EFFECTS OF ADOLESCENT NICOTINE EXPOSURE ON ADULT COCAINE REWARD, AVERSION AND SELF-ADMINISTRATION

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ABSTRACT

Rationale. These studies examined adolescent nicotine pre-exposure on the rewarding and aversive effects of cocaine and the relationship of these changes to adult cocaine self-administration.

Methods. In Experiment 1, male rats on postnatal day (PND) 28 were divided into two groups and given once daily 0.6 mg/kg injections of nicotine or vehicle (until PND 43). They were then allowed to age untreated to adulthood (PND 66), exposed to habituation and pretests (PND 67-77) and then tested for the aversive and rewarding effects of cocaine in a combined conditioned taste avoidance (CTA)/place preference (CPP) procedure (PND 78-90). Briefly, rats were placed in wire mesh hanging cages, given access to a novel saccharin solution, injected with cocaine (5.6, 10 or 18 mg/kg) or vehicle then placed in CPP chambers.

In Experiment 2, rats were pre-exposed to nicotine as described above with the addition of baseline and locomotor (every third injection day) tests to examine nicotine-induced locomotor sensitization. They were then allowed to age untreated to adulthood (PND 76), implanted with jugular catheters and allowed to recover for at least 5 days. Beginning on PND 90, rats were tested for cocaine self-administration (0.25 or 0.75 mg/kg), progressive ratio (PR) responding, extinction and cue-induced reinstatement.

Results. In Experiment 1, rats showed a significant CTA Trial x Drug interaction (p = .012) with cocaine groups consuming less saccharin over Trials, but no Pre-exposure or Dose group differences. For place preferences, there was no main effect of Pre-exposure or Dose or
any interactions. Therefore, data were collapsed across Pre-exposure and Dose. There was a significant main effect of Drug (p=.008) such that the cocaine group spent more time on the drug paired side (DPS) than the vehicle group. In Experiment 2, the nicotine group displayed sensitized locomotor activity over nicotine injections when compared to controls, showing that the nicotine was behaviorally active. All rats in Experiment 2 showed clear, dose-dependent responding during cocaine acquisition, PR, extinction and reinstatement with no effect of nicotine pre-exposure.

Conclusions. These studies suggest that adolescent nicotine pre-exposure does not have an impact on adult cocaine self-administration or its affective properties, at least under these specific parametric conditions. Further research is needed to clarify the conditions under which adolescent nicotine induces changes in the adult affective properties and self-administration patterns of cocaine. A clearer understanding of these factors could lead to better drug abuse treatment and prevention strategies in the future.
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CHAPTER 1
GENERAL INTRODUCTION

In 2011, approximately 8,400 adolescents a day tried an illicit drug for the first time (NIDA, 2012) with many of these new users continuing to abuse drugs daily. The 2013 Monitoring the Future survey stated that 50% of young people have used an illicit drug and approximately 30% have used a “hard” drug such as cocaine, heroin or methamphetamine before leaving high school. Given that adolescents are using drugs, it is essential to understand several issues regarding this use. The first concern is how such use affects the individual in the present. Short-term consequences could include risky sexual behavior, decreased inhibitions, conduct disorders and other delinquent behaviors (Winters and Anderson, 2000; Vitaro, Brendgen, Ladouceur, and Tremblay, 2001; Guo et al., 2002). A second area of concern is how adolescent drug use impacts the individual’s use and abuse of drugs in adulthood. Specifically, it is important to understand if, how and to what extent adolescent use impacts the future long-term vulnerability to drugs of abuse.

In addressing this latter question, much of the clinical literature has focused on the impact of adolescent alcohol consumption, finding that early initiation of alcohol use correlates with chronic adult use (Guttmannova et al., 2011) and showing that adolescent alcohol abuse predicts not only adult alcohol dependence (Gruber, DiClemente, Anderson, and Lodico, 1996; Guo et al., 2000; Grant et al., 2006) but also the use of other illicit drugs (McGue, Iacono, Legrand, Malone, and Elkins, 2001; Zhou, King and Chassin, 2006; Hicks, Iacono and McGue, 2010). However, such vulnerability changes are not limited to experience with alcohol. In fact, Degenhardt et al. (2011) suggested that early onset use of any illicit substance is related to the risk for later drug dependence, depending on the extent of prior usage and the age of initiation. Since adolescent abuse patterns correlate with future use, adolescent drug history may be an
important factor related to subsequent drug vulnerability (Toumbourou et al., 2007; Iacono, Malone, and McGue, 2008).

Potential changes in vulnerability have been supported by preclinical studies which report that drug history impacts subsequent self-administration. This altered responding has been described for drugs such as cocaine (Zhang and Kosten, 2007), alcohol (O’Dell et al., 2004) and nicotine (Levin, Rezvani, Montoya, Rose, and Swartzwelder, 2003; Chen, Matta and Sharp, 2007). Prior drug history alters the self-administration not only of the same drug, but also of different drugs of abuse as shown with combinations such as methamphetamine to cocaine (Crawford et al., 2011) and MDMA to cocaine (Fletcher, Phil, Robinson, and Slippoy, 2001). Under such conditions, a history with one drug frequently increases the self-administration of the second drug. The basis for these changes in self-administration following drug exposure is not known, but may be a function of alterations in the drug’s rewarding and aversive effects. In this context, it is important to note that both the rewarding and aversive effects of drugs can change with prior drug history (Brandon et al., 2001; Andersen et al., 2002; Hutchison and Riley, 2008).

Although it is clear that adolescent history with certain drugs of abuse is related to drug use in adulthood, surprisingly little is known about similar relationships with one of the most commonly used drugs in adolescence, i.e., nicotine (Johnston et al., 2012). What is known suggests that adult nicotine use is significantly impacted by adolescent nicotine use, even at low doses (DiFranza et al., 2007). A one year study of 681 seventh graders found that the first symptoms of nicotine dependence occur very quickly, often within days or weeks of the start of occasional use (DiFranza et al., 2000) and this dependence is a strong predictor of nicotine dependence in adulthood (Doubeni, Reed and DiFranza, 2010). Further, DiFranza et al. (2004) reported that feelings of relaxation following first cigarette inhalation strongly predicted nicotine dependence. Interestingly, dizziness and nausea also predicted future nicotine dependence,
although it is not clear why this was the case. Buchmann et al. (2011) also reported that adolescents who began smoking at an early age and found the experience of the first cigarette pleasurable were more likely to become regular smokers in adulthood. Taken together, age and experience of the first cigarette, combined with adolescent use patterns, clearly appear to impact future nicotine abuse.

Such findings are also supported by preclinical work. For example, Levin et al. (2003) found that female rats exposed to nicotine during adolescence subsequently (up to PND 82) displayed a rate of nicotine self-administration that was almost double that of rats exposed to nicotine in adulthood. The same increased responding has also been reported in male rats (Levin et al., 2007). Recently, two studies have examined adolescent nicotine pretreatment on self-administration of another drug, namely cocaine (Anker and Carroll, 2011; Dickson, Miller, Rogers, Blaha and Mittleman, 2012). In both studies, a nicotine history impacted cocaine self-administration, e.g., rats exposed to nicotine during adolescence showed evidence of higher rates of responding for cocaine (Dickson et al., 2012.) or greater reinstated responding by cocaine and cocaine-associated cues (Anker and Carroll, 2011).
CHAPTER 2

EXPERIMENT 1: NICOTINE HISTORY ON CONDITIONED TASTE AVOIDANCE/ PLACE PREFERENCE

Introduction

The demonstrations that adolescent nicotine exposure impacts adult cocaine self-administration are important for several reasons. As discussed, there is a limited amount of research examining the future consequences of adolescent nicotine use. Given the evidence from human data, it is clear that adolescents are using nicotine, starting at a young age. While Monitoring the Future data suggest that rates of adolescent tobacco use may be declining (although nicotine is still in the top three mostly commonly used drugs), the introduction of alternate means of administering nicotine such as the e-cigarette may lead to an increased number of new nicotine users in the future (Bunnell et al., 2015; Ramo, Young-Wolff, and Prochaska, 2015). The lasting consequences of e-cigarettes on drug use initiation and future use, specifically of cocaine, are still not clear.

Simply determining whether cocaine use is affected by adolescent nicotine history, however, does not indicate the processes mediating such changes. An understanding of these mediating processes may be imperative in controlling and modifying future drug taking. It is, therefore, important to discuss self-administration in general and the factors that impact drug intake to understand a possible basis for the effects of an adolescent drug history. While there may also be co-occurring changes in the underlying biological mechanisms responsible for drug taking, the current project seeks to demonstrate changes in the behavioral mechanisms that may lead to changes in self-administration patterns. In this context, self-administration is typically considered to be a function of the rewarding effects of a drug, with these effects responsible for
initial use and maintenance. This is generally reflected in dose-response functions that illustrate the dose-related changes in drug intake (i.e., self-administration) and their relation to the drug’s rewarding effects (see Figure 1). Although the drug’s rewarding effects are critical for drug self-administration (Stewart, de Wit, and Eikelboom, 1984; Self and Nestler, 1995), it is important to note that self-administration is also influenced by another affective property of the drug, i.e., its aversive effect. In fact, it has been argued that overall drug intake is a function of the balance of these two affective properties with the drug’s rewarding effects supporting self-administration and the aversive effects limiting intake (see Figure 1; for a discussion, see Mariathasan and Stolerman, 1994; Riley, 2011).

Figure 1: Hypothetical Model of the Balance of Reward and Aversion and How it Impacts Self-administration of a Drug (adapted from Riley, 2011).

Although the above model is a hypothetical one, it has been reported that most drugs of abuse have both of these affective properties (for alcohol, see Spear and Varlinkskaya, 2010, for morphine, see Hurwitz, Merluzzi, and Riley, 2013, for MDMA, see Albaugh, Rinker, Baumann, Sink, and Riley, 2011, for cocaine, see Zakharova, Leoni, Kichko, and Izenwasser, 2009 and for
nicotine, Wilmouth and Spear, 2004). Further, both of these effects can change with prior drug history (Brandon et al., 2001; Andersen et al., 2002; Hutchison and Riley, 2008). For example, Lett (1989) demonstrated that repeated exposure to drugs such as amphetamine, morphine and cocaine increased conditioned place preferences for that drug, suggesting that a drug history sensitized animals to its rewarding effects (see also Simpson and Riley, 2005; Tzschentke, 2007). This sensitization is not limited to the drug given during pre-exposure as others have found similar sensitization with multiple combinations of cocaine, amphetamine, alcohol and nicotine (see Tzschentke, 2007 for a review). Drug history has also been shown to affect the drug’s aversive effects, attenuating its own (as well as other drugs’) ability to condition taste avoidance (for a review, see Riley and Simpson, 2001; for nicotine, see Iwamoto and Williamson, 1984). Importantly, it has been shown that the rewarding and aversive effects are dissociable, meaning that the same drug can induce independent changes in these affective properties and these changes can occur in different directions or to varying degrees (Figure 2; for a review, see Verendeev and Riley, 2011). Together, these studies support the position that previous drug experience influences both the rewarding and aversive effects of a drug, which may impact its subsequent self-administration. Interestingly, given the pre-exposure results reported in the aforementioned studies, it might be expected that animals pre-exposed to a drug would display increased self-administration as a function of the reported changes in the rewarding (increased) and/or aversive (decreased) effects of the drug (see Figure 2).
Although it is clear that pre-exposure can impact the affective properties of drugs, an important question relevant to adolescent drug history is whether any changes that might occur in adolescence are long lasting and remain evident in adulthood. Limited preclinical research suggests that early drug exposure can impact these affective properties in adults. For example, adolescent pre-exposure to nicotine, cocaine, methamphetamine or MDMA (Vastola et al., 2002; Badanich, Adler, and Kirstein, 2006, Zakharova et al., 2009; Aberg et al., 2007) enhanced conditioned place preference to these same drugs in adulthood. Similarly, adolescent pre-exposure to cocaine (Schramm-Sapyta, Morris, and Kuhn, 2006) or alcohol (Diaz-Granados and Graham, 2007) attenuated aversions induced by these same drugs in adult animals. As with adults, these changes in reward and aversion can also be seen when the pre-exposure and conditioning drugs are different (for reward see: Andersen, et al., 2002, Achat-Mendes, Anderson, and Itzhak, 2003; for aversion see: Graham and Diaz-Granados, 2006, Hutchinson and Riley, 2008). It is of interest to note that pre-exposure does not always produce changes in adult responses, suggesting that pre-exposure effects may be dependent on the drug and pre-exposure regimens used (for reward, see Schramm-Sapyta, Pratt, and Winder, 2004; for aversions, see Cunningham, Tull, Rindal, and Meyer, 2002; Wetzell and Riley, 2012).
As previously discussed, there is limited research exploring the effects of adolescent nicotine consumption on adult use of other drugs, specifically cocaine. The few studies that have directly addressed these pre-exposure induced changes in reward and aversion have found conflicting results. For example, Kelley and Middaugh (1999) reported that adolescent nicotine pre-exposure decreased the cocaine-induced place preference conditioning in adults (see also Kelley and Rowan, 2004), whereas McMillen et al. (2005) found that adolescent nicotine pre-exposure increased cocaine-induced preferences. When exploring aversions, Hutchinson and Riley (2008) found no effect of nicotine pre-exposure on adult cocaine-induced taste avoidance. These results may be due to procedural differences in adolescent nicotine exposure, nicotine and/or cocaine dose, conditioning duration and species, making it unlikely that the results from these studies can be directly compared. Another consideration is the fact that these studies of reward and aversion were done in separate groups of animals. This is important because if it is the balance of reward and aversion that determines drug intake, it is impossible to assess the impact of changes in each of these two factors when they are examined in two different groups of animals. When the two effects are examined independently of one another, the adolescent nicotine pre-exposure induced changes in reward and/or aversions cannot be directly compared and thus one cannot talk about the relative contribution of the two properties or the potential impact on self-administration (as illustrated in Figure 1).

Given the effects of adolescent nicotine history on cocaine self-administration (as reported by Anker and Carroll, 2011 and Dickson et al., 2012), Experiment 1 examined the effects of adolescent nicotine exposure on the rewarding (conditioned place preference) and aversive (conditioned taste avoidance) effects of cocaine in adult subjects. Importantly, these changes were assessed in the same animals using a combined conditioned taste avoidance/place
preference procedure (Simpson and Riley, 2005; Verendeev and Riley, 2011) in which animals were given 20-minutes access to saccharin, injected with their assigned drug dose and placed in their non-preferred side of the place preference chamber (DPS; drug paired side). On the next day, animals were given 20-minutes access to water, injected with vehicle and placed on their preferred side of the place preference chamber (nDPS; non-drug paired side). This conditioning cycle was repeated three more times, followed by a final place preference and taste avoidance test. This method may allow for a determination of any potential alterations in cocaine’s affective properties following an adolescent history with nicotine and, in turn, may provide insight into potential treatment and prevention strategies for drug use and abuse in adulthood.

Methods

Subjects and Housing

Sixty-four experimentally naive male Sprague-Dawley rats arrived at the on-site animal colony on postnatal day 21 (PND 21). Animals were randomly divided into nicotine or saline pre-exposure groups and housed in groups of three or four in OptiRat Plus housing bins (38.9 x 56.9 x 26 cm; 1,181 sq. cm) until procedures began. Animals were then housed two per OptiRat Plus bin, separated by a Plexiglas divider, for the remainder of the project. They were given ad libitum access to food and water and maintained on a 12:12 h light/dark cycle and at an ambient temperature of 23°C. All procedures were conducted under the guidelines established by the Institutional Animal Care and Use Committee at American University and the Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011).

Drugs

Nicotine hydrogen tartrate salt (Sigma Aldrich Co., St. Louis, MO) was dissolved in 0.9% saline to a concentration of 1 mg/5 ml. Saccharin (0.1% sodium saccharin, Sigma Chemical
Co) was prepared as a 1 g/l solution in tap water. Cocaine hydrochloride salt (NIDA) was prepared as a 10 mg/ml solution in 0.9% saline. Vehicle injections were saline and were matched in volume to corresponding drug.

Apparatus

*Conditioned Place Preference:* The conditioned place preference apparatus (San Diego Instruments Place Preference System, San Diego, CA) is made up of two main chambers (28 x 21 x 34.5 cm) connected by a smaller middle chamber (14 x 21 x 34.5 cm). One main chamber consists of white walls and a white aluminum, diamond patterned floor. The other side is made up of black walls and a black plastic, haircell-textured floor. The middle, connecting chamber has gray walls and a steel rod floor. Each place preference apparatus has an individual LED overhead white light and features a 16 x 4 photo beam array for recording seconds spent in each chamber. The room in which place preferences were assessed was lit with a 25-watt red light mounted in the ceiling, and a white noise generator was used to mask background noise. Eight identical chambers were used for testing.

Procedure

*Pre-Exposure.* Beginning on PND 28, rats were weighed and subcutaneously injected once daily with 0.6 mg/kg nicotine (Group Nicotine) or 0.9% saline (Group Saline). Dosage and route of administration were based on previous studies of adolescent nicotine exposure (Horger, Giles, and Schenk, 1992; Vastola, Douglas, Varlinskaya, and Spear, 2002, Adriani et al., 2006; McQuown, Belluzzi, and Leslie, 2007). Injections were repeated daily until PND 42. This timeframe is commonly regarded as the early through late adolescence period in rats (Spear, 2000). Rats were then allowed to age undisturbed to early adulthood, approximately PND 66. Ad libitum food and water were available throughout this entire period.
**Water Habituation.** On PND 66, water was removed from the animals prior to the initial water adaptation session. On the following day, the rats were placed in wire-mesh testing cages (24.9 x 19 x 18 cm) and given 20-min access to water. Immediately after this period, they were returned to their home cages with no access to water until the following day during which they were again given 20-min water access in the test cages. This procedure was repeated daily until all animals began drinking within 2 sec of water presentation.

**CPP Pretest.** After water consumption was stable (i.e., all rats drank within 2 seconds of bottle presentation and average consumption did not vary by more than 2 ml with no consistent increase or decrease), all rats were placed in the conditioned place preference (CPP) apparatus for 15 min and allowed to roam freely. Location in the chamber was recorded to assess any pre-existing side preferences.

**CTA/CPP Conditioning.** Eight subjects at a time were run during the light phase of the light/dark cycle. On Day 1 of this phase, the fluid-deprived animals were given 20-min access to a novel saccharin solution in the test cages. Immediately following this exposure, they were injected intraperitoneally (IP) with cocaine (5.6, 10 or 18 mg/kg) or vehicle and placed into their non-preferred CPP chamber (DPS; drug-paired side) for 30 min. After conditioning, animals were returned to their home cages. On the next conditioning day (Day 2), animals received 20-min access to water in the test cages, followed by an injection of vehicle, and were placed in their preferred CPP compartment (nDPS; non-drug-paired side). This cycle was repeated three additional times. Upon completion of conditioning, animals were tested for both CPP and CTA. For the CPP test, rats were first placed in the test cages and given 20-min access to water. The rats were then placed in the middle gray compartment and given 15 min to explore the apparatus in a drug-free state. No injections were given on this day. On the next day, rats were placed into
the test cages and given 20-min access to both water and saccharin for a two-bottle taste avoidance test. Specifically, animals were first presented a bottle (containing either saccharin or water) on the left side of the cage and allowed to sample. The bottle was removed, and the animals were then presented with a second bottle (containing the alternative solution) on the right side of the cage. The tube was then removed and both bottles (containing water and saccharin) were placed simultaneously onto the cage on their respective sides. The order of presentation and bottle placement was counterbalanced between groups. The amount of saccharin and water consumed for each animal was recorded. No injections were given following the test. Body weight and fluid consumption were recorded for all sessions. Animals remained on 23\(\frac{2}{3}\) h water deprivation throughout the 10 testing days.

Data Analysis

In order to monitor the animal’s health during testing, body weight differences following nicotine or saline pre-exposure were assessed using a 2 x 15 repeated measures ANOVA with Day (PND 28-42) as the within-subjects factor and Pre-exposure (nicotine or vehicle) as the between-subjects factor.

For taste avoidance conditioning, the amount of saccharin consumed over conditioning was analyzed using a 2 x 4 x 4 repeated measures ANOVA with Trial (conditioning days 1-4) as the within-subjects factor and Pre-exposure (nicotine or vehicle) and Dose (0.0, 5.6, 10 or 18 mg/kg) as between-subjects factors.

For place preference conditioning, percent time spent on the DPS was analyzed using a 2 x 2 x 4 repeated measures ANOVA with Test (baseline and final) as the within-subjects factor and Pre-exposure (nicotine or vehicle) and Dose (0.0, 5.6, 10 or 18 mg/kg) as the between-
subjects factors. A one-way ANOVA with Drug as the independent variable and percent change in time spent on the DPS as the dependent variable was also performed.

For analyses in which significant main effects and interactions were obtained, Tukey’s *post hoc* tests were performed. Statistical significance was set at $\alpha = 0.05$ for all analyses.

**Results**

**Adolescent Nicotine Exposure**

*Body Weight.* The 2 x 15 repeated measures ANOVA on body weight during pre-exposure revealed a significant effect of Day [$F(14,868) = 7858.565, \ p < .05$] such that all rats gained weight over the course of injections. There was also a significant Pre-exposure x Day interaction [$F(14,868) = 5.655, \ p < .05$]. Nicotine-exposed rats weighed significantly less than saline controls on PND 30-42 (see Figure 3).

![Figure 3: Mean Weight (±SEM) for Nicotine and Saline Groups (n=32 per group). *indicates significant difference between Groups.](image)

**Adult Cocaine Conditioned Taste Avoidance**

A 2 x 4 factorial ANOVA revealed a significant effect of Dose [$F(3,56) = 19.172, \ p < .05$] such that saccharin consumption decreased as the cocaine dose increased, but no effect of Pre-exposure and no Pre-exposure x Dose interaction (see Figure 4). Subsequent one-way
ANOVAs and Tukey’s *post hoc* tests revealed that Group 10.0 and Group 18.0 drank significantly less than Group 0 and Group 5.6, when collapsed across Pre-exposures.

Figure 4: Percent Saccharin Consumed in Two-Bottle Test (*n*=16 per group). *indicates Group 0.0 drank significantly more saccharin than Group 5.6, 10.0, and 18.0. **indicates that Group 0.0 and 5.6 drank significantly more saccharin than Group 10.0 and 18.0.

Adult Cocaine Conditioned Place Preference

The 2 x 2 x 4 repeated measures ANOVA revealed a significant main effect of Trial [F(1, 56) = 17.877, *p* < .05] such that time spent on the DPS increased from pretest to posttest (see Figure 5). There was no main effect of Pre-exposure or Dose, nor any significant interactions. Due to the lack of a Pre-exposure or Dose main effect, groups were collapsed across Pre-exposure and Dose (see Figure 6). A one-way ANOVA and Bonferroni *post hoc* tests revealed a significant main effect of Drug [F(1, 63) = 7.534, *p* = .008] such that the cocaine group spent more time on the DPS than the vehicle group (see Figure 6).
Figure 5: Percent Change in Time Spent on the Drug-Paired Side from Pretest to Posttest, Separated by Pre-exposure (n=16 per group).

Figure 6: Percent Change in Time Spent on the Drug-Paired Side from Pretest to Posttest, Collapsed across Pre-Exposure and Dose. *indicates significant difference between animals exposed to vehicle (n=16) and animals exposed to cocaine (n=48) during conditioned taste avoidance/place preference conditioning.

Discussion

As noted, an adolescent history with nicotine appears to impact adult cocaine self-administration (Anker and Carroll, 2011; Dickson et al., 2012). What remains unknown is how this change is mediated. If the self-administration of a drug is a function of the balance of its rewarding and aversive effects (Mariathasan and Stolerman, 1994; Riley, 2011; Verendeev and Riley, 2013) then changes in either or both of these affective properties may be altered by a
nicotine history. Understanding how these factors are important to the eventual self-administration of cocaine may allow for better control and possible modification of future drug use. Experiment 1 assessed this issue by examining any changes in the rewarding and aversive effects of cocaine in animals with an adolescent history of nicotine (see Kelley and Middaugh, 1999; Kelley and Rowan, 2004; McMillen et al., 2005; Hutchison and Riley, 2008). Specifically, adolescent rats were exposed to nicotine, allowed to age to adulthood and then tested for any impact of adolescent nicotine use on the affective properties of cocaine in adults using a combined conditioned taste avoidance/place preference procedure (Simpson and Riley, 2005; Verendeev and Riley, 2011). As described, cocaine induced significant taste avoidance and place preferences, indicative of the multiple stimulus properties of cocaine (Walsh and Cunningham, 1997, Ettenberg, 2004). Although subjects displayed both cocaine-induced reward and avoidance, there was no effect of nicotine pre-exposure on either index.

The present work with cocaine-induced avoidance replicates Hutchison and Riley (2008), who also demonstrated dose-dependent cocaine-induced taste avoidance with no effects of nicotine history. Using parameters similar to those in the present experiment, Hutchison and Riley pre-exposed male, Sprague-Dawley rats to nicotine (0.4 mg/kg) during adolescence (PND 35-44) then tested for cocaine-induced taste avoidance (10, 18 or 32 mg/kg) in adulthood. After four conditioning trials (acquisition), cocaine induced avoidance at all doses that was comparable in subjects pretreated with nicotine or vehicle during adolescence. However, during extinction of CTA conditioning, they reported that nicotine pre-exposed animals displayed delayed extinction relative to controls (at the two highest conditioning doses of cocaine). The present experiment did not examine CTA extinction, thus direct comparisons in that aspect cannot be made.
Although the present experiment confirms the lack of adolescent pre-exposure effects on the acquisition of cocaine-induced taste avoidance in adults, the effects of adolescent nicotine exposure on cocaine-induced place preferences are less clear. For example, Kelley and Middaugh (1999; see also Kelley and Rowan, 2004) reported that adolescent nicotine pre-exposure reduced cocaine-induced place preference conditioning, whereas McMillen et al. (2005) found an enhancement of cocaine CPP in animals with a history of adolescent nicotine. This study found no effects of nicotine history on adult cocaine-induced place preferences. The basis for these differing results with place preference conditioning is unknown, but it is likely due to differences in species (rats or mice), dose (0.3 mg/kg up to 1 mg/kg), pre-exposure duration (10-35 days) and drug-free duration (approximately 12-30 days) between pre-exposure and cocaine administration, among other experimental parameters. Due to the wide variety of parameters used to examine the impact of nicotine pre-exposure on cocaine place preference, it is difficult to directly compare and explain the varying results obtained. Minimally, the effects of nicotine history on cocaine reward (as assessed with place preference conditioning) are inconsistent and parameter dependent.

There are several possible explanations for the lack of a nicotine effect on cocaine aversion and reward. First, the nicotine dose given during pre-exposure may not have been behaviorally active, i.e., the dose was too low to have any behavioral effects. This explanation seems unlikely given the significantly lower body weight of the nicotine group over pre-exposure sessions and the fact that others have obtained similar effects of pre-exposure on other behavioral indices (Horger et al., 1992; Vastola et al., 2002; Adriani et al., 2006). Secondly, it is also possible that the cocaine dose given was not subject to modification with the given pre-exposure routine. This may account for the similarities in the present findings to those of
Hutchison and Riley (2008), which used the same species-rats, a similar nicotine dose (0.4mg/kg) and pre-exposure (PND 35-44) and drug-free (PND 45-69) duration as well as similar cocaine CTA doses (10, 18, 32 mg/kg). However, Kelley and Middaugh (1999; see also Kelley and Rowan, 2004) used a similar pre-exposure dose (0.3 and 1.0 mg/kg) and comparable doses of cocaine (5, 10 and 20 mg/kg) during place preference conditioning. It is important to note that they used mice, a much longer pre-exposure duration (PND 25-60) and a shorter drug-free duration (12 days), which may account for the reported pre-exposure induced changes in reward. McMillen et al (2005) also used rats with a similar pre-exposure dose (0.4 mg/kg), duration (PND 35-44), and drug free period (approximately 45 days). However, only one dose of cocaine (3.0 mg/kg) was used for preference testing. Therefore, it is possible that a larger cocaine dose during preference testing would yield results similar to those found in the current study. Given the results of the aforementioned studies, it is likely that the pre-exposure and testing parameters are important for determining any nicotine effects on adult cocaine responding.

A more likely possibility is that the timing of the nicotine pre-exposure may have impacted the ability of adolescent nicotine to affect cocaine. Although relatively little research exists assessing the timing of adolescent drug pre-exposure on adult vulnerability, recent work by Kandel and Kandel (2014) in adult mice suggests that timing may be important in general when examining the interaction of drugs (and specifically nicotine and cocaine). In an analysis of the effects of nicotine exposure on the subsequent interaction of nicotine and cocaine in motor sensitization and place preference conditioning, they reported that while a nicotine history (7 days) significantly impacted the subsequent effects of co-administration of nicotine and cocaine, such effects were no longer evident with a delay of 14 days between pre-exposure and testing (see also Kelley and Middaugh, 1999; Slawecki, Gilder, Roth, and Ehlers, 2003). Given this, it is
possible that the amount of time between nicotine pre-exposure and cocaine conditioning (approximately 30 days) in the present study was too long to produce a lasting effect and differences may have been seen if pretreatment was continued up to and concurrently with testing. Evidence from adult nicotine history to cocaine studies also support the notion that nicotine pre-exposure must be more proximal to exert an effect. For example, Levine et al. (2011) showed that nicotine pretreatment (24 hours and 7 days), followed immediately by cocaine (20 mg/kg) produced locomotor sensitization and increased cocaine place preferences. However, there are also studies to suggest that proximal pre-exposure exerts an effect only if initiated during adolescence. For example, Collins and Izenwasser (2004) demonstrated that nicotine pre-exposure (7 days) sensitized cocaine’s locomotor effects in male rats only when pre-exposure began in periadolescence. These results indicate that there may be differing behavioral responses, depending not only on the proximity of nicotine and cocaine exposure but also on the timing of pre-exposure initiation (adolescent vs. adult). Continuing nicotine pre-exposure up to and concurrently with cocaine administration may also provide a more accurate model for human drug taking, given that adolescents who continue drug taking in adulthood do not typically stop nicotine use after adolescence then begin to use cocaine after an extended drug-free period (Chen and Jacobson, 2012). Further testing is required to determine if more proximal nicotine pre-exposure in adolescence would impact cocaine’s affective properties long term.
CHAPTER 3

EXPERIMENT 2: NICOTINE HISTORY ON COCAINE SELF-ADMINISTRATION

Introduction

Experiment 1 demonstrated that nicotine history does not impact the rewarding or aversive effects of cocaine in adults. In this context, the results found by Anker and Carroll (2011) and Dickson et al. (2012) are a bit surprising. As previously mentioned, if neither the rewarding nor aversive effects of a drug are affected, one would expect no change in self-administration of the drug. Since Experiment 1 demonstrated that cocaine produces both rewarding and aversive effects which are not impacted by a nicotine history, one would argue that nicotine history would also have no impact on adult cocaine self-administration. However, Dickson et al. reported that mice with an adolescent history of nicotine use self-administered more cocaine at all but the highest (1.8 mg/kg) dose during the maintenance phase of their study and Anker and Carroll reported nicotine pre-exposure differences in reinstatement in rats.

Although these earlier reports are suggestive of an effect of adolescent nicotine exposure on adult cocaine intake, there are a number of issues in the work that may limit this conclusion. First, both reports used animal models genetically predisposed to cocaine self-administration. Specifically, Anker and Carroll (2011) used a phenotype bred for high saccharin preference, which have been shown to be more vulnerable to the acquisition (Perry et al., 2007), maintenance (Anker et al., 2008) and reinstatement (Perry et al., 2006) of cocaine self-administration. Similarly, Dickson et al. (2012) used B6 mice, which have been reported to more quickly learn to consistently self-administer cocaine and administer it at a higher rate than DBA/2J mouse controls (Carney, Landrum, Cheng, and Seale, 1991) and shown to acquire the task faster, regardless of cocaine dose (0.5, 1.0 or 2.0 mg/kg) with a higher asymptotic rate of
responding than DBA/2J mouse strains (Grahame and Cunningham, 1995). To what extent the results from genetic strains highly sensitive to cocaine generalize to other strains or outbred animals remains unknown (Crawley et al., 1997; Thomsen and Caine, 2011). Also, in neither report was there a significant difference in the rate of acquisition or in the level of cocaine self-administration between the vehicle and nicotine pre-exposed subjects. Although Anker and Carroll reported faster extinction in the nicotine pre-exposed rats, no differences were seen in the Dickson et al. report. Thus, while there was an effect of nicotine on cocaine, these effects were evident only under specific conditions. Finally, Dickson et al. exposed mice to the nicotine via osmotic minipumps which delivered nicotine continuously throughout each day over PND 28-56, i.e., the mice had 28 days of continuous nicotine exposure and presumably constant, elevated blood levels (see below), a pattern that differs from the punctate delivery in humans (Everett et al., 1999; Rubenstein, Thompson, Benowitz, Shiffman, and Moscicki, 2007). These procedural issues may account for the ability of nicotine pre-exposure to impact cocaine self-administration despite the fact that Experiment 1 showed no changes in the affective properties of cocaine in nicotine pretreated animals.

Given that nicotine affected neither the rewarding nor aversive effects of cocaine (Experiment 1) and the various procedural issues in the abovementioned studies, Experiment 2 re-examined the effects of adolescent nicotine pre-exposure on adult cocaine self-administration. Briefly, rats were injected (SC) once daily with nicotine (0.6 mg/kg) or vehicle during adolescence (PND 28-43), allowed to age until adulthood (PND 75), implanted with jugular catheters (PND 76-80) and tested for adult cocaine self-administration (PND 90). To further extend and clarify the Dickson et al. (2012) findings, both PR and cue-induced reinstatement were examined, in addition to acquisition and extinction of cocaine self-administration. This
allows for measurement of not only drug intake, but also the motivation to take the drug (PR) and the inability to refrain from drug seeking (extinction/reinstatement), both of which are considered crucial components in the study of addictive behaviors (Belin et al., 2008). To eliminate the issue of continuous exposure to nicotine during adolescence, Experiment 2 used an intermittent (once daily injection, SC) nicotine pre-exposure dosing design that more accurately models human adolescent nicotine consumption of approximately one cigarette per day (Caraballo, Giovino, and Pechacek, 2004). To address the possibility that genetic differences in B6 mice might alter nicotine and/or cocaine responsivity, Experiment 2 used outbred Sprague-Dawley rats. Using this outbred strain eliminates the possible limited generalizations associated with testing differentially sensitive genetic strains.

**Methods**

**Subjects and Housing**

Sixty-four experimentally naive male Sprague-Dawley rats arrived at the on-site animal colony on postnatal day 21 (PND 21). Animals were randomly divided into nicotine or saline pre-exposure groups and housed in groups of three or four in OptiRat Plus housing bins (38.9 x 56.9 x 26cm, 1,181 sq. cm) until procedures began. Animals were then housed two per OptiRat Plus bin, separated by a Plexiglas divider, for the remainder of the project. They were given ad libitum access to food and water and maintained on a 12:12 h light/dark cycle and at an ambient temperature of 23°C. All procedures were conducted under the guidelines established by the Institutional Animal Care and Use Committee at American University and the Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011).
Drugs

Nicotine hydrogen tartrate salt (Sigma Aldrich Co., St. Louis, MO) was dissolved in 0.9% saline at a concentration of 1 mg/5 ml. Cocaine hydrochloride salt (NIDA) was prepared as a 10 mg/ml solution in 0.9% saline. Vehicle injections were saline and were matched in volume to corresponding drug.

Apparatus

**Locomotor Testing:** To ensure that the nicotine given was behaviorally active, locomotor testing was conducted using the conditioned place preference chambers. The conditioned place preference apparatus (San Diego Instruments Place Preference System, San Diego, CA) is made up of two main chambers (28 x 21 x 34.5cm) connected by a smaller middle chamber (14 x 21 x 34.5cm). One main chamber consists of white walls and a white aluminum, diamond patterned floor. The other side is made up of black walls and a black plastic, haircell-textured floor. The middle, connecting chamber has gray walls and a steel rod floor. Each chamber has an individual LED overhead white light and features a 16 x 4 photo beam array for recording seconds spent in each chamber. The CPP room was lit with a 25-watt red light mounted in the ceiling, and a white noise generator was used to mask background noise. Eight identical chambers were used for testing. In order to test for locomotor behavior in these chambers, the walls and floors were replaced with solid gray panels and the center dividers were removed to allow the rats to roam freely amid the whole testing box. This assessment was performed, and results compared to previous literature, to ensure that the nicotine was behaviorally active.

**Self-Administration:** Ten identical self-administration chambers were used for testing. Each chamber (Coulbourn Instruments, Whitehall, PA) measures 24 x 29 x 29 cm and has aluminum front walls, rear walls and ceilings. Each box had clear Plexiglas side walls, a grid
floor and was housed inside of a sound and light attenuating chamber (Coulbourn Instruments), equipped with two non-retractable levers (3.4 x 1.6 cm). The levers were positioned approximately 6 cm above the floor on the left and right side of the center food cup, located on the right wall of each box. All self-administration testing and data collection were controlled by a desktop computer running Med Associates software (MED-PC for Windows). A swivel, located above the center of each chamber, with an attached spring arm leash, was used to attach the drug line to the backmount for cocaine administration. The terminal end of the leash had a plastic nut which screwed onto the animal’s backmount. This permitted the rat to move freely in the chamber without putting strain on the polyethylene (PE) drug delivery tubing. The swivel was connected via PE tubing (Plastics One; 0.044 mm ID, 0.814 mm OD) to a 10 ml syringe containing cocaine, which was driven by a Med-Associates (St Albans, VT) syringe pump. A white-noise generator was used in the self-administration room to mask background noise.

Procedure

**Nicotine Pre-Exposure.** Beginning on PND 28, rats were weighed and subcutaneously injected once daily with 0.6 mg/kg nicotine (Group Nicotine) or 0.9% saline (Group Saline). Injections were repeated daily until PND 43. During pre-exposure, rats were also tested for nicotine-induced locomotor sensitization. On PND 27, all rats were placed into the locomotor chambers for 1 h to establish baseline activity. On PND 28 and every subsequent 3rd injection day, rats were placed into the locomotor chambers immediately following injection. Both gross (consecutive beam breaks) and fine (repeated breaks of the same beam) motor activity was recorded for each pre-exposure group (nicotine or saline).

**Catheter Implantation Surgery and Recovery.** Between PND 76-80, rats were implanted with jugular catheters. Aseptic surgical procedures were conducted according to the
recommendations of the National Institutes of Health Guidelines for Survival Rodent Surgery. Prior to surgery, all surgical tools were autoclaved. Immediately upon removal from the autoclave, tools were placed in a broad-spectrum germicide/bactericide/virucide/fungicide disinfectant (Cetylcide II) and allowed to soak for the duration of the surgery to ensure sterility. Beaded catheters (SAI) and mesh back mounts (Plastics One) were soaked in Cetylcide II as well. Animals were given a pre-operative anti-inflammatory (ketoprofen, 5 mg/kg, SC) followed by anesthesia (1.1 ml/kg, IP, of solution containing 3 ml of 100 mg/ml ketamine and 2.5 ml of 20 mg/ml xylazine). The surgical sites were clipped of fur and disinfected with Betadine and 70% ethanol. The animal’s eyes were protected with lubricating ophthalmic gel, and its tongue was gently cleared from its airway using forceps. Next, the animal was placed stomach-down on a sterile absorbent surgery pad (which was draped over a low-heat generating mat) and a small incision was made between the shoulder blades for backmount placement. Curved hemostats were inserted into the incision and worked subcutaneously just rostral of the shoulder to the jugular area to separate the skin from the underlying tissue. The back incision was then covered with cotton soaked in sterile saline, the animal turned to lie on its back and a small incision was made just above the jugular area, which was visualized by observing the rat’s pulse. A curved hemostat was used to widen the incision, and curved forceps were used to carefully separate extra tissue and expose the jugular vein. A curved 22G needle was inserted through one side of the vein into the center lumen and reverse-action vein-spreaders were used to maintain the opening as the catheter was inserted into the vein up to the first of the catheter’s two beads. Using monofilament suturing thread on either side of the beads, the catheter was attached to the jugular vein and surrounding tissue and locked into place before being passed through the previously cleared subcutaneous channel to the animal’s back with a trocar, where it exited from
the previous incision between the shoulder blades. Stainless-steel wound clips were used to close the jugular incision. The catheter was then trimmed to an approximate length of 22 mm and attached securely to the backmount, which was then sutured into the back, closing the wound. During suturing, care was taken to incorporate the back mount’s mesh to encourage fusion with the animal’s skin for security. A minimum of four sutures were used to secure each back mount.

At the completion of the procedure, the animal was given a second injection of ketoprofen for analgesia (5 mg/kg, SC). The first dose of ketoprofen was given before surgery to prevent inflammation prior to disturbing the tissue. The second dose was given after completion of the surgery to prolong the anti-inflammatory/analgesic effects.

For five days following the surgery, animals were given ketoprofen (5 mg/kg, SC) to alleviate post-operative pain or distress and to minimize inflammation. Catheters were flushed daily with a heparin/gentamycin solution (0.2 ml/infusion of 10 mg/ml gentamycin and 50 U/ml heparin) for the duration of the study. All animals were allowed to recover for a minimum of 5 days before self-administering cocaine. The patency of the catheters was checked as needed by administering Brevital sodium (5 mg/kg of 5 mg/ml solution, IV). A catheter was judged to be patent if ataxia was observed immediately after infusion. Wound clips/sutures were removed 10-14 days after surgery if needed.

**Cocaine Self-Administration Acquisition.** Four rats died during or following surgery leaving 60 subjects to begin cocaine self-administration acquisition. After recovery (starting on PND 90), rats were allowed to self-administer intravenous cocaine (0.25 or 0.75 mg/kg) on an FR-1 schedule of reinforcement during daily 2-h sessions. Rats were placed into the chambers with the house light turned off and a cue light illuminated above the active lever to signal the availability of cocaine. After each cocaine infusion, the house light turned on and the cue light
above the active lever turned off to signal the beginning of a 20-sec timeout period. During this time, any lever responding did not result in a cocaine infusion. The time elapsed between each earned infusion and the subsequent lever press (post-infusion pause) was recorded. After the timeout period elapsed, the house light turned back off and the cue light turned on to indicate that the lever was once again active. This phase was repeated until rats reached acquisition criterion (10 or more active lever for 5 consecutive sessions and greater than 2:1 ratio of active/inactive lever responding) and stability criterion (2 sessions of active lever responding with no more than 20% variance), at which point the rat was moved to the progressive ratio phase. Rats were given 13 sessions maximum to acquire the task. Six rats were excluded due to failure to learn the task and one rat was excluded because the catheter lost patency leaving 53 subjects that moved to progressive ratio self-administration.

**Progressive Ratio Cocaine Self-Administration.** After meeting acquisition criterion, two progressive ratio sessions (2 h) were given. Over the session, the number of responses required for each reinforcer progressively increased. Cocaine delivery was dependent on increased responding during the session [(1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc.; see (Richardson and Roberts, 1996)]. Following PR assessments, two FR-1 stability days were given. Responding was recorded and analyzed to ensure responding was back to pre-PR levels (no more than 10% variance from last two FR-1 acquisition session’s average lever presses). Responding was stable for all rats after two FR-1 stabilization days. Rats were then moved on to the extinction phase. During PR, two rat’s backmount began to leak, leading to exclusion of those subjects from the study. Therefore, 51 rats progressed to the extinction procedures.
**Cocaine Self-Administration Extinction.** Following stable responding, rats were subjected to the same conditions as given during the acquisition phase, with the exception of 1) the cue light was never illuminated and 2) active lever pressing no longer resulted in a cocaine infusion. All house light parameters remained identical to those in the acquisition phase and the same lever pressing data was recording. This phase was repeated until rats reached extinction criterion (20% or less of the four previous session’s average active lever responding) at which point the rat was moved to the reinstatement phase. If a rat failed to meet the criterion after 14 days, the rat was excluded. Two rats were excluded for failure to extinguish; therefore, 49 rats were moved to reinstatement.

**Cue Induced Reinstatement.** After meeting extinction criterion, rats were placed in self-administration chambers for a one trial reinstatement test under the exact same cue and house light conditions as the acquisition phase. The cue light above the previously active lever was illuminated. However, when rats pressed the previously active lever, no cocaine infusion was received. As before, the number of active and inactive lever presses was recorded.

Data Analysis

Body weight differences during nicotine and saline pre-exposure were assessed using a 2 x 17 repeated measures ANOVA with Day (PND 27-43) as the within-subjects factor and Pre-exposure (nicotine or vehicle) as the between-subjects factor.

Locomotor data over the 1-h sessions during the pre-exposure phase were analyzed using a 2 x 7 repeated measures ANOVA with Trial (baseline and 1-6) as the within-subjects factor and Pre-exposure (nicotine or vehicle) as the between-subjects factor.

Mean sessions to acquisition and percent of subjects reaching criterion were analyzed using a factorial ANOVA with Pre-exposure (nicotine or saline) or Dose (0.25 or 0.75 mg/kg) as
the between-subjects factors. Lever presses during the first five days of acquisition were
analyzed using a 2 x 2 x 5 repeated measures ANOVA with the within-subjects factor of
Sessions (1-5) and the between-subjects factors of Pre-exposure (nicotine or vehicle) and Dose
(0.25 or 0.75 mg/kg). Both lever presses and infusions earned during progressive ratio testing
were analyzed using a 2 x 2 x 2 repeated measures ANOVA with Session (1-2) as a within-
subjects factor and Pre-exposure (nicotine and vehicle) and Dose (0.25 or 0.75 mg/kg) as
between-subjects factors. Data from the two stability days following PR were compared using a
2 x 2 x 2 repeated measures ANOVA with Session (1 and 2) as a within-subjects factor and Pre-
exposure (nicotine or saline) and Dose (0.25 or 0.75 mg/kg) as between-subjects factors. Mean
sessions to extinction criterion were analyzed using a factorial ANOVA with Pre-exposure
(nicotine or saline) or Dose (0.25 or 0.75 mg/kg) as the between subjects factors. Similar to the
acquisition data, extinction presses and percent of subjects reaching criterion were analyzed
using a 2 x 2 x 5 repeated measures ANOVA with the within-subjects factor of Sessions (1-5)
and the between-subjects factors of Pre-exposure (nicotine or vehicle) and Dose (0.25 or 0.75
mg/kg). Responding during the reinstatement phase was compared using a 2 x 2 ANOVA with
Pre-exposure (nicotine or saline) and Dose (0.25 or 0.75 mg/kg) as between-subjects factors. To
analyze the reinstatement lever presses in relationship to previous responding, a 2 x 2 x 2
repeated measures NOVA was run with Session (average last four extinction session responding
or reinstatement session) as within-subjects factors and Pre-exposure (nicotine or saline) and
Dose (0.25 or 0.75 mg/kg) as between-subjects factors.

Any main effects or interactions were further analyzed by one-way ANOVAs and/or
Bonferroni post hoc tests as needed. Statistical significance was set at α = 0.05 for all measures.
Results

Adolescent Nicotine Exposure

*Body Weight.* The 2 x 17 repeated measures ANOVA on body weight during drug pre-exposure revealed a significant effect of Day \([F(15,930) = 4260.265, p < .05]\) such that all rats gained weight over the course of injections. There was also a significant Pre-exposure x Day interaction \([F(15,930) = 5.562, p < .05]\). Nicotine-exposed rats weighed significantly less than saline controls on PND 35 and PND 39-43 (see Figure 7).

![Figure 7: Mean Weight (±SEM) for Nicotine and Saline Groups \((n=32\text{ per group})\). *indicates significant difference between Groups.](image)

*Locomotor Activity.* The 2 x 7 repeated measures ANOVA on motor activity during drug pre-exposure revealed a significant main effect of Day \([F(6,372) = 25.783, p < .05]\), a significant main effect of Pre-exposure \([F(1,62)=93.171, p < .05]\) and a significant Day x Pre-exposure interaction \([F(6,372) = 20.56, p < .05]\). The nicotine treatment group increased locomotor activity over Days 2-6 compared to controls, indicating that nicotine was behaviorally active (see Figure 8).
Figure 8: Total Locomotor Activity (Gross+ Fine) for Nicotine and Saline Groups (n = 32 per group). *indicates significant difference between Groups on Test Days 2-6.

Adult Cocaine Self-Administration

*Acquisition.* Figure 9 illustrates the percentage of subjects in each group reaching criteria (10 or more active lever for five consecutive sessions and greater than 2:1 ratio of active/inactive lever responding) over cocaine self-administration sessions. As illustrated, all rats met the acquisition criterion by Session 14. A factorial ANOVA revealed a main effect of Dose [F(1, 50) = 4.066, p = .049], such that the high dose cocaine group met criterion faster than the low dose group. There was no main effect of Pre-exposure [F(1, 50) = .065, p = .799] nor a significant Pre-exposure x Dose interaction [F(3, 50) = .674, p = .416].
A 2 x 2 x 5 repeated measures ANOVA on the number of active lever presses over the first five sessions of cocaine access revealed a significant effect of Session \([F(4,188) = 3.101, p = .017]\) and Dose \([F(1,47) = 16.403, p < .05]\), as well as a significant Session x Dose interaction \([F(4,188) = 2.414, p = .050]\). There was no main effect of Pre-exposure \([F(1, 47) = .860, p = .359]\) nor was there a Pre-exposure x Dose interaction \([F(1, 47) = 1.533, p = .222]\). When considering inactive lever presses, there was a significant effect of Session \([F(4,188) = 2.890, p = .024]\) such that inactive presses decreased over sessions. There was no main effect of Pre-exposure \([F(1, 47) = 1.091, p = .302]\) or Dose \([F(1, 47) = .812, p = .372]\) on inactive lever presses, nor any significant interactions. The number of active lever presses was significantly greater than the number of inactive lever presses on Sessions 2-5 (see Figure 10). Only the first five sessions of acquisition were analyzed because some subjects in each Pre-exposure and Dose group met criterion (see above) by Session 6, e.g., three of thirteen (23%) nicotine low dose group, three of fourteen (21%) nicotine high dose group, two of thirteen (15%) saline low dose group and six of eleven (55%) saline high dose and were moved to progressive ratio self-administration.
Figure 10: Active (Top Panel) and Inactive (Bottom Panel) Lever Pressing Over First Five Acquisition Trials *indicates (A) significant difference in active lever responses between Groups N-0.25 (n=13) and S-0.25 (n=13) and Groups N-0.75 (n=14) and S-0.75 (n=14) on Sessions 3-5 and (B) significant differences between Session 1 and Sessions 2-5 for all groups.

Progressive Ratio Responding. A 2 x 2 x 2 repeated measures ANOVA revealed no significant effect of Session or Pre-exposure on number of active lever presses \( F(1,47) = 3.419, p = .071 \) or number of infusions earned \( F(1,47) = 3.209, p = .634 \). However, there was a significant main effect of Dose on active lever presses \( F(1,49) = 54.096, p < .05 \) and number of infusions \( F(1,49) = 243.986, p < .05 \), such that the high dose groups made significantly more active lever presses and earned more infusions than the low dose cocaine group, regardless of Pre-exposure (see Figure 11). There was no significant interaction for lever presses \( F(1, 47) = .346, p = .559 \) or number of infusions \( F(1, 47) = .205, p = .653 \). There was no significant main effect of Session \( F(1, 47) = .859, p = .359 \), Pre-exposure \( F(1, 47) = .171, p = .681 \) or Dose \( F(1, 47) = .474, p = .495 \) on inactive lever presses, nor were there any significant interactions.
Figure 11: Number of Active Lever Presses (A) and Number of Infusions Earned (B) During PR Sessions. * indicates significant difference between Groups N-0.75 (n=14) and S-0.75 (n=11) and Groups N-0.25(n=13) and S-0.25(n=13).

**FR-1 Stability.** A 2 x 2 x 2 repeated measures ANOVA on lever pressing during the FR-1 stability phase revealed an effect of Dose [F(1, 47) = 52.239, p < .05], such that the low dose group responded significantly more on the active lever than the high dose group, and of Pre-exposure [F(1, 47) = 52.4.684, p = .036, such that the nicotine pre-exposed group performed more active lever presses than the saline group. There was no effect of Session [F(1, 47) = .212, p = .648]. There was a significant Pre-exposure x Dose interaction [F(1, 47) = 4.183, p = .046].

Post hoc tests showed that the nicotine low dose group performed more active lever presses then the saline low dose group, as well as both high dose groups on Session 2 (all ps < 0.05). There was no significant difference in inactive lever presses on either session. Responding on these stability sessions met criterion (no more than 10% variance from last two FR-1 acquisition session’s average lever presses), showing responding was stable and rats could proceed to extinction (see Figure 12).
Figure 12: Active Lever Presses Following PR Testing. *indicates significant difference between Group N-0.25 and all other groups on Day 2.

Extinction. The percentage of subjects in each group meeting criterion (20% or less of the four previous session’s average active lever responding) over extinction sessions is shown in Figure 13. Inactive lever presses were minimal and were therefore not included in the criterion. A factorial ANOVA revealed a significant effect of Dose \[ F(1, 48) = 10.371, \ p = .002 \], such that the low dose cocaine group met criterion faster than the high dose group. There was no effect of Pre-exposure \[ F(1, 48) = .160, \ p = .691 \] nor a significant interaction between Dose and Pre-exposure \[ F(1, 48) = .728, \ p = .398 \].

Figure 13: Percentage of Subjects in Each Group Reaching Criteria over Extinction Sessions.
A 2 x 2 x 4 mixed measures ANOVA performed on active lever pressing during extinction revealed a significant effect of Session \( F(3,135) = 31.194, p < .05 \), such that each group decreased active lever responding over test session. There was no main effect of Pre-exposure \( F(1, 45) = .055, p = .816 \) or Dose \( F(1,45) = 2.574, p = .166 \) nor a significant Pre-exposure x Dose interaction \( F(1,45) = .078, p = .782 \). When considering inactive lever presses, there no significant effect of Session \( F(3,135) = 1.803, p = .150 \), Pre-exposure \( F(1,45) = .283, p = .597 \) or Dose \( F(1,45) = .388, p = .536 \), nor any significant interactions. Only the first four sessions of extinction were analyzed because five of twelve (42%) subjects in the nicotine low dose group, eight of thirteen (62%) subjects in the nicotine high dose group, four of thirteen (31%) subjects in the saline low dose group and ten of eleven (91%) subjects in the saline high dose animals met the extinction criterion on Session 5 and were moved to reinstatement (see Figure 14).

Figure 14: Active Lever Presses During Extinction Over the First Four Sessions.*indicates significant difference between Session 1 and Sessions 2-4 for all groups.

*Cue-induced reinstatement. A 2 x 2 ANOVA performed on the active number of lever presses during reinstatement revealed no significant effect of Pre-exposure \( F (1,48) = .167, p = .685 \) or Dose \( F(1,48) = .595, p = .444 \) nor any interaction \( F(1,48) = .109, p = .743 \). There

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was no significant effect of Pre-exposure \[F(1,48) = .448, p = .506\] or Dose \[F(1,48) = .913, p = .344\] nor an interaction \[F(1,48) = .2.331, p = .134\] in regards to inactive lever presses. To analyze the reinstatement active lever presses in relationship to previous extinction responding, a 2 x 2 x 2 mixed measures ANOVA revealed a significant effect of Session \[F (1,45) =155.133, p < .05\] (see Figure 16), such that all groups made significantly more lever presses during the reinstatement session than were made during extinction sessions (average of the last four extinction sessions). This suggests that the cue light was sufficient to restore responding, even with the continued absence of the drug. However, there was no significant effect of Pre-exposure \[F(1,45) =.114, p = .737\] or Dose \[F(1,45) = .488, p = .488\], nor was there a significant interaction \[F(1,45) = .066, p = .799\].

![Figure 15: Active Lever Presses During Reinstatement by Pre-exposure and Cocaine Dose Group.](image-url)
Figure 16: Active Lever Presses Compared Between Extinction (Mean Responding During Last Four Trials) and Reinstatement. *indicates significant difference between extinction and reinstatement responding for each group.

Discussion

As noted, only a few studies have examined the effects of an adolescent nicotine history on cocaine self-administration in which it was reported that although adolescent nicotine pre-exposure had no impact on the acquisition of cocaine self-administration, it did increase responding for various doses of cocaine once stable responding had been established (Dickson et al., 2012) and resulted in greater cue- and cocaine-dependent reinstatement (Anker and Carroll, 2011). Although adolescent nicotine exposure impacted adult cocaine self-administration in these studies, there were several concerns that limited the generality of these findings (see above). Given that nicotine had no effect on either the rewarding or aversive effects of cocaine (Experiment 1), Experiment 2 re-examined the effects of adolescent nicotine pre-exposure on adult cocaine self-administration. Specifically, Experiment 2 pre-exposed rats to either nicotine (0.6 mg/kg, SC) or vehicle from PND 28-43 and examined cocaine self-administration in adulthood (approximately PND 90). As described, adolescent nicotine exposure had no effect on cocaine self-administration.

These results agree with previous research demonstrating that nicotine does not impact the acquisition of cocaine self-administration. However, when comparing results from the other
self-administration phases, the conclusions are mixed. For example, Anker and Carroll (2011) reported no differences in the maintenance of cocaine responding, while Dickson et al. (2012) reported that nicotine pre-exposed rats displayed greater cocaine self-administration at a number of test doses of cocaine during the maintenance sessions. Maintenance responding was not assessed in the present study, so no direct comparisons can be made. In regards to extinction, Dickson et al. and the current study report no differences in the extinction of cocaine responding, while Anker and Carroll reported that nicotine pre-exposed rats made significantly more non-reinforced lever presses than controls during extinction, although the data were not presented so it cannot be determined to what extent the various groups differed. Our reinstatement results also differ from those found by Anker and Carroll, who reported that cue- and cocaine-induced reinstatement was greater in the nicotine pre-exposed subjects. Although the basis for these conflicting results is unknown, it is most likely a function of species, self-administration phase parameters, a continuous nicotine pre-exposure regiment (osmotic mini pumps) and the use of animals genetically predisposed to self-administer cocaine. Although nicotine history can clearly impact some facets of cocaine self-administration, e.g., maintenance responding and reinstatement, under certain conditions, it is clear that such effects are not consistently seen and other effects are consistently absent (acquisition). The present results, combined with those of Anker and Carroll and Dickson et al., suggest that the overall effects of nicotine history are relatively weak and parameter dependent.

This general weakness is a bit surprising when examined in the context of work with other drugs on the effects of adolescent pre-exposure and drug self-administration. Specifically, a history with a variety of drugs such as cocaine (Zhang and Kosten, 2007), alcohol (O’Dell, Roberts, Smith, and Koob, 2004) and heroin (Doherty and Frantz, 2012) increases self-
administration of that same drug. This is also true when examining different drug combinations. For example, methylphenidate (Crawford et al., 2011), MDMA (Fletcher et al., 2001) and ethanol (Hutchison and Riley, 2010) have all been shown to increase cocaine self-administration (see also, Ellgren, Spano and Hurd, 2007; Wooters, Neugebauer, Rush, and Bardo, 2008 for other drug combinations). Given these reports, it is possible that the pre-exposure effects seen with nicotine are a function of nicotine itself. However, this is unlikely given that adolescent nicotine pre-exposure increases adult nicotine self-administration in both female and male rats (Levin et al., 2003; 2007). It also does not appear that the effect is limited only to the similarity of the pre-exposed and subsequently tested drug given that adolescent nicotine pre-exposure alters the aversive (Rinker et al., 2011) and rewarding effects of alcohol (Le et. al, 2003).

Given that an adolescent drug history produces an effect on subsequent self-administration with other drug combinations, it remains unknown why there was no effect of nicotine pre-exposure in this experiment (and others). Perhaps the simplest possibility is that the nicotine dose given during adolescence was not sufficient to exert an effect. As described in Experiment 1, this is not likely given that previous studies using the same pre-exposure parameters have shown various nicotine induced changes (Horger et. al., 1992; Vastola et. al., 2002; Adriani et.al., 2006) and similar weight differences between saline and nicotine pre-exposed animals (Trauth, Seidler and Slotkin, 1999; Faraday, Elliot and Grunberg, 2001). Additionally, the rats in the present experiment showed clear sensitization to the locomotor effects of nicotine during pre-exposure, an effect similar to that reported in other research of motor sensitization in adolescent nicotine exposed rats (Belluzzi et.al, 2004; Elliott, Faraday, Phillips, and Grunberg, 2004). Taken together, the lack of a pre-exposure effect was not likely
due to the use of a behaviorally inactive dose of nicotine, although other doses may have impacted cocaine self-administration differently.¹

The present failure to see any effects of nicotine history on cocaine self-administration is consistent with the results of Experiment 1, in which adolescent nicotine exposure had no impact on the affective properties of cocaine. Given that drug use is thought to be a function of the balance of the rewarding and aversive properties of the given drug (Riley, 2011), it would be expected that a similar pre-exposure history would not affect cocaine self-administration. In this context, changes in self-administration would likely be evident only if cocaine’s affective properties were impacted in different directions (or to different degrees). These results help to further reinforce the position that there may be something unique about this combination of nicotine and cocaine that does not allow for long term effects of adolescent pre-exposure. As in Experiment 1, it is likely that the drug-free duration of approximately 50 days was too long to produce lasting changes in the behavioral response to cocaine. This study provides further support for Kandel and Kandel’s (2014) suggestion that nicotine pre-exposure must be relatively close (7 days) to and combined with subsequent cocaine administration to exert a priming effect. It is possible that an effect of nicotine would have been detected if nicotine was given up to and/or concurrently with cocaine self-administration testing. This idea is further supported by Horger, Giles, and Schenk (1992) who pre-exposed adult rats to nicotine (0.6 mg/kg) then tested for cocaine self-administration 24 hours after the last nicotine injection. Their results show that the nicotine pre-exposed rats more rapidly acquired the self-administration task when compared

¹ It is important to note that while there was no group effect of nicotine pre-exposure, there may be individual differences in the responsivity to both adolescent nicotine and later cocaine exposure. However, the self-administration regiment used here, whereby animals were moved to the each phase upon meeting predetermined criteria does not allow for such comparisons to be made given the varying individual training histories.
to saline pre-exposed controls. Further testing is required to determine if this effect persists when tested in adolescence.
CHAPTER 4

CONCLUSIONS

The aim of the current study was to determine if adolescent nicotine pre-exposure has any lasting impact on adult cocaine reward, aversion and self-administration. Experiment 1 pre-exposed adolescent rats to nicotine or saline, allowed them to age drug-free to adulthood and tested for cocaine conditioned taste avoidance and place preferences using a combined CTA/CPP procedure. Under these conditions, nicotine failed to produce any pre-exposure effects, although animals showed dose-dependent cocaine avoidance and a place preference. Given that self-administration may be a product of the balance of rewarding and aversive effects and nicotine pre-exposure did not alter either of these affective properties, it was then hypothesized that there would be no effects of adolescent nicotine pre-exposure on various aspects of cocaine self-administration. Accordingly, Experiment 2 pre-exposed adolescent rats using the same regiment as Experiment 1. To ensure that the nicotine was behaviorally active, locomotor testing during pre-exposure was also conducted. The animals were allowed to age drug-free to adulthood, then tested for cocaine self-administration acquisition, PR, extinction and cue-induced reinstatement. Again, rats showed cocaine dose-dependent responses, with no effect of pre-exposure. Under the conditions of the current study, adolescent nicotine did not produce any lasting alterations in the affective properties or self-administration of cocaine in adulthood.

The design used in these studies was appropriate for studying adolescent drug effects on adult responding given that the purpose was to examine nicotine use only during adolescence and to test adult responsivity. However, when examining the human literature, many adolescents who abuse drugs continue to use the drugs into adulthood. Therefore, continuing nicotine administration up to and/or concurrently with cocaine testing may be a more appropriate design to model adolescent behavior. However, there are multiple confounds with this approach that
may make the design difficult to interpret in an animal model. First, there may be an issue with interpreting any results obtained as a pre-exposure effect, given that the pre-exposure was continued up to testing. Also, giving only a short period of time between nicotine exposure and cocaine testing may allow for the potentially confounding influence of nicotine withdrawal, which has been shown to impact preferences, aversions and self-administration (Bardo and Bevins, 2000; Kenny and Markou 2001; Cohen, Koob, and George, 2012; though see Harris, Pentel, Burroughs, Staley, and LeSage, 2011). Despite these confounds, it could be argued that this method would produce a more externally valid model of adolescent drug abuse. Further assessment of the parameters influencing nicotine pre-exposure and its impact on cocaine responsivity is needed to extend and clarify the consequences of adolescent nicotine use. Continued research in this area may lead to better understanding of the factors that impact drug use and may in turn result in better prevention and treatment strategies.

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2 It could also be argued that the use of outbred animal strains is not the most valid model for studying drug use given that only a small subset of drug users go on to abuse the drug. In this context, it is possible that the animals genetically predisposed to drug abuse may more accurately model the percentage of the population that continues to abuse drugs from adolescence to adulthood.
REFERENCES


