally, when viral vectors were used to re-express the missing $\alpha 4$ or $\alpha 6$ subunits specifically in the VTA in the KO mice, they acquired nicotine self-administration. $\beta 2$ KO mice also did not self-administer nicotine, until the $\beta 2$ subunit was virally re-expressed in the VTA. Re-expression of $\beta 2$ in the SN, however, did not result in nicotine-self administration. Together, these results show that $\alpha 4\beta 2$ and $\alpha 6\beta 2$ -containing nAChRs, but not $\alpha 7$ -containing nAChRs, must be present specifically in the VTA for nicotine self-administration to take place.

Although this previous study didn't indicate a role for $\alpha 7$ -containing nAChRs in nicotine reward, other work has done so. For example, intra-VTA administration of nicotine was shown to be rewarding in a conditioned place preference procedure (Laviolette and van der Kooy 2003a). When the $\alpha 7$ -nAChR-selective antagonist methyllycaconitine (MLA) was co-infused with nicotine, the motivational valence was switched from rewarding to aversive.

To summarize this section, the study performed here demonstrates that rats will reliably self-administer nicotine directly into the pVTA in a dose-dependant manner, and that sites adjacent to the pVTA do not support nicotine self-administration behavior. These results pinpoint the pVTA as an important

neuroanatomical substrate for the reinforcing properties of nicotine. Results from earlier studies indicate that the reinforcing action of nicotine in the pVTA seen here is likely predicated on activation of $\alpha 4\beta 2$ and $\alpha 6\beta 2$ -containing nAChRs, although, as discussed in this section and in the Introduction, it is probable that $\alpha 7$ homopentameric nAChRs are involved in the local reinforcing actions of nicotine as well.

B. Adolescent exposure to nicotine results in persistent changes in the mesolimbic DA system

The second and third aims of this dissertation were to assess the persistent effects of adolescent nicotine exposure on the mesolimbic DA system both neurobiologically and behaviorally. Using the finding from the first aim that the pVTA is a neuroanatomical site that mediates the reinforcing properties of nicotine, and based on earlier work demonstrating that systemic nicotine self-administration increases DA output in the NAc shell to a greater extent than in the NAc core (Lecca et al. 2006), here we examined the effects of a series of intra-pVTA microinjections on DA release within the shell of the NAc in rats that either did or did not receive repeated injections of nicotine during adolescence. The microinjection procedure was set up to mimic a pattern of infusions that a rat might self-administer in our ICSC paradigm, so as to provide insight into the conditions that may be present during the response-contingent behavior.

The major finding of the second aim of this dissertation was that exposure to nicotine during adolescence resulted in enhanced nicotine-stimulated DA release in the shell of the NAc during adulthood. This enhancement was predicated on increases in both the amplitude and the duration of increased DA release (Fig. 8). Following the 10-minute series of microinjections of either 100 or 200 μ M nicotine into the pVTA, DA levels in the NAc shell increased to approximately 150% of pre-injection baseline amounts in the rats treated with saline during adolescence. By the 3rd post-injection time point, 60 minutes following the injection, DA levels in the NAc were no longer significantly different from baseline in these rats. In the rats treated with nicotine during adolescence, however, DA levels in the NAc were elevated approx. 300% above pre-injection baseline in the 20 minutes following the nicotine microinjection, which is a significant increase above the baseline for this group. At the second post-injection time point, DA levels in the adolescent nicotine rats remained significantly higher than pre-injection baseline, by approximately 200%. DA levels remained elevated above baseline for 80 minutes after the pVTA nicotine injection in this group, and returned to baseline levels at 100 minutes post-injection.

The finding that intra-VTA nicotine microinjections result in increases in DA in the NAc is consistent with the overall idea that drug reinforcement is predicated on accumbal DA release. Since the first part of this dissertation demonstrated that nicotine is reinforcing within the pVTA, it was expected that pVTA microinjections would induce DA release in the NAc. The current findings

are also supported by previous studies showing extracellular DA elevations in the NAc following systemic nicotine administration, through intravenous, subcutaneous, and central means, and at doses known to support self-administration in rats (Pontieri et al. 1996; Benwell and Balfour 1992; Tizabi et al. 2002). The current study serves to pinpoint the pVTA as a specific site regulating this neurobiological effect. Further support for the current results comes from a study demonstrating that blocking nAChRs in the VTA decreases NAc DA release stimulated by systemic nicotine (Nisell et al. 1994a).

The finding that the sensitivity of mesoaccumbens DA projections to local nicotine infusions is enhanced following adolescent nicotine exposure is in line with previous work demonstrating that nicotine exposure during adolescence can result in changes in brain regions associated with nicotine reinforcement and dependence. This will be discussed, together with the results of the third aim of this dissertation, later in this section.

To summarize thus far, these data demonstrate evidence for persistent sensitization of the dopaminergic projections from the VTA to the shell of the NAc in adult rats as a result of prior exposure to nicotine during adolescence. In response to a series of intra-VTA microinjections of nicotine, the increase of DA in the NAc is enhanced in rats with adolescent nicotine exposure. While only the no-net-flux technique of microdialysis can obtain true quantitative measures of extracellular DA, the traditional microdialysis procedure was successfully used here to demonstrate clear percent increases in extracellular DA amounts in the NAc compared to baseline levels within individual animals. Further studies using

the no-net-flux technique should be conducted to explore quantitatively whether adolescent nicotine exposure affects baseline extracellular DA levels within the NAc.

Next, the persistent effects of adolescent nicotine exposure on behavioral sensitization to nicotine were investigated. The primary finding of the third part of this dissertation is that adolescent nicotine exposure results in enhanced behavioral sensitization to the locomotor-increasing properties of nicotine during adulthood in a dose-dependant manner. Following the initial adulthood nicotine injection (Fig. 10), rats treated with nicotine during adolescence showed an enhanced locomotor response compared to those that were previously nicotine-naïve. Compared with the adolescent nicotine-naïve group, adolescent nicotine-treated rats showed an accentuated increase in daily total activity levels across the 10 test sessions (Fig. 11). Furthermore, these elevated activity levels persisted longer within each 1-hr test session in adolescent nicotine-treated rats than in saline controls, and this tendency strengthened across days (Figs. 12-14). These findings suggest that nicotine exposure during adolescence yields persistent changes in the neurobiological substrates underlying nicotine-induced behavioral sensitization.

In rats treated with saline during adolescence, there was a modest effect of nicotine on LMA across the 10 test sessions (Fig. 11, top panel). Previous studies indicated a more robust development of nicotine locomotor sensitization during adulthood in nicotine-naïve rats (Clarke and Kumar 1983a,b; Clarke et al. 1988; Faraday et al. 2003). Detailed temporal analysis of the data did indicate

significant nicotine-induced LMA increases in rats treated with saline during adolescence (Figs. 12-14, top panels). However, the more modest effects of nicotine on LMA reported in the present study could also be the result of differences in rat species, or the lesser drug exposure paradigm used in the present study compared to previous studies (Berg and Chambers 2008).

Nicotine treatment during adolescence resulted in elevated locomotion during the 1 hr of pre-injection activity recording that took place immediately before the first adulthood injection (Figs. 9 and 10). Since this was the rats' first experience in the activity chambers, these data reflect an elevation in response to a novel environment in the rats with adolescent history of nicotine treatment, suggesting persistent alterations in circuits regulating novelty-seeking behavior. The present findings are consistent with observations from previous research (Adriani et al. 2006).

Adolescent nicotine treatment also resulted in differences in LMA induced by the first nicotine injection during adulthood. As seen in Fig. 10, both of the adolescent nicotine exposures resulted in significantly higher activity in response to the initial adulthood dose of 0.25 mg/kg or 0.5 mg/kg nicotine. These data indicate that adolescent nicotine exposure resulted in a long-lasting elevation in response to the stimulating effects of nicotine, which might reflect enhanced sensitivity to the reinforcing effects of nicotine. If nicotine exposure during adolescence had similar effect in humans, then increased sensitivity to the reinforcing effects of nicotine would likely promote smoking in adulthood.

In contrast to the adolescent saline-treated rats, rats that received nicotine (0.25 or 0.5 mg/kg) during adolescence showed significant overall effects and dose-dependent enhancements of sensitization to the locomotor-activating properties of nicotine. The increases of daily LMA levels were more robust and appeared earlier in the 10-day study in the rats that had received 0.5 mg/kg nicotine during adolescence (Fig. 11, bottom panel) than those that had received 0.25 mg/kg nicotine (Fig. 11, middle panel). Not only were there differences in total daily distances traveled, but when the temporal data were examined within each locomotor session, the elevated LMA in response to daily nicotine injections persisted longer into each 60-minute session on successive days (Figs. 12-14). The observation that nicotine-induced behavioral sensitization was both increased across sessions and prolonged within sessions may shed light on the underlying neurobiological changes produced by the adolescent nicotine pretreatment.

A working hypothesis in our laboratory is that drug exposure during adolescence results in long-lasting changes in sensitivity to drugs of abuse, which increases the propensity to use and/or abuse drugs during adulthood. This hypothesis applies to both the second and third aims of this dissertation. The results discussed above are supported by the literature. Adolescents have shown differential responses to nicotine when compared with adults in studies of conditioned place preference (Vastola et al. 2002; Beluzzi et al. 2004) and behavioral sensitization (Faraday et al. 2003; Adriani et al. 2006). Given the results of the second aim of this dissertation, demonstrating increased sensitivity

of the mesolimbic DA system to nicotine following prior nicotine exposure during adolescence, it is plausible that that same system underlies the observed increases in behavioral sensitization in the third aim. The results of these two studies are very consistent with each other, especially in regards to the temporal nature of the increased nicotine sensitivity. In both studies, response to nicotine (measured by NAc DA release or behavioral response to nicotine) was not only elevated in amplitude, but also increased in duration. NAc DA was increased above baseline levels for twice as long in adolescent nicotine-treated rats as adolescent saline-treated rats, and similarly, adolescent nicotine-treated rats showed elevated locomotor activity longer into each locomotor recording session than adolescent saline-treated rats. So while nicotine administration yielded significant responses in all groups in these two studies, the responses were both amplified and prolonged in the rats with an adolescent nicotine history.

Previous work lends support to the hypothesis that adolescent drug exposure can increase sensitivity to drugs later in life. Indeed, in humans, data indicate that adolescent smoking increases the risk of later dependence on nicotine as well as other drugs (Yamaguchi and Kandel 1984). Previous work showed that rats that consumed EtOH during adolescence showed increased acquisition of operant EtOH self-administration, resistance to extinction, and susceptibility to relapse during adulthood (Rodd-Henricks et al. 2002). Also, it has been shown that adolescent EtOH consumption by alcohol-preferring (P) rats increases both basal and EtOH-induced mesolimbic dopamine neurotransmission in adults (Sahr et al. 2004). These findings all support the

hypothesis that drug exposure during adolescence can result in persistent changes in sensitivity to certain behavioral effects of drugs, and that alterations in sensitivity are dependent on changes within the mesolimbic DA system. Given that the mesolimbic DA system has been shown to underlie the locomotor stimulant action of nicotine (Clarke et al. 1988), the same principles likely apply to the current studies discussed here.

Previous research has indicated that many neurobiological changes may take place following adolescent exposure to nicotine. For example, nAChRs in several brain areas, including the midbrain, were upregulated to a greater and more persistent extent in adolescent rats treated with nicotine than in adult rats that received similar nicotine exposure (Trauth et al. 1999; Adriani et al. 2003). It is clear that the density of nAChRs in various brain regions, especially the midbrain, could influence the way the mesolimbic DA system reacts to a nicotine challenge and therefore the locomotor activity which it mediates. Adolescent nicotine exposure has also been shown to reduce DA transporter densities in the striatum, including the NAc (Collins et al. 2004b). Reductions in DA transporter density could decrease DA reuptake and thereby increase the amount of DA in the synapse, which could in turn increase the response of the system as a whole to nicotine. However, the extracellular DA increases observed in the current study are more likely due to increased DA release rather than decreased reuptake, given the observation that DA clearance in the NAc is actually increased by nicotine, rather than decreased (Ksir et al. 1995).

The previous findings that nAChR levels are up-regulated in the midbrain, including the VTA, in adult rats following adolescent treatment with nicotine may underlie the observations in the current studies. Higher levels of nAChRs in the VTA could result in the observed increase in neurobiological and behavioral sensitivity to nicotine, as a function of stronger activation of dopaminergic projections in response to systemic nicotine injections.

To summarize the second and third parts of this dissertation, adolescent nicotine exposure results in enhanced sensitivity to the effects of nicotine during adulthood, suggesting that nicotine exposure during adolescence can result in persistent changes in the mesolimbic DA system and the behaviors that it mediates. This is an indication that adolescent nicotine exposure may cause enhanced vulnerability to the reinforcing effects of nicotine and promote smoking in humans.

C. Adolescent nicotine exposure enhances adulthood EtOH drinking in Wistar and P rats

After identifying long-lasting changes in the mesolimbic DA system and a behavior mediated by that system as a result of nicotine exposure during adolescence, the final step was to assess the influence of adolescent nicotine exposure on subsequent drug-taking behavior. Since nicotine and EtOH are so widely co-abused in human populations, EtOH drinking behavior was used as the behavioral measure for this study.

The fourth part of this dissertation presents the primary finding that nicotine exposure during adolescence enhances subsequent EtOH intake during adulthood in both Wistar and alcohol-preferring P rats (Figs. 16 and 18). In rats with access to 5% and 10% EtOH solutions, EtOH intake during the continuous access phase of the experiment was significantly increased in adults that were exposed to the higher (0.5 mg/kg) nicotine dose during adolescence, as compared to nicotine-naïve rats. In the case of the Wistar rats, the elevated EtOH intake was apparent during the acquisition phase of EtOH drinking; that is, the initial 4 weeks of the 8-week continuous EtOH access period (Fig. 16). For the P rats with access to 5% and 10% EtOH, the elevated EtOH intake was observed across all 8 weeks of continuous EtOH access (Fig. 18). This difference in the pattern of enhancement of EtOH intake may indicate that the adolescent nicotine exposure had a more prolonged effect in the P rats, such that the effects on EtOH intake persisted through all weeks of continuous EtOH access. In Wistar rats that were exposed to nicotine as adults, no differences in subsequent EtOH intake were observed (Fig. 34). Together, these findings suggest that exposure to nicotine during adolescence yields persistent changes in the neurobiological substrates underlying EtOH drinking, both in a non-selected rat line and in rats selectively bred for EtOH preference.

Although nicotine-related changes in EtOH intake over the 8-week continuous access period were observed, there were no effects of adolescent nicotine treatment on EtOH intake during the initial 7 days of access for either Wistar or P rats (Fig. 15). Whereas the three groups of rats (Wistars, P rats

drinking lower EtOH concentrations, and P rats drinking higher EtOH concentrations) demonstrated markedly different EtOH intake levels even on the first day of EtOH access (approximately 2-4 g/kg, 6-7 g/kg, and 8-10 g/kg, respectively), adolescent nicotine exposure had no effects on EtOH intake during this first week.

It is interesting to note that adolescent nicotine exposure resulted in changes in EtOH preference in the P rats but not in the Wistar rats. In the Wistar rats, there were no significant effects of adolescent nicotine treatment on preference for either 5%, 10%, or total EtOH (Fig. 17). In the P rats, however, the elevation in EtOH intake by the 0.5 mg/kg nicotine-treated rats was predicated on a higher preference for 5% EtOH as well as total EtOH as a percentage of total fluid intake (Fig. 19). These rats were consuming EtOH solutions for approximately 90% of their total fluid intake, whereas the 0.25 mg/kg nicotine and saline groups were consuming EtOH solutions for under 80% of their total fluid intake (Fig. 19, bottom panel). This indicates that the underlying changes induced by adolescent nicotine exposure may result in increased preference for EtOH. Since this effect was only observed in rats selectively bred for high EtOH preference, this lends support to the idea that the reinforcing actions of nicotine and EtOH may neurobiologically related and/or influenced by some common genes.

P rats were first tested in this study with access to the same EtOH concentrations as the Wistar rats, 5% and 10% EtOH. This was done to allow for direct comparison between the rat strains, without needing to account for

differences in EtOH intake as a percentage of total fluid intake. However, the EtOH intake levels achieved by this cohort of P rats did not quite reach those observed in previous studies, especially during the EtOH deprivation and reinstatement cycles (Rodd-Henricks et al. 2000c and 2001; Bell et al. 2008). Thus, a separate cohort of P rats received the same adolescent treatment as the previous groups, only this time given access to 15% and 30% EtOH during adulthood. Although overall EtOH intake levels by these P rats were higher than in the previous group of P rats (8-9 g/kg/day vs. 5-6 g/kg/day), adolescent nicotine exposure did not have an effect on either the acquisition (first 4 weeks) or maintenance (second 4 weeks) phases of EtOH drinking in these rats (Fig. 20). Similarly, there were also no significant effects of adolescent nicotine exposure on preference for 15%, 30%, or total EtOH in these P rats (Fig. 21). Together, these results indicate that the effects of adolescent nicotine exposure on the neurocircuitry regulating EtOH drinking may be masked when EtOH consumption levels are high.

Another finding of this study is that nicotine exposure during adolescence did not have an effect on the expression of an alcohol deprivation effect (ADE) in any of the cohorts of rats. The ADE is defined as a temporary increase in EtOH preference over baseline drinking conditions, when EtOH is reinstated following a period of deprivation (Sinclair and Senter 1967). It has been proposed as a model of relapse-like drinking and, in animals, is considered to be a model of alcohol craving (Sinclair and Li 1989). The expression of the ADE can be influenced by the length of EtOH deprivation and also by repeated deprivations

(Rodd-Henricks et al. 2000b and 2000c). The ADE was investigated here to determine whether adolescent exposure to nicotine might influence EtOH craving as indicated by elevated relapse-like drinking. Although an ADE was seen in all cohorts of rats (Wistar and P rats drinking both the lower and higher EtOH concentrations), there were no differences among adolescent treatment groups in g/kg EtOH consumed during the reinstatement days. Wistar rats showed a more prolonged ADE than either group of P rats, with elevated EtOH drinking levels persisting for the first 3 re-exposure days (Fig. 22). In both groups of P rats, while significant increases above baseline EtOH drinking levels were observed, these increases only lasted for the first re-exposure day (Figs. 26 and 30). Taken together with the other results of this study, this finding indicates that the neurobiological substrates regulating relapse-like EtOH drinking behavior may be distinct from those regulating acquisition and maintenance of EtOH drinking, since acquisition of EtOH drinking was affected by adolescent nicotine exposure, whereas relapse EtOH drinking was not.

Interestingly, the post-deprivation increase in EtOH intake in P rats drinking 5% and 10% EtOH was modest (approximately 1 g/kg/day increase), which is consistent with a previous study from our laboratory showing only a modest ADE in P rats (Rodd-Henricks et al. 2000c; Bell et al. 2008), whereas the observed increase in EtOH intake in the P rats drinking 15% and 30% EtOH was much higher (approximately 3.5 g/kg/day increase).

When data from the three cycles of repeated EtOH deprivation and reinstatement were analyzed for EtOH preference, some interesting results

emerged. In the Wistar rats, no effects of adolescent nicotine treatment were observed in EtOH preference following the first EtOH deprivation (Fig. 23). However, following the second EtOH deprivation, there was a significant Day x Adolescent Treatment interaction for both 5% and 10% EtOH preference (Fig. 24, top and middle panels). Interestingly, the rats in both adolescent nicotine groups showed an elevated preference for the lower EtOH concentration (5%) and a lower preference for the higher EtOH concentration (10%) compared with the adolescent saline-treated rats. Following the third EtOH deprivation, the enhanced preference for 5% EtOH continued in the adolescent nicotine-treated rats (Fig. 25, middle panel). This seems contradictory to the results reported earlier, indicating that adolescent nicotine treatment enhances EtOH intake during adulthood. It is also contradictory to earlier work showing that rats shifted their preference from lower to higher concentrations following multiple EtOH deprivations (Rodd et al. 2009), although in that case, high-alcohol-drinking (HAD) rats were used, and drawing comparisons between selected and non-selected lines is difficult. However, it is possible that the increased overall EtOH consumption demonstrated by adolescent nicotine-treated Wistar rats is due to enhanced sensitivity to EtOH, which may result in those rats preferring the lower EtOH concentration compared to adolescent saline-treated rats. If so, it appears that this effect is only apparent in the relapse-like conditions brought on by repeated EtOH deprivations.

Analysis of the EtOH preference data for the cohort of P rats drinking 5% and 10% EtOH also revealed significant effects of adolescent nicotine exposure.

Following the first EtOH deprivation, the significant main effect of adolescent nicotine treatment on preference for both 5% and 10% EtOH was the result of the adolescent nicotine-treated rats (particularly the 0.5 mg/kg nicotine dose) showing a greater preference for 5% EtOH and a lower preference for 10% EtOH (Fig. 27, top and middle panels). This mirrored the effect observed in the Wistar rats. However, there were no significant effects of adolescent nicotine exposure on EtOH preference following the second and third periods of EtOH deprivation (Figs. 28 and 29), so in the case of the P rats, the effect was not brought out by repeated EtOH deprivations.

Adolescent nicotine exposure had no significant effects on EtOH preference under relapse-like drinking conditions in the cohort of P rats drinking the higher concentrations (15% and 30%) of EtOH (Figs. 31-33). This is consistent with the earlier conclusion that the effects of adolescent nicotine exposure on the neurocircuitry regulating EtOH drinking, and in this case EtOH preference, may be masked when EtOH intake levels are high.

It is a widely held hypothesis that drug exposure during adolescence results in persistent changes in sensitivity to drugs of abuse, which increases the likelihood of drug use and/or abuse in adulthood. The results of previous sections of this dissertation have lent support to this hypothesis. It has been shown that adolescent EtOH consumption in P rats yields changes in adulthood EtOH self-administration including increased acquisition of operant responding for EtOH and susceptibility to relapse following extinction training (Rodd-Henricks et al. 2002). Previous work has also linked adolescent nicotine exposure to an

increased propensity for adulthood nicotine self-administration in rats (Adriani et al. 2003). Additionally, chronic nicotine exposure, while not necessarily during adolescence, has been shown to result in subsequent increases in EtOH consumption (Blomqvist et al. 1996; Le et al. 2000; Clark et al. 2001). The current study adds an important element to the literature by showing that nicotine exposure during adolescence can result in a persistent enhancement in the acquisition of EtOH drinking behavior during adulthood. The fact that this effect seems to be prolonged in P rats compared to Wistar rats is supported by the previous findings that P rats are more sensitive to certain effects of nicotine (Gordon et al. 1993; Le et al. 2006).

Two previous studies have investigated the effects of adolescent nicotine exposure on subsequent EtOH drinking, which were described in the Introduction. To review briefly, one study concluded that adulthood EtOH drinking was not affected by peri-adolescent nicotine exposure (Smith et al. 2002). However, several important differences exist between that study and the current one. First, nicotine exposure (PD 35-56) extended beyond the adolescent timeframe and overlapped with the start of EtOH access. The method of nicotine exposure also differs between that study and the current one. Smith and colleagues used a subcutaneous 21-day time-release pellet containing a specific dose of nicotine, versus the daily nicotine injections (intermittent exposure) given in the current study. Additionally, EtOH intake was only measured for 21 days, which may not have been long enough for nicotine-induced changes in drinking behavior to be observed. Finally, the previous study

used female Sprague-Dawley rats, and the current study uses male Wistar and P rats, so strain and gender differences could play a role in divergent results.

A second, more recent published study used alcohol-preferring AA rats to study the effects of adolescent nicotine exposure on adulthood EtOH intake (Kemppainen et al. 2009). That study also concluded that adulthood EtOH intake was unaffected by adolescent nicotine exposure. Continuous access to 10% EtOH (in addition to water) commenced on PD 93 and lasted for four weeks. No differences among adolescent treatment groups (nicotine or saline) were observed. This could have been due to the single concentration of EtOH, as rats drink higher overall amounts of EtOH when given access to multiple EtOH concentrations. Again, the duration of the EtOH drinking study was much shorter than the study in this dissertation, at only 28 days. Thus, it may have been too short of a study to observe differences in EtOH drinking behavior between adolescent treatment groups. Additionally, although AA rats were selectively bred from Wistar rats, there could be genetic differences between AA rats and the Wistar and P rats used in the current study, which confer divergent responses to adolescent nicotine exposure.

As mentioned earlier, previous work has demonstrated that nicotinic cholinergic receptor (nAChR) levels are up-regulated in the midbrain, including the ventral tegmental area (VTA), in adult rats following adolescent treatment with nicotine (Trauth et al. 1999; Abreu-Villaca et al. 2003; Adriani et al. 2003). The VTA is an essential neural substrate for EtOH self-administration, as evidenced studies demonstrating intracranial self-administration of EtOH into the

pVTA but not areas surrounding the pVTA in Wistar rats (Rodd-Henricks et al. 2000a). Studies have also shown indicated a role for VTA DA activity in EtOH consumption both by operant and free-choice drinking means, as activation of inhibitory D2 receptors decreased EtOH intake (Hodge et al. 1996; Nowak et al. 2000). Up-regulated nAChR levels in the VTA may play a role in the observed enhancement in EtOH drinking by adolescent nicotine-treated rats, not just because of their location at a site demonstrated here to be involved in adolescent nicotine-induced changes in responses to adulthood nicotine, but because previous research has implicated nAChRs for a role in EtOH stimulation of the mesolimbic DA system. Additionally, this dissertation has revealed that the pVTA is also a site at which nicotine stimulates NAc DA release and supports self-administration.

Together, these results provide support for a role for nAChRs in EtOH consumption. Considering the results presented in this section, it is plausible that adolescent nicotine exposure results in long-lasting changes in nAChRs in brain areas regulating EtOH drinking behavior. Nicotinic mechanisms may also underlie the expression of an ADE, given that adolescent-nicotine-induced changes in preference for different EtOH concentrations were observed following periods of EtOH deprivation.

In summary, adolescent exposure to nicotine enhances acquisition of EtOH drinking behavior during adulthood in Wistar rats and in P rats with access to lower concentrations of EtOH. This effect was masked in P rats when EtOH intake levels were higher, and effects on the ADE were limited to changes in

preference between multiple EtOH concentrations rather than total EtOH intake. This suggests that nicotine exposure during adolescence can result in persistent changes in the neurobiological substrates regulating EtOH drinking behavior and EtOH preference, which indicates that teenage smoking may promote subsequent alcohol drinking in humans.

D. Other possible mechanisms by which adolescent nicotine exposure could influence adulthood neurobiology and behavior

Due to the complex and widespread actions of nicotine in the brain, and the wide scope of neurological changes that the developing adolescent brain undergoes, the number of possible mechanisms underlying the persistent effects of adolescent nicotine demonstrated here are virtually limitless. In the previous sections, nAChRs have been focused on as a likely substrate for the observed changes in adulthood measures following nicotine treatment during adolescence, due to the strong support from the literature for their involvement in all of the endpoints studied. However, there are many other possible mechanisms by which adolescent nicotine treatment could exert its lasting effects.

Other neurotransmitters in addition to DA are likely involved in the development and expression of the observed sensitization to nicotine. For example, studies have indicated that excitatory amino acids are also involved in behavioral sensitization to psychomotor stimulants (reviewed by Kalivas, 1995). Recall also the mechanism of nAChR activation and desensitization within the

VTA proposed by Mansvelder and McGehee (2002) and discussed earlier in this dissertation, wherein both glutamate and GABA are implicated to play a role in long-lasting nicotine stimulation of VTA DA projections. Other studies have demonstrated that stimulating VTA or NAc shell metabotropic glutamate 2/3 (mGlu2/3) receptors, which are presynaptic and therefore inhibit glutamate transmission when activated, decreased i.v. nicotine self-administration (Liechti et al. 2007). Nicotine self-administration has also been shown to up-regulate NMDA receptor subunit expression in the VTA (Kenny et al. 2009). Based on these collective findings, it is possible that DA and excitatory and inhibitory amino acids are all involved in the up-regulated responses to nicotine in adolescent nicotine-treated animals observed in the current studies as part of a complex mechanism.

Serotonin is another neurotransmitter system that may be involved in the long-lasting effects of adolescent nicotine exposure. Serotonin transporter densities in the striatum were reduced following repeated nicotine administration, both acutely and in a persistent fashion (Collins et al. 2004b; Xu et al. 2001). This could lead to increased levels of serotonin within the synapse and therefore enhanced serotonergic input at postsynaptic sites. In a more recent study, nicotine was administered throughout gestation in rats, and then the responses of serotonin receptors and transporters to adulthood nicotine treatment were studied (Slotkin et al. 2007). Prenatal exposure to nicotine effectively reprogrammed the way the serotonergic system responded to nicotine, resulting in differing up- and down-regulation of specific serotonin receptor subtypes.

Although the nicotine pre-exposure in this case was during gestation rather than adolescence, the previous study demonstrates the vulnerability of a developing system to persistent drug-induced changes.

The mu opioid system has also been implicated in nicotine self-administration behaviors. Mu opioid receptors are located in the VTA, primarily on GABA interneurons and on feedback projections from the NAc (Kalivas 1993). The effects of intra-VTA infusion of the mu opioid agonist DAMGO, as well as the GABA_A agonist muscimol and the GABA_B agonist baclofen, were tested on i.v. self-administration of nicotine (Corrigall et al. 2000). DAMGO only attenuated nicotine self-administration at the highest dose, in contrast to both GABA agonists, which showed a much more pronounced decrease of nicotine self-administration. So, while mu opioid receptors may be involved in nicotine self-administration, it is likely via a more indirect mechanism than disinhibition of the VTA DA neurons by interfering with local GABA interneuron signaling. Alternatively, nicotine itself might reduce the amount of GABA signaling in the VTA, rendering mu opioid activity less able to result in disinhibition of the VTA DA neurons. It is possible that adolescent nicotine exposure could alter the way mu opioid and/or GABA receptors function to recruit GABAergic feedback to the VTA. This is an example of yet another neurotransmitter system with complex involvement in nicotine reinforcement.

Even more basic intracellular signaling molecules have been altered by adolescent nicotine exposure. Adenylyl cyclase (AC) activity was studied in response to adulthood nicotine administration, and numerous alterations in the

signaling cascade were observed, in brain areas including the cerebral cortex and brainstem, in rats exposed to nicotine during adolescence compared to nicotine-naïve rats (Slotkin et al. 2008). Both basal and nicotine-induced AC activity were affected, although sometimes differently between males and females. Clearly, exposure to nicotine during the vulnerable adolescent time period can result in a myriad of complex changes throughout the brain, many of which may influence various responses to nicotine later in life.

One or more of these systems could be impacted in adulthood by changes in gene expression triggered by nicotine during adolescence. Recently, microarray technology is beginning to be used to identify genes whose expression is altered by nicotine. One such study investigated nicotine's effects on genes specifically during the adolescent timeframe (Polesskaya et al. 2007). Nicotine was administered continuously via osmotic minipumps, and gene expression in different brain areas was assessed at four different time points: PD 25 (just prior to adolescence), PD 35 (during adolescence), PD 45 (post-adolescence), and PD 55 (early adulthood). Genes whose expression was changed regulated everything from cell metabolism to signal transduction to vesicular trafficking, but the overall conclusion was that the majority of the genes studied showed dramatic peaks in nicotine responses at the PD 35 time point. These results were consistent with the behavioral literature showing that adolescent rodents are particularly vulnerable to nicotine's effects. Clearly, there is much more research to be done into the gene expression changes influenced

by adolescent nicotine, as this could represent the underpinnings of so many of the persistent effects mentioned here.

E. Summary and future directions

The objectives of this dissertation were to 1) determine whether nicotine would be self-administered into the posterior pVTA, a neuroanatomical component of the mesolimbic DA system, which is known to be involved in reward and reinforcement; 2) investigate whether adolescent nicotine exposure would alter the sensitivity of the mesolimbic DA system as measured by DA release in the NAc in response to nicotine microinjections into the pVTA; 3) examine the effects of adolescent nicotine exposure on behavioral sensitization to nicotine in adulthood; and 4) investigate whether adulthood alcohol drinking behavior, in both Wistar and P rats, would be augmented by nicotine exposure during adolescence. The results of this dissertation have demonstrated that 1) the pVTA is a neuroanatomical site that supports nicotine self-administration; and that adolescent nicotine exposure results in 2) increased nicotine-stimulated DA release in the NAc during adulthood; 3) augmented behavioral sensitization to nicotine in adult animals; and 4) enhanced acquisition of alcohol drinking behavior in adult Wistar and P rats.

The results of this dissertation provide insight into the persistent changes, on both neurobiology and behavior, caused by exposure to nicotine during the critical developmental period of adolescence. While nicotine has complex effects

throughout many brain regions, one consistent theme identified here is the likely involvement of nAChRs in the neurobiological and behavioral effects that were observed. It is plausible that nicotine administered during adolescence alters the functionality of nAChRs in brain regions important for drug reinforcement in such a way as to increase sensitivity to subsequent drug exposure during adulthood.

Clearly, much future research is necessary to determine the specific effects on nAChRs and the mesolimbic DA system, and also what other receptors and systems might be involved. With a better understanding of the changes that take place in the brain following adolescent drug exposure, it may become possible to more successfully treat or even prevent subsequent drug addictions in people whose first experience with substances of abuse came during the critical adolescent window.

V. LITERATURE CITED

Abreu-Villaca Y, Seidler FJ, Slotkin TA (2003) Impact of adolescent nicotine exposure on adenylyl cyclase-mediated cell signaling, enzyme induction, neurotransmitter-specific effects, regional selectivity, and the role of withdrawal. *Brain Res* 988:164-72

Adriani W, Deroche-Gamonet V, Le Moal M, Laviola G, Piazza PV (2006) Preexposure during or following adolescence differently affects nicotine-rewarding properties in adult rats. *Psychopharmacology* 184:382-90

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* 23:4712-6

Anthony JC and Petronis KR (1995) Early-onset drug use and risk of later drug problems. *Drug Alcohol Depend* 40:9-15

Balfour DJ (2004) The neurobiology of tobacco dependence: a preclinical perspective on the role of the dopamine projections to the nucleus accumbens. *Nicotine Tob Res* 6:899-912

Bell RL, Rodd ZA, Schultz JA, Peper CL, Lumeng L, Murphy JM, McBride WJ (2008) Effects of short deprivation and re-exposure intervals on the ethanol drinking behavior of selectively bred high alcohol-consuming rats. *Alcohol* 42:407-16

Belluzzi JD, Lee AG, Oliff HS, Leslie FM (2004) Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology* 174:389-95

Benwell ME, Balfour DJ (1992) The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. *Br J Pharmacol* 105:849-56

Berg SA, Chambers RA (2008) Accentuated behavioral sensitization to nicotine in the neonatal ventral hippocampal lesion model of schizophrenia. *Neuropharmacology* 45:1201-7

Blomqvist O, Engel JA, Nissbrandt H, Söderpalm B (1993) The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. *Eur J Pharmacol* 249:207-13

- Blomqvist O, Ericson M, Johnson DH, Engel JA, Söderpalm B (1996) Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. *Eur J Pharmacol* 314:257-67
- Bonci A, Melenka RC (1999) Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. *J Neurosci* 19:3723-30
- Bozarth MA, Wise RA (1980) Electrolytic microinfusion transducer system: an alternative method of intracranial drug application. *J Neurosci Methods* 2:273-5
- Bozarth MA, Wise RA (1981) Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life Sci* 28:551-5
- Cain ME, Saucier DA, Bardo MT (2005) Novelty seeking and drug use: contribution of an animal model. *Exp Clin Psychopharmacol* 13:367-75
- Centers for Disease Control and Prevention. *Quick facts: economic and health burden of chronic disease*. <http://www.cdc.gov/nccdphp/press/index.htm>, 2007
- 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
- Changeux JP, Bertrand D, Corringier PJ, Dehaene S, Edelstein S, Lena C, Le Novère N, Marubio L, Picciotto M, Zoli M (1998) Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. *Brain Res Brain Res Rev* 26:198-216
- Chen CY, Storr CL, Anthony JC (2009) Early-onset drug use and risk for drug dependence problems. *Addictive Behav* 34:319-22
- Chen J, Millar WJ (1998) Age of smoking initiation: implications for quitting. *Health Rep* 9:39-46
- Chevrette J, Stellar JR, Hesse GW, Markou A (2002) Both the shell of the nucleus accumbens and the central nucleus of the amygdale support amphetamine self-administration in rats. *Pharmacol Biochem Behav* 71:501-7
- Cicero TJ (1979) A critique of animal analogues of alcoholism. In: Majchrowicz E, Noble EP, editors. *Biochemistry and pharmacology of ethanol*, vol. 2. New York: Plenum Press; 533-60
- Clark A, Lindgren S, Brooks SP, Watson WP, Little HJ (2001) Chronic infusion of nicotine can increase operant self-administration of alcohol. *Neuropharmacology* 41:108-17

Clarke PB, Fu DS, Jakubovic A, Fibiger HC (1988) Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. *J Pharmacol Exp Ther* 246:701-8

Clarke PB, Hommer DW, Pert A, Skirboll LR (1985) Electrophysiological actions of nicotine on substantia nigra single units. *Br J Pharmacol* 85:827-35

Clarke PB, Kumar R (1983a) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol* 78:329-37

Clarke PB, Kumar R (1983b) Characterization of the locomotor stimulant action of nicotine in tolerant rats. *Br J Pharmacol* 80:587-94

Collins SL, Montano R, Izenwasser S (2004a) Nicotine treatment produced persistent increases in amphetamine-stimulated locomotor activity in periadolescent male but not female or adult male rats. *Dev Brain Res* 153:175-87

Collins SL, Wade D, Ledon J, Izenwasser S (2004b) Neurochemical alterations produced by daily nicotine exposure in periadolescent vs. adult male rats. *Eur J Pharmacol* 502:75-85

Corrigall WA, Coen KM (1991) Selective dopamine antagonists reduce nicotine self-administration. *Psychopharmacology* 104:171-6

Corrigall WA, Franklin KB, Coen KM, Clarke PB (1992) The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology* 107:285-9

Corrigall WA, Coen KM, Anderson KL (1994) Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res* 653:278-84

Corrigall WA, Coen KM, Adamson KL, Chow BLC, Zhang J (2000) Response of nicotine self-administration in the rat to manipulations of the mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology* 149:107-14

Daeppen JB, Smith TL, Danko GP, Gordon L, Landi NA, Nurnberger Jr. JI, Bucholz KK, Raimo E, Shuckit MA (2000) Clinical correlates of cigarette smoking and nicotine dependence in alcohol-dependent men and women. *Alcohol and Alcoholism* 35:171-5

Dani JA, Heinemann S (1996) Molecular and cellular aspects of nicotine abuse. *Neuron* 16: 905-8

David V, Besson M, Changeux JP, Granon S, Cazala P (2006) Reinforcing effects of nicotine microinjections into the ventral tegmental area of mice: dependence on cholinergic nicotinic and dopaminergic D1 receptors. *Neuropharmacology* 50:1030-40

Devine DP, Wise RA (1994) Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J Neurosci* 14:1978-84

Di Chiara G (2000) Role of dopamine in the behavioral actions of nicotine related to addiction. *Eur J Pharmacol* 393:295-314

DiFranza JR, Guerrera MP (1990) Alcoholism and smoking. *J Stud Alcohol* 51:130-5

Ding ZM, Rodd ZA, Engleman EA, McBride WJ (2009) Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of ethanol. *Alcohol Clin Exp Res* 33:1-11

Ericson M, Blomqvist O, Engel JA, Söderpalm B (1998) Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *Eur J Pharmacol* 358:189-96

Ericson M, Löf E, Stomberg R, Chau P, Söderpalm B (2008) Nicotinic acetylcholine receptors in the anterior, but not posterior, ventral tegmental area mediate ethanol-induced elevation of accumbal dopamine levels. *J Pharmacol Exp Ther* 326:76-82

Faraday MM, Elliott BM, Phillips JM, Grunberg NE (2003) Adolescent and adult male rats differ in sensitivity to nicotine's activity effects. *Pharmacol Biochem Behav* 74:917-31

Gatto GJ, McBride WJ, Murphy JM, Lumeng L, Li TK (1994) Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. *Alcohol* 11:557-64

Goeders NE, Smith JE (1983) Cortical dopaminergic involvement in cocaine reinforcement. *Science* 221:773-5.

Goeders NE, Smith JE (1986) Reinforcing properties of cocaine in the medial prefrontal cortex: primary action on presynaptic dopaminergic terminals. *Pharmacol Biochem Behav* 25:191-9

Goeders NE, Smith JE (1987) Intracranial self-administration methodologies. *Neurosci Biobehav Rev* 11:319-29

Gordon TL, Meehan SM, Schechter MD (1993) Differential effects of nicotine but not cathinone on motor activity of P and NP rats. *Pharmacol Biochem Behav* 44:657-9

Grant BF, Hasin DS, Chou SP, Stinson FS, Dawson DA (2004) Nicotine dependence and psychiatric disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. *Arch Gen Psychiatry* 61:1107-15

Hodge CW, Haraguchi M, Chappelle AM, Samson HH (1996) Effects of ventral tegmental microinjections of the GABAA agonist muscimol on self-administration of ethanol and sucrose. *Pharmacol Biochem Behav* 53:971-7

Hoebel BG, Monaco AP, Hernandez L, Aulisi EF, Stanley BG, Lenard L (1983) Self-injection of amphetamine directly into the brain. *Psychopharmacology* 81:158-63

Ikemoto S, Murphy JM, McBride WJ (1998) Regional differences within the rat ventral tegmental area for muscimol self-infusions. *Pharmacol Biochem Behav* 61:87-92

Ikemoto S, Qin M, Liu ZH (2006) Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area. *J Neurosci* 26:723-30

Ikemoto S, Wise RA (2002) Rewarding effects of the cholinergic agents carbachol and neostigmine in the posterior ventral tegmental area. *J Neurosci* 22:9895-904

John U, Meyer C, Rumpf HJ, Hapke U (2003) Probabilities of alcohol high-risk drinking, abuse or dependence estimated on grounds of tobacco smoking and nicotine dependence. *Addiction* 98:805-14

Jones S, Sudweeks S, Yakel JL (1999) Nicotinic receptors in the brain: correlating physiology with function. *Trends Neurosci* 22:555-61

Kalivas PW (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res Brain Res Rev* 18:75-113

Kalivas PW (1995) Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. *Drug Alcohol Depend* 37:95-100

Kalivas PW, Duffy P, Barrow J (1989) Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. *J Pharmacol Exp Ther* 251:378-87

Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HBM (1988) Development of the dopaminergic innervation of the prefrontal cortex of the rat. *J Compar Neurol* 269:58-72

Kandel DB, Yamaguchi K, Chen K (1992) Stages of progression in drug involvement from adolescence to adulthood: further evidence for the gateway theory. *J Stud Alcohol* 53:447-57

Karan L, Dani JD, Benowitz N (2003) The pharmacology of nicotine and tobacco. *Principles of Addiction Medicine* 3rd Ed. 225-48

Kemppainen H, Hyytia P, Kiianmaa K (2009) Behavioral consequences of repeated nicotine during adolescence in alcohol-preferring AA and alcohol-avoiding ANA rats. *Alcohol Clin Exp Res* 33:340-9

Kenny PJ, Chartoff E, Roberto M, Carlezon WA, Markou A (2009) NMDA receptors regulate nicotine-enhanced brain reward function and intravenous nicotine self-administration: role of ventral tegmental area and central nucleus of the amygdala. *Neuropsychopharmacology* 34:266-81

Kiianmaa K, Tuomainen P, Makova N, Seppa T, Mikkola JA, Petteri Piepponen T, Ahtee L, Hyytia P (2000) The effects of nicotine on locomotor activity and dopamine overflow in the alcohol-preferring AA and alcohol-avoiding ANA rats. *Eur J Pharmacol* 407:293-302

Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP (2001) Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J Neurosci* 21:1452-63

Kohl RR, Kather JS, Chernet E, McBride WJ (1998) Ethanol and negative feedback regulation of mesolimbic dopamine release in rats. *Psychopharmacology* 139:79-85

Ksir C, Mellor G, Hart C, Gerhardt GA (1995) Nicotine enhances dopamine clearance in C rat nucleus accumbens. *Prog Neuro-Psycho-pharmacol Biol Psychiatry* 19:151-6

Lanca AJ, Adamson KL, Coen KM, Chow BL, Corrigall WA (2000) The pedunculo-pontine tegmental nucleus and the role of cholinergic neurons in nicotine self-administration in the rat: a correlative neuroanatomical and behavioral study. *Neuroscience* 96:735-42

Larsson A, Svensson L, Söderpalm B, Engel JA (2002) Role of different nicotinic acetylcholine receptors in mediating behavioral and neurochemical effects of ethanol in mice. *Alcohol* 28:157-67

- Larsson ML, Frisk M, Hallström J, Kiviloog J, Lundbäck B (2001) Environmental tobacco smoke exposure during childhood is associated with increased prevalence of asthma in adults. *Chest* 120:711-7
- Laviolette SR, van der Kooy D (2003a) The motivational valence of nicotine in the rat ventral tegmental area is switched from rewarding to aversive following blockade of the $\alpha 7$ -subunit-containing nicotinic acetylcholine receptor. *Psychopharmacology* 166:306-13
- Laviolette SR, van der Kooy D (2003b) Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. *Mol Psychiatry* 8:50-9
- Le AD, Corrigall WA, Harding JW, Juzytsch W, Li TK (2000) Involvement of nicotinic receptors in alcohol self-administration. *Alcohol Clin Exp Res* 24:155-63
- Le AD, Wang A, Harding S, Juzytsch W, Shaham Y (2003) Nicotine increases alcohol self-administration and reinstates alcohol seeking in rats. *Psychopharmacology* 168:216-21
- Le AD, 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* 26:1872-9
- Lecca D, Cacciapaglia F, Valentini V, Gronli J, Spiga S, Di Chiara G (2006) Preferential increase of extracellular dopamine in the rat nucleus accumbens shell as compared to that in the core during acquisition and maintenance of intravenous nicotine self-administration. *Psychopharmacology* 184:435-46
- Leikla-Pelho T, Jackson DM (1992) Preferential stimulation of locomotor activity by ventral tegmental microinjections of (-)-nicotine. *Pharmacol Toxicol* 70:50-2
- Leonard S, Bertrand D (2001) Neuronal nicotinic receptors: from structure to function. *Nicotine Tobacco Res* 3:203-23
- Levin ED, Rezvani AH, Montoya D, Rose JE, and Schwartzwelder HS (2003) Adolescent-onset nicotine self-administration modeled in female rats. *Psychopharmacology* 169:141-9
- Liechti ME, Lhuillier L, Kaupmann K, Markou A (2007) Metabotropic glutamate 2/3 receptors in the ventral tegmental area and the nucleus accumbens shell are involved in behaviors relating to nicotine dependence. *J Neurosci* 27:9077-85

- Lopez-Moreno JA, Trigo-Diaz JM, Rodriguez de Fonseca F, Gonzales Cuevas G, Gomez de Heras R, Crespo Galan I, Navarro M (2004) Nicotine in alcohol deprivation increases alcohol operant self-administration during reinstatement. *Neuropharmacology* 47:1036-44
- Lumeng L, Hawkins TD, Li Tk (1977) New strains of rats with alcohol preference and non-preference. *Alcohol and Aldehyde Metabolizing Systems, Vol. III*, New York, Academic Press, 537-44
- Mansvelder HD, Keath JR, McGehee DS (2002) Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron* 33:905-19
- Mansvelder HD, McGehee DS (2000) Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* 27:349-57
- Mansvelder HD, McGehee DS (2002) Cellular and synaptic mechanisms of nicotine addiction. *J Neurobiol* 53:606-17
- Martin CA, Kelly TH, Rayens MK, Brogli BR, Brenzel A, Smith WJ, Omar HA (2002) Sensation seeking, puberty, and nicotine, alcohol, and marijuana use in adolescence. *J Am Acad Child Adol Psych* 41:1495-502
- McBride WJ, Murphy JM, Ikemoto S (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res* 101:129-52
- McKinzie DL, McBride WJ, Murphy JM, Lumeng L, Li TK (2002) Effects of amphetamine on locomotor activity in adult and juvenile alcohol-preferring and -nonpreferring rats. *Pharmacol Biochem Behav* 71: 29-36
- McKinzie DA, Rodd-Henricks ZA, Dagon CT, Murphy JM, McBride WJ (1999) Cocaine is self-administered into the shell region of the nucleus accumbens in Wistar rats. *Ann NY Acad Sci* 877:788-91
- McMillin BA (1997) Toward a definition of a valid animal model of alcoholism: multiple animal models for multiple diseases. *Alcohol* 3:331-6
- Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li TK (2002) Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav Genetics* 32:363-88
- Museo E, Wise RA (1994) Sensitization of locomotion following repeated ventral tegmental injections of cytosine. *Pharmacol Biochem Behav* 48:521-4

National Institute on Drug Abuse (2006) NIDA InfoFacts: Cigarettes and other tobacco products.

National Institute on Drug Abuse Research Report - Tobacco Addiction. NIH Publication No. 06-4342, Printed 1998, Reprinted 2001, Revised 2006

Nisell M, Nomikos GG, Svensson TH (1994a) Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse* 16:36-44

Nisell M, Nomikos GG, Svensson TH (1994b) Infusion of nicotine in the ventral tegmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. *Pharmacol Toxicol* 75:348-52

Nowak KL, McBride WJ, Lumeng L, Li TK, Murphy JM (2000) Involvement of dopamine D2 autoreceptors in the ventral tegmental area on alcohol and saccharin intake of the alcohol-preferring P rat. *Alcohol Clin Exp Res* 24:476-83

Panagis G, Nisell M, Nomikos GG, Chergui K, Svensson TH (1996) Nicotine injections into the ventral tegmental area increase locomotion and Fos-like immunoreactivity in the nucleus accumbens of the rat. *Brain Res* 730:133-42

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th Ed. New York, Academic Press

Perry KW, Fuller RW (1992) Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. *Life Sci* 50:1683-90

Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* 391:173-7

Pidoplichko VI, DeViasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. *Nature* 390:401-4

Polesskaya OO, Fryxell KJ, Merchant AD, Locklear LL, Ker K-F, McDonald CG, Eppolito AK, Smith LN, Wheeler TL, Smith RF (2007) Nicotine causes age-dependent changes in gene expression in the adolescent female rat brain. *Neurotox Teratol* 29:126-40

Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, Changeux JP, Maskos U, Fratta W (2008) Crucial role of $\alpha 4$ and $\alpha 6$ nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci* 28:12318-27

Pontieri FE, Tanda G, Orzi F, Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarities to those of addictive drugs. *Nature* 382: 255-7

Pothoff AD, Ellison G, Nelson L (1983) Ethanol intake increases during continuous administration of amphetamine and nicotine, but not several other drugs. *Pharmacol Biochem Behav* 18:489-93

Rahman S, Zhang J, Corrigall WA (2004) Local perfusion of nicotine differentially modulates somatodendritic dopamine release in the rat ventral tegmental area after nicotine preexposure. *Neurochem Res* 29:1687-93

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-salience theory of addiction. *Brain Res Rev* 18:247-91

Rodd ZA, Bell RL, Kic KA, Murphy JM, Lumeng L, McBride WJ (2009) Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of high-alcohol-drinking (HAD) rats. *Addict Biol* 14:152-64

Rodd ZA, Toalston JE, Ding ZM, Oster SM, Davids MR, Bell RL, Engleman EA, McBride WJ (2007) Ethanol-activation of ventral tegmental area (VTA) dopamine neurons is mediated by local serotonin-3 receptors: program no. 553.2. *Neuroscience Meeting Planner*; 2007 Nov 3-7; San Diego, CA

Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, Li TK (2001) Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcohol-preferring (P) rats. *Alcohol Clin Exp Res* 25:747-53

Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, Li T-K (2002) Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: I. Periadolescent exposure. *Alcohol Clin Exp Res* 26:1632-41

Rodd-Henricks ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ (2000a) Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. *Psychopharmacology* 149:217-24

Rodd-Henricks ZA, McKinzie DL, Murphy JM, McBride WJ, Lumeng L, Li TK (2000b) The expression of an alcohol deprivation effect in the high-alcohol-drinking replicate rat lines is dependent on repeated deprivations. *Alcohol Clin Exp Res* 24:747-53

Rodd-Henricks ZA, McKinzie DL, Saame RS, Murphy JM, McBride WJ, Lumeng L, Li TK (2000c) Alcohol deprivation effect is prolonged in the alcohol-preferring P rat after repeated deprivations. *Alcohol Clin Exp Res* 24:8-16

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* 28:702-11

SAMHSA (2003) Results from the 2002 National Survey on Drug Use and Health: National Findings. U.S. Office of Applied Studies.

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

Sharpe AL, Samson HH (2002) Repeated nicotine injections decrease operant ethanol self-administration. *Alcohol* 28:1-7

Sinclair JD, Li TK (1989) Long and short alcohol deprivation: effects on AA and P alcohol-preferring rats. *Alcohol* 6:505-9

Sinclair JD, Senter RJ (1967) Increased preference for ethanol in rats following deprivation. *Psychon Sci* 8:11-12

Slawecki CJ, Gilder A, Roth J, Ehlers CL (2003) Increased anxiety-like behavior in adult rats exposed to nicotine as adolescents. *Pharmacol Biochem Behav* 75:355-61

Slotkin TA, Ryde IT, Mackillop EA, Bodwell BE, Seidler FJ (2008) Adolescent nicotine administration changes the responses to nicotine given subsequently in adulthood: adenylyl cyclase cell signaling in brain regions during nicotine administration and withdrawal, and lasting effects. *Brain Res Bull* 76:522-30

Slotkin TA, Ryde IT, Tate CA, Seidler FJ (2007) Lasting effects of nicotine treatment and withdrawal on serotonergic systems and cell signaling in rat brain regions: separate or sequential exposure during fetal development and adulthood. *Brain Res Bull* 73:259-72

Slotkin TA (2002) Nicotine and the adolescent brain: insights from an animal model. *Neurotoxicol Teratol* 24:369-84

Smith AM, Kelly RB, Chen WA (2002) Chronic continuous nicotine exposure during periadolescence does not increase ethanol intake during adulthood in rats. *Alcohol Clin Exp Res* 26:976-9

Smith LN, McDonald CG, Bergstrom HC, Brielmaier JM, Eppolito AK, Wheeler TL, Falco AM, Smith RF (2006) Long-term changes in fear conditioning and anxiety-like behavior following nicotine exposure in adult versus adolescent rats. *Pharmacol Biochem Behav* 85:91-7

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. *Neurosci and Biobehav Rev* 24:417-63

Stolerman IB and Shoaib M (1991) The neurobiology of tobacco addiction. *Trends Pharmacol Sci* 12:467-73

Tarazi FI, Baldessarini RJ (2000) Comparative postnatal development of dopamine D(1), D(2), and D(4) receptors in rat forebrain. *Int J Dev Neurosci* 18:29-37

Tizabi Y, Copeland RL Jr, Louis VA, Taylor RE (2002) Effects of combined systemic alcohol and central nicotine administration into ventral tegmental area on dopamine release in the nucleus accumbens. *Alcohol Clin Exp Res* 26:394-9

Trauth JA, Seidler FJ, McCook EC, Slotkin TA (1999) Adolescent nicotine exposure causes persistent upregulation of nicotinic cholinergic receptors in rat brain regions. *Brain Res* 851:9-19

Tribollet E, Bertrand A, Marguerat A, Raggénbass M (2004) Comparative distribution of nicotini receptor subtypes during development, adulthood and aging: an autoradiographic study in the rat brain. *Neuroscience* 12:405-20

Vastola BJ, Douglas LA, Varlinskaya EI, Spear LP (2002) Nicotine-induced conditioned place preference in adolescent and adult rats. *Physiol Behav* 77:107-14

Vidal C (1996) Nicotinic receptors in the brain. Molecular biology, function, and therapeutics. *Mol Chem Neuropathol* 28:3-11

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. *Psychol Rev* 94, 469-92

Wonnacott S (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci* 20:92-8

Wooltorton JRA, Pidoplichko VI, Broide RS, Dani JA (2003) Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci* 23:3176-85

Xu Z, Seidler FJ, Ali SF, Slikker WJ, Slotkin TA (2001) Fetal and adolescent nicotine administration: effects on CNS serotonergic systems. *Brain Res* 914:166-78

Yamaguchi K, Kandel DB (1984) Patterns of drug use from adolescence to young adulthood: III. Predictors of progression. *Am J Public Health* 74:673-81

CURRICULUM VITAE

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EDUCATION

- June 2003 – August 2009
Indiana University, Indianapolis, IN
Medical Neuroscience program
Doctor of Philosophy
- August 2002 – May 2003
Emory University, Atlanta, GA
Molecular and Systems Pharmacology PhD program
Predoctoral student
- August 1998 – May 2002
DePauw University, Greencastle, IN
Biology (German minor)
Bachelor of Arts

RESEARCH EXPERIENCE

- Indiana University School of Medicine, Indianapolis, IN, June 2003 – July 2009
 - Medical Neuroscience PhD program – PhD dissertation research
 - Effects of nicotine exposure in adolescent rats on acquisition of alcohol drinking and response to nicotine in adulthood
 - Mentor: William J. McBride, Ph.D.
- Emory University, Atlanta, GA, January 2003 – May 2003
 - Molecular and Systems Pharmacology PhD program – research rotation
 - Characterization of behavioral effects of neuropeptides in rats
 - Mentor: Michael Kuhar, Ph.D.
- Emory University, Atlanta GA, August 2002 – December 2002
 - Molecular and Systems Pharmacology PhD program – research rotation
 - Characterization of effects of various agonists on subtypes of the NMDA receptor NR2 subunit
 - Mentor: Stephen Traynelis, Ph.D.

- Eli Lilly & Company, Indianapolis, IN, May 2002 – August 2002
 - Neuroscience discovery research department
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- Eli Lilly & Company, Indianapolis, IN, May 2001 – August 2001
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AWARDS & HONORS

- RSA Junior Investigator Meeting Award, 2009
- NIAAA Travel Award to attend “Alcoholism and Stress: A Framework for Future Treatment Strategies” conference in Volterra, Italy; 2008
- Joseph A. Hingtgen Travel Award for Medical Neuroscience graduate program, 2004

AFFILIATIONS

- Research Society on Alcoholism, member since 2007
- Society for Neuroscience, member since 2004

ABSTRACTS & PRESENTATIONS

Kostrzewa AL, Rodd ZA, Bell RL, Murphy JM, McBride WJ. Nicotine is self-administered into the posterior ventral tegmental area (VTA) of Wistar rats. Society for Neuroscience Annual Meeting, October 2004; San Diego, CA

Bracken AL, Rodd ZA, Bell RL, Chambers RA, McBride WJ. Nicotine treatment during peri-adolescence enhances acquisition of ethanol consumption during adulthood in Wistar rats. Research Society on Alcoholism Annual Meeting, July 2007; Chicago, IL

Bracken AL, Chambers RA, Berg SA, Rodd ZA, McBride WJ. Nicotine treatment during peri-adolescence enhances behavioral sensitization to nicotine during adulthood in Wistar rats. Society for Neuroscience Annual Meeting, November 2007; San Diego, CA

Bracken AL, Rodd ZA, Bell RL, Chambers RA, McBride WJ. Exposure to drugs of abuse during adolescence facilitates self-administration and sensitization during adulthood. Alcohol and Stress: A Framework for Future Treatment Strategies, May 2008; Volterra, Italy

Bracken AL, Rodd ZA, Chambers RA, Bell RL, McBride WJ. Peri-adolescent exposure to nicotine enhances ethanol consumption during adulthood in alcohol-preferring (P) rats. Research Society on Alcoholism Annual Meeting, July 2008; Washington DC

Cook JB, Bracken AL, Rodd ZA, Toalston JE, Lumeng L, Murphy JM, McBride WJ, Bell RL. The effects of ethanol consumption during peri-adolescence or adulthood on subsequent ethanol-induced motor impairment in alcohol-preferring (P) rats. Research Society on Alcoholism Annual Meeting, July 2008; Washington DC

Hauser SR, Ding Z-M, Getachew B, Dhaher R, Bracken AL, McBride WJ, Rodd ZA. Microinjection of nicotine into the posterior ventral tegmental area (p-VTA) increases alcohol-seeking behavior in alcohol preferring (P) rats. Society for Neuroscience Annual Meeting, November 2008; Washington DC

Bracken AL, Oster SM, Toalston JE, McQueen VK, McBride WJ, Rodd ZA. Positive contrast and sustained ethanol self-administration in alcohol-preferring (P) rats given concurrent operant access to ethanol and sucrose. Research Society on Alcoholism Annual Meeting, June 2009; San Diego, CA

Getachew B, Hauser SR, Dhaher R, Bracken AL, McBride WJ, Rodd ZA. Selective breeding for high alcohol preference is associated with increased sensitivity of the posterior ventral tegmental area to the reinforcing properties of nicotine. Society for Neuroscience Annual Meeting, November 2009; Chicago, IL

Hauser SR, Ding Z-M, Getachew B, Bracken AL, McBride WJ, Rodd ZA. Nicotine self-administration into the posterior ventral tegmental area (p-VTA) is reduced by co-administration of a 5HT-3 receptor antagonist. Society for Neuroscience Annual Meeting, November 2009; Chicago, IL

PUBLICATIONS

Bracken AL, Chambers RA, Berg SA, Rodd ZA, McBride WJ (submitted, undergoing revisions) Nicotine exposure during adolescence enhances behavioral sensitivity to nicotine during adulthood in Wistar rats.

Bracken AL, Rodd ZA, Chambers RA, Bell RL, McBride WJ (in preparation) Nicotine exposure during adolescence enhances ethanol consumption during adulthood in Wistar and alcohol-preferring (P) rats.

Bracken AL, Hauser SR, Getachew B, Chambers RA, McBride WJ, Rodd ZA (in preparation) Nicotine is self-administered into the posterior ventral tegmental area: Effects of pretreatment with nicotine.

Getachew B, Bracken AL, Hauser SR, Ding Z-M, Berg SA, Chambers RA, McBride WJ, Rodd ZA (in preparation) Divergent locomotor response to nicotine in alcohol-preferring (P) and non-preferring (NP) rats.