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Examination Of The Neurochemical Mechanisms That Mediate Nicotine Withdrawal In Adolescent And Adult Rats

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EXAMINATION OF THE NEUROCHEMICAL MECHANISMS THAT MEDIATE NICOTINE WITHDRAWAL IN ADOLESCENT AND ADULT RATS

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Lovingly dedicated to my parents, Pedro and Margarita Natividad, my six siblings, and Christina, my beautiful wife.

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by

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Abstract

Introduction: The mechanisms that mediate nicotine withdrawal are presently unclear and age group differences in the neurochemical effects of withdrawal have been largely unexplored. Previous studies in our laboratory demonstrated that adult rats display a decrease in extracellular levels of dopamine in the nucleus accumbens (NAcc) during nicotine withdrawal and this decrease is reduced in adolescent rats (Natividad et al., 2010). The goal of this dissertation was to examine whether these age group differences in dopamine during withdrawal are mediated via excitatory and inhibitory mechanisms that modulate dopamine in the cell body region of the ventral tegmental area (VTA). **Methods:** Adolescent and adult rats (n=7-14) were prepared with subcutaneous pumps that delivered an equivalent dose of nicotine. On day 13 of nicotine exposure, the rats were implanted with microdialysis probes into the NAcc and the ipsilateral VTA. Dialysates collected from the NAcc were assayed for dopamine and the results were previously published (Natividad et al., 2010). The data presented here reflect dialysates collected from the VTA of the same rats. On day 14 of nicotine exposure, extracellular levels of glutamate and gamma-aminobutyric acid (GABA) in the VTA were monitored following administration of escalating doses of the nicotinic receptor antagonist mecamylamine to precipitate withdrawal. To examine whether mecamylamine alone produced neurochemical changes, a group of drug-naïve adolescent and adult rats (n=6 per group) received a sham surgery and were then monitored for extracellular levels of dopamine in the NAcc following the same dose regimen of mecamylamine. **Results:** Naïve rats of both age groups did not exhibit any alterations in NAcc dopamine levels following mecamylamine administration, suggesting that our pharmacological tool to precipitate withdrawal alone does not alter neurochemical measures. In adult rats, nicotine withdrawal produced a decrease in VTA glutamate levels (44% maximal decrease) and an

increase in GABA levels (38% increase), consistent with an overall inhibition of dopamine cell activity. In contrast, adolescents did not exhibit significant changes in either VTA glutamate or GABA levels during nicotine withdrawal. In order to examine the relationship between NAcc dopamine and VTA amino acid transmission, regression analyses were performed for each age group before and after withdrawal. Following mecamylamine, decreases in dopamine were positively correlated with decreases in glutamate in adults, whereas adolescents did not exhibit any significant correlation. The relationship between dopamine and glutamate was significantly stronger in adult versus adolescent rats. Similarly, decreases in dopamine were negatively correlated with increases in GABA in adults, whereas adolescents did not exhibit any significant correlation. The relationship between dopamine and GABA was also significantly stronger in adult versus adolescent rats. **Discussion:** Overall, the results revealed that adults display a decrease in dopamine during nicotine withdrawal. This effect appears to be related to a decrease in excitatory and an increase in inhibitory mechanisms that modulate dopamine transmission in the cell body region of the VTA. On the other hand, adolescents appear to be resistant to these neurochemical changes that follow nicotine withdrawal. Specifically, adolescents do not exhibit deficits in VTA glutamate and elevations in VTA GABA that contribute to the decreases in NAcc dopamine during withdrawal in adults. Taken together, these results indicate that adolescents display resistance to withdrawal-related neurochemical processes that inhibit mesolimbic dopamine function in adults experiencing nicotine withdrawal. The findings provide a potential mechanism involving VTA amino acid neurotransmission that may begin to explain age group differences in nicotine withdrawal.

Table of Contents

Dedication…….....iii

Acknowledgements…….……...……..v

Abstract…….……..……....……....vi

List of Figures……..……...…….....……....…….....x

Chapter 1: Introduction……1

I. Human Tobacco Use……1

II. Animal Studies..…….....6

III. Mechanisms of Nicotine Withdrawal……..11

IV. Dissertation Questions…...…….....20

V. Background Information Regarding Adolescence in Rats…….……...22

VI. Background Information for Neurochemical Methods……23

Chapter 2: Methods……29

I. Experimental Overview…...……...29

II. General Methods.…….....30

III. Dialysis Methods..……...31

IV. Neurochemical Methods…….....33

V. Histology Methods…….....34

VI. Statistical Methods……...34

Chapter 3: Results……..36

Chapter 4: Discussion……39

I. Summary…….....39

II. Mechanisms of Withdrawal in Adults……..…….....40

III. Age Group Differences in the Mechanisms of Withdrawal..……...42

IV. Clinical Relevance..…….....49

V. Future Directions.…….....50

References……..52

Curriculum Vitae……...81

List of Figures

Figure 1: Mecamylamine did not alter NAcc dopamine transmission in naive adolescent or adult rats……..…….…….....76

Figure 2: Nicotine withdrawal produced a decrease in VTA glutamate transmission that was larger in adult versus adolescent rats…….……77

Figure 3: Nicotine withdrawal produced an increase in VTA GABA transmission that was larger in adult versus adolescent rats……78

Figure 4: Nicotine-treated adults experiencing withdrawal display a stronger correlation between VTA glutamate and NAcc dopamine versus adolescents……..79

Figure 5: Nicotine-treated adults experiencing withdrawal display a stronger correlation between VTA GABA and NAcc dopamine versus adolescents……..……80

Chapter 1: Introduction

I. Human Tobacco Use

Tobacco use is a major health and economic concern in the United States. Tobacco use remains the leading cause of preventable death and disease in the United States. In 2010, an estimated 70 million Americans reported daily use of tobacco products [National Survey on Drug Use and Health (NSDUH), 2011]. The high rates of tobacco use are alarming given that many chronic diseases are closely linked to tobacco use, including lung cancer, ischemic heart disease and chronic obstructive pulmonary disease [Centers for Disease Control and Prevention (CDC), 2008]. In fact, it is estimated that 443,000 Americans die every year from illnesses associated with tobacco use. Moreover, for every one person who dies from a tobacco-related disease, 20 others suffer from a major health issue related to their tobacco use (CDC, 2003). Consequently, this poses a significant burden on the American economy, as medical treatment for tobacco users is projected to cost $96 billion a year (CDC, 2008). Efforts to reduce tobacco use are important, as many epidemiological studies demonstrate that tobacco-related mortality and hospitalizations have decreased with the recent implementation of smoking ordinances (CDC, 2009a, 2009b, and 2011).

Adolescent tobacco use is especially concerning. Tobacco use among adolescents is pervasive. This is concerning because adolescents display a stronger propensity to experiment with and then become addicted to tobacco. Adolescents are also more likely to become long-term tobacco users if they initiate use during adolescence (Brook et al., 2008; Janson, 1999; Riggs et

al., 2007; Taioli and Wynder, 1991). In fact, 80% of tobacco use begins before the age of 18 (NSDUH, 2009). While there has been a slight decline in tobacco use in the United States, the rates of smoking initiation remain high among adolescents (CDC, 2010). Every day, approximately 3,800 young people in the United States try their first tobacco product, and of these first-time users, 1,000 of them will go on to become daily users (NSDUH, 2011). Given that tobacco use leads to deleterious health consequences, young people that smoke are at heightened risk of developing tobacco-related diseases later in life. Despite the myriad of concerns with adolescent tobacco use, little is known regarding the mechanisms that mediate their enhanced vulnerability to tobacco use.

Tobacco use is motivated in large part by the rewarding effects of nicotine. Nicotine is a natural pyridine alkaloid found in tobacco leaves and is most commonly burned and inhaled or consumed via chewing tobacco-containing products (Pogocki et al., 2007). Studies investigating the biological factors that motivate tobacco use have demonstrated that nicotine is the primary reinforcing ingredient in tobacco products. This conclusion is based largely on the finding that nicotine is readily self-administered by humans, primates, dogs and rodents (Corigall and Coen, 1989; Goldberg et al., 1981; Henningfield et al., 1985; Risner and Goldberg, 1983). In humans, nicotine elicits positive emotional states such as elation and subjective feelings of euphoria that enhance the likelihood of continued use (i.e., positive reinforcement). As an example, human smokers given intravenous access to nicotine reported positive effects following infusions of nicotine (Henningfield et al., 1983; Le Foll and Goldberg, 2009). Moreover, tobacco products containing reduced nicotine levels were rated as less rewarding relative to cigarette brands containing more nicotine (Rose and Behm, 2004a and 2004b).

Tobacco use is also motivated by avoiding the negative effects of withdrawal. Repeated exposure to tobacco leads to nicotine dependence as defined in the *Diagnostics and Statistical Manual IV-TR*. One of the hallmark characteristics of this disorder is the manifestation of a withdrawal syndrome during periods of abstinence from tobacco. Adult smokers display adverse withdrawal symptoms including craving, anxiety and negative affect. The emergence of these subjective effects is closely associated with the severity of dependence and the likelihood of smoking relapse (Hughes, 2007; Piper et al., 2011a and 2011b; Ríos-Bedoya et al., 2008; West et al., 2006). The negative effects of withdrawal can be alleviated with pharmacological agents that have been shown to promote abstinence (Bolt et al., 2012; Buchhalter et al., 2008; Lerman et al., 2006). Given that withdrawal induces negative affective states, relapse behavior is thought to be due to negative reinforcement processes driven by the avoidance of withdrawal states.

To summarize, adult tobacco use is mediated via experiencing the positive rewarding effects of nicotine and avoiding the negative aversive consequences of nicotine withdrawal. Thus, tobacco addiction in adults involves at least two processes. Initially, smoking is driven by the strong positive rewarding effects of nicotine that promote continued use. With repeated tobacco use, nicotine dependence develops and abstinence produces craving, which can lead to relapse. Thus, an addicted state in adults is thought to reflect a dynamic shift in motivational systems from positive to negative reinforcement processes that promote compulsive use of tobacco and loss of control over drug use (Koob and Le Moal, 2001). Given the importance of nicotine withdrawal in motivating tobacco use and relapse behavior, more research is needed to understand the underlying mechanisms in the brain that mediate the nicotine withdrawal syndrome.

Adolescent humans display enhanced rewarding effects of nicotine. To our knowledge, the rewarding effects of nicotine have not been directly compared in adolescents versus adults in a clinical setting. However, adult smokers that initiated smoking during adolescence reported more pleasant effects and fewer unpleasant effects (e.g., dizziness and sickness) following their first smoking episode. On the other hand, adults that initiated smoking in adulthood reported more adverse symptoms following their first smoking episode (see Eissenberg and Balster, 2000; Pomerleau et al., 1993, 1998, and 1999). There is also a myriad of social factors that contribute to tobacco use during adolescence. The risk factors that promote tobacco use during adolescence include enhanced risk-taking, peer pressure, media influence, and weight concerns (Henningfield et al., 2011; Hussaini et al., 2011; Nizami et al., 2011). More research is needed to directly compare the rewarding effects of nicotine in adolescents versus adults.

Adolescent humans display reduced nicotine withdrawal. A study comparing adolescent smokers versus non-smokers found that young smokers exhibited mild symptoms during withdrawal (i.e., anger and craving) that were not associated with self-reports of dependence or biological markers of cigarette use (Smith et al., 2008a). A separate report from this laboratory also found that withdrawal symptoms on the quit day were not related to relapse behavior in adolescent smokers (Smith et al., 2008b). The latter findings suggest that nicotine withdrawal may not be the primary motivating factor that drives adolescent tobacco use. Furthermore, treatment strategies that focus on alleviating the negative effects of nicotine withdrawal, such as the nicotine patch, do not improve abstinence rates in adolescent smokers (Bailey et al., 2012; Grimshaw and Stanton, 2006; Hanson et al., 2003; Moolchan et al., 2005). Studies examining the ability of a variety of tobacco cessation medications to produce abstinence in adults reported

success rates in the range of 19.0-36.5% whereas studies in adolescents reported success rates in the range of 4.5-20.6% (see Bailey et al., 2012; Hudmon et al., 2010). One possible explanation for these age group differences is that adolescents experience lower negative effects of withdrawal during abstinence from nicotine. As a result, treatments that target withdrawal may not reduce tobacco use in adolescents. These differences in withdrawal symptoms and treatment outcomes suggest that the mechanisms that mediate nicotine withdrawal are fundamentally different in adolescents versus adults.

To summarize, the factors that drive adult tobacco use involve experiencing the positive rewarding effects of nicotine and avoiding the negative aversive effects of nicotine withdrawal. A summary of the reviewed clinical data may suggest that adolescent tobacco use is driven by motivational factors that are different from those described in adults. Namely, a hypothesis was presented by O'Dell (2009) suggesting that enhanced vulnerability to tobacco use in adolescents is driven by two factors: 1) the positive rewarding effects of nicotine are greater, and 2) the negative aversive effects of nicotine withdrawal are reduced in magnitude relative to adults. Thus, it is proposed that an inadequate balance between the strong positive rewarding effects of nicotine that are unopposed by minimal negative effects during withdrawal enhances the motivation to use tobacco products during adolescence. The proposed model of adolescent tobacco use is supported by animal studies showing that adolescent rodents display enhanced rewarding effects of nicotine and reduced aversive effects of withdrawal as compared to adults.

II. Animal Studies

Adolescent rodents display enhanced rewarding effects of nicotine. Rodent studies provide strong evidence that the rewarding effects of nicotine are enhanced during adolescence. For example, adolescents displayed enhanced preference for an environment repeatedly paired with nicotine as compared to adults across a wide range of experimental protocols, nicotine doses, and routes of administration (Belluzzi et al., 2004; Kota et al., 2007; Shram et al., 2006; Shram and Le, 2010; Torres et al., 2008 and 2009; Vastola et al., 2002). Also, studies using both intravenous and oral self-administration procedures have shown that nicotine intake is higher in adolescent versus adult rats (Chen et al., 2007; Levin et al., 2003, 2007, and 2011; Nesil et al., 2011) and mice (Adriani et al., 2002). Nicotine self-administration is also greater in adolescents versus adult rats across a range of nicotine doses (Natividad, unpublished data). *Taken together, as suggested in the human clinical studies, this pre-clinical evidence supports the hypothesis that the rewarding effects of nicotine are enhanced in adolescent versus adult rodents.*

Animal models of withdrawal. Nicotine withdrawal has been widely studied in rodents using chronic nicotine administration via subcutaneous osmotic mini-pumps (Kenny and Markou, 2001; Malin, 2001; O'Dell et al., 2004, 2006 and 2007). Most studies examining nicotine withdrawal use a protocol whereby rats are first prepared with pumps that deliver nicotine for 5-7 days to produce dependence. Withdrawal is then assessed following the cessation of nicotine via pump removal (i.e., spontaneous withdrawal) or administration of a nicotinic acetylcholine receptor antagonist to disrupt nicotine binding on these receptors (i.e., precipitated withdrawal). The advantage of using nicotinic receptor antagonists such as

mecamylamine is that these drugs produce a discrete withdrawal with a well-established time of onset that can be repeatedly induced. The onset of mecamylamine-induced withdrawal symptoms begins within 10 minutes of administration in nicotine-treated rats (Malin et al., 1994). Mecamylamine has a 1.2-hour blood half-life in rats, suggesting that it is quickly eliminated (Debruyne et al., 2003). Studies in our laboratory have verified that withdrawal effects can be repeatedly induced with mecamylamine in the same animals in a dose-dependent manner (Natividad et al., 2010). Lastly, mecamylamine does not produce a withdrawal-like phenotype in animals that are nicotine-naïve (O'Dell et al., 2004, 2006, and 2007; Hildebrand et al., 1998 and 1999). Collectively, these findings suggest that pharmacological blockade of nicotinic receptors is a valid approach to study the effects of nicotine withdrawal.

Studies have demonstrated that the nicotine withdrawal syndrome is comprised of both physical and affective components, as discussed in the following sections. For example, nicotine-treated adult rats display physical signs of withdrawal that include abdominal constrictions (e.g., writhes and gasps), facial fasciculation (e.g., teeth chattering and cheek tremors), eye blinks, body shakes, head shakes, and ptosis (see Malin, 2001). These physical signs have also been reliably demonstrated during withdrawal from other drugs of abuse such as morphine and cocaine (Malin et al., 1987 and 2000).

Adolescent rodents display reduced nicotine withdrawal. Initial studies examining age group differences in nicotine withdrawal demonstrated that mecamylamine precipitated fewer physical signs of withdrawal in adolescent versus adult rats that received the same nicotine dose in their pumps (O'Dell et al., 2004). Further studies that adjusted for metabolic differences to nicotine verified that adolescents displayed fewer withdrawal signs versus adults with

comparable levels of blood nicotine (O'Dell et al., 2006). This is consistent with a report showing that adolescent rats displayed fewer signs of nicotine withdrawal following mecamylamine administration relative to adult rats that received equivalent nicotine doses to produce dependence (Shram et al., 2008). The latter study also demonstrated that although precipitated withdrawal produced robust age group differences, adolescent and adult rats displayed similar physical signs of withdrawal during spontaneous withdrawal (i.e., pump removal). Thus, it appears that precipitated withdrawal, which produces intense physical symptoms, is a more sensitive method for detecting age group differences relative to spontaneous withdrawal that only produces mild effects.

The affective properties of withdrawal have been studied using intracranial self-stimulation (ICSS) procedures. In ICSS studies, rodents are allowed to self-administer small amounts of electrical current to the brain via an electrode placed in the medial forebrain bundle that consists of efferent projections to several reward-related structures. The stimulation is highly reinforcing and can be used to generate a threshold measure of the activity of brain reinforcement circuitry. For example, it has been shown that withdrawal from many drugs of abuse such as cocaine, alcohol and nicotine elicited an increase in self-administered current (Chartoff et al., 2012; Chester et al., 2006; Watkins et al., 2000). The need for higher current levels via increases in ICSS thresholds is believed to reflect a decrease in brain reward function because the animal has difficulty experiencing pleasure during withdrawal.

Using ICSS procedures, changes in current intensity thresholds were compared in adolescent and adult rats experiencing both precipitated and spontaneous nicotine withdrawal (O'Dell et al., 2006). The results revealed that adult rats showed an increase in ICSS current thresholds (which is interpreted as a decrease in brain reward function) following

mecamylamine-precipitated and spontaneous withdrawal conditions relative to naïve controls. However, this effect was absent in adolescent rats. These data support the hypothesis that adolescents experience less negative affect during nicotine withdrawal relative to adults. Further, the negative affective properties of nicotine withdrawal were lower during spontaneous withdrawal from nicotine, suggesting that the behavioral effects do not appear to depend on differential sensitivity to mecamylamine.

In addition to ICSS procedures, the affective properties of nicotine withdrawal have been studied using conditioned place aversion (CPA) procedures. In these studies, animals receive chronic nicotine via osmotic pumps for 5-7 days. During conditioning, the animals receive a nicotinic receptor antagonist to precipitate withdrawal and are then confined to one side of the apparatus. On alternating days, the animals receive saline in the other compartment. Following conditioning, nicotine-treated adult rats were observed to display an aversion to the compartment where they experienced withdrawal (Ise et al., 2000; Suzuki et al., 1996 and 1999; Watkins et al., 2000). Using these procedures, work in our laboratory demonstrated that the ability of nicotine withdrawal to produce CPA was lower in adolescent versus adult rats (O'Dell et al., 2007).

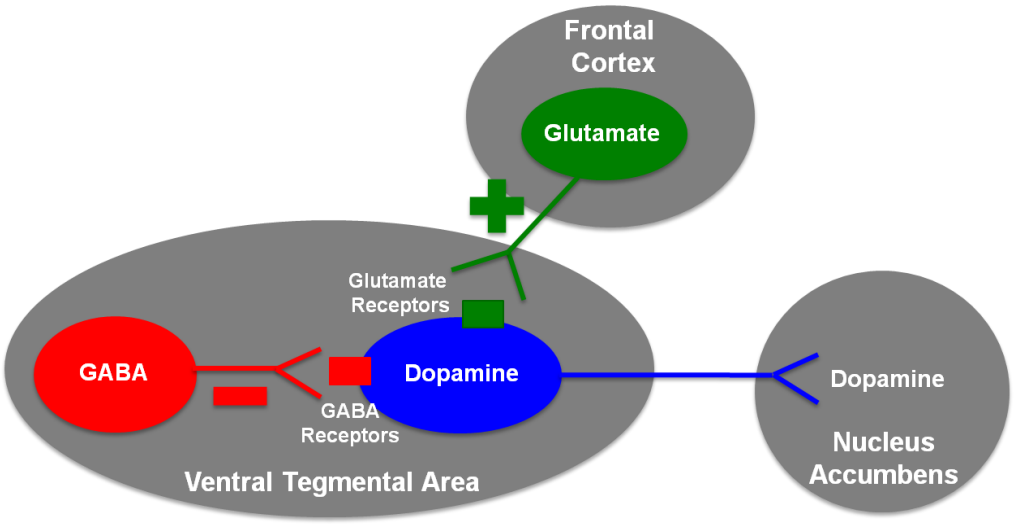
The affective properties of nicotine withdrawal have also been examined with another technique that measures anxiety-like behavior in rodents using elevated plus maze procedures. This procedure assesses how rats respond to an approach-avoidance situation involving open elevated spaces that are normally avoided versus enclosed safe areas that are inherently preferred. Adult rodents experiencing nicotine withdrawal display an increase in anxiety-like behavior, as indicated by a decrease in open-arm time relative to controls. However, the latter effect was lower in adolescent rats (Wilmouth and Spear, 2006) and mice (Kota et al., 2007) as compared to adults.

Taken together, as suggested in the human clinical studies, pre-clinical evidence supports the hypothesis that the physical and negative affective properties of nicotine withdrawal are reduced in adolescent versus adult rodents. The converging lines of evidence in clinical and pre-clinical work also suggest that rodent models are a valid approach to study the motivational factors that drive human tobacco use.

Rationale for focusing on withdrawal mechanisms. The reviewed literature supports the hypothesis that the rewarding effects of nicotine are *enhanced* whereas the aversive effects of withdrawal from this drug are *reduced* during adolescence. Although the rewarding effects of nicotine are a critical factor to study, the purpose of this dissertation was to explore age group differences in the neurochemical mechanisms underlying the aversive effects of nicotine withdrawal. This was done for several reasons. First, work in our laboratory showed that the behavioral effects of nicotine withdrawal are lower in adolescent versus adult rats (O'Dell et al., 2004, 2006 and 2007). By extension of these behavioral findings, the present studies explored the hypothesis that the neural mechanisms mediating nicotine withdrawal are fundamentally different in adolescents versus adults. Second, the present research reflects an extension of previous work done by Natividad et al. (2010) showing that age group differences within mesolimbic dopamine systems modulate age group differences in nicotine withdrawal. Lastly, given the importance of nicotine withdrawal in motivating tobacco use and relapse behavior, more research is needed to understand the underlying mechanisms in the brain that mediate nicotine withdrawal. A better understanding of these mechanisms may contribute to the development of specialized pharmacological tools to aid in tobacco cessation.

This dissertation thesis addressed the following questions. Question #1: What are the underlying neural mechanisms that mediate nicotine withdrawal? Question #2: Are there age group differences in these mechanisms?

III. Mechanisms of Nicotine Withdrawal



This illustration depicts the mesolimbic pathway and inhibitory and excitatory mechanisms that modulate dopamine transmission as discussed in the following sections.

Mesolimbic dopamine pathway. The mesolimbic pathway is generally thought to mediate behavioral responses involving motivational processes for exogenous stimuli such as food, sex, and drugs of abuse (see Wise, 2002). Dopamine transmission in this pathway is part of a larger motivational circuit that mediates behavioral responses driven by positive and negative reinforcement processes. This pathway, one of four major dopaminergic pathways in the brain, originates in the ventral tegmental area (VTA) where a large cluster of dopamine neurons project their axons forward to the nucleus accumbens (NAcc).

The present experiments focused on studying mechanisms in the VTA that modulate NAcc dopamine transmission during nicotine withdrawal. The rationale for focusing on mechanisms in the VTA is based on research showing that nicotine-treated rats displayed a decrease in extracellular levels of NAcc dopamine following intra-VTA, but not intra-NAcc, infusions of mecamylamine (Hildebrand et al., 1999; Hildebrand and Svensson 2000). Moreover, intra-VTA infusions of nicotine produced a higher and more sustained increase in NAcc dopamine levels relative to intra-NAcc infusions of nicotine (Nisell et al., 1994). The latter study also showed that the ability of nicotine to increase NAcc dopamine was reversed by intra-VTA, but not intra-NAcc, infusions of mecamylamine. Collectively, these findings support the rationale for focusing on the VTA to study the neural mechanisms of nicotine withdrawal.

Dopamine transmission in the NAcc is influenced by dopamine cell excitability in the VTA (Vos et al., 1999). In this regard, much interest has been expressed in studying the various factors that influence excitability of the VTA dopamine cell bodies. Overall, this large body of work has shown that NAcc dopamine transmission is modulated in the VTA by excitatory and inhibitory mechanisms, as described below.

Excitatory mechanisms regulate mesolimbic dopamine. Glutamate is the most abundant excitatory amino acid neurotransmitter in the brain (Meldrum, 2000). Glutamate binds to 2 main receptor subtypes including N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Glutamate activates the opening of ion channels that allow the passage of cations, such as sodium and potassium, to enter the cell following their concentration gradient. This influx of positive current elevates the membrane potential of the cell from its resting state (i.e., depolarization) toward the threshold of generating action potentials and, as a result, is excitatory (Neuroscience: Exploring the Brain, 2001).

Increased frequency of action potentials generated via glutamatergic signaling produces increased release of neurotransmitter.

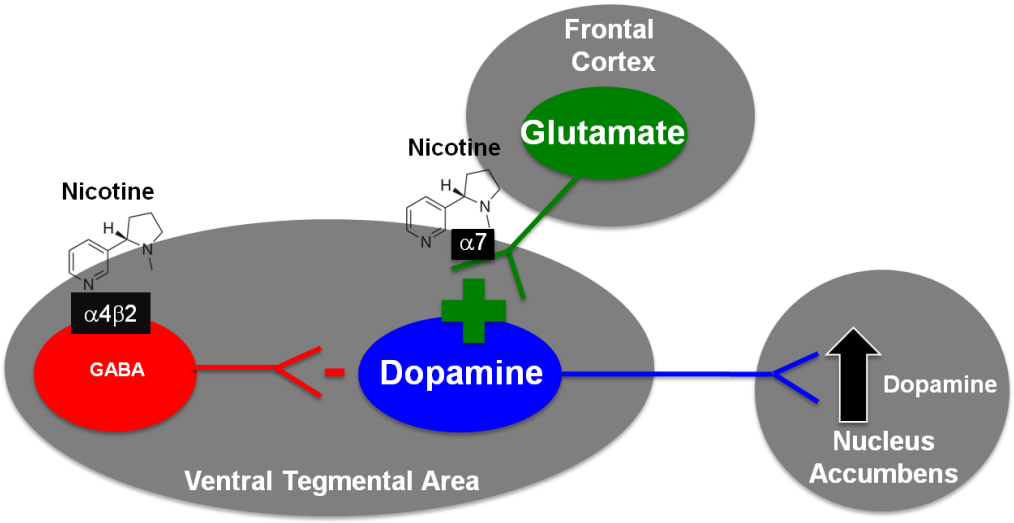
The glutamatergic inputs to the VTA arise primarily from the prefrontal cortex, which provides excitatory control of VTA neuron activity and enhances dopamine transmission in the NAcc (Johnson et al., 1992; Kalivas et al., 1989; Mansvelder et al., 2003; Sesack and Pikel, 1992; Suaud-Chagny et al., 1992; Taber and Fibiger, 1995). The ability of glutamate to enhance dopamine transmission is due to NMDA and AMPA receptors that are found on the somas of the dopamine neurons in the VTA (Choi et al., 2011; Johnson et al., 1992; Mao et al., 2011; Paquet et al., 1997; Schilstrom et al., 2006; Schumann et al., 2009). Local administration of glutamate receptor agonists in the VTA induce dopamine burst firing (Harnett et al., 2009; Sombers et al., 2009) and elevate extracellular levels of dopamine in the NAcc (Karreman et al., 1996; Kretschmer, 1999; Westerink et al., 1996). On the other hand, glutamate antagonist administration into the VTA reduces NAcc dopamine levels (Sombers et al., 2009; Westerink et al., 1996). Collectively, these studies show that NAcc dopamine transmission is under excitatory control of glutamate systems that innervate dopamine cell bodies in the VTA.

Inhibitory mechanisms also regulate mesolimbic dopamine. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain (Watanabe et al., 2002). GABA binds to 2 main receptor subtypes including $GABA_A$ and $GABA_B$ receptors. GABA activates the opening of ion channels that allow the passage of anions, such as chloride, to enter the cell following their concentration gradient. This influx of negative current reduces the membrane potential of the cell from its resting state (i.e., hyperpolarization) away from the threshold of generating action potentials and, as a result, is inhibitory (Neuroscience: Exploring the Brain,

2001). Decreased frequency of action potentials generated by GABAergic signaling produces decreased release of neurotransmitter.

Dopamine transmission in the NAcc is inhibited by GABAergic mechanisms in the VTA via a population of GABA interneurons. In addition, there are also GABAergic projections back from the NAcc and ventral pallidum that form synapses onto the VTA dopamine neurons (Johnson and North, 1992; Kalivas, 1993; Mansvelder and McGehee, 2002; Mansvelder et al., 2002; Rahman and McBride, 2002). The ability of GABA to inhibit dopamine transmission is due to $GABA_A$ and $GABA_B$ receptors that are found on the somas of the dopamine neurons in the VTA (Criswell et al., 1993; Cruz et al., 2004; Johnson et al., 1992; Johnson and North, 1992; Labouèbe et al., 2007; Okada et al., 2004). Electrophysiological studies have shown that VTA dopamine activity is elevated in the presence of GABA antagonists, and diminished with GABA agonists (Xi and Stein, 1998). In agreement with these findings, microdialysis studies reported that intra-VTA infusions of a GABA receptor antagonist increased extracellular dopamine levels in the NAcc (Ikemoto et al., 1997; Natividad et al., 2010; Westerink et al., 1996). On the other hand, GABA agonist administration into the VTA decreased NAcc dopamine levels (Westerink et al., 1996). Collectively, these studies show that NAcc dopamine transmission is under inhibitory control of GABA systems that innervate dopamine cell bodies in the VTA.

To summarize, the mesolimbic dopamine pathway consists of a collection of dopamine cell bodies that are innervated by excitatory and inhibitory amino acid mechanisms in the VTA. Dopamine cell excitability is altered in the VTA via excitatory glutamate and inhibitory GABA systems, and changes in levels of these amino acids alter dopamine transmission in the NAcc. The subsequent sections describe the effect of nicotine on the circuits of the mesolimbic pathway.



This illustration depicts the anatomical substrates by which nicotine alters dopamine transmission as described in the following sections.

Nicotine alters mesolimbic dopamine. Nicotine binds to nicotinic acetylcholine receptors (nAChRs) that consist of pentameric membrane proteins of homomeric or heteromeric complexes of α or β subunits (see Mansvelder et al., 2002). Stimulation of these ligand-gated receptors activates the opening of ion channels that allow the passage of cations to enter and depolarize the cell, thereby inducing an excitatory response. nAChRs that are located on the pre-synaptic terminal have particular influence on neurotransmitter release because they stimulate the opening of voltage-sensitive calcium channels that regulate exocytosis (McGehee and Role, 1995; Pidoplichko et al., 2004; Rathouz et al., 1996; Schilstrom et al., 2000).

To date, 12 isoforms have been identified (α2-10 and β2-4), and the various subunit combinations give rise to functional differences in binding affinity, channel activation, calcium permeability, and desensitization produced by nAChR agonists in general. The two most pervasive and well-described nAChR subtypes in the brain are α7 and α4β2 nAChRs (Picciotto et al., 2008). It has been shown that nicotine and other nAChR agonists display higher affinity

binding for α4β2 relative to α7 subtypes (Lippiello et al., 1987; McGehee and Role, 1995; Papke and Thinschmidt, 1998; Picciotto et al., 2008; Wonnacott, 1986). The significance of high affinity binding onto α4β2 subtypes is that these receptors are highly susceptible to desensitization. Thus, following prolonged agonist stimulation, the channel membrane associated with α4β2 receptors remains in a closed conformational state, and agonists are no longer able to activate these channels (see Jones and Westbrook, 1996).

nAChRs are anatomically positioned to alter dopamine transmission in the mesolimbic pathway. These receptors are abundantly present in the VTA because of cholinergic projections that arise from the pedunculopontine and laterodorsal tegmentum and innervate the VTA dopamine neurons (see Omelchenko and Sesack, 2006). Three cell types in the VTA have been shown to express nAChRs: dopamine neurons, GABA interneurons, and glutamate pre-synaptic terminals (Mansvelder and McGehee, 2002; Xu et al., 2006). Dopamine neurons express α2-10 and β2-4 subunits (Mansvelder et al., 2003). Glutamate terminals in the VTA contain α7 nAChRs, whereas GABA cell bodies largely contain α4β2 sites (see Marchi et al., 2002; Nomikos et al., 2000). The ability of these receptor subtypes to modulate NAcc dopamine transmission has been demonstrated in the findings that intra-VTA administration of α7 or α4β2 selective antagonists blocked nicotine-induced increases in NAcc dopamine levels (Ericson et al., 2003; Fu et al., 2000; Schilstrom et al., 1998).

Cholinergic transmission onto VTA GABA neurons can be reduced via the blockade of nAChRs or via desensitization during nicotine exposure. When nicotine is administered, α4β2 nAChRs desensitize within minutes and recover very slowly from desensitization (Gentry et al., 2003; Mansvelder and McGehee, 2002; Pidoplichko et al., 2004; Wang and Sun, 2005). In fact, in the first 15 minutes after nicotine exposure, GABA neurons do not respond to nicotine

application, and they take approximately 1 hour to return to normal levels of sensitivity (Mansvelder et al., 2002). Thus, as a result of nicotine exposure, the VTA dopamine neurons receive less inhibitory GABA transmission, and this decrease in inhibitory tone results in enhanced action potential firing of the dopaminergic neurons. In contrast, α7 nAChRs that modulate glutamate transmission are much less susceptible to desensitization during nicotine exposure relative to α4β2 nAChRs (Wooltorton et al., 2003). The latter study was conducted at low concentrations of nicotine (20-80 nM) to model the plasma levels of nicotine reported in human smokers. In fact, the doses of nicotine that are self-administered in both humans and animals often result in low nM brain concentrations, and this is believed to induce desensitization of α4β2 to a larger degree than α7 nAChRs (see Grady et al., 2007; Buccafusco et al., 2009; Mansvelder et al., 2002; Pidoplichko et al., 2004; Wooltorton et al., 2003).

Although the β2 heteromeric nAChRs became rapidly desensitized with low nicotine concentrations, α7 sites on afferent glutamate terminals remain functional and facilitate the release of glutamate onto the VTA dopamine cell bodies (Dani et al., 2001; Pidoplichko et al., 2004; Schilstrom et al., 2000). Nicotine, therefore, induces a shift from inhibition towards excitation of the VTA dopamine neurons that result in enhanced dopamine transmission in reward circuits of the NAcc. Consistent with this, Yin and French (2000) demonstrated that VTA GABAergic neurons displayed a lower electrophysiological response to nicotine, and in sequential exposure, a more profound desensitization relative to those observed in dopamine neurons. This rapid desensitization has been purported to lead to a disinhibition of the dopamine neurons, thereby facilitating a more sustained increase in mesolimbic dopamine transmission following nicotine. Collectively, these studies suggest that desensitization produced by nicotine differentially affects α4β2 relative to α7 nAChRs in the VTA. The consequences of this

desensitization is an imbalance of excitatory/inhibitory signaling, such that α4β2 receptors located on GABAergic interneurons rapidly desensitize and attenuate inhibitory transmission. On the other hand, α7 receptors located on glutamate pre-synaptic terminals do not desensitize and instead facilitate excitatory transmission.

To summarize, nicotine binds to α4β2 receptors located on GABAergic interneurons, and following chronic nicotine stimulation, these receptors desensitize and reduce GABA inhibition of VTA dopamine neurons. On the other hand, nicotine binding to α7 receptors facilitates excitatory glutamatergic transmission on the dopamine neurons. These reciprocal effects on GABA and glutamate transmission converge on the dopamine cell bodies to enhance dopamine cell excitability and facilitate an increase in NAcc dopamine transmission following nicotine administration.

Neurochemical mechanisms of nicotine withdrawal. The allostasis theory of addiction posits that drug-seeking behavior is mediated by the emergence of negative emotional states produced by counteradaptive opponent processes that are left unopposed in the absence of the drug (Koob and Le Moal, 2001). Following chronic drug use, Koob and Le Moal suggest that the counteradaptive opponent processes that limit reward function fail to return to a normal homeostatic range. This forms an allostatic state that represents a chronic deviation of the reward set point that drives an enhanced state of vulnerability and results in loss of control over drug use. Further, the theory proposes that neuroadaptations of withdrawal occur within the same brain circuits that mediate the rewarding effects of the drug, but are *opposite* to the effects produced by the drug itself. In light of the previous studies on nicotine, this model provides a foundation for considering potential neurochemical processes that mediate nicotine withdrawal.

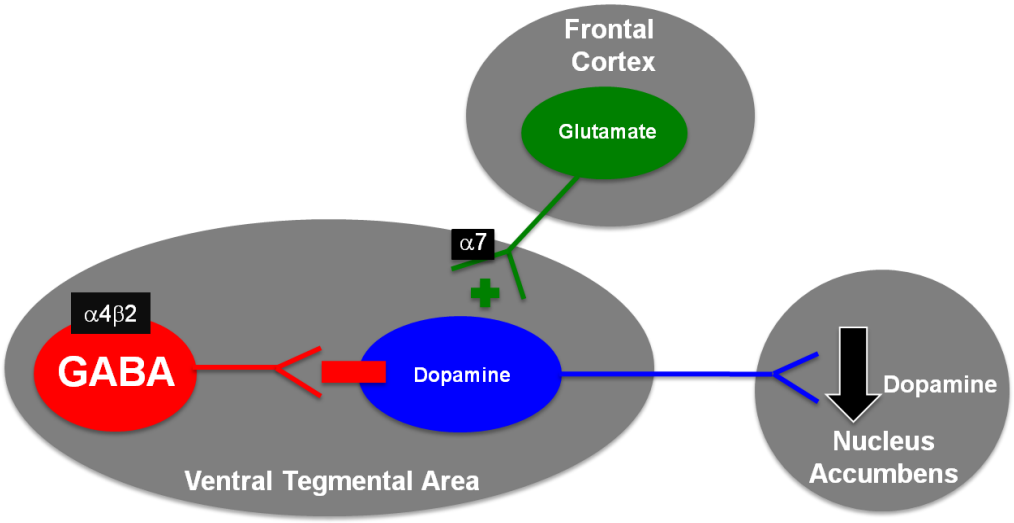
As predicted by the allostasis theory, the increase in NAcc dopamine produced by drugs of abuse (e.g., alcohol, opiates and cocaine) is followed by a decrease in NAcc dopamine once the drug is removed and the direct effects of the drug subside (Pothos et al., 1991; Rada et al., 2004; Weiss et al., 1992). This withdrawal effect has also been observed in nicotine-treated adult rats that displayed a 20-35% decrease in extracellular levels of NAcc dopamine following nAChR blockade with mecamylamine (Carboni et al., 2000; Gaddnas et al., 2002; Hildebrand et al., 1998 and 1999; Natividad et al., 2010; Rada et al., 2001). The ability of mecamylamine to reduce NAcc dopamine levels is believed to be due to the non-selective blockade of nAChRs located on dopaminergic and non-dopaminergic neurons in the VTA. This is based on the finding that intra-VTA, but not intra-NAcc infusions of mecamylamine, produced decreases in NAcc dopamine and concomitant increases in physical signs of withdrawal (Hildebrand et al., 1999; Hildebrand and Svensson, 2000).

To summarize, nicotine elicits increases in NAcc dopamine; however, withdrawal from this drug produces a counteradapative decrease in NAcc dopamine. This finding reflects a neuroadaptation in reward circuitry that is opposite to the effects produced by nicotine itself. *Currently, there is a significant knowledge gap regarding the neurochemical mechanisms that mediate decreases in NAcc dopamine observed during nicotine withdrawal.* In this regard, candidate systems may include amino acid mechanisms in the VTA based on previous findings that glutamate and GABA modulate VTA dopamine cell activity that project to the NAcc.

IV. Dissertation Questions

Dissertation Question #1: Are the decreases in NAcc dopamine observed during withdrawal modulated via amino acid mechanisms in the VTA? Nicotine produced elevations in NAcc dopamine via amino acid mechanisms in the VTA (Mansvelder et al., 2002; Mansvelder and McGehee, 2002). By extension, it was hypothesized that changes within the same mechanisms regulate decreases in NAcc dopamine, but in a drug-opposite manner (i.e., opposite to the effects produced by nicotine) during withdrawal. In accordance with the allostasis theory, it was hypothesized that during nicotine withdrawal, excitatory transmission on the dopamine cell bodies in the VTA is reduced (less glutamate in the VTA) whereas inhibitory transmission is enhanced (more GABA in the VTA). Thus, these two simultaneous effects are hypothesized to converge on the dopamine cell bodies to reduce NAcc dopamine levels during withdrawal.

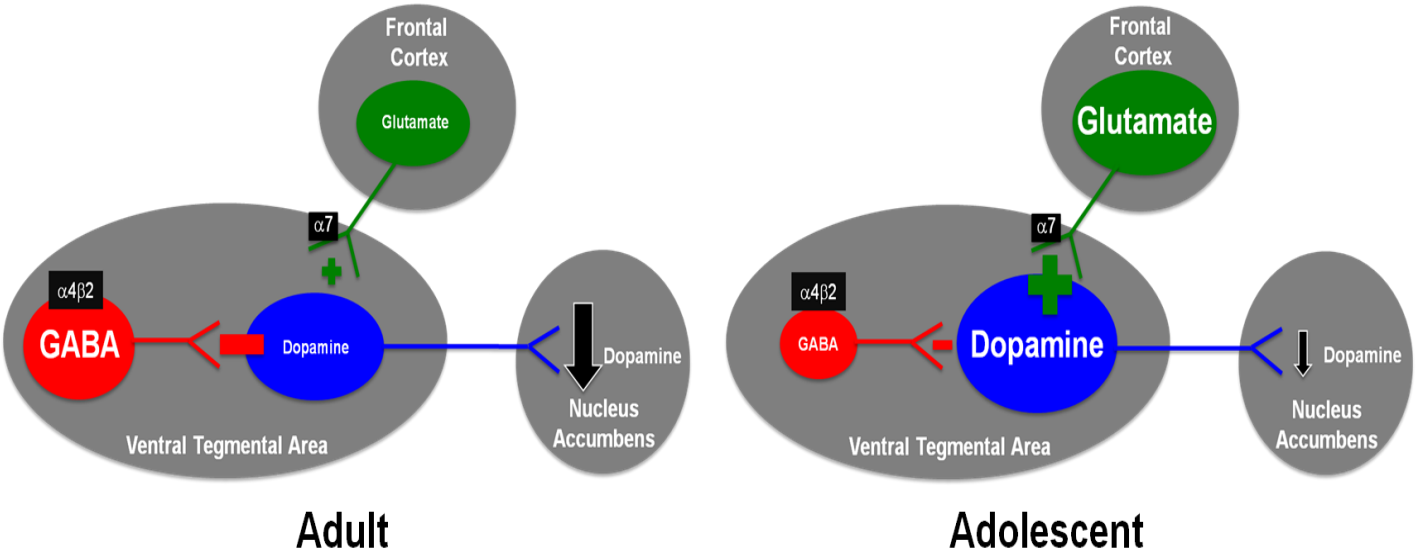
A withdrawal mechanism involving reduced glutamate transmission is supported by the finding that intra-VTA administration of a glutamate antagonist elicits morphine withdrawal (Wang et al., 2004). Further, morphine withdrawal enhances presynaptic inhibitory mechanisms in the VTA that regulate glutamate release, an effect that suggests that glutamate transmission is reduced during withdrawal (Manzoni and Williams, 1999). Other work has demonstrated that GABA transmission in the VTA is enhanced during withdrawal from several drugs of abuse, including amphetamine (Giorgetti et al., 2002), cocaine, and morphine (Bonci and Williams, 1996 and 1997; Madhaven et al., 2012). Collectively, these studies suggest that VTA amino acid systems play a role in mediating mesolimbic dopamine responses during drug withdrawal.



This illustration depicts the hypothesized changes during nicotine withdrawal.

To summarize, it was hypothesized that during nicotine withdrawal, excitatory glutamatergic transmission is *reduced* and inhibitory GABAergic transmission is *enhanced* in the VTA. The effects of GABA and glutamate transmission are hypothesized to converge on the dopamine cell bodies to *reduce* VTA dopamine cell excitability and decrease dopamine transmission in the NAcc.

Dissertation Question #2: Is the amino acid modulation of dopamine during withdrawal age dependent? Previous work in our laboratory demonstrated that mecamylamine precipitated a lower magnitude of a decrease in NAcc dopamine levels of nicotine-treated adolescent versus adult rats (Natividad et al., 2010). These data suggested that adolescent rats were *less* sensitive to the neurochemical changes produced by nicotine withdrawal. The goal of this dissertation was to extend these findings by examining whether age group differences in NAcc dopamine during withdrawal were mediated by amino acid systems in the VTA.



The illustration depicts the hypothesis that amino acid mechanisms underlie age group differences in NAcc dopamine during nicotine withdrawal.

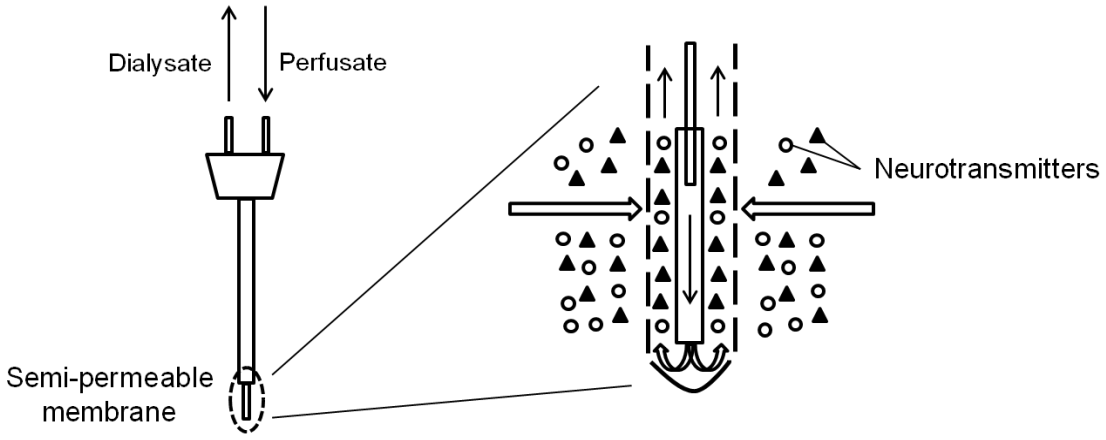
Specifically, it was hypothesized that diminished decreases in NAcc dopamine of adolescent rats experiencing nicotine withdrawal is related to *enhanced* excitatory glutamate (denoted as a larger glutamate neuron) and *reduced* inhibitory GABA (denoted as a smaller GABA neuron) transmission in adolescent relative to adult rats. This age group difference in amino acid transmission during nicotine withdrawal was hypothesized to minimize reductions in dopamine cell excitability (denoted as a larger dopamine neuron) and prevent large decreases in NAcc dopamine of adolescent versus adult rats.

V. Background Information Regarding Adolescence in Rats

In assessing the boundaries of the adolescent period in rodents, most researchers agree that the prototypic age range for adolescence conservatively ranges from postnatal day (PND) 28

to 45 (see Spear, 2000). Historically, it has been difficult to define an exact time frame of adolescence since this phase of development is a transitional period that encompasses a series of events with no single discrete event that signals its onset or termination, such as puberty that signals sexual maturation. Adolescence reflects a period of "soft" events during which age-specific behavioral discontinuities from younger and older animals are most evident (Pickles et al., 1998). Some of the behavioral characteristics that have established the period of adolescence in rats include accelerated growth rates, emergence from the protected nest (Galef, 1981) and enhanced social interaction (Primus and Kellogg, 1989), risk-taking and novelty-seeking behavior (Spear et al., 1980, Spear and Brake, 1983). These innate age group differences are important to consider when evaluating the behavioral effects of drugs of abuse in rats from different stages of development.

VI. Background Information for Neurochemical Methods

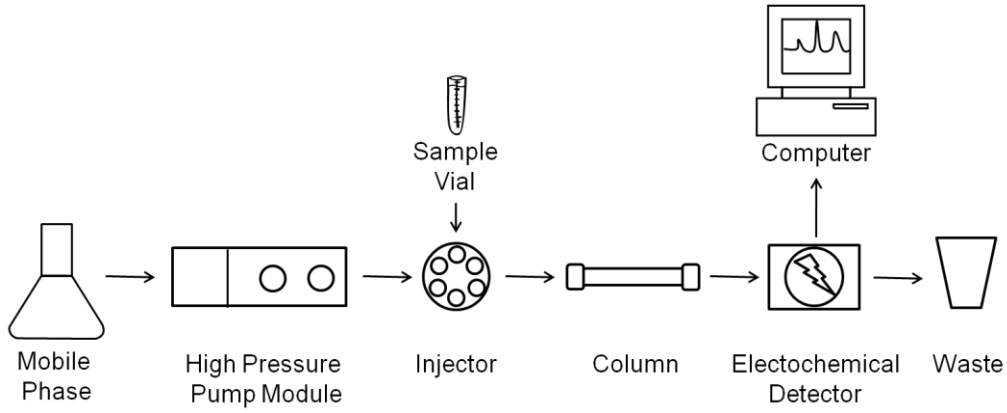


The illustration depicts a microdialysis probe used to extract samples from the extracellular environment in freely-moving animals (adapted from Chaurasia et al., 2007).

In vivo microdialysis procedures. Microdialysis procedures allow an experimenter to estimate changes in neurotransmission in the synapse of a discrete brain region. A probe consisting of a semi-permeable membrane tip is inserted into the brain region of interest. Artificial cerebral spinal fluid (ACSF) is delivered through the inlet of the probe at a constant rate via a syringe pump. This creates a concentration gradient across the microdialysis membrane such that molecules of a small enough size enter the probe tip via diffusion properties. The ACSF containing the neurotransmitters (i.e., dialysate) is collected in an outlet vial at the probe. The passage of molecules across the membrane tip is determined by the concentration of neurotransmitter in the area where the probe is implanted, the flow rate of the perfusate, and the size of the pores on the membrane tip. Typically, the external diameter of the membrane is about 250-300 µm with molecular weight cut-off of 6,000-20,000 Daltons. Dialysate samples that were retrieved from the NAcc were examined for dopamine, and samples from the VTA were examined for amino acid (i.e., glutamate and GABA) neurotransmitter content. It should be noted that different techniques were employed to assay and quantify the neurotransmitters, as described in the sections below.

***Neurochemical methods for dopamine and amino acids*.** Different methods were used to quantify dopamine and the amino acids in the present studies. High performance liquid chromatography (HPLC) was used to measure dopamine because temporal resolution remains high with this neurochemical assay without requiring a large amount of brain dialysate to be injected into the system (Zapata et al., 2009). Capillary electrophoresis (CE) was used to separate the small and highly polarized amino acid molecules that would have required a larger dialysate volume (i.e., more sampling time) to assay with conventional HPLC methods. Thus, CE

technology was used to ensure that similar dialysate collection strategies could be employed across all neurochemical measures.

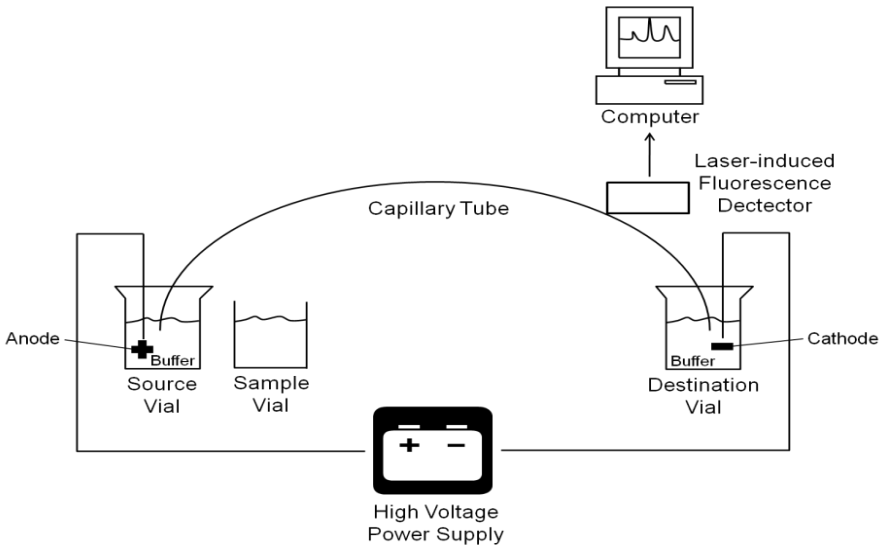


This illustration depicts how HPLC produces separation of analytes in a dialysate sample (adapted from Lambertson, 2012).

Separation method for dopamine. HPLC is used to separate components of a sample (i.e., analytes) via reverse-phase ion-pairing retention on a chromatographic column. This occurs via the use of a pump apparatus that circulates a mobile phase solution at high pressure speed to deliver the sample through a separation column. The column consists of tightly packed silicon beads containing alkyl-carbon chains (i.e., stationary phase) that interact with the analyte based on the polarity of the molecule. Thus, the elution time of the analyte from the column depends on its ability to associate with the non-polarized elements of the stationary phase. For example, the less polar the analyte, the better the electrons of this molecule will interact with those of the column packing to enhance elution time from the column. The elution time is also a function of the alkyl-carbon chain length in the column packing (e.g., 18-carbon chain length for dopamine separation), the concentration of ion-pairing detergent (e.g., sodium dodecyl sulfate), the concentration of the organic modifiers in the mobile phase (e.g., methanol), and the flow rate

applied to the system. The time at which a specific analyte elutes is called retention time and is considered to be a unique characteristic of the analyte.

Detection method for dopamine. HPLC is coupled with electrochemical detection for quantification of the individual analytes such as dopamine. The electrochemical detector consists of two electrodes that carry an electrical potential which is set to oxidize in one electrode and reduce in the other as the separated analytes pass through the pre-set field potentials. Oxidation and reduction produced by the field potentials then generate a measurable current via the release and gain of electrons that reflects the quantity of the analytes. The electrical current is translated into a computerized image of the reaction in peak format, which can be quantified and compared against a current that is generated from a standard containing a known concentration of dopamine. The area-under-the-curve or peak heights from a series of standard injections are linearly related to the amount of analyte. Thus, a linear regression of these standard injections is used to predict the amount of analyte in a sample containing an unknown quantity.



This illustration depicts how CE separates analytes in a dialysate sample (adapted from Principles of Instrumental Analysis, 2007).

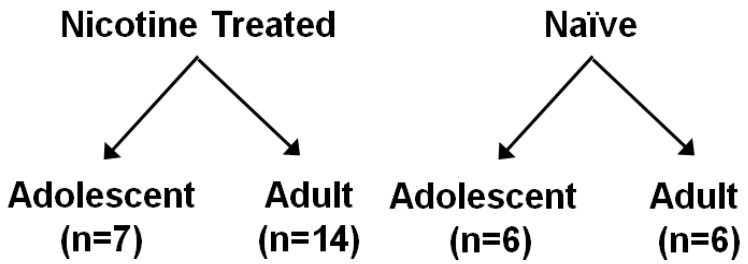
Separation methods for amino acids. The CE method involves the use of a high voltage supply to generate an electrical potential between two conductive buffers that are separated by a silicon capillary tube. These buffers generate an electrostatic drive that causes positively charged ions (i.e., cations) to migrate from a sample towards the buffer containing a negative charge (i.e., cathode), whereas negatively charged ions (i.e., anions) are strongly retained in the positively charged buffer (i.e., anode). The size of the analyte also allows for smaller molecules to migrate faster along the capillary tube relative to larger molecules (i.e., hydrodynamic radius). Moreover, the modification of chemical constituents along the capillary wall forms a secondary layer of ionic species that interact with the charged elements of the analyte to facilitate electrophoretic mobility (i.e., electro-osmotic flow). As an example, the addition of sodium hydroxide (that carries a negative charge) along the capillary wall allows analytes that are positively charged to migrate against the repelling forces of the anode so that they may be separated distinctly from other cation molecules. This chemical adjustment is helpful in the separation of amino acids that display low electrophoretic mobility. The order in which the separated analytes migrate forward to the detector at the end of the capillary tube is considered a unique characteristic of each molecule.

Detection methods for the analysis of amino acids. CE technology is often paired with laser-induced fluorescence (LIF) detection to quantify amino acid compounds. Typically, the detection of amino acids such as glutamate and GABA requires a derivatizing process that combines the use of reducing and fluorescent chemical agents to attach a fluoroform to the amino acid. The derivatized molecule is then excited by a focused laser beam that causes the fluoroform to emit light at a specific wavelength which is captured by a detector. The intensity of

the fluorescence, which is linearly related to the concentration of the fluoroform, is sent to a computer interface that visualizes the data in peak format, and this can be quantified and compared against the wavelength generated from standards containing a known concentration of the amino acid of interest. The area-under-the-curve or peak heights from a series of standards are linearly related to the amount of analyte. Thus, a linear regression of these standards is used to predict the amount of analyte in a sample containing an unknown quantity.

Chapter 2: Methods

I. Experimental Overview



This illustration depicts the experimental cohorts that were used in the dissertation experiments.

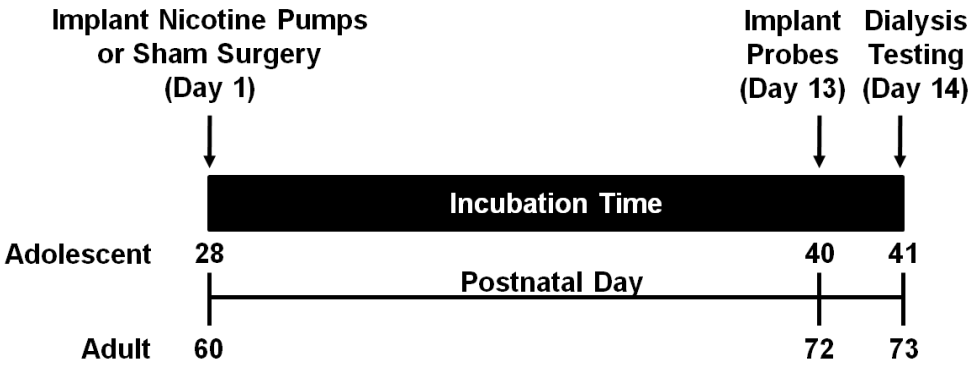
The first experimental cohort included a group of nicotine-treated adolescent and adult animals experiencing nicotine withdrawal with a dialysis probe in the NAcc to measure dopamine and another probe in the VTA to measure amino acids. The NAcc dopamine data were published as an important first step in elucidating the role of mesolimbic dopamine in mediating age group differences in nicotine withdrawal (Natividad et al., 2010). The current studies extended the proposed neurochemical hypothesis by assessing concomitant measures of VTA amino acids in order to explain age group differences in NAcc dopamine during withdrawal. An additional cohort of animals was used to examine whether our method of precipitating nicotine withdrawal (i.e., mecamylamine) alone produced neurochemical changes. To address this question, a group of nicotine naïve adolescent and adult rats were included to examine changes in NAcc dopamine levels following mecamylamine administration. The procedures used for these cohorts are described below.

II. General Methods

Animals. Male Wistar adolescent and adult rats (n=6-14 per group) were used. Adolescents were PND 28 and adults were PND 60 at the start of the experiment. All rats were handled for 5 days prior to the start of experimentation and were given free access to food and water throughout the study. Rats were housed in groups of 2-3 per cage in a humidity- and temperature-controlled (20-22^{o}C) vivarium using a 12-/12-hour light/dark cycle with lights on at 8:00 AM. The home cages consisted of a rectangular Plexiglas® hanging cage (41.5 cm long x 17 cm wide x 21 cm high) with pine bedding. The food and water were located above the animals' living spaces on a wire platform encased within a filtered top cover. Testing procedures were conducted during the light phase of the rats' light/dark cycle. The rats were bred in the Psychology Department from a stock of out bred Wistar rats from Harlan, Inc (Indianapolis, IN). All procedures were approved by the UTEP Animal Care and Use Committee and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs. The drugs used in these experiments were (-) nicotine-hydrogen tartate and mecamylamine-hydrochloride purchased from Sigma Aldrich Inc. (St. Louis, MO). Mecamylamine was dissolved in 0.9% sterile saline and injected via the intraperitoneal (ip) route of administration in a volume of 1 ml/kg. To minimize pain and discomfort following surgeries, animals were given a 2.5 mg/kg dose of flunixin meglumine via the subcutaneous route of administration (Vedco, St Joseph, MO).

III. Dialysis Methods



This illustration depicts the timeline of experimental procedures and ages of the animals.

Rats were first anesthetized with an isofluorane/oxygen mixture (1-3% isofluorane) prior to undergoing sham surgery or surgical implantation of 14-day Alzet osmotic pumps purchased from Durect Corporation (Alzet model 2ML2; 5.0 µl/hour; Cupertino, CA) that were implanted subcutaneously on the back of the animal, parallel to the spine. Pumps were filled with an adjusted concentration of nicotine (4.7 mg/kg/day for adolescents or 3.2 mg/kg/day for adults; expressed as base). The concentration of nicotine in the pump was adjusted according to the weight of the rat at the time of the surgery. The nicotine doses were based on previous work showing that adolescent rats require approximately 1.5 times more nicotine to display similar plasma nicotine levels as adults (O'Dell et al., 2006). After surgery, the surgical incision was closed with 9-mm stainless steel wound clips and treated with a topical antibiotic ointment, and the animals received an injection of flunixin (sc) to minimize pain and discomfort.

Thirteen days after pump implantation, the rats were implanted unilaterally with 2 probes into the NAcc and the ipsilateral VTA. All rats were implanted with probes between postnatal day 40 for adolescents and postnatal day 72 for adults. The probes were purchased from CMA-Microdialysis (model CMA 11; Solna, Sweden) with an active membrane length of 2 mm in the

NAcc and 1 mm in the VTA. The probes were perfused for at least 1 hour prior to implantation at a rate of 0.5 µl/minute with ACSF composed of 145 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl2, 1.2 mM MgCl2, 5.4 mM d-glucose, and 0.25 mM ascorbic acid and adjusted to a pH of 7.2-7.4. The probes were stereotaxically implanted using the following coordinates for the NAcc from bregma [adolescent placement- anterior-posterior (AP) = +2.2, medial-lateral (ML) = ±1.0, dorsal-ventral (DV) = -7.1; and adult placement- AP = +1.7, ML = ±1.4, DV = -8.1] and the ipsilateral VTA [adolescent placement- AP = -4.0, ML = ±0.6, DV = -7.4; and adult placement- AP = -4.8, ML = ±0.8, DV = -8.5]. The hemisphere that was implanted with the probe was randomized across treatment groups to control for possible hemispheric differences across age groups.

Following surgery, adolescent and adult animals were transferred to similar sized test cages (24 cm long x 24 cm wide x 31 cm high) with pine bedding. The perfusate flow rate was increased to 1 µL/minute for 1 hour to allow equilibration of the probes. Samples were then collected in 10-minute intervals for 1 hour to establish a baseline period, and then for 3 separate 1-hour collection periods following a systemic injection of saline and then 2 doses of mecamylamine in increasing order (1.5 and 3.0 mg/kg, expressed as salt; ip). Microdialysis testing was done in the continuous presence of nicotine that was distributed via the pumps. Dialysate samples collected from the NAcc probe in naïve animals were diluted with 10 µl of perchloric-acid (0.05 N) in order to preserve the samples and prevent degradation of dopamine. After collection, the samples were immediately frozen on dry ice and were then stored in a -80^{o}C freezer until they were analyzed.

IV. Neurochemical Methods

Dialysate dopamine content was estimated from a 10-µl sample injected into a HPLC system equipped with an ESA HR-80 80x4.6 mm column (3 µm BetaBasic packing material, carbon-18 stationary phase, Chelmsford, MA) and eluted using a mobile phase composed of a 60 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM sodium-EDTA, 17% methanol, and 0.035 mM sodium dodecyl sulfate at pH 3.75 delivered at 1 ml/minute by an ESA model 582 syringe pump (Chelmsford, MA). Quantification was achieved via an ESA Coulochem II detector equipped with a coulometric sensor containing dual glassy carbon working electrodes (model 5011A; Chelmsford, MA) set to oxidate on the first channel at +350 mV and reduce on the second channel at -150 mV. The extracellular levels of dopamine were estimated using external calibration curves with standards containing known concentrations of these neurochemicals.

Dialysate amino acid content was estimated using CE technology. Derivatization of the amino acids was achieved by mixing 6 µL of dialysate with 9 µL of 40 mM borate buffer (pH 10.5) containing 3.8 mM potassium cyanide and 1 µL of 5 mM naphthalene-2,3-dicarboxaldehyde in methanol. The derivatized dialysate was then loaded onto a 90 cm fused silica capillary and the amino acids were separated using +15 kV and a background electrolyte solution consisting of 100 mM borate buffer (pH 9.2) containing 30 mM sodium dodecyl sulfate and 2 mM hydroxypropyl-β-cyclodextrin. The amino acids were detected using a LIF detector (Zetalif, Picometrics, Ramon Ville, France) equipped with a 442 nm helium-cadmium laser (30 mW, Melles Griot, Carlsbad, California). External calibration standards were run in duplicate and were interspersed throughout the sample run.

V. Histology Methods

At the end of the experiment, rats were deeply sedated with pentobarbital (100 mg/kg, salt; ip) and perfused using 0.85% saline and then a 4% paraformaldehyde solution. Following the perfusion, the brains were extracted and stored in formalin solution until they were sectioned. Verification of the probe placements was achieved during tissue sectioning using the Paxinos and Watson (1986) atlas. The probe placements were focused in the NAcc core region for both adolescents and adults, as determined during sectioning of the brain tissue. The VTA placements were all confined in this small brain region. In addition, an intra-VTA perfusion of bicuculline-methochloride (100 μM) using reverse dialysis procedures produced strong ipsilateral turning in all of the animals, and this effect provided neuroanatomical verification since other studies have reported similar results (Grubb et al., 2002; Westerink et al., 1996).

VI. Statistical Methods

Repeated-measures analysis of variance (ANOVA) was first conducted to examine whether there were age group differences across the 6 baseline samples of dopamine, glutamate, and GABA. The data revealed that there were no age group differences in basal levels of these neurochemical measures. The subsequent analyses were conducted on values that were converted to % change from baseline [i.e., (dialysate value/average baseline value) x 100%] in order to demonstrate the magnitude of changes in these neurochemicals on a similar scale and also to clearly illustrate group differences across experimental conditions. The dialysate data as % change from baseline were then analyzed using repeated-measures ANOVA with age group

(adolescent and adult) as a between-subject factor and time (10-minute intervals) as a within-subject factor. Wherever appropriate, significant interaction effects were further analyzed using Fisher's protected least significant difference tests with a modified Bonferroni correction factor for alpha inflation ($p \leq 0.05$).

To examine whether amino acid levels in the VTA modulate age group differences in NAcc dopamine during nicotine withdrawal, regression analyses was performed between % change in VTA glutamate or GABA, against % change in NAcc dopamine in the samples collected before (i.e., baseline and saline) and after both doses of mecamylamine. A comparison of amino acid with dopamine transmission was conducted only in the nicotine-treated animals that had concomitant measures of these neurochemicals (n=7 per age group). Pearson correlations (r) were conducted on the average of % baseline value for each dialysate fraction of amino acid against each dialysate fraction of dopamine before and after mecamylamine administration. To compare age group differences, the Pearson correlations were converted to Fisher's z scores and then compared by age group.

Chapter 3: Results

Figure 1 illustrates % change in extracellular levels of NAcc dopamine (± SEM) following mecamylamine administration in nicotine-naïve adolescent and adult rats. This experimental cohort examined whether mecamylamine alone produced changes in NAcc dopamine in drug-naïve adolescent and adult rats. Overall, the results revealed that mecamylamine did not produce any alterations in NAcc dopamine across control groups of both age groups. Baseline dopamine levels were not different between adult (2.3 ± 0.2 nM) and adolescent (1.9 ± 0.3 nM) rats [$F(1,10)=1.3$; p=ns]. There were no main effects of time [$F(23,230)=0.1$; p=ns] or age group [$F(1,10)=0.04$ p=ns]. An interaction between time and age group was also not observed [$F(23,230)=0.3$; p=ns].

Figure 2 illustrates % change in extracellular levels of VTA glutamate (± SEM) in nicotine-treated adolescent and adult rats experiencing nicotine withdrawal. The analyses reflect a separate experimental cohort that examined age group differences in VTA amino acid levels in adolescent and adult animals experiencing nicotine withdrawal. Overall, the results revealed that mecamylamine produced a decrease in VTA glutamate in nicotine-treated adult but not adolescent rats. Baseline glutamate levels were not different between adult (881.5 ± 135.1 nM) and adolescent (952.4 ± 82.8 nM) rats [$F(1,19)=0.2$; p=ns]. A significant main effect of time [$F(23,437)=10.0$; $p\leq0.05$], age group [$F(1,19)=8.5$; $p\leq0.05$], and interaction between time and age group [$F(23,437)=3.5$; $p\leq0.05$] was observed, with adults, but not adolescents displaying a decrease in glutamate following mecamylamine. Specifically, adult rats displayed a maximal decrease in VTA glutamate (43.6 ± 6.9% from baseline) that was larger relative to adolescents that displayed a small but non-significant decrease in glutamate (10.4 ± 9.7% from baseline).

Subsequent post-hoc analyses revealed that adult rats displayed a significant decrease in glutamate relative to baseline at the 3rd-12th time points (*p≤0.05) and as compared to adolescents at the 6th-12th time points after mecamylamine (†p≤0.05).

Figure 3 illustrates % change in baseline extracellular levels of VTA GABA (± SEM) in nicotine-treated adolescent and adult rats experiencing nicotine withdrawal. Overall, the results revealed that mecamylamine produced an increase in VTA GABA in nicotine-treated adult but not adolescent rats. Baseline GABA levels were not different between adult (44.4 ± 3.7 nM) and adolescent (38.3 ± 4.4 nM) rats [$F(1,19)=0.8$; p=ns]. An interaction between time and age group [$F(23,437)=1.8$; $p\leq0.05$] was observed, with adults, but not adolescents displaying an increase in GABA following mecamylamine. Specifically, adult rats displayed a maximal increase in VTA GABA (38.2 ± 17.2% from baseline) and adolescents were unchanged. Subsequent post-hoc analyses revealed that adult rats displayed a significant increase in GABA levels relative to baseline at the 1st and 9th time points (*p≤0.05) and as compared to adolescents at the 1st, 4th, and 9th time points after mecamylamine (†p≤0.05).

The overall goal of these studies was to provide a better understanding of age group differences in the neurochemical mechanisms of nicotine withdrawal. The following regression analyses were done in order to help guide an overarching hypothesis regarding the manner in which VTA amino acids regulate age group differences in NAcc dopamine.

Figure 4 is a scatter plot illustrating % change in baseline extracellular levels of VTA glutamate plotted against NAcc dopamine in nicotine-treated adult versus adolescent rats before and after mecamylamine administration. Overall, the results revealed that neither age group displayed a significant correlation between VTA glutamate and NAcc dopamine before mecamylamine administration. Following mecamylamine administration, however, adults

displayed a significant correlation in VTA glutamate and NAcc dopamine, and this effect was not observed in adolescent rats. Specifically, adults displayed a positive correlation between VTA glutamate and NAcc dopamine, such that decreases in glutamate were associated with decreases in NAcc dopamine ($r= 0.90$; $p\leq0.05$). Also, the correlation between glutamate and dopamine was stronger in adults ($p\leq0.05$), and accounted for a larger proportion of the variance ($R^2= 0.82$) relative to adolescents ($R^2= 0.30$).

Figure 5 is a scatter plot illustrating % change in baseline extracellular levels of VTA GABA plotted against NAcc dopamine in nicotine-treated adult versus adolescent rats before and after mecamylamine administration. Overall, the results revealed that neither age group displayed significant correlations of VTA GABA and NAcc dopamine before mecamylamine administration. Following mecamylamine administration, however, adult rats displayed a significant correlation in VTA GABA and NAcc dopamine, and this effect was not observed in adolescent rats. Specifically, adults displayed a negative correlation such that increases in VTA GABA were associated with decreases in NAcc dopamine ($r= -0.66$; $p\leq0.05$). In contrast, adolescents did not display a significant correlation of these neurotransmitters. Also, the correlation between GABA and dopamine was stronger in adults ($p\leq0.05$) and accounted for a larger proportion of the variance ($R^2= 0.42$) as compared to our analysis in adolescents ($R^2= 0.14$).

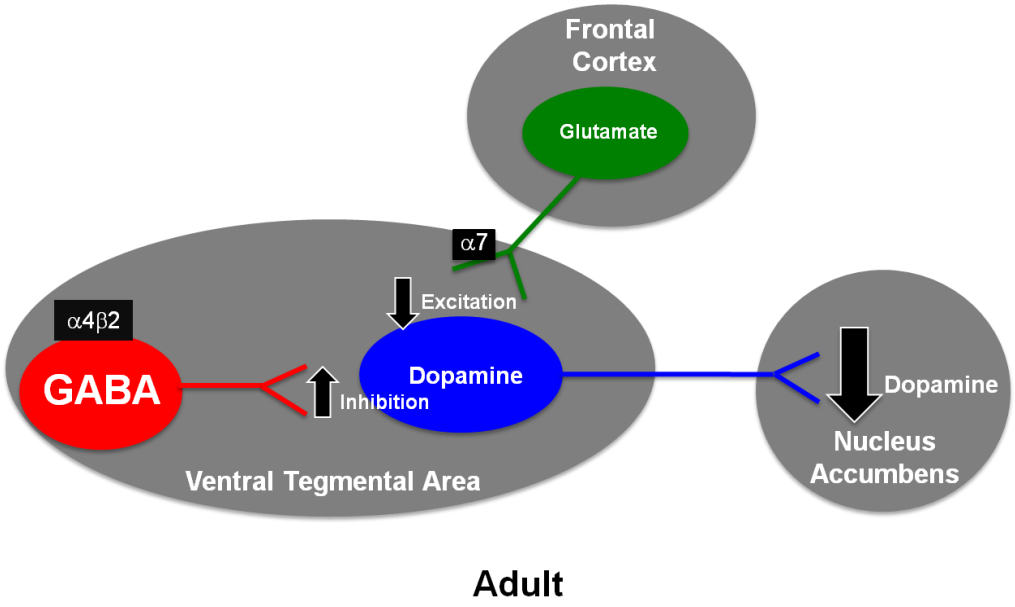
Chapter 4: Discussion

I. Summary

Previous work in our laboratory demonstrated that nicotine withdrawal produces a decrease in extracellular NAcc dopamine levels that is larger in adult versus adolescent rats. The present studies expanded on these initial findings in three important ways. First, mecamylamine did not produce alterations in NAcc dopamine levels in nicotine naïve adult or adolescent rats. This validates the use of mecamylamine as a pharmacological tool to precipitate withdrawal in nicotine-treated rats because the use of mecamylamine alone does not appear to confound the results of the observed neurochemical measures. Second, nicotine withdrawal produces a *decrease* in glutamate and an *increase* in GABA in the VTA of adults. On the other hand, adolescents do not display significant changes in either of these amino acids in the VTA during nicotine withdrawal. Third, as expected, due to the excitatory effects of glutamate on dopamine cell bodies, decreases in VTA glutamate and decreases in NAcc dopamine were *positively* correlated in adult rats, whereas adolescents did not display a significant correlation during withdrawal. This correlation was *stronger* in adults versus adolescents. Also, as expected, due to the inhibitory effects of GABA on dopamine cell bodies, increases in VTA GABA and decreases in dopamine were *negatively* correlated in adults, whereas adolescents did not display a significant correlation during withdrawal. This correlation was also *stronger* in adults versus adolescents. Taken together, the results indicate that adult rats display changes in the neurochemical processes that inhibit mesolimbic dopamine function during nicotine withdrawal. However, adolescents show less pronounced amino acid control of the VTA dopamine cell

bodies and lower withdrawal-related inhibition of mesolimbic dopamine function as compared to adults.

II. Mechanisms of Nicotine Withdrawal in Adults



This illustration depicts the hypothesized mechanisms of nicotine withdrawal in adults.

The decreases in NAcc dopamine observed during withdrawal are modulated via amino acid mechanisms in the VTA. Adult rats experiencing nicotine withdrawal display reduced extracellular glutamate and enhanced GABA levels in the VTA. A significant proportion of variance in the decreases in NAcc dopamine were accounted for by decreases in VTA glutamate (R^2= 0.82) and increases in VTA GABA (R^2= 0.42). These effects likely reflect adaptations in the mechanisms that modulate VTA cell excitability in response to long-term nicotine-induced dopamine cell activation. The proposed theoretical framework suggests that upon clearance of nicotine (or blockade of nicotinic receptors), these neuroadaptations are left unopposed, and this results in an overall inhibition of VTA cell activity and a reduction in NAcc dopamine levels.

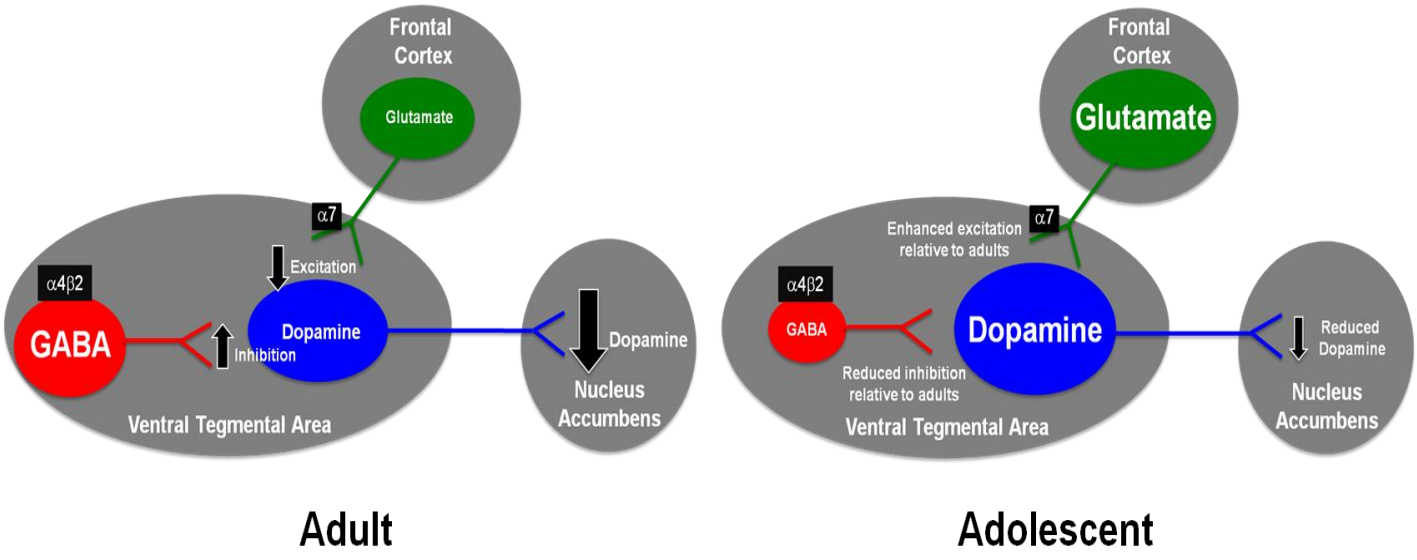
These observations in adult rats are consistent with previous reports showing a similar pattern of changes in glutamate and GABA in the VTA during withdrawal from other drugs of abuse. For example, a withdrawal mechanism involving reduced glutamate transmission is supported by the finding that intra-VTA administration of a glutamate antagonist elicited morphine withdrawal (Wang et al., 2004). Further, morphine withdrawal enhanced pre-synaptic inhibitory mechanisms in the VTA that regulate glutamate release, an effect that suggests that glutamate transmission is reduced during withdrawal (Manzoni and Williams, 1999).

Other work has demonstrated that GABA transmission in the VTA is enhanced during withdrawal from several drugs of abuse, including amphetamine (Giorgetti et al., 2002), cocaine, and morphine (Bonci and Williams, 1996 and 1997; Madhavan et al., 2010). These studies suggest that VTA amino acid systems play a role in mediating mesolimbic dopamine responses during nicotine withdrawal. Specifically, these data suggest that during withdrawal from drugs of abuse, dopamine cell bodies in the adult VTA are subject to diminished excitatory glutamate signaling and enhanced inhibitory GABAergic tone. Collectively, these processes likely contribute to reduced activity of mesolimbic dopamine projection neurons and decreases in NAcc dopamine levels.

It is unclear whether nicotine withdrawal precipitated by mecamylamine produces neurochemical differences that are distinct from those accompanied by spontaneous withdrawal. Mecamylamine was used in this experiment because nicotinic receptor blockade elicits a discrete and profound withdrawal in rats. Thus, relative to the removal of chronic nicotine that elicits a mild withdrawal, mecamylamine produced a maximal withdrawal to study the underlying mechanisms that mediate nicotine withdrawal. It is expected that similar neurochemical results in adult rats (albeit, at a lower magnitude) would be observed under spontaneous nicotine

withdrawal, since tissue dopamine content in the nucleus accumbens of adult rats has been shown to decrease 24 hours following the removal of chronic nicotine (Fung et al. 1996).

III. Age Group Differences in the Mechanisms of Withdrawal



This illustration depicts our hypothesis regarding age group differences in amino acid regulation of NAcc dopamine during nicotine withdrawal.

Amino acid modulation of dopamine during withdrawal is age dependent. A major finding of the present study is that adolescent rats do not exhibit overall increases in VTA inhibitory tone during precipitated nicotine withdrawal. Specifically, in contrast to adults, there were no significant changes in either VTA glutamate or GABA transmission in adolescents during withdrawal. Moreover, adolescents did not display significant correlations between NAcc dopamine and VTA glutamate or GABA. This suggests that underdeveloped inhibitory regulation of NAcc dopamine in adolescents may be responsible for age group differences in

withdrawal. Relative to an adult experiencing withdrawal, adolescents exhibited *enhanced* excitatory and *reduced* inhibitory modulation of the dopamine cell bodies in the VTA. This age group difference in amino acid transmission during nicotine withdrawal is suggested to minimize reductions in dopamine cell excitability and prevent large decreases in NAcc dopamine of adolescent versus adult rats.

Rationale for enhanced glutamate and reduced GABAergic modulation of dopamine during adolescence. Developmental studies have suggested that the period of adolescence is characterized by *enhanced* excitatory and *reduced* inhibitory systems. Thus, it is possible that this age-related imbalance toward excitation in adolescents buffers against the decreases in excitation and increases in inhibition observed in the VTA during nicotine withdrawal in adults.

With respect to *enhanced* excitatory systems during adolescence, excitatory amino acid pathways undergo transient overshoots and overproduction of glutamate terminals during adolescence (de Graff-Peters and Hadders-Algra, 2006; McDonald and Johnston, 1990). For example, glutamate innervation of the frontal cortex is overproduced during early postnatal periods in rats (7-14 days) and humans (1-2 years; see Herlenius and Lagercrantz, 2004). Although pruning of excitatory projections occurs during adolescence, increased density of glutamate receptors and overproduction of synapses support the hypothesis that adolescence reflects a period of enhanced glutamate cortical innervation (Dunah et al., 1996; Herlenius and Lagercrantz, 2004). Other research has shown that the presence of glutamate receptors such as NMDA is ubiquitously expressed in the immature brain to drive synaptic plasticity (Lujan et al., 2005). In fact, the NR2B NMDA receptor subunit is highly expressed in early development, and is suggested to enhance activation of cation channels and increase the ability of glutamate to

strengthen synapses. Thus, enhanced excitatory regulation of the mesolimbic pathway may partly explain why adolescents experience lower deficits in NAcc dopamine transmission relative to adults during nicotine withdrawal.

With respect to *reduced* GABA systems during adolescence, before taking on its role as an inhibitory neurotransmitter, GABA serves as a trophic factor during early development of neural organization (see Kellogg, 1998). Although GABA is the main inhibitory transmitter in the mature brain, paradoxically, it is an excitatory neurotransmitter in early postnatal life (Ben-Ari et al., 1997; Cherubini et al., 1991; Lujan et al., 2005; Rivera et al., 1999). This is due to an inverted chloride gradient early in development (Herlenius and Lagercrantz, 2004). In young nerve cells, the chloride concentration is high such that when GABA activates chloride channels, these anions exit the cell following their concentration gradient and induce an excitatory depolarization response. However, during maturation, the chloride concentration reverses so that these anions are present at higher concentrations outside the cell. Thus, the opening of chloride channels by GABA allows these anions to enter the cell following their concentration gradient to produce an inhibitory hyperpolarization response. In this way, GABA switches from being an excitatory to inhibitory neurotransmitter in the developing brain.

Electrophysiological studies have also demonstrated that during adolescence, inhibitory postsynaptic potentials (IPSP) in GABA neurons are slower, less frequent, and weaker in response to GABA agonists relative to neurons from more mature animals (see Cohen et al., 2000). The latter report demonstrated that by postnatal day 21, GABA agonist-induced IPSPs are still weak reflecting continued immaturity in synaptic structure and function persisting through adolescence. Also, GABA-mediated inhibition by postsynaptic $GABA_B$ receptors is not functional early in life and GABA currents in neonatal rat neurons are insensitive to

benzodiazepine activation of $GABA_A$ receptors, suggesting that there is immaturity in synaptic function during early development (Cherubini et al., 1991). The excitatory role of GABA switches to inhibition around 1 week after birth in the hippocampus and about postnatal day 7-14 in the frontal cortex of rat brain (Miles, 1999; Rivera et al., 2005). Although this time frame precedes the period of adolescence described here, it is suggested that GABA systems in adolescents may not be fully developed to inhibit VTA dopamine neurons. To further support this suggestion, intra-VTA administration of a $GABA_A$ antagonist in adolescents produced a lower increase in extracellular levels of NAcc dopamine relative to adults (Natividad et al., 2010). Also, the ability of toxic doses of ethanol to enhance IPSPs in GABA neurons was reduced in adolescent relative to adult rats (Li et al., 2006). Thus, reduced GABA-mediated inhibition of the mesolimbic pathway may partly explain why adolescents experience lower deficits in NAcc dopamine transmission relative to adults during nicotine withdrawal.

Alternative explanations for age group differences during withdrawal. The following section provides a consideration of alternative explanations/mechanisms than those presented above. Given that adolescence is characterized by enhanced excitatory and diminished inhibitory transmission, it is surprising that age group differences in basal levels of the amino acids were not observed. This quandary may reflect innate age group differences in the metabolism/uptake of these amino acids, and/or neurotoxic-specific effects of nicotine that may have normalized age group differences in basal neurochemistry. It is possible that our findings during withdrawal relate to changes in glial release of amino acids. In this regard, structural remodeling of neuroglial networks has been reported in the prefrontal cortex of adolescent rats repeatedly treated with methylphenidate (Cavaliere et al., 2012). Thus, nicotine-induced changes in glial

glutamate transporters may play a role in mediating age group differences in the neurochemical effects of nicotine withdrawal. It may also be possible that other mechanisms involved in nicotine-induced neuroadaptations are underdeveloped in the adolescent brain. For example, the onset of dopamine D2-mediated regulation of prefrontal cortex network activity occurs during or after adolescence (Tseng and O'Donnell, 2007) and this late-developing mechanism may also provide important inhibitory regulation of excitatory glutamate projections to the VTA (Harte and O'Connor, 2004).

The role of nicotinic receptors in mediating age group differences in the neurochemical effects of nicotine withdrawal is not well understood. Electrophysiological studies have shown that nicotine alters VTA glutamate and GABA transmission via binding to α7 and α4β2 nAChRs. Specifically, nicotinic binding to α7 receptors located on VTA glutamate terminals facilitates glutamate transmission, whereas nicotine quickly desensitizes α4β2 receptors located on VTA GABA interneurons (Dani et al., 2000; Mansvelder and McGehee, 2002; Mansvelder et al., 2002; Pidoplichko et al., 1997). The net effect is an imbalance of amino acid transmission characterized by enhanced VTA glutamate and reduced GABA transmission that facilitates NAcc dopamine transmission. The findings of this report during withdrawal show a drug-opposite pattern in these amino acid systems of adult rats (i.e., reduced VTA glutamate and enhanced GABA levels). The finding that these effects were otherwise absent in adolescents may suggest that age group differences in nicotinic receptors underlie diminished changes in amino acids levels during withdrawal. In fact, chronic nicotine has been shown to enhance nicotinic receptor binding on α7 and α4β2 receptors to a larger magnitude in adolescents versus adults (Doura et al., 2008; Levin et al., 2007; Trauth et al., 1999; Slotkin et al., 2004). Thus, enhanced nicotinic receptors that have been previously shown to modulate amino acid activity in the VTA

may begin to describe why adolescents display resistance to decreases in NAcc dopamine during nicotine withdrawal. Future studies might examine the involvement of α7 and α4β2 receptors in mediating age group differences in the neurochemical effects of nicotine withdrawal.

Are all nicotine-induced effects diminished during adolescence? It is important to note that not all nicotine-induced adaptations are impaired in adolescent rodents. For example, unpublished studies in our laboratory have shown that extracellular levels of acetylcholine in the NAcc are increased in a similar manner during nicotine withdrawal in adolescent and adult rats. Although withdrawal-induced increases in cholinergic transmission were similar across these age groups, baseline levels of NAcc acetylcholine were higher in nicotine-treated adolescents versus adults. In the same regard, adolescents display enhanced neurochemical and molecular responses in the mesolimbic reward pathway following nicotine as compared to adults. For example, acute nicotine enhanced long-term potentiation processes modulated by AMPA receptors in the VTA of adolescent versus adult mice (Placzek et al., 2009). Nicotine also stimulated a greater release of [^{3}H] dopamine in striatal synaptosomes of adolescent versus adult rats (Azam et al., 2007). Lastly, nicotine increased extracellular levels of NAcc dopamine to a larger magnitude in adolescent versus adult rats (Shearman et al., 2008). Thus, it is suggested that adolescents may have heightened expression of mechanisms involved in mediating the positive rewarding effects of nicotine.

Are the hypothesized mechanisms sufficient to understand age group differences in nicotine? The proposed framework provides a hypothesis regarding age group differences in nicotine withdrawal. This mechanism involves dopamine transmission in the mesolimbic

pathway and its regulation via excitatory and inhibitory mechanisms in the cell body region of this pathway. This hypothesis is based on existing work that emphasizes the importance of the mesolimbic pathway and it builds upon this literature by suggesting that amino acid systems in the VTA may contribute to age group differences in nicotine withdrawal.

Although this framework focuses specifically on one neural system, it is recognized that the proposed mechanisms are not likely sufficient to explain the complex mechanisms that modulate age group differences in the behavioral effects of nicotine and withdrawal from this drug. For example, emerging data suggests that stress systems play an important role in mediating nicotine dependence (see Koob and Kreek, 2007). Several studies have shown that adolescent animals display lower anxiety-like responses to nicotine and withdrawal from this drug, suggesting that differences in stress responses may modulate age group differences in nicotine. Other work has demonstrated that opioid receptor antagonists precipitate withdrawal signs (Biala et al., 2005; Malin et al., 1993) and induce CPA in nicotine-treated adult rats (Ise et al., 2000; Watkins et al., 2000). In the same regard, unpublished studies in our laboratory have shown that administration of a kappa-opioid receptor agonist triggers nicotine withdrawal in adult but not adolescent animals. These findings suggest that age group differences in opioid systems may also contribute to age group differences in nicotine withdrawal. Further, it is recognized that other dopamine pathways in the brain contribute to withdrawal states. For example, a microdialysis study examining the neurochemical effects of mesocortical dopamine during morphine withdrawal revealed that noradrenaline modulated an increase in dopamine levels of the prefrontal cortex (Devoto et al., 2002). These findings suggest that not all dopamine pathways of the brain are regulated in a manner to produce deficits in dopamine during

withdrawal. Thus, more studies are needed to unveil the complex milieu of neural systems that modulate enhanced vulnerability to tobacco abuse during adolescence.

IV. Clinical Relevance

There are several clinical implications of the proposed hypothesis regarding the rewarding effects of nicotine. First, nicotine produces strong rewarding effects that drive tobacco use during adolescence. Also, it is likely that tobacco use enhances the positive effects of other substances, such as alcohol that is commonly abused in adolescents (Schmid et al., 2007). Enhanced positive effects of nicotine may also facilitate affective states produced by social interaction and risk-taking behavior that elicit positive affective states in adolescents. Thus, the most effective avenue for reducing smoking behavior may involve avoidance or preventative strategies that minimize tobacco use during adolescence.

There are also several clinical implications of the proposed hypothesis regarding nicotine withdrawal. First, the current diagnostic criteria for nicotine dependence are steeped in terms that are used to diagnose negative affective states during nicotine withdrawal in adults that are long-term smokers. However, these diagnostic criteria may need to be reconsidered for adolescent tobacco users that experience less nicotine withdrawal compared to adults. Second, adolescents may be less likely to consider quitting because they are less aware of their dependence upon nicotine due to a lack of withdrawal symptoms. However, it should be noted that adolescents may also be less likely to consider quitting if they experience high levels of withdrawal that maintain continued use. Lastly, the current treatment strategies for smoking cessation focus on alleviating withdrawal via

pharmacological approaches or nicotine replacement therapies. However, a lack of withdrawal during adolescence implies that these treatments may be less effective in adolescent tobacco users. Consistent with this suggestion, clinical studies in adolescents have found that long-term abstinence rates were not closely associated with nicotine replacement therapies (Hanson et al., 2003: Hurt et al., 2000; Moolchan et al., 2005). Furthermore, there is some evidence to suggest that the nicotine patch did not prevent the expression of nicotine withdrawal symptoms in adolescent smokers (Killen et al., 2001).

V. Future Directions

The hypotheses presented in this dissertation are meant to provide a framework for the study of the psychobiological substrates mediating adolescent tobacco use. Although the neurochemical hypotheses focused on cross-sectional differences between adolescents and adults, future developmental studies may examine a broader range of many ages. Moreover, the findings here may present an inroad for determining the mechanisms that confer enhanced vulnerability to nicotine addiction in adults that were exposed to nicotine during adolescence.

The present studies elucidated the neurochemical effects modulating age group differences in nicotine withdrawal. However, it is not well-understood whether similar mechanisms mediate age group differences in the neurochemical effects produced by nicotine administration. Age group differences might be expected since a microdialysis study showed that NAcc dopamine levels were higher in adolescents versus adults following nicotine (Shearman et al., 2008). Moreover, acute nicotine enhanced long term potentiation

to a larger degree in the VTA dopamine neurons of adolescent versus adult mice (Placzek et al., 2009). Thus, it is possible that the proposed neurochemical hypothesis that modulates age group differences in withdrawal may be responsible for mediating age group differences in the neurochemical effects produced by nicotine. Based on the present findings, it may be hypothesized that *enhanced* glutamate and *reduced* GABA transmission facilitates a *larger* increase in NAcc dopamine transmission of adolescent versus adult rats following nicotine.

Finally, more studies are needed to functionally validate the proposed role of VTA amino acid mechanisms in modulating age group differences in nicotine and withdrawal from this drug. For example, based on the present findings, one might expect that glutamate receptor expression or binding may be lower, whereas GABA receptor activity may be higher in the VTA of adults versus adolescents experiencing withdrawal. Reversing the inhibitory effects with glutamate agonists or GABA antagonists may present an avenue for testing novel therapeutics to treat withdrawal in adults. Future experiments might also elucidate the role of nicotinic receptors in mediating age group differences in the rewarding effects of nicotine. As an example, studies might use α7 antagonists to reduce excitatory processes (i.e., glutamate transmission) that are believed to facilitate the rewarding effects of nicotine, and this would be expected to reduce tobacco use in adolescents more so than adults. Recently approved medications have already begun to explore such possibilities with a partial agonist of the α4β2 receptor site (i.e., varenicline) that has been reported to reduce withdrawal symptoms and the rewarding effects of nicotine (Rollema et al., 2007). The extent to which these medications may alter amino acid transmission in an age dependent manner to promote long-term abstinence is a future avenue of research to be followed.

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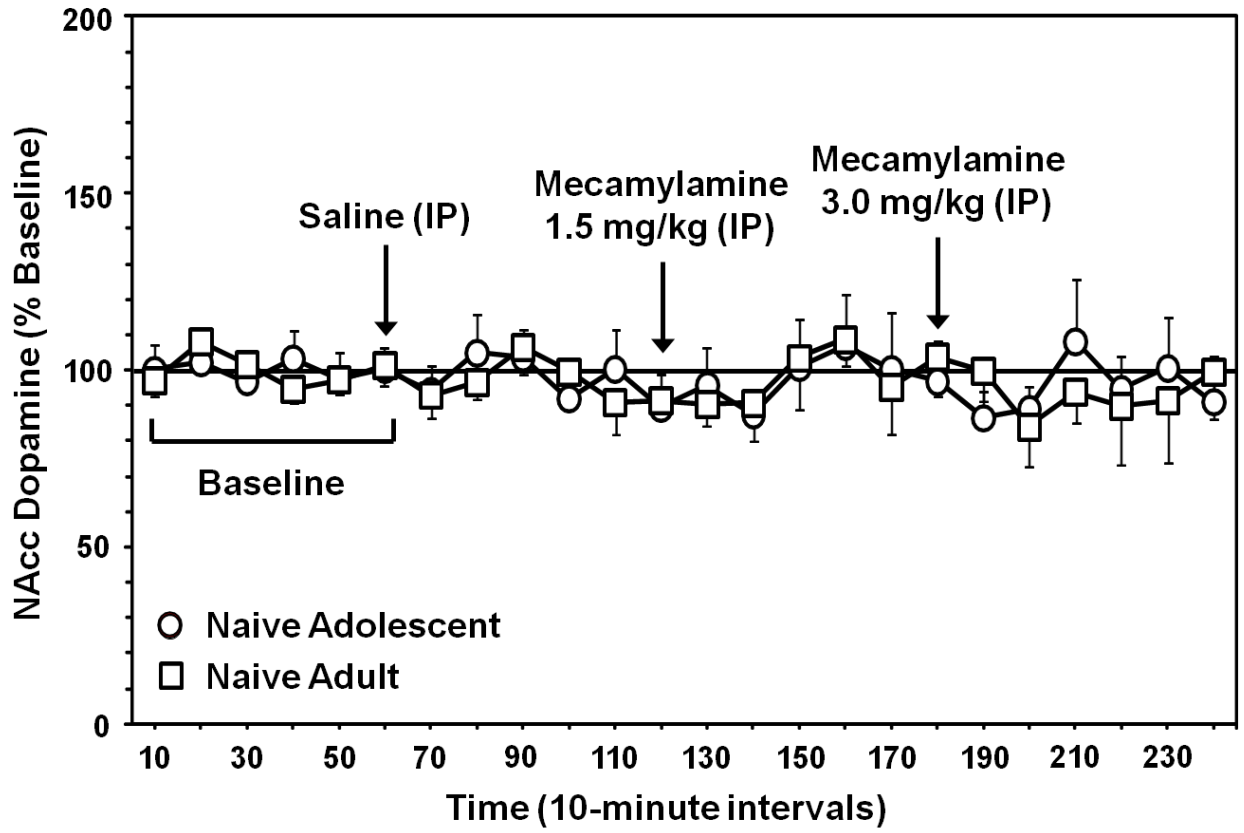

Figure 1: Mecamylamine did not alter NAcc dopamine transmission in naive adolescent or adult rats. Data reflect % change in extracellular levels of NAcc dopamine (± SEM) plotted across 10-minute sample collections during baseline and following administration of saline and then 2 doses of mecamylamine in nicotine-naïve adolescent (n=6) and adult (n=6) rats. The arrows indicate the onset of drug administration. Mecamylamine did not produce any significant changes in NAcc dopamine relative to baseline in either age group.

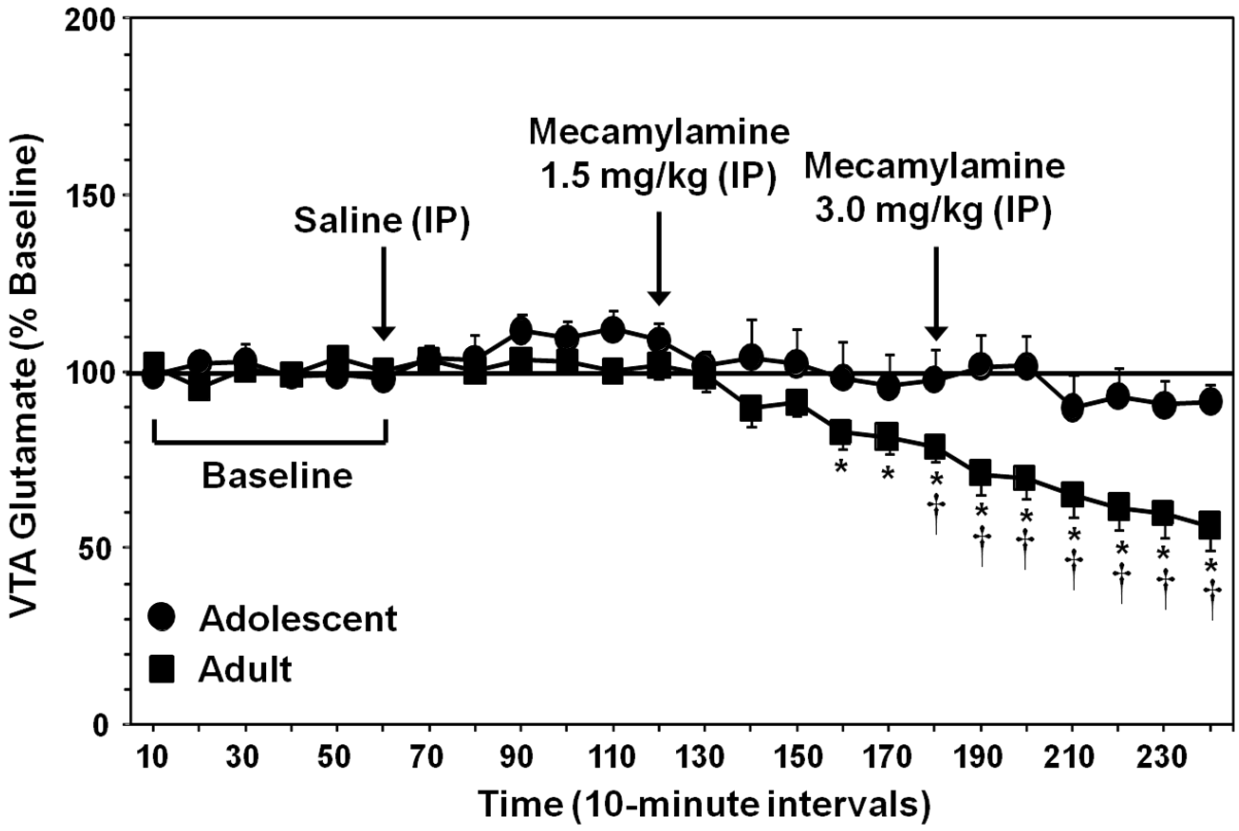

Figure 2: Nicotine withdrawal produced a decrease in VTA glutamate transmission that was larger in adult versus adolescent rats. Data reflect % change in extracellular levels of VTA glutamate (± SEM) plotted across 10-minute sample collections during baseline and following administration of saline and then 2 doses of mecamylamine to precipitate withdrawal in nicotine-treated adolescent (n=7) and adult (n=14) rats. The arrows indicate the onset of drug administration. Mecamylamine produced a decrease in VTA glutamate relative to baseline in adult (*$p<0.05$) rats that was more robust relative to adolescents (†$p<0.05$).

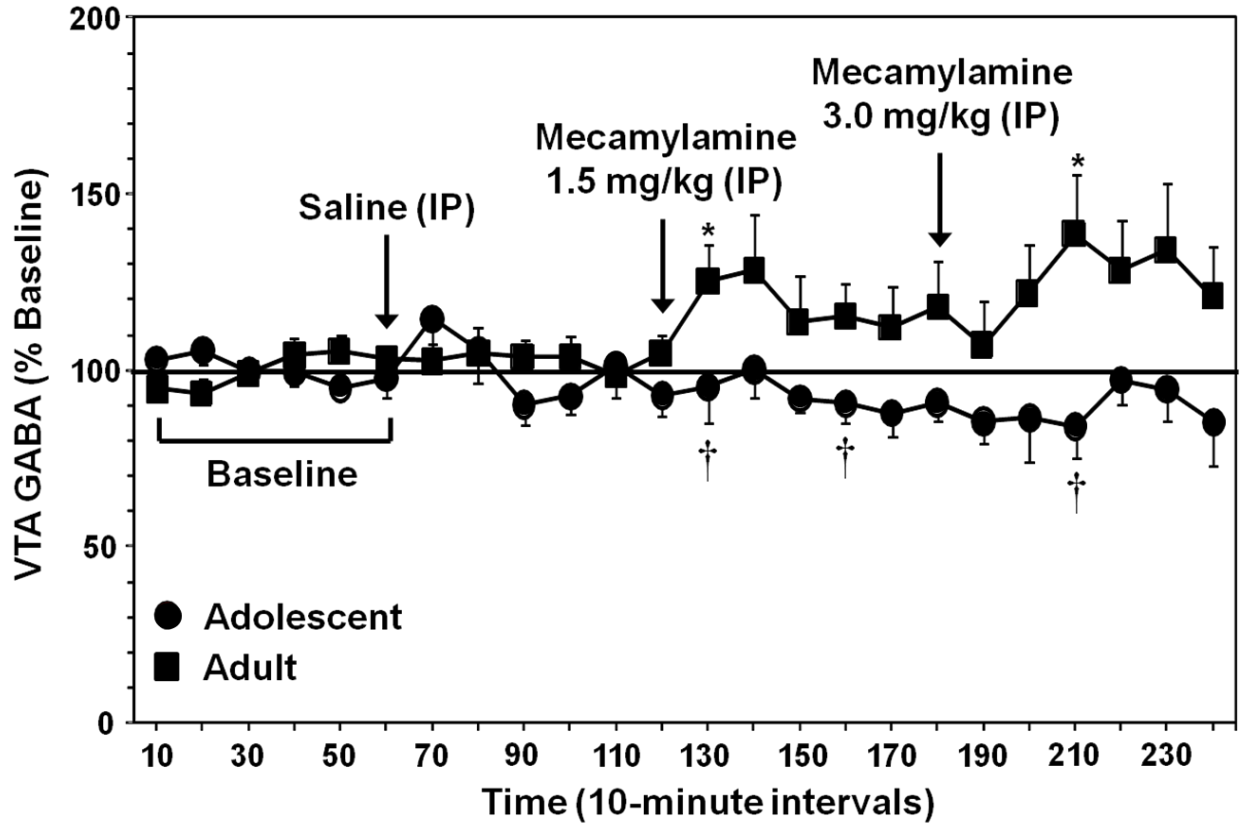

Figure 3: Nicotine withdrawal produced an increase in VTA GABA transmission that was larger in adult versus adolescent rats. Data reflect % change in extracellular levels of VTA GABA (± SEM) plotted across 10-minute sample collections during baseline and following administration of saline and then 2 doses of mecamylamine to precipitate withdrawal in nicotine-treated adolescent (n=7) and adult (n=14) rats. The arrows indicate the onset of drug administration. Mecamylamine produced an increase in VTA GABA relative to baseline in adult (*$p<0.05$) rats that was more robust relative to adolescents (†$p<0.05$).

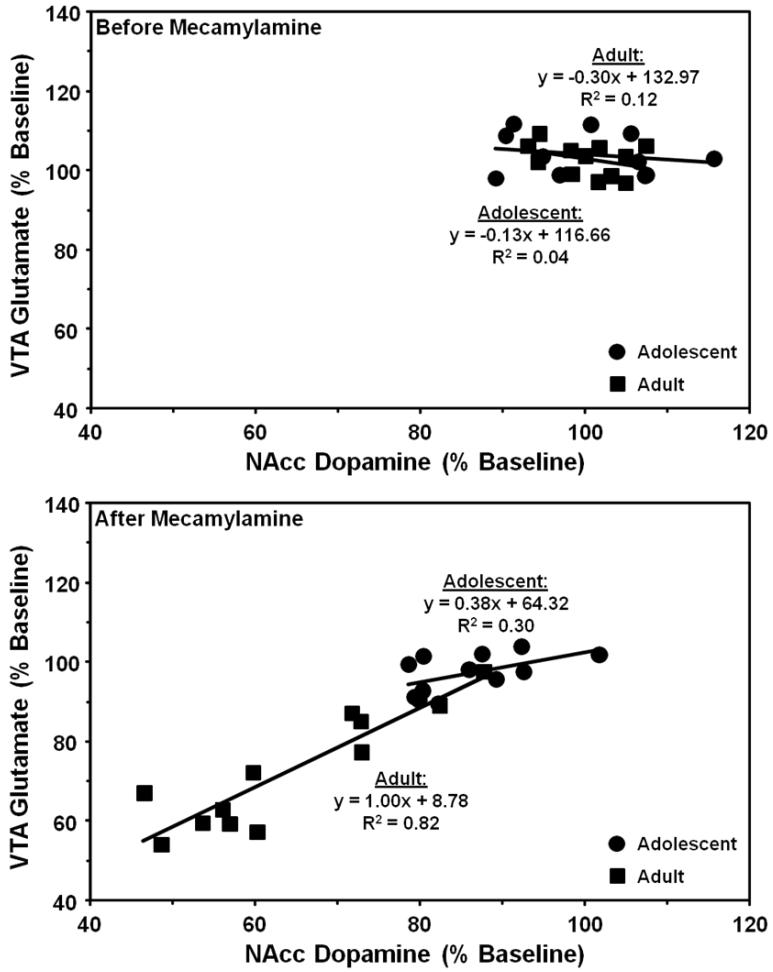

Figure 4: Nicotine-treated adults experiencing withdrawal display a stronger correlation between VTA glutamate and NAcc dopamine versus adolescents. Data reflect % change from baseline in VTA glutamate plotted against NAcc dopamine in nicotine-treated adolescent (n=7) and adult (n=7) rats. Each point reflects an average value across all rats for dialysate fractions that were collected before (top panel) and after (bottom panel) mecamylamine administration. Thus, the data points per age group reflect an average of the 12 points collected before mecamylamine (i.e., baseline and saline) and 12 points after the 2 doses of mecamylamine. Dopamine and glutamate were positively correlated in adults (R^2=0.82; $p \leq 0.05$) following mecamylamine administration, whereas adolescents did not display a significant correlation (R^2=0.30). The correlation was stronger in adults as compared to adolescents ($p \leq 0.05$).

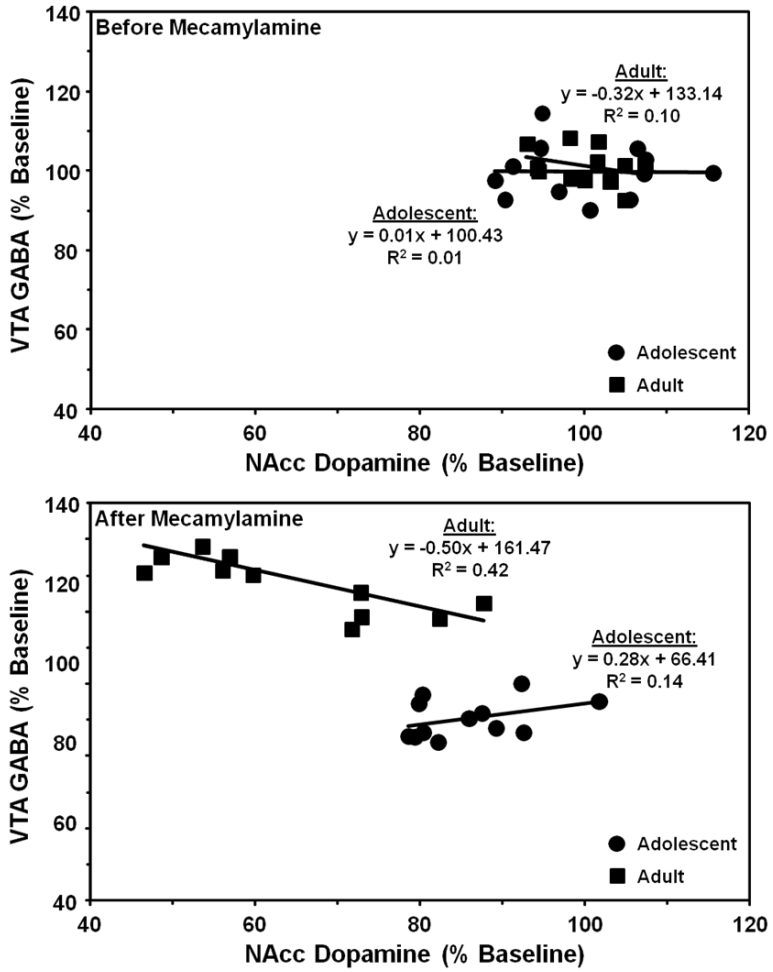

Figure 5: Nicotine-treated adults experiencing withdrawal display a stronger correlation between VTA GABA and NAcc dopamine versus adolescents. Data reflect % change from baseline in VTA GABA plotted against NAcc dopamine in nicotine-treated adolescent (n=7) and adult (n=7) rats. Each point reflects an average value across all rats for dialysate fractions that were collected before (top panel) and after (bottom panel) mecamylamine administration. Thus, the data points per age group reflect averages of the 12 points collected before mecamylamine (i.e., baseline and saline) and 12 points after the 2 doses of mecamylamine. Dopamine and GABA were negatively correlated in adults (R^2=0.42; $p \leq 0.05$) following mecamylamine administration, whereas adolescents did not display a significant correlation between these measures (R^2=0.14). The correlation was stronger in adults as compared to adolescents ($p \leq 0.05$).

Curriculum Vitae

Luis Alberto Natividad was born to Pedro and Margarita in El Paso, Texas on January 9th, 1980. He graduated from Stephen F. Austin High School in May, 1998 and entered the University of Texas at El Paso (UTEP). During this time, Luis was an Americorp National Service member where he mentored underprivileged students. Thereafter, he entered the University of Texas at Austin to complete his undergraduate studies in Psychology. He became interested in Neuroscience and began research work with Dr. Adriana Alcantara whose laboratory focused on studying the neural mechanisms of alcohol addiction. Luis received his Bachelor of Arts degree in May, 2002 and obtained an internship as a drug addiction counselor. He became interested in age-related issues in drug abuse, particularly the rampant drug use among teenagers. This inspired him to explore the neural basis of adolescent vulnerability to drug addiction. Thus, he entered the Social, Cognition, and Neuroscience program at UTEP in August, 2005 where he received training from Dr. Laura O'Dell. Her laboratory combines behavioral and biochemical tools to study the mechanisms that drive nicotine addiction in rats of different ages. Luis received his Master's of Arts degree in May, 2009. He has presented 43 poster abstracts and 6 talks in nation-wide conferences. He published a first-author paper in *Synapse* and is a co-author on 3 other publications. He is the recipient of 2 fellowships from the National Institute of Health. He received recognitions of excellence from the National Hispanic Science Network and recently from UTEP via a dissertation fellowship award. Dr. Natividad plans to continue studying the neural basis of drug addiction. He will begin his postdoctoral work at the Scripps Research Institute in La Jolla, California in the laboratory of Dr. Loren Parsons.

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Education
1. Ph.D. (2012): UTEP- Psychology with emphasis in Neuroscience
2. M.A. (2009): UTEP- Experimental Psychology
3. B.A. (2002): University of Texas at Austin- Psychology

Grants
1. January 2012- current: UTEP- Natalicio Dissertation Fellowship Award
2. June 2008- June 2011: National Institute of Health, Ruth L. Kirschstein National Research Service Award Pre-doctoral Fellowship- National Institute on Drug Abuse (F31-DA021133)
3. May 2006- April 2007: American Psychological Association, Diversity Program in Neuroscience Fellowship- National Institute of Mental Health (T32-MH018882)

Honors and Awards
1. April, 2011: UTEP- Student representative in the steering committee for the Outstanding Teaching Award by a Graduate Student
1. May, 2011: Texas Tech Medical School Research Colloquium- Outstanding Student Presentation Award
2. February, 2011- current: UTEP- Student representative in the Graduate Program Committee
3. October, 2009: National Hispanic Science Network on Drug Abuse- Excellence in Research by a Graduate Student Award
4. June, 2009: National Hispanic Science Network on Drug Abuse and the National Institute of Health (NIH)- Summer Internship Training Fellow
6. June 2006: Marine Biological Laboratories- Summer Program in Neuroscience, Ethics and Survival (SPINES) and Post-course Research Fellow
7. May 2005: National Hispanic Science Network on Drug Abuse- Interdisciplinary Research Training Institute on Hispanic Drug Abuse Fellow
8. April 2005: UTEP- Graduate Excellence Scholarship Award
9. August 1999; June 2000: Americorp National Service Award- National Service Education Award
10. August 1998: UTEP- Undergraduate University Academic Scholarship

Travel Awards

1. August, 2011: National Institute on Drug Abuse to attend meeting at Society for Neuroscience conference
2. August, 2011: National Hispanic Science Network meeting
3. March, 2011: Behavior, Biology and Chemistry meeting
4. August, 2010: National Institute on Drug Abuse to attend meeting at Society for Neuroscience conference
5. March, 2010: Behavior, Biology and Chemistry meeting
6. August, 2009: National Institute on Drug Abuse to attend the American Psychological Association meeting
7. March, 2009: Behavior, Biology and Chemistry meeting
8. March 2008: Primm-Singleton Underrepresented Population Committee to attend the College on Problems of Drug Dependence meeting

Research Experience

1. January 2005- Current: UTEP
Title: Ph.D. student in the Social, Cognition, and Neuroscience program
Training: Conducted research examining the behavioral and neurochemical effects that mediate developmental sensitivity to nicotine addiction in rats. I gained research experience in behavioral techniques such as conditioned place procedures, physical assessment of drug withdrawal, and intravenous self-administration. I also learned biochemical techniques for assaying neurochemicals in the brain using high performance liquid chromatography.
Contact: Laura E. O'Dell, Ph.D., Graduate Mentor and Associate Professor
UTEP- Department of Psychology in El Paso, Texas
E-mail: lodell@utep.edu

2. June, 2009- August, 2009: National Institute on Drug Abuse
Title: Summer Internship Fellow
Training: Participated in neurochemical projects studying opioid-receptor modulation of the neurochemical effects of cocaine in rats. I gained experience in capillary electrophoresis coupled with laser-induced fluorescence detection for assaying amino acids.
Contact: Toni Shippenberg, Ph.D., Section Chief of Integrative Neuroscience
National Institute on Drug Abuse- Intramural Research Program in Baltimore, Maryland
E-mail: tshippen@intra.nida.nih.gov

3. June, 2006- July, 2006: Marine Biological Laboratories
Title: Summer Research Fellow
Training: Participated in a series of whole cell, patch-clamp experiments studying the effects of noradrenaline in granule cells of the olfactory bulb in mice. I gained experience in basic theory and methods in electrophysiology.
Contact: Ricardo C. Araneda, Assistant Professor
University of Maryland- Department of Biology in College Park, Maryland
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4. September 2001-May 2002: University of Texas at Austin
Title: Laboratory Assistant

Training: Participated in research studying ethanol-induced changes in expression of dopamine receptors in rats. I gained research experience in immunocytochemistry techniques and brain preparation.
Contact: Dr. Adriana Alcantara, Ph.D., Undergraduate Mentor and Associate Professor
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E-mail: aaalcant@central.uh.edu

Publications
1. Natividad, L.A., Tejeda, H.A., Orfila, J. E., Torres, O.V., O'Dell, L.E. Dysregulation of kappa-opioid receptor systems by chronic nicotine modulate the nicotine withdrawal syndrome in an age-dependent manner. Psychopharmacology. Currently under review.
2. Natividad, L.A., Parsons, L.H., Torres, O.V., O'Dell, L.E. Adolescent rats are resistant to adaptations in excitatory and inhibitory mechanisms that modulate mesolimbic dopamine during nicotine withdrawal. Journal of Neurochemistry. Currently under review.
3. Natividad, L.A. (2011). Developmental differences in nicotine withdrawal: Implications for understanding adolescent vulnerability to tobacco abuse. Published in the El Faro online newsletter sponsored by the National Hispanic Science Network on Drug Abuse, April 3(4).
4. Natividad, L.A., Tejeda, H.A., Torres, O.V., O'Dell, L.E. (2010). Nicotine withdrawal produces a decrease in extracellular levels of dopamine in the nucleus accumbens that is lower in adolescent versus adult male rats. Synapse, 64(2):136-145.
5. Torres, O.V., Natividad, L.A., Tejeda, H.A., Van Weelden, S.A., O'Dell, L.E. (2009). The rewarding and aversive effects of nicotine in female rats are age-, hormone-, and sex-dependent. Psychopharmacology, 206(2):303-12.
6. Torres, O.V., Tejeda, H.A., Natividad, L.A., O'Dell, L.E. (2008). Enhanced vulnerability to the rewarding effects of nicotine during the adolescent period of development. Pharmacology, Biochemistry and Behavior, 90: 658-663.
7. O'Dell, L.E., Torres, O.V., Natividad, L.A., Tejeda, H.A. (2007). Adolescent nicotine exposure produces less affective measures of withdrawal relative to adult nicotine exposure in male rats. Neurotoxicolgy and Teratology, 29(1): 17-22.

Abstract Presentations
1. Jackson, J.A., Natividad, L.A., Torres, I.D., Nazarian, A. O'Dell, L.E. The rewarding effects of nicotine are enhanced in diabetic rats, an effect that appears to be mediated via suppressed dopamine systems. Behavior, Biology, Chemistry (BBC), 2012.
2. Jackson, J.A., Natividad, L.A., Torres, I.D., Nazarian, A. O'Dell, L.E. The rewarding effects of nicotine are enhanced in diabetic rats. College on Problems of Drug Dependence (CPDD), 2012.
3. Natividad, L.A., Parsons, L.H., Orfila, J.E., Torres, O.V., O'Dell, L.E. Periadolescent rats are resistant to adaptations in excitatory and inhibitory mechanisms that modulate mesolimbic dopamine during nicotine withdrawal. Society for Neuroscience (SFN), 2011.
4. Orfila, J.E., Torres, I.D., Natividad, L.A., Castañeda, E., and O'Dell, L.E. Examination of cholinergic levels in the nucleus accumbens during nicotine exposure and withdrawal. SFN, 2011.
5. O'Dell, L.E., Natividad, L.A., Escalante, E., Torres, I.D., Nazarian, A. The rewarding effects of nicotine are enhanced in diabetic rats. SFN, 2011.

6. Natividad, L.A., Orfila, J.E., Torres, O.V., Parsons, L.H., O'Dell, L.E. Adolescent rats are resistant to adaptations in excitatory and inhibitory mechanisms that modulate mesolimbic dopamine during nicotine withdrawal. National Hispanic Science Network (NHSN) meeting, 2011.
7. Natividad, L.A., Escalante, E., Mangubat, M., Chang-Sung., S., Torres., O.V., Friedman, T.C., and O'Dell, L.E. Age differences in food-intake and the weight-suppressant effects of self-administered nicotine. Endocrine Society, 2011.
8. Torres, O.V., Natividad, L.A., Muñiz, A.K., Byers, D.M., O'Dell, L.E. Behavioral, biochemical and molecular indices of nicotine withdrawal: differential impact of sex on stress-related markers. CPDD, 2011.
9. Natividad, L.A., Orfila, J.E., Torres, O.V., Parsons, L.H., O'Dell, L.E. Developmental differences in nicotine withdrawal are mediated via enhanced excitatory and reduced inhibitory mechanisms that regulate dopamine transmission in the mesolimbic pathway. BBC, 2011.
10. Orfila, J.E., Torres, I., Natividad, L.A., Castañeda, E., and O'Dell, L.E. Cholinergic levels in the nucleus accumbens (NAcc) are enhanced in adolescent versus adult rats exposed to nicotine but are similar in both age groups following nicotine withdrawal. BBC, 2011.
11. Torres, O.V., Natividad, L.A., Byers, D.M., O'Dell, L.E. Developmental and sex differences in the expression of the molecular targets in a rat model of nicotine withdrawal. BBC, 2011.
12. Natividad, L.A., Escalante, E., Torres, O.V., Tejeda, H.A., Friedman, T.C., O'Dell, L.E. Age differences in the rewarding and weight-suppressant effects of nicotine. SFN, 2010.
13. Orfila, J.E., Torres, I.D., Natividad, L.A., Castañeda, E., and O'Dell, L.E. Cholinergic levels in the nucleus accumbens (NAcc) are enhanced in adolescent versus adult rats exposed to nicotine but are similar in both age groups following nicotine withdrawal. SFN, 2010.
14. Torres, O.V., Natividad, L.A., Walker, E.M., Muñiz, A.K., and O'Dell, L.E. Differential impact of sex on stress-related markers during nicotine withdrawal. SFN, 2010.
15. Orfila, J.E., Torres, I.D., Natividad, L.A., Castañeda, E., and O'Dell, L.E. Nicotine withdrawal produces similar changes in cholinergic transmission in the nucleus accumbens of adolescent versus adult rats. NHSN, 2010.
16. Orfila, J.E., Torres, I.D., Natividad, L.A., Castañeda, E., and O'Dell, L.E. Cholinergic transmission in the nucleus accumbens is lower in adolescent versus adult rats experiencing nicotine withdrawal. CPDD, 2010.
17. Orfila, J.E., Torres, I.D., Natividad, L.A., Castañeda, E., and O'Dell, L.E. Nicotine withdrawal produces similar changes in cholinergic transmission in the nucleus accumbens of adolescent versus adult rats. BBC, 2010.
18. Escalante, E., Natividad, L.A., Roman, F., and O'Dell, L.E. The rewarding effects of nicotine are enhanced in diabetic rats. BBC, 2010.
19. Torres, O.V., Natividad, L.A., Walker, E.M., Muñiz, A.K., and O'Dell, L.E. Nicotine withdrawal enhances anxiety-like behavior in female versus male rats. BBC, 2010.
20. Natividad, L.A., Roman, F., Torres, O.V., Tejeda, H.A., and O'Dell, L.E. Exposure to nicotine during adolescence alters intake of the drug later in adulthood. NHSN, 2009.
21. Torres, O.V., Muniz, A., Roman, F., Beas, B.S., Natividad, L.A., and O[1]Dell, L.E. Nicotine withdrawal is diminished during adolescence in female and male rats. NHSN, 2009.
22. Natividad, L.A., Tejeda, H.A., Torres, O.V., Castañeda E., and O'Dell, L.E. The neurochemical effects of nicotine withdrawal on dopamine transmission in the nucleus accumbens are lower in adolescent relative to adult rats. American Psychological Association, 2009.

23. Torres, Oscar V., Natividad, L.A., Byers, Donna M., Tejeda, Hugo A. and O'Dell, Laura E. Nicotine withdrawal enhances anxiety-like behavior and expression of stress-related genes in female versus male rats. BBC, 2009.
24. Orfila J.E., Tejeda H.A., Natividad L.A., Torres O.V., Castañeda E., and O'Dell L.E. The behavioral and neurochemical effects produced by kappa-opioid receptor stimulation are diminished in nicotine-dependent adolescent versus adult rats. BBC, 2009.
25. Tejeda, H.A., Torres, O.V., Natividad, L.A., Orfila, J.R., Castañeda, E., and O'Dell, L.E. Stimulation of kappa opioid receptors elicits nicotine withdrawal in adult but not adolescent rats. NHSN, 2008.
26. Torres, O.V., Natividad, L.A., Tejeda, H.A., and O'Dell, L.E. The rewarding effects of nicotine are age-, hormone- and sex-dependent in rats. NHSN, 2008.
27. Natividad, L.A., Tejeda, H.A., Torres, O.V., Castañeda, E., and O'Dell, L.E. Robust developmental differences to the neurochemical effects of nicotine withdrawal are not observed following nicotine administration in adolescent versus adult rats. SFN, 2008.
28. Tejeda, H.A., Natividad, L.A., Torres, O.V., Castañeda, E., and O'Dell, L.E. The behavioral and neurochemical effects produced by kappa-opioid receptor stimulation are diminished in nicotine-dependent adolescent versus adult rats. SFN, 2008.
29. Torres, O.V., Van Weelden, S.A., Natividad, L.A., Tejeda, H.A., B.S., Beas, and O'Dell, L.E. The rewarding effects of nicotine are enhanced in female adolescent rats relative to adults that display rewarding or aversive effects in a hormone-dependent manner. SFN, 2008.
30. Natividad, L.A., Tejeda, H.A., Torres, O.V., and O'Dell, L.E. Diminished neurochemical effects of nicotine withdrawal in adolescent versus adult rats. CPDD, 2008.
31. Tejeda, H.A., Torres, O.V., Natividad, L.A., Beas, B.S., and O'Dell, L.E. Stimulation of kappa-opioid receptors induces the behavioral effects of nicotine withdrawal in nicotine-dependent adult but not adolescent rats. Society for Research on Nicotine and Tobacco (SRNT), 2008.
32. Byers, D.M., Natividad, L.A., Tejeda, H.A., Torres, O.V., and O'Dell, L.E. Developmental and sex differences in the expression of key molecular targets during nicotine withdrawal. SRNT, 2008.
33. Torres, O.V., Natividad, L.A., Tejeda, H.A., and O'Dell, L.E. The rewarding effects of nicotine are enhanced during adolescence in both male and female rats. SRNT, 2008.
34. Natividad, L.A., Torres, O.V., Tejeda, H.A., and O'Dell, L.E. Pre-exposure to nicotine during adolescence facilitates nicotine self-administration in adult rats given intermittent access to escalating nicotine doses. SFN, 2007.
35. Tejeda, H.A., Natividad, L.A., Torres, O.V., and O'Dell, L.E. Stimulation of kappa-opioid receptors elicits nicotine withdrawal in adult but not adolescent rats. SFN, 2007.
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38. Byers, D.M. Natividad, L.A., Irwin, L.N., and O'Dell, L.E. Molecular targets of nicotine withdrawal are differentially expressed in adolescent and adult rats. CPDD, 2007.
39. Torres, O.V., Tejeda, H.A., Natividad, L.A., and O'Dell, L.E. Reduced nicotine withdrawal may contribute to enhanced tobacco use during adolescence. NHSN, 2006.

40. Natividad, L.A., Torres, O.V., Tejeda, H.A., and O'Dell, L.E. Nicotine withdrawal produces a decrease in dopamine release in the nucleus accumbens of adult, but not adolescent rats. SFN, 2006.
41. Torres, O.V., Tejeda, H.A., Natividad, L.A., and O'Dell, L.E. Enhanced nicotine reward and diminished nicotine withdrawal in adolescent versus adult rats. SFN, 2006.
42. O'Dell, L.E., Natividad, L.A., Torres, O.V., and Tejeda, H.A. The affective properties of nicotine withdrawal are diminished in adolescent versus adult rats. CPDD, 2005.
43. Torres, O.V., Natividad, L.A., Tejeda, H.A., and O'Dell, L.E. Diminished nicotine withdrawal in adolescent rats: Implications for vulnerability to addiction. Faculty for Undergraduate Neuroscience at the SFN, 2005.

Oral Presentations

1. Natividad, L.A., Orfila, J.E., Torres, O.V., Parsons, L.H., O'Dell, L.E. Adolescent rats are resistant to adaptations in excitatory and inhibitory mechanisms that modulate mesolimbic dopamine during nicotine withdrawal. Presented as a data-blitz at the National Hispanic Science Network meeting, 2011.
2. Natividad, L.A., Orfila, J.E., Torres, O.V., Parsons, L.H., O'Dell, L.E. The mechanisms that mediate developmental sensitivity to nicotine withdrawal involve amino acid regulation of mesolimbic dopamine systems. Texas Tech Research Colloquium, 2011. El Paso, Texas
3. Natividad, L.A., Torres, O.V., Escalante, E., O'Dell, L.E. The rewarding effects of nicotine are enhanced in adolescent rats and adults that were pre-exposed to nicotine during adolescence. Behavior, Biology and Chemistry, 2010. San Antonio, Texas.
4. Natividad, L.A., Roman, F., Torres, O.V., Tejeda, H.A., and O'Dell, L.E. Exposure to nicotine during adolescence alters intake of the drug later in adulthood. Presented as a data-blitz at the National Hispanic Science Network on Drug Abuse, 2009. Miami, Florida.
5. Natividad, L.A., Roman, F., Tejeda, H.A., Torres, O.V., Castañeda E., and O'Dell, L.E. Diminished neurochemical effects of nicotine withdrawal in adolescent versus adult rats. Behavior, Biology and Chemistry, 2009. San Antonio, Texas.
6. Natividad, L.A., Torres, O.V., Tejeda, H.A., Castañeda, E., and O'Dell, L.E. The neurochemical effects of nicotine withdrawal are different in adolescent and adult rats. National Hispanic Science Network on Drug Abuse, 2008. Washington, D.C.

Academic References

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