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THE IMPACT OF ADOLESCENT NICOTINE EXPOSURE ON DRUG DEPENDENCE IN
ADULTHOOD

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science at Virginia Commonwealth University.

by

MAI ALAJAJI
Bachelor of Pharmaceutical Sciences
King Saud University, 2004

Director: M. IMAD DAMAJ, PH.D
PROFESSOR, DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

Virginia Commonwealth University
Richmond, Virginia
July, 2010

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Table of Contents

	Page
Acknowledgements.....	ii
List of Figures.....	iv
List of Abbreviations.....	v
Abstract.....	vi
Chapter	
1. General Introduction	
1.1. Adolescent development.....	1
1.2. Adolescent Brain development.....	2
1.3. Adolescent Drug Use.....	3
1.4. Adolescence and Smoking.....	4
2. Materials and Methods	
2.1. Subjects.....	8
2.2. Drugs.....	9
2.3. Injection Protocol.....	9
2.4. Conditioned place preference.....	10
2.5. Acute Locomotor Activity.....	12
2.6. Cocaine Locomotor Sensitization.....	12
3. Studies	
3.1. methods.....	14
3.1. Results.....	16
3.2. Discussion.....	31
3.3. Future studies.....	38
Literature Cited.....	40
Vita.....	45

LIST OF FIGURES

Figure 1: Effects of Early Adolescent Nicotine Exposure on Cocaine-Induced Conditioned Place Preference.....	19
Figure 2: Effects of Late Adolescent and adult Nicotine Exposure on Cocaine-Induced Conditioned Place Preference.....	20
Figure 3: The Onset of the Cocaine Enhancement.....	21
Figure 4: The Effects of early adolescent cocaine exposure on nicotine-induced CPA in adulthood.....	22
Figure 5: The Effects of early adolescent mecamylamine-nicotine exposure on cocaine-induced CPP in adulthood.....	23
Figure 6: Effects of early adolescent nicotine exposure on morphine and amphetamine-induced CPP in adulthood.....	25
Figure 7: Effects of Early Adolescent Nicotine Exposure on Cocaine-Induced Locomotor Activity.....	27
Figure 8: Effects of Adulthood Nicotine Exposure on Cocaine-Induced Locomotor Activity.....	28
Figure 9: Effects of Early Adolescent Nicotine Exposure on Locomotor Sensitization to Cocaine.....	30

List of Abbreviations

ANOVA	analysis of variance
CPP	condition place preference
CPA	condition place aversion
DA	dopamine
Fig	figure
HIP	hippocampus
i.p.	intraperitoneal
Inj	injection
MCL	mesocorticolimbic reward pathway
mg/kg	milligrams/kilogram
min	minutes
MPE	maximal percent effect
NAC	nucleus accumbens
nAChR	nicotinic acetylcholine receptor
PFC	prefrontal cortex
PND	postnatal day
s.c.	subcutaneous
SE	standard error
Sec	seconds
SEM	standard error of the mean
STR	striatum
VTA	ventral tegmental area

Abstract

THE IMPACT OF ADOLESCENT NICOTINE EXPOSURE ON DRUG DEPENDENCE IN ADULTHOOD

By Mai alajaji, B.Pharm.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2010

Major Director: M. Imad Damaj, Ph.D
Professor, Pharmacology and Toxicology

Nicotine is one of the first and most commonly abused drugs in adolescence. According to The Center for Disease Control, every day more than 6000 adolescents try their first cigarette and over 3000 of them become daily smokers. Smoking among adolescents is a strong predictor of future drug abuse and dependence in adulthood. A number of studies has suggests that adolescents pre-exposed to nicotine may suffer permanent disruption of the brain's reward systems through changes in dopamine receptor function. We hypothesize that nicotine exposure during adolescence causes long lasting neurobiological alterations that increase the likelihood of cocaine use in adulthood. Furthermore, it activates a neurobiological mechanism that is shared by many drugs of abuse, which will increase susceptibility to their rewarding effects. The work in this thesis contributes to the further understanding of this critical developmental period. Conditioned-place-preference, acute locomotor and locomotor sensitization pardigms were used to examine changes in cocaine sensitivity in adulthood. Testing was performed on adult ICR mice that were

exposed to nicotine (0.1 or 0.5 mg/kg, S.C., b.i.d.) or saline during adolescence (postnatal days 28 or 46) or adult (postnatal day 70). Data showed that a 7-day exposure to the higher dose of nicotine (0.5 mg/kg) altered cocaine-induced responses. In contrast, neither 1 day exposure nor a low dose of nicotine (0.1 mg/kg) elicited this effect. A follow-up study was undertaken to determine if this enhancement generally applies to other drugs of abuse. Pre-exposure to 0.5mg/kg nicotine during early adolescence demonstrated significant enhancement to morphine reward, but it failed to increase d-amphetamine preference in a CPP model. Further research will be required in order to more fully examine the mechanisms of action for the observed changes in cocaine rewards. In summary, these findings suggest that early adolescent nicotine exposure leads to changes in cocaine reward and sensitivity during adulthood in both dose and duration matters. Indeed, the adolescent brain is uniquely vulnerable to the effects of nicotine on subsequent drug reward.

Introduction

1.1. Adolescent development:

Adolescence, defined as approximately ages 12 to 18 in humans and 28 to 60 postnatal days in mice and rats, is the final developmental period leading to adulthood (Spear, 2000). During this critical period a transition occurs from a fully-dependent child to an independent adult. This transition involves many changes in a variety of areas, such as physical growth, cognition, social skills, physiology, and emotions. This development maturation allows the individual to reach independence from parental care. Adolescence is generally associated with puberty (sexual maturation). However, puberty can be exactly defined in physiological terms; adolescence boundaries are less precisely defined and include both psychological and social factors (Laviola, 2003). Furthermore, adolescence stage is defined by certain behavioral changes observed in this time frame including increases in social interaction, risk-taking and novelty or reward seeking. These changes are universal across a variety of species (Spear, 2000). Indeed, over fifty percent of adolescents exhibit an increase in risk-taking behaviors such as novel experiences involving drugs, alcohol and sexual activity. Usually, risky behavior is viewed as exciting and rewarding (Arnett 1992). Similar to humans, adolescent mice have shown hyperactive behavior in novel environments (Darmani et al., 1996).

1.2. Adolescent Brain development:

The adolescent brain is unique and in a state of transition as it undergoes marked maturation that may play a role in subsequent drug abuse (Spear 2000). An adolescent brain is anatomically and neurochemically different from that of an adult brain. The adult male brain is approximately 10% larger than an adolescent brain. Human MRI images have shown a linear increase in white matter and an inverted U-shaped change in gray matter volume. Consequent to gray matter, the synaptic connections increased during the early adolescent and rapidly pruned back in late adolescence (Giedd, 2004). The adolescent brain goes through an increase in myelination and synaptic pruning to allow more efficient neural signaling. It has been predictable that as many as 50% of the average number of synapses are lost during adolescence. This appears to be associated with the marked maturation. One reason for synapse elimination is to decrease unnecessary excitatory stimuli to the brain since many of the synapses in adolescence are excitatory (Rakic et al. 1994). Moreover, the adolescent brain shows remarkable alterations in neurochemical transmission. Distinctively, the mesocorticolimbic dopamine system goes through significant modeling during adolescent periods .The balance between mesocortical and mesolimbic dopamine systems varies across a variety of species (Spear 2000). These developments are responsible for the integration of the external environment with internal drives to produce motivated behavior (Chambers et al., 2003).The prefrontal cortex (PFC) Volume decline is in humans (Sowell et al. 1999) and rats (Van Eden et al. 1990).

Moreover, density of spines on pyramidal cells in the human PFC decline (Mrzljak et al. 1990). Dopaminergic innervation of the prefrontal cortex increases in density during adolescence peak at levels well above those seen earlier or later in life (Lewis 1997; Brenhouse et al. 2008). Also, the DA transporters number increase (Akbari et al. 1992). There is also a transient increase in the number of DA receptors that has been reported (Seeman et al. 1987). In spite of that, transformations of neural circuitry are not limited to the DA system, these changes are thought to play a critical role in the rewarding and reinforcing effects of many drugs of abuse, including nicotine and cocaine. These various studies suggest that adolescence is a unique period of intense neurological development, and many of the changes that are ongoing during this period may contribute to a heightened susceptibility to substance abuse.

1.3. Adolescent Drug Use:

The age of adolescence is often the time for novelty seeking and risk taking behaviors. It is also during this period that they are introduced to the world of tobacco, alcohol, and illicit drugs. According to the National Survey on Drug Use and Health (2007), about 2.8 million children, aged 12 and above have tried illicit drugs for the first time. In fact, in 2006, the number of cocaine initiates, or those who have tasted cocaine for the first time, reached about 918 adolescents a day (NSDUH, 2007). Based on epidemiological studies, adolescents who are exposed to tobacco and alcohol at an early age are most likely to use illicit drugs later on in their lives (Kandel and Logan, 1984). Furthermore, those

who started at an early age have a harder time quitting, thus leading to a heavier consumption of illicit drugs, tobacco and alcohol (Breslau and Peterson, 1996). Individuals under the age of 15 who smoke cigarettes are eighty times more likely to use illegal drugs as compared to those who don't (Breslau and Peterson, 1996). Epidemiological studies have led to the hypothesis that nicotine may serve as a "gateway" drug that leads to an increased likelihood of dependence on other drugs (Kandel et al., 1992). Animal studies have been conducted to evaluate the "gateway" theory, since it allows for a more controlled experiment and can identify the underlying mechanism for the progression of drug use. In contrast, epidemiological studies in humans have been unable to control factors such as environment, genetics, and others that confound the analysis.

When an adolescent is exposed to nicotine at an early age, it leads to a neurochemical alteration that may persist into adulthood, thus enhancing further the need to smoke (Adriani et al., 2003). In fact, changes in the mesocorticolimbic dopaminergic signaling due to illicit drug use at an early age can increase a person's vulnerability to other classes of abused drugs (Trauth et al., 2001).

1.4. Adolescent Smoking:

The long-term impact of tobacco use in adolescence is documented. 90% of adult smokers report their first use of tobacco prior to age 18 (Chassin et al. 1990). Another study found that students who have tried a single cigarette by

age 11 remain vulnerable to future smoking, up to 3 years later (Fidler et al. 2006). Over 6,000 teenagers begin smoking every day (American Lung Association Statistics 2002). Initiating smoking during adolescence correlates with greater addiction liability, higher daily consumption, and reduced likelihood of quitting (Colby et al. 2000; Kandel and Chen 2000). Indeed, an adolescent smoking only two to four cigarettes per week is at risk of becoming addicted in early adulthood (Riggs et al. 2007). Among American adolescents the number of smokers has been rising sharply since 1992, while the age of initiation for smoking has been declining (Johnston et al. 1998). Nicotine, the primary addictive component in tobacco, acts on the brain to produce both rewarding and aversive effects (Castane et al. 2005). Many adolescents become dependent on nicotine despite the fact that initial exposure to nicotine has been shown to be unpleasant (Eissenberg and Balster 2000). Despite the fact that nicotine reaches the brain rapidly, it does not have long lasting acute effects; the short half-life of nicotine of only 1 to 2 hours is likely to contribute to its repeated and consistent use (Viveros et al. 2006). Adolescent smoking is different than adult smoking and occurs in stages. The average number of cigarettes smoked per day is 5.2 among adolescent smokers aged 12 to 17 (NHSDA ,2003). Adolescent smokers also experience signs of withdrawal such as cravings, nervousness, and the inability to concentrate (Rojas et al. 1998; Killen et al. 2001). Indeed, this group of teenagers reports frequent unsuccessful attempts to quit due to cravings and withdrawal symptoms (Johnson 1982; Biglan and Lichtenstein

1984). Without a doubt, factors such as social pressure, environment, stress, biological effects, reinforcing effects, and aversive withdrawal symptoms contribute to an adolescent's decision to maintain a regular level of smoking.

Hypothesis

We hypothesize that nicotine exposure during adolescence causes long lasting neurobiological alterations that increase the susceptibility to cocaine reward in adulthood. Furthermore, it will activate a neurobiological mechanism that is shared by many drugs of abuse, which will increase susceptibility to their rewarding effects.

Dissertation Objectives

The research in this thesis focuses on the effects impact of adolescent nicotine exposure on the subsequent behavioral of cocaine. Based on preliminary data and previous literature, we hypothesized that adolescent who are exposed to low doses of nicotine would demonstrate increased vulnerability to cocaine reward as compared to adults. Our first specific aim was to characterize the impact of the effects of nicotine exposure during adolescence with regards to cocaine. Both dose and duration of nicotine exposure were investigated. Rewarding effects, changes in locomotor activity and locomotor sensitization to cocaine were evaluated. The second and final specific aim was to examine whether adolescent nicotine exposure effect generalizes to other typically-abused drugs such as morphine and amphetamine.

Materials and Methods

2.1. Subjects

Experimentally, naïve male adolescents and adult ICR mice were purchased from Harlan Laboratories (Indianapolis, IN.). ICR mice are an out-bred strain which have been used extensively in pharmacological studies. Adolescent animals were obtained from different litters to avoid any effects that may have confounded the result. Adolescent mice have been classified by the use of three age intervals, early adolescence (PND 28-to-34), middle adolescence (PND 34-to-46), and late adolescence (PND 47-to-59), (Spear 2000; Laviola 2003). These divisions are based on the similarities in physical, sociological, and biological development in both rodents and humans. These divisions have been carefully assessed in rodents and are assumed to correlate well with aspects of human adolescence. For all studies, adolescent mice arrived on postnatal day (PND) 21 and weighed approximately 18-23 grams at the start of the experiment; adult mice arrived on PND 65 and weighed approximately 30-35 grams. The animals were housed in groups of four mice per cage, and allowed to acclimate for seven days, the cages had small houses and toys. The mice were handled for three days prior to the experiment with unlimited access to food and water, except during the experimental sessions. All mice were housed in a humidity and temperature controlled (22 °C) vivarium on a 12-hr light/dark cycle (lights on at 6 a.m., off at 6 p.m.). Testing was conducted during the light phase of the cycle. At the end of each experiment, the animals were euthanized by

way of CO₂ inhalation. Animals were maintained in a facility approved by the American Association for Accreditation of Laboratory Animal Care, and all procedures were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.2. Drugs

The drugs used in these experiments were (–)-Nicotine hydrogen tartrate salt[(–)-1-methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt] and mecamylamine hydrochloride [2-(methylamino) isocamphane hydrochloride], purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA); and d-amphetamine, morphine and cocaine HCl, obtained from the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). All drugs were dissolved in 0.9% sterile saline (0.9% sodium chloride) and prepared fresh before each experiment. All compounds were injected subcutaneously (s.c.) except for the cocaine, which was injected intraperitoneally (i.p.) at a volume of 10 ml/kg body weight. Doses are expressed as the free base of the drug. Control groups received saline injections at the same volume and by the same route.

2.3. Injection Protocol

Mice received nicotine during early adolescence (PND 28), middle adolescence (PND 34), late adolescence (PND 47), or adulthood (PND 70+). Based on previous work done by our lab, we choose to use either a short pattern (one day) or a long pattern (7 days) of exposure. Depending upon the experiment conducted, nicotine (0.1 and 0.5 mg/kg), cocaine (10 mg/kg), or

saline was administered twice daily, with injections approximately 6 hours apart (8 a.m. and 2 p.m.). After treatment, the adolescent mice were kept in their home cages for 42 days to allow them to reach adulthood, at which point they were evaluated in paradigms as described below. Adult mice were kept for similar time periods as the adolescent mice.

2.4. Conditioned Place Preference

Conditioned place preference is a method which has been used widely to evaluate the rewarding effects of a drug by pairing a drug with a particular context (Bardo et al. 1995; Tzschentke 1998). Place conditioning boxes consisted of two equal-sized compartments (20 cm long x 20 cm wide x 20 cm high), separated by a grey central area with an opening that allowed access to either side of the chamber. The opening in the partition could be closed off for pairing days. The compartments have different-colored walls (one black, one white) and distinct floor textures (grid rod floor in the black compartment and mesh in the white one). The CPP protocol was conducted over the course of five days in an unbiased fashion. The CPP procedure consisted of three phases: an initial preference test, three conditioning days, and a final preference test. Animals showing great initial preferences for one of the compartments were eliminated from the study, because it is difficult to detect a shift in time spent in a compartment when an animal had a strong initial bias prior to conditioning. This is particularly important for drugs such as nicotine, which has a weak reinforcing property.

Handling habituation: On Friday through Sunday of the week prior to the start of the place-conditioning procedure, mice in the CPP studies were handled once per day for approximately two minutes each. Previous work done by our lab demonstrated that handling experience plays an important role in the ability of nicotine to produce a conditioned place preference (Grabus et al. 2006).

On day one: An initial preference test; animals were placed in the boxes and allowed to roam freely from side to side for 15 minutes. Time spent in each side was recorded using Med Associates interface and software. These data were used to separate the animals into groups of approximately equal bias.

On day 2-4: Conditioning phase animals were paired for 20 minutes, the saline group received saline on both sides of the boxes. Depending on the experiment, the drug groups received nicotine, cocaine, morphine or d-amphetamines on one side and saline on the opposite side of the boxes. Drug paired sides were randomized among all groups. Conditioning lasted for three days, with animals in the drug group receiving drugs each day.

On day 5: The final preference test was administered, no injections were given. Animals were placed in the boxes and allowed to roam freely from side to side for 15 minutes. The time spent on each side was recorded, and the data were calculated based on time spent on the drug paired side minus time spent on the saline paired side. An increase in time spent in the initially favored compartment was indicated as a preference for the drug paired side, while a reduction or

negative number indicated an aversion (CPA) to the drug paired side. A number at or near zero indicated no partiality for either side.

2.5. Acute Locomotor Activity:

Pretreated mice were placed into individual Omnitech photocell activity cages, (Columbus, OH; 28 x 16.5 cm), 10 minutes after the i.p. administration of cocaine. Interruptions of the photocell beams, which assess walking and rearing, were then recorded for the next 30 minutes. Data were computed as the number of photocell interruptions.

2.6. Cocaine Locomotor Sensitization:

In this study, only early adolescent mice (PND 28) were used. Mice were pretreated at adolescence with saline or nicotine (0.5 mg/kg) s.c. injections twice daily for seven days; the injections were approximately six hours apart. Our protocol was based on the study completed by Biala, (2003). Once the mice reached PND 70, a 13 day cocaine sensitization procedure was launched.

On Day 1: Mice received a saline injection (i.p.) and were then placed in locomotor activity chambers for a 30 minute habituation period while activity counts were recorded. Immediately the mice were removed from the locomotor boxes and randomly divided into three groups: saline-saline, saline-cocaine, and cocaine-cocaine (the group names represent the acquisition-day drug, followed by the challenge day drug). The mice were then given another injection of either saline or cocaine 20 mg/kg (i.p.), depending on their assigned group, and placed in the chambers again for a 30-minute acquisition period.

Days 2–5: The mice received an i.p. injection of either saline or cocaine 20 mg/kg, depending on their assigned group, and placed in the chambers again for a 30-minute acquisition period.

Days 6–12: A drug-free week; the animals received no injections or exposure to the chambers.

Day 13: Challenge day; the mice were tested again in the same way as described for days 1–5, but the cocaine mice received a challenge-dose of cocaine of 5 mg/kg (i.p.). Counts were recorded for a 30-minute test period.

2.7. Statistical analysis

For all data, statistical analyses were performed using StatView ® (SAS, Cary, NC, USA). Statistical analysis of all behavioral studies was performed with mixed-factor ANOVA with post-hoc Tukey's test when appropriate. P-values of less than 0.05 were considered to be statistically significant.

Studies

3.1 Methods

3.1.1. The Effect of Adolescent Nicotine Exposure on Cocaine-Induced Conditioned Place Preference

Early adolescent mice (PND 28) and adults were divided into two groups. One group received a short (1-day) nicotine exposure protocol, while the other group received a long (7-day) protocol. Furthermore, each group was subdivided, eight animals to each group. Two dose of nicotine were tested (0.1 or 0.5 mg/kg, s.c.) As a control, adult ICR mice (PND=70) received the same treatment protocol as the adolescents. When the adolescent mice reached young adulthood (PND 70), and again at PND 112, they were tested for cocaine reward using conditioned place preference. As previously described, mice have an *initial preference phase* which is a drug-free assessment of baseline preference in a three-compartment chamber. This is followed by a *conditioning phase*, which includes three days of conditioning to cocaine (10 mg/kg i.p.). After the conditioning period, the last day of the paradigm is the *final preference phase*, during which preference is assessed. Preference scores are expressed as time spent on the drug-paired side minus time spent on the saline-paired side. A positive number indicated a preference for the drug-paired side, while a negative number indicated an aversion to the drug-paired side. A number at or near zero indicated no preference for either side.

3.1.2. Influence of the Age of Nicotine Exposure on the Enhancement of Cocaine Reward

Only late adolescent mice (PND 47) were used in this study. Mice were injected with either nicotine (0.5 mg/kg s.c.) or saline twice a day for one week, then put in their cages to reach adulthood. Once the adolescent mice had reached PND 89, they were tested for cocaine reward using conditioned place preference.

3.1.3. To Determine the Onset of the Cocaine Enhancement

For this study we used only early adolescent (PND 28) mice. The mice were injected with either 0.5mg/kg nicotine or saline twice daily for a week. At PND 36, the mice were tested for cocaine preference (10mg/kg, i.p.) as described previously. Separate groups of mice received the same pretreatment protocol and were tested for cocaine CPP at late adolescence (PND 50).

3.1.4. To Determine the Impact of the Sequential Order between Nicotine and Cocaine

Early adolescent mice (PND 28) received cocaine (10mg/kg i.p.) or saline twice daily for a week. Once adolescent mice had reached adulthood (PND 70), they were tested for nicotine (0.5mg/kg s.c.) reward using conditioned place preference.

3.1.5. To Determine if the Enhancement of Cocaine Reward by Nicotine is Receptor-Mediated

Male ICR mice (PND 28) were randomly divided into groups: saline-saline, mecamylamine-saline, mecamylamine-nicotine and saline-nicotine (groups represent the first treatment followed by the second treatment). Depending on

the group, mice were injected with mecamylamine (2mg/kg s.c.—a dose well known to block most behavioral effects of nicotine in rodents), nicotine (0.5 mg/kg s.c.), or saline. At adulthood (PND 70), mice were tested for cocaine (10 mg/kg i.p.) reward using CPP animal model

3.1.6 Effect of Adolescent Nicotine Exposure on Morphine and Amphetamine-Induced Conditioned Place Preference

To determine whether the early adolescent nicotine pretreatment effect generalizes to other illicit drugs, adolescents, aged postnatal day 28, were given two daily injections of saline or nicotine (0.5 mg/kg, s.c.). At PND 70, mice were tested with a morphine (5mg/kg s.c.) reward, and another group were tested with amphetamine (5mg/kg s.c.) reward using conditioned place preference.

3. 1.7. Effects of Adolescent Nicotine Exposure on Cocaine-Induced Hyperactivity

Mice were tested for cocaine-induced hyperactivity using locomotor chambers after reaching adulthood. For this study, early adolescent (PND 28) and adult (PND 70) ICR male mice received 0.5 mg/kg nicotine or saline s.c. injection twice daily for 7 days, with injections approximately 6 hours apart. On PND 70 and 112 respectively, Mice were injected i.p with either saline or various doses of cocaine (5, 10, and 20 mg/kg) and then placed into individual Omnitech photocell activity cages (Columbus, OH; 28 x 16.5 cm) 10 minutes after injection. Interruptions of the photocell beams, which assess walking and rearing, were then recorded for the next 10 minutes. The data are expressed as the number of photocell interruptions.

3.2. RESULTS

3.2.1 Effect of Adolescent Nicotine Exposure on Cocaine-Induced Conditioned Place Preference

Figures 1 and 2 show cocaine-induced CPP in nicotine pretreated mice over all stages of adolescence and adulthood. It was important first to determine the dose and length of nicotine exposure that is required to produce cocaine enhancement in adulthood. Figures 1-a and 1-b show respectively the mice that received either a short 1-day, or long 7-day exposure to nicotine during early adolescence. All mice conditioned with cocaine in the CPP model developed significant preference for the cocaine-paired side when compared to their respective saline controls. An overall two-way ANOVA (pretreatment x exposure duration) showed that only mice that had a 7-day exposure to the higher dose of nicotine (0.5 mg/kg) displayed a significantly enhanced level of preference, compared to those mice pretreated with saline. Interestingly, the short exposure to nicotine failed to produce a significant enhancement of cocaine when compared to the saline pretreated mice, even with the higher dose of nicotine.

Next, we wanted to determine the influence of the age of nicotine exposure on the enhancement of cocaine reward. In Figure 1 and 2, age differences were seen when cocaine was given (two-way ANOVA: age x pretreatment), with only early adolescents exhibiting greater preference in response to 10 mg/kg of cocaine based on pretreatment status (shown in Figure 1-b). The results of cocaine-induced CPP following late adolescent and adult nicotine exposure are shown in Figures 2-a and 2-b respectively. Neither late

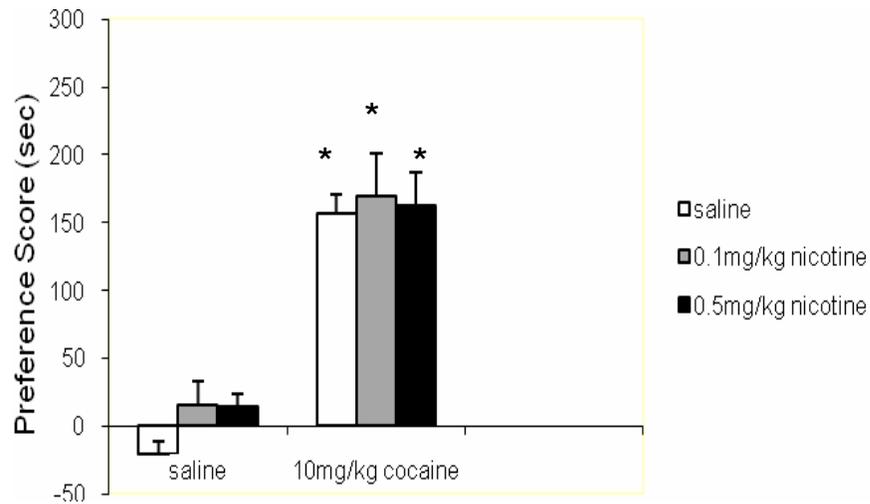
adolescent nor adult mice displayed any significant differences based on pretreatment status in a 7-day exposure protocol.

Also, it was important to determine the onset of this enhancement. Results from Figure 3-a and b show that significant enhancement peak in mice tested in CPP model at PND 50 and continue to PND 70 (two-way ANOVA: age × pretreatment). In contrast, mice tested for cocaine-induced reward at PND 35 displayed approximately equal levels of preference for cocaine despite varying pretreatment groups.

Moreover, we wanted to determine the impact of the sequential order between nicotine and cocaine. Mice were pretreated with various dose of cocaine (10 or 20 mg/kg) in early adolescence and conditioned with nicotine in the CPP model in the adulthood. Results revealed that nicotine produced significant preference in saline pretreated mice compare to saline control. On the other hand, 10 and 20 mg/kg cocaine pre-exposure in adolescent mice demonstrated no nicotine preference compared to saline pretreated mice (fig. 4).

Finally, determining if the enhancement of cocaine rewards or nicotine rewards is receptor-mediated was a priority. Figure 5 shows that enhancement in pretreated nicotine mice disappeared when mice received mecamylamine before nicotine. These data suggest that early adolescence is the most critical stage for cocaine-induced rewarding effects, and that this enhancement is affected by dose and duration of nicotine exposure.

a. 1-day exposure



b. 7-day exposure

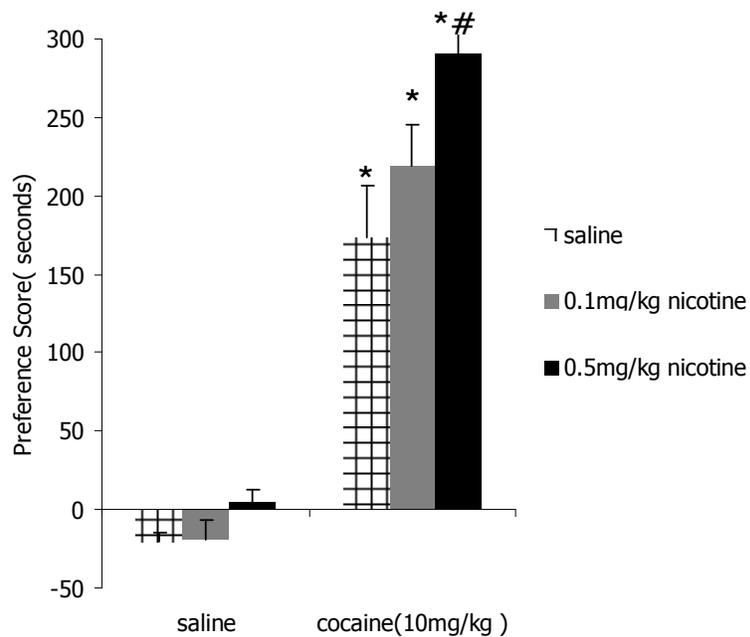


Figure 1. Effects of early adolescent nicotine exposure on cocaine-induced CPP in adulthood (a) 1-day (two injections) (b)7-day(14 injection).The y-axis represents preference score and the x-axis expresses adolescent treatment in the CPP paradigm. Each bar represents the mean \pm SEM of seven to eight mice. * $p < .05$ from respective saline control; # $p < .05$ from saline-cocaine

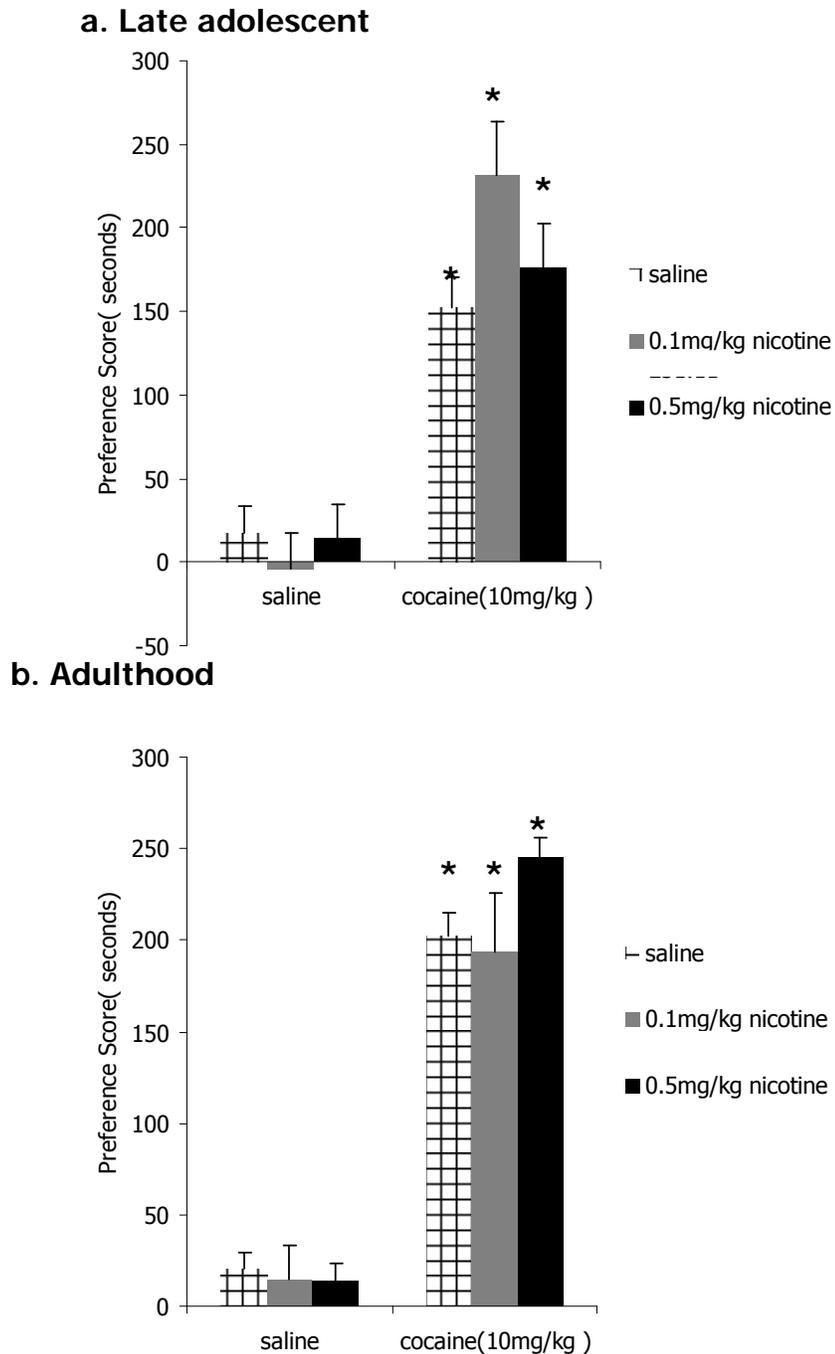
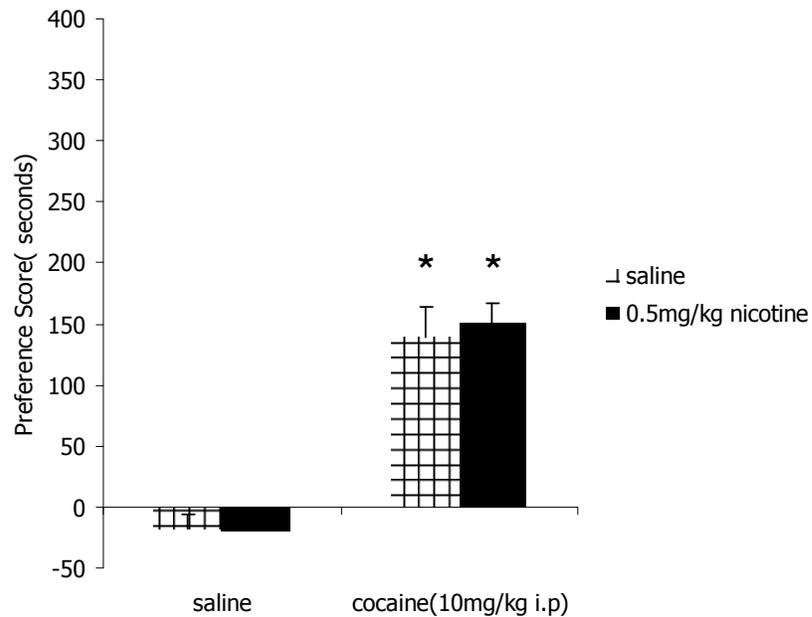


Figure 2. Effect of late adolescent and adulthood nicotine exposure on cocaine-induced reward. The y -axis represents preference score and the x -axis expresses adolescent treatment in the CPP paradigm. A frequent pattern (7-day) of nicotine exposure in late adolescence (a) and adulthood (b) was tested. Each bar represents the mean \pm SEM of eight mice. * $p < 0.05$ from respective saline control.

a. Early Adolescence



b. Late Adolescence

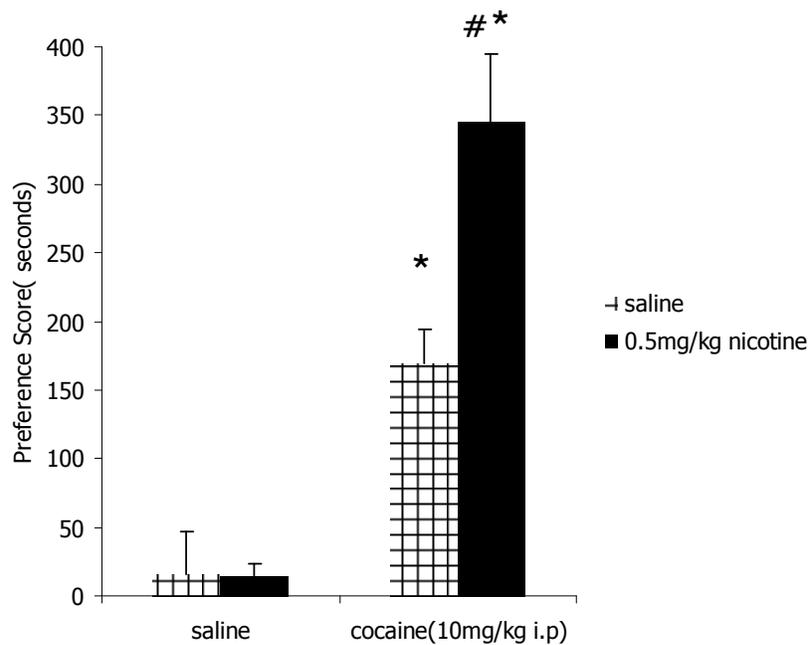


Figure 3. The Onset of the Cocaine Enhancement. The y-axis represents preference score and the x-axis expresses adolescent treatment followed by treatment in the CPP paradigm. a. CPP at early adolescence. b. CPP at late adolescence. * $p < .05$ from respective saline control; # $p < .05$ from saline-cocaine group in the same graph.

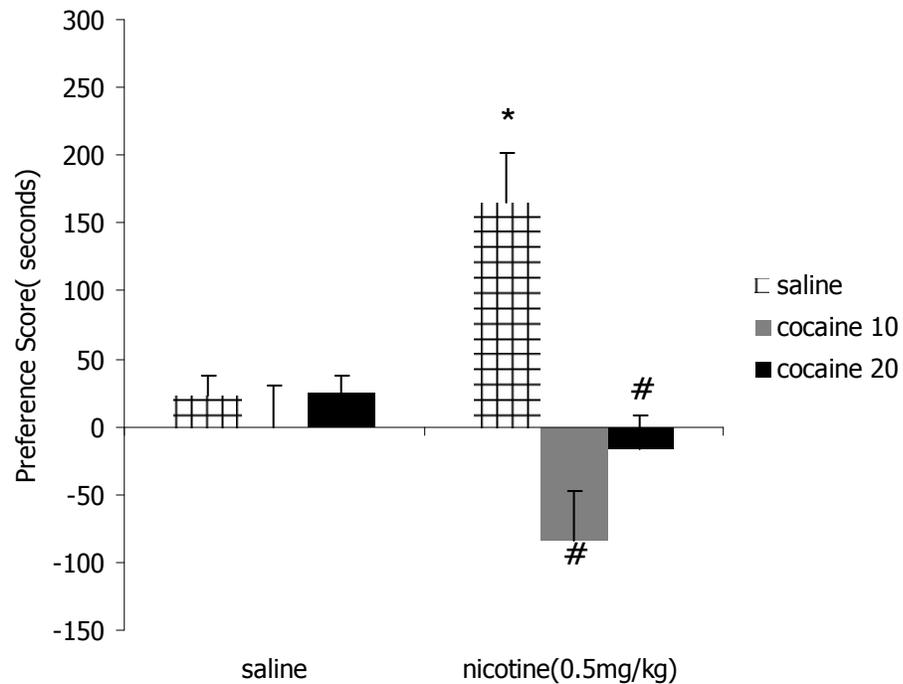


Figure 4. The Effects of early adolescent cocaine exposure on nicotine-induced CPA in adulthood. The y-axis represents preference score and the x-axis expresses adolescent treatment in the CPP paradigm. Each bar represents the mean \pm SEM of seven to eight mice. * $p < .05$ from respective saline control; # $p < .05$ from saline-nicotine.

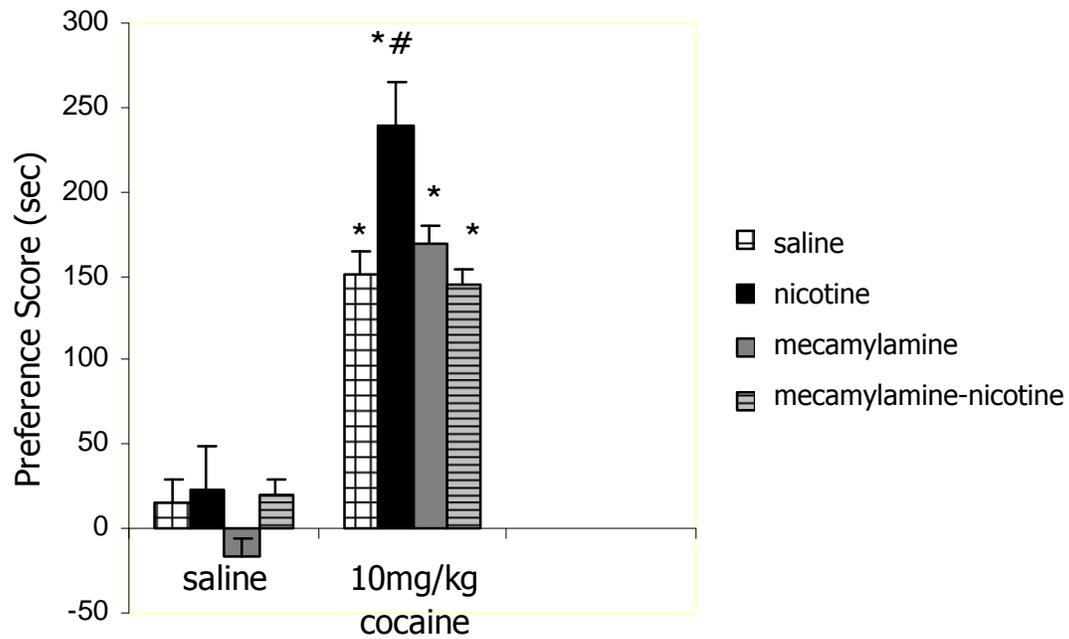


Figure 5. The Effects of early adolescent mecamlamine-nicotine exposure (7-day) on cocaine-induced CPP in adulthood. The y-axis represents preference score and the x-axis expresses adolescent treatment in the CPP paradigm. Each bar represents the mean \pm SEM of seven to eight mice. * $p < .05$ from respective saline control; # $p < .05$ from saline-cocaine.

3.2.2 Effect of Adolescent Nicotine Exposure on Morphine and Amphetamine-Induced Conditioned Place Preference

Figure 6, shows that all mice, which were conditioned with morphine or amphetamine in the CPP model, developed significant preference for the drug-paired side as compared to their respective saline controls. Interestingly, mice which were pretreated with nicotine during adolescence and had morphine in adulthood displayed a significantly enhanced level of preference as compared to those mice which were pretreated with saline. In contrast to the morphine data, the amphetamine (5 mg/kg) did not produce a significant enhancement of reward.

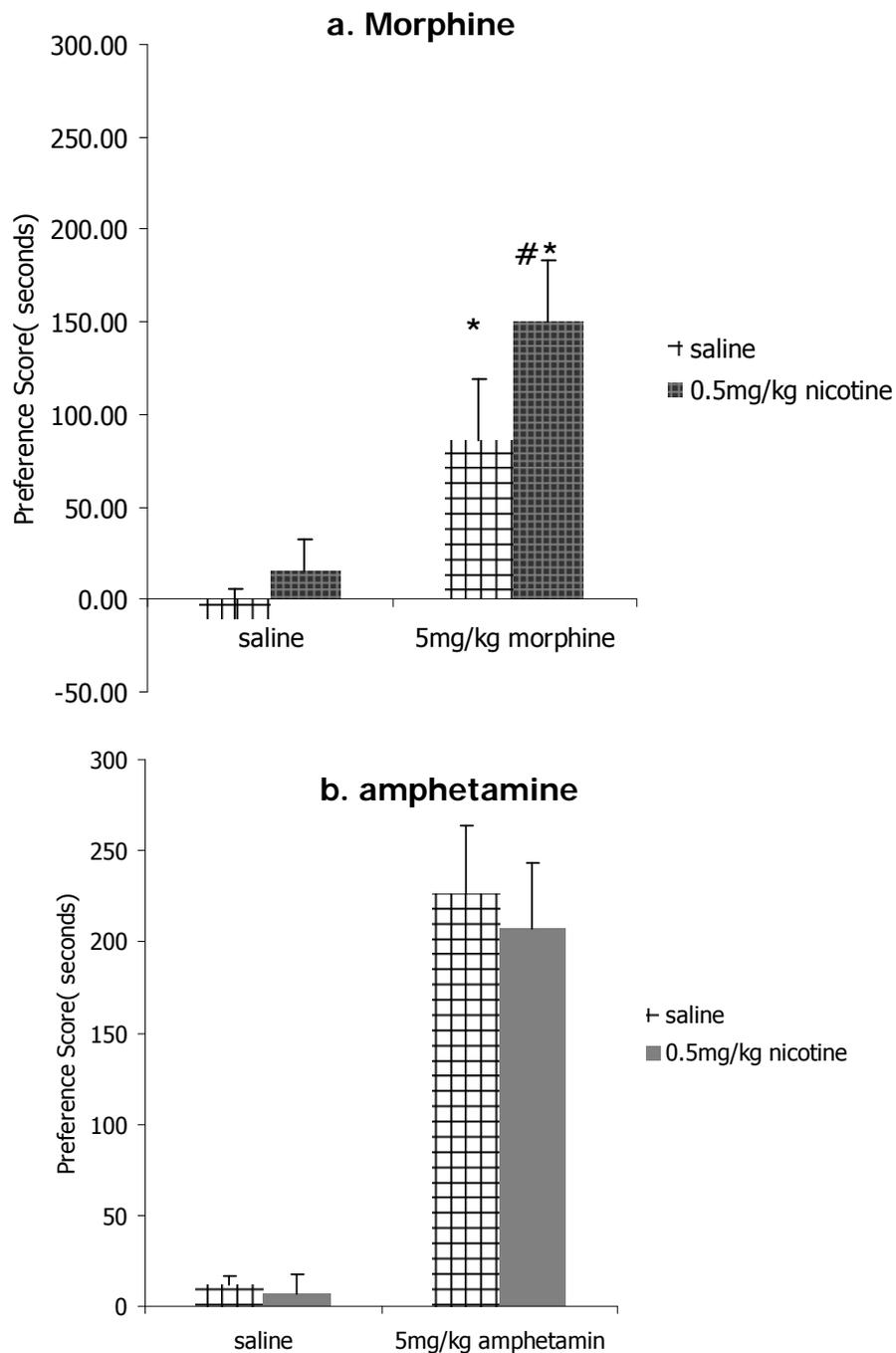
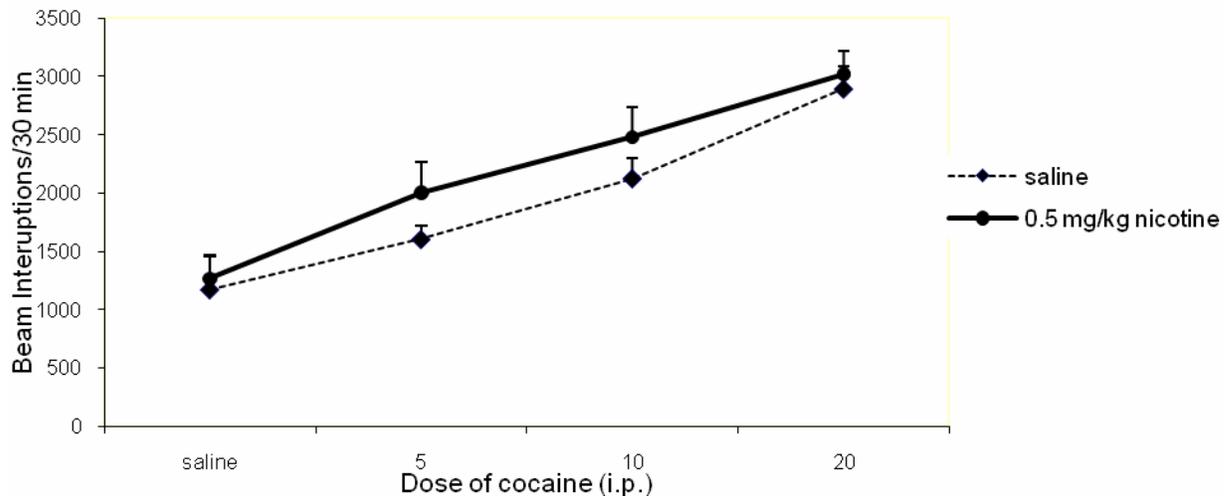


Figure 6. Effects of early adolescent nicotine exposure on morphine and amphetamine-induced CPP in adulthood (a) morphine (b) amphetamine. The y-axis represents preference score and the x-axis expresses adolescent treatment in the CPP paradigm. Each bar represents the mean \pm SEM of seven to eight mice. * $p < .05$ from respective saline control; # $p < .05$ from saline-morphine.

3.3.3. Effects of Adolescent Nicotine Exposure on Cocaine-Induced Hyperactivity

In this study, we examined the effects of early adolescent exposure to low doses of nicotine (0.5mg/kg) on cocaine's acute effects, using a locomotor activity test. Figures 7 and 8 show the results of these studies. All age groups displayed a dose-responsive increase in locomotor activity in when given cocaine. No significant changes were observed after the short (one day) or long (seven day) nicotine exposure protocol during early adolescence as compared to those pretreated with saline. Figures 8-a and 8-b show the results from studies where pretreatment occurred in adulthood. The results were the same, no significant differences were seen based on the adult group that received pretreatment.

a. Effect of 1-day Nicotine Exposure in Early Adolescence



b. Effect of 7-day Nicotine Exposure in Early Adolescence

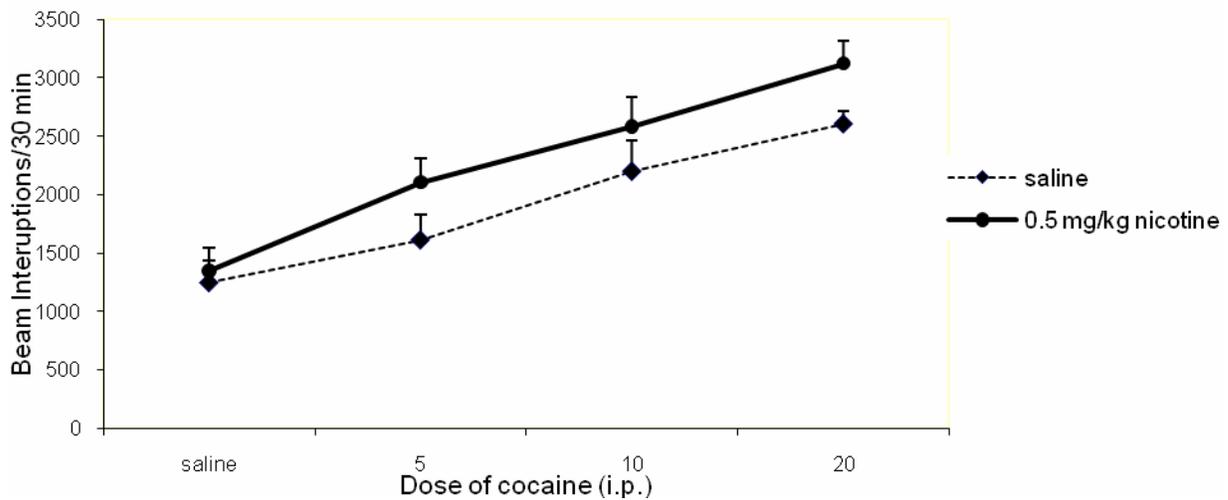


Figure 7. Cocaine-induced hyperactivity following nicotine exposure in early adolescence. Mice were pretreated with saline or nicotine during early adolescence either acutely (1 day) or repeatedly (7 days) and were tested for cocaine hyperactivity in adulthood. $n=6/\text{group}$.

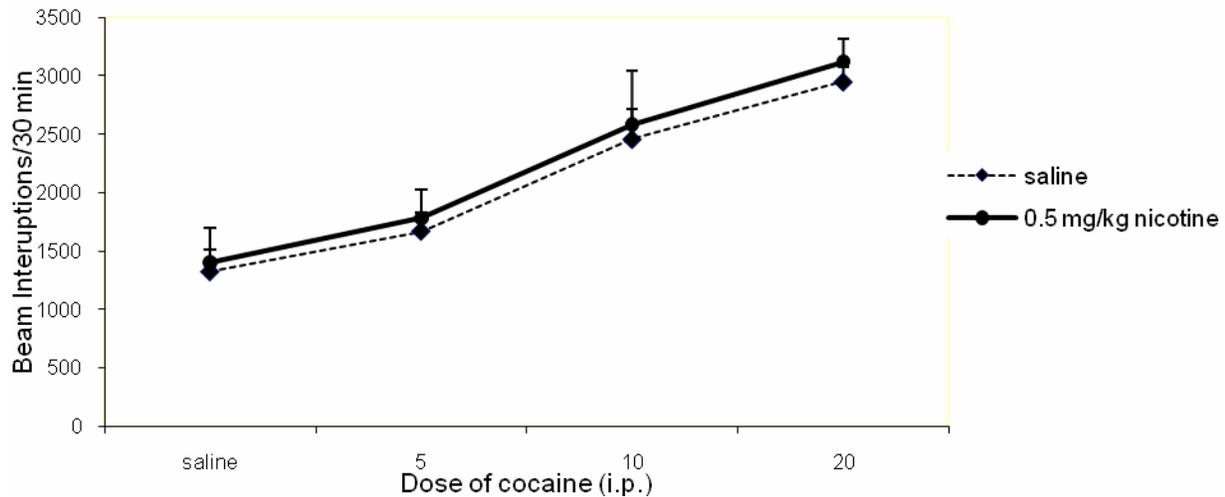
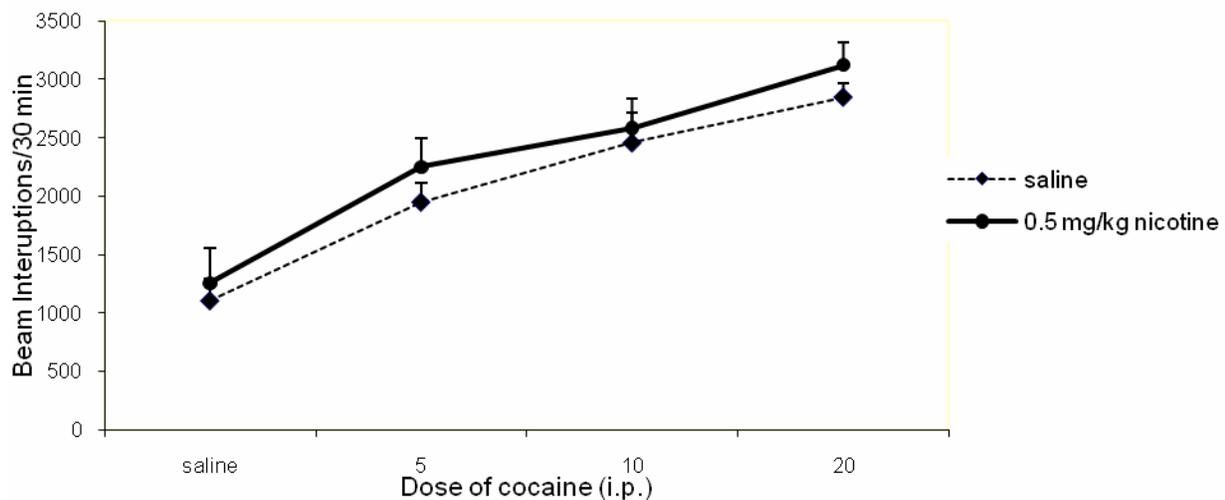
a. Effect of 1-day Nicotine Exposure in Adulthood**b.** Effect of 7-day Nicotine Exposure in Adulthood

Figure 8. Cocaine-induced hyperactivity following nicotine exposure in Adulthood Mice were pretreated with saline or nicotine during early adolescence either acutely (1 day) or repeatedly (7 days) and were tested for cocaine hyperactivity in adulthood. n=6/group.

3.3.4 Effects of Adolescent Nicotine Exposure on Locomotor Sensitization to Cocaine

In Figure 9, mice that received low doses of nicotine in adolescence are depicted with solid bars while the mice pretreated with saline are displayed with non-solid bars. During the acquisition period, mice that were treated with cocaine (20 mg/kg) showed an increase in locomotor activity, as expected, with no differences due to adolescent pretreatment (* $p < .05$ as compared to sal-sal). On challenge day, two groups received an injection of cocaine i.p. (5 mg/kg). Mice pretreated with both saline and nicotine and mice treated with cocaine, during acquisition, displayed an enhanced locomotor activity compared to mice treated with saline only. However, mice that were pretreated with nicotine in adolescence demonstrated a significant increase in cocaine-induced locomotor activity in comparison to the animals pretreated with saline. These results established that we were able to induce locomotor sensitization to cocaine, and that early adolescent nicotine exposure enhances this effect.

On challenge day two groups received an injection of cocaine i.p. (5 mg/kg). Both saline and nicotine pretreated mice who were treated with cocaine during acquisition displayed enhanced locomotor activity as compared to those mice treated with saline during acquisition. However, mice which were pretreated with nicotine in adolescence demonstrate a significant increase in cocaine-induced locomotor activity as compared to saline pretreated animals. These results established that we were able to induce locomotor sensitization to cocaine and that early adolescent nicotine exposure enhances this effect.

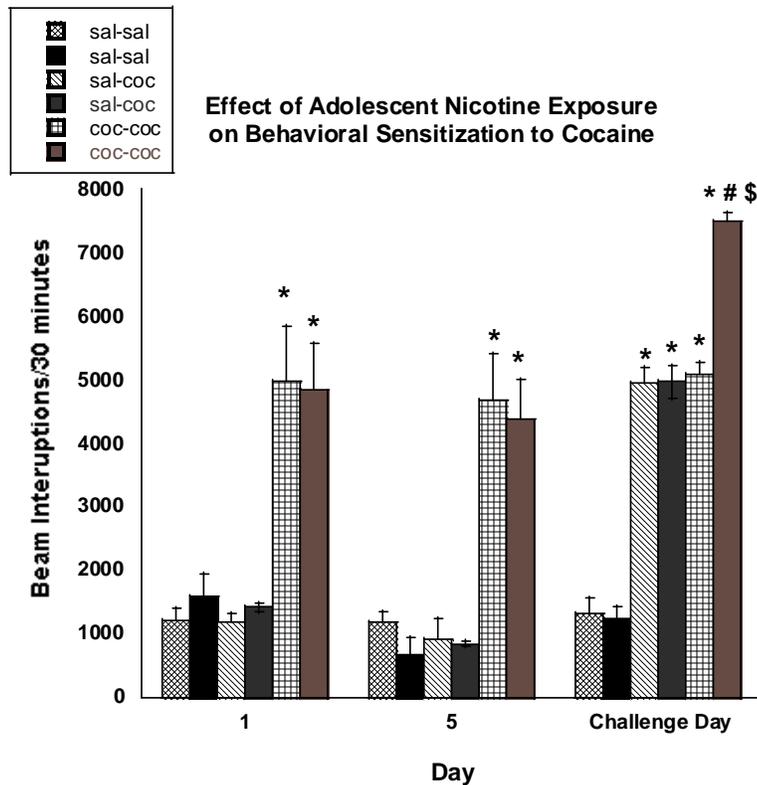


Figure 9. Cocaine-sensitization in ICR male mice. Early adolescent mice were pretreated with either saline (non-solid bars) or nicotine (solid bars) for 7 days and were tested for cocaine-induced locomotor sensitization in adulthood. Treatment groups are represented by acquisition drug-challenge drug in the legend (ex. sal-coc = saline during acquisition and cocaine on challenge day) * $p < .05$ from sal-sal control on the same day; # $p < .05$ from sal-coc group; \$ $p < .05$ from saline pretreated coc-coc group. (done by Dena Kota)

Discussion

We hypothesized that adolescent nicotine exposure causes long-lasting neurobiological alterations that increase susceptibility to cocaine use in adulthood. Furthermore, by activating a neurobiological mechanism shared by many commonly abused drugs, the effect of pre-exposure to nicotine during adolescence may enhance rewards derived from a variety of other substances, which in turn may increase susceptibility to abuse these drugs.

The present study of nicotine use in adolescence finds that exposure to nicotine enhances the experienced reward of cocaine, but this is dependent on the dose, the duration of nicotine exposure and the age of the subject. Our data showed that a 7-day exposure to (0.5 mg/kg) nicotine during early adolescent was able to alter cocaine-induced responses. In contrast, neither a 1-day exposure nor a lower dose of nicotine (0.1 mg/kg) was able to elicit this effect. This suggests that a more chronic pattern of adolescent nicotine exposure is required to induce lasting changes in subsequent behavioral responses. Since data in our first experiment suggested early adolescence was a critical period for nicotine reward, we decided to focus on this phase of development for subsequent studies. Similar to the effects seen with reward, exposure of early adolescent mice to nicotine also enhanced locomotor sensitization to cocaine in adulthood. However, an enhancement of cocaine-induced hyperactivity did not occur upon acute or chronic injection of the drug in early adolescent and adult mice pre-treated with nicotine.

This differential enhancement of cocaine's behavioral effects suggests that nicotine exposure in adolescence has an impact only on long-term neuroadaptations after chronic/repeated administration to nicotine. Our data strongly suggest that nicotine intake during adolescence may act to cross-sensitize the brain to cocaine's long-term changes in the brain.

Many drugs of abuse share reward circuitry in the brain: the mesocorticolimbic reward pathway, which has been implicated in many of the rewarding and reinforcing effects of drugs of abuse (Nestler 2001; Kobb and Le Moal 2001). This pathway originates in the ventral tegmental area and sends projections to the nucleus accumbens (NAc) (Nestler 2001; Hyman and Malenka 2001). In fact, animals with lesions in these regions demonstrate a loss of drug utilization (Robinson and Berridge 2001; Nestler 2004). Dopamine is the most common and essential neurotransmitter involved in this pathway.

Azam et al. (2007) report that nicotine-stimulated dopamine release is significantly higher during the early adolescent period in the male rat. Nicotine, in particular, is able to activate VTA dopaminergic neurons directly via stimulation of nicotinic cholinergic receptors, or indirectly via stimulation of its receptors on glutamatergic neurons, which then innervate dopamine cells. Early-adolescent nicotine exposure significantly elevates nAChR function in adulthood (Kota 2009). Repeated stimulation by nicotine may promote maturation and facilitate cocaine-induced plasticity of the mesocorticolimbic system. Our results show that nicotine-induced enhancement of cocaine's effects is mediated by

neuronal nicotine receptors since mecamylamine, a nicotinic receptor antagonist, blocked the enhancement. It is not clear which specific nicotinic subtypes are blocked, because mecamylamine is a non-selective antagonist. Our data suggest that the high preference of cocaine following nicotine pretreatment results from activation of neuronal nicotinic receptors during the pretreatment phase, because the enhancement “portion” of cocaine preference was blocked .

It is also clear from our results that the animals’ age of exposure has a great impact. Indeed, nicotine exposure in early, but not late, adolescence enhanced cocaine’s rewarding effects, suggesting that early adolescence is a critical period for the behavioral plasticity induced by nicotine. Furthermore, control animals receiving nicotine during adulthood did not show enhancement of cocaine’s rewarding effects.

Finally, cross-sensitization to the rewarding effects of cocaine in the CPP after nicotine pre-exposure was observed in late adolescence and continued to adult age. Although the time-course of this enhancement was not fully determined, our results suggest that the behavioral plasticity observed is long and may well extend beyond PND 70.

We have used an intermittent pattern of nicotine exposure over a brief period (7-days), and a low dose of nicotine (0.5 mg/kg) that is known to produce CPP. These protocols were selected in order to mimic patterns of adolescent experimentation with cigarette smoking, namely short/acute and intermittent exposure. The dose was administered by subcutaneous injection, which more

closely mimics early teenage smoking. The pattern of adolescent smoking is different to that of adults, as it occurs in stages. It usually involves repeated, albeit irregular, use over an extended period. This ranges from 3 to 5 cigarettes per week in an irregular manner for occasional and experimental smokers, to 3 to 5 cigarettes per week, every week, for regular smokers who might later move to a state of nicotine dependence. In fact, some youths will advance to dependence before leaving high school. The smoking pattern in adolescence is further complicated by the fact that it is affected by specific events, such as parties and weekends. Therefore, mimicking the human pattern of nicotine exposure in an adolescent mouse model is not an easy task, since the adolescence period in rodents is very short. We therefore chose a low dose regimen (0.1 and 0.5mg/kg) and an intermittent pattern of nicotine exposure over a short period (7-days) for our studies. Subcutaneous injection better reflects the intermittent pattern of nicotine administration. Although oral administration (nicotine in drinking water) of nicotine is stress free, the absorption of nicotine is affected by the first pass metabolism, which leads to variable absorption. For our studies, we have attempted to mimic the amount of nicotine that an adolescent is exposed to daily, which is an equivalent of 5.2 cigarettes. A dose of 0.5 mg/kg of nicotine is comparable to the amount of nicotine inhaled from smoking two to four cigarettes, (Benowitz N.L. ,1990).

Our data agree with a study conducted using rats where the investigators utilized intravenous pre-treatments containing low doses of nicotine in

adolescents over a four-day period, (McQuown, 2007). This nicotine exposure resulted in an enhanced cocaine-sensitization response. Similarly, rats given nicotine at PND 35 for 10 days showed an enhancement of cocaine-induced reward using a CPP paradigm, (McMillen et al., 2005). Similar to our data on cocaine sensitization, it has recently been shown that exposure to nicotine in adolescent rats for seven days led to an enhanced sensitization to cocaine; as opposed to those exposed only to saline, (McQuown, 2009).

In contrast, another study found that C57BL/6J mice demonstrated a decline in cocaine-induced preferences, as measured by CPP after 25 days of nicotine exposure in adolescents, (Kelley and Rowan, 2004). This inconsistency could be due to the difference in mouse strain, C57BL/6J vs. ICR, as well as the length of time of exposure. In addition, it was found that nicotine pre-exposure led to an increase in cocaine's motor activating effects, whereas our data demonstrates no change in the acute locomotor study. Research has shown mixed results regarding the effect of cocaine rewarding properties from nicotine exposure in adolescents as compared to that of adults. It is clear that a number of factors may be responsible for the differences between these studies; such as species, drug dosage, length of pre-exposure, and timing of the testing. Since any of the variables, or a combination thereof, may be responsible for the difference in results; more work needs to be done to establish how the long-term effects of adolescent nicotine exposure may be affected by these variables.

Exposure to nicotine during this period of brain development may lead to

persistent, long lasting changes in the brain. Furthermore, the enhancement in cocaine reward may be replicated with other drugs of abuse. A study done by Kota et al. suggested early adolescent nicotine exposure significantly elevates the nAChR function in adulthood in the brain. Indeed, pre-exposure to 0.5mg/kg nicotine during early adolescence demonstrated significant enhancement to the morphine's reward, but it failed to increase d-amphetamine preference in a CPP model (fig.6). Adolescent nicotine exposure has long-lasting effects on the development of various pharmacological systems, specifically the dopaminergic system. Amphetamine, cocaine (psychostimulants), morphine (opiates), and nicotine (cholinergic agonists) preferentially increase synaptic dopamine concentrations in the mesolimbic dopaminergic system (Di Chiara G). Cocaine acts as an indirect dopamine agonist. It increases synaptic DA levels in the nucleus accumbens via its actions at the DA transporter, inhibiting uptake into the presynaptic terminals (Harris and Baldessarini, 1973). Morphine, through the mu-opioid receptor activation, is known to excite dopamine neurons in the VTA by the inhibition of the GABA-ergic inhibitory interneurons and, thereby, increases dopamine transmission to the NAC (Rezayof et al., 2007).

The dopaminergic pathway is a likely candidate for observed cross-sensitization as mentioned previously; many studies have shown that illicit drugs tend to enhance dopamine transmission from the ventral tegmental area to the nucleus accumbens (Koob and Le Moal, 1997. Dani, 2003). Also, other receptors may be involved in our behavioral observations. Glutamatergic receptors are

known to be involved in nicotinic effects as well. A study shows that adolescent, but not adult, nicotine exposure down-regulated mGluR2/3 subunits in the hippocampus and striatum. This same study also showed changes in NMDA NR2A/B subunits regardless of the time of exposure, suggesting the involvement of NMDA receptors in certain aspects of nicotine dependence (Adriani et al. 2004). These findings imply that other receptors may also be involved and should be further examined.

Surprisingly, 10 mg. of cocaine pretreatment during early adolescence demonstrates condition-place aversion to nicotine during adulthood. These results may correlate with the establishment of drug dependence and an increased risk of relapse after a period of withdrawal. They also further implicate a role for dopamine in cross-sensitization to other drugs of abuse. Taken together, our data suggests that adolescent nicotine exposure may cause molecular alterations which lead to enhanced vulnerability to drug dependence later in life. Preventing adolescent experimentation with tobacco is extremely important as it can rapidly cause persistent changes in drug-induced behavioral responses.

Future Studies

Our findings suggest that early adolescent nicotine exposure results in long-lasting alterations in behavioral response to cocaine and other drugs of abuse in adulthood. The rewarding effects of cocaine and morphine are elevated in a dose- and duration-dependent manner. In our studies, relatively low levels of nicotine and short patterns of exposure during early adolescence resulted in long-lasting changes in the rewarding properties of cocaine and morphine. Our data imply that the adolescent brain is uniquely vulnerable to the effects of nicotine on subsequent drug reward. Even short periods of exposure to cigarette smoking, which are often seen in the adolescent population, could have long-lasting and detrimental effects on smoking and drug abuse behavior.

Although drugs of abuse target several brain areas, enhanced dopamine transmission from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is a key element in the reward (Koob and Le Moal 1997; Dani 2003). It is known that adolescent nicotine exposure has long-lasting effects on the development of various pharmacological systems, and it is likely that the dopaminergic system is one that is greatly affected. Since many drugs of abuse are known to affect levels of dopamine in the brain, this pathway is a likely candidate for the observed cross-sensitization. The mechanisms underlying this "cross-sensitization" are still being elucidated, and additional studies would be useful for determining these pathways. For example, nicotine may alter number of dopamine receptors or function or level of dopamine transporters; therefore,

studies measuring DA receptor function and binding of DA ligands as well as DAT binding should be conducted. Specifically, D1 and D2 ligands are of particular interest.

These findings also raise the question of how exposure to secondhand smoke in adolescence may affect sensitivity to drug abuse reward. We have shown that relatively short periods of nicotine exposure and at low levels can cause alterations in important regulatory systems. Children with parents or friends who smoke may be exposed to levels of nicotine that can detrimentally affect the development of neurological systems. These changes are likely to affect the reinforcing and aversive properties of nicotine and other drugs of abuse and may lead to increased vulnerability in these areas. The effect of exposure to secondhand smoke on nicotine dependence in those children has yet to be explored, and our results could have important implications for prevention messages

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VITA

Mai Abdullah Alajaji was born on October 18, 1981 in Riyadh, Saudi Arabia. Mai graduated in May 2004 Mai obtained her Bachelor of Pharmaceutical Science degree in Pharmacy in May 2004 from King Saud University. Mai came to Virginia Commonwealth University in August 2008 and joined the Department of Pharmacology and Toxicology. She entered the lab of Dr. M. Imad Damaj in August 2008 and began her research on adolescent drugs abuse.