Age-related differences in cocaine place conditioning and cocaine-induced dopamine

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Age-Related Differences in Cocaine Place Conditioning and Cocaine-Induced Dopamine

By

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A thesis submitted in partial fulfillment of the requirements for the degree of
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# Table of Contents

<table>
<thead>
<tr>
<th>Chapter One: Introduction</th>
<th>Chapter Two: Early Adolescents are More Sensitive to the Rewarding Effects of Cocaine than Late Adolescent and Young Adult Rats</th>
<th>Chapter Three: Basal and Cocaine-Induced Extracellular Dopamine In the Nucleus Accumbens Septi During Adolescence and Young Adulthood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theories of Drug Addiction</td>
<td>Abstract</td>
<td>Abstract</td>
</tr>
<tr>
<td>Drugs of Abuse and the Mesolimbic Dopamine system</td>
<td>Introduction</td>
<td>Introduction</td>
</tr>
<tr>
<td>Expectancy and Drugs of Abuse</td>
<td>Method and Materials</td>
<td>Method and Materials</td>
</tr>
<tr>
<td>Cocaine and the Mesolimbic Dopamine System</td>
<td>Subjects</td>
<td>Subjects</td>
</tr>
<tr>
<td>Drugs of Abuse and Adolescence</td>
<td>Apparatus</td>
<td>Apparatus</td>
</tr>
<tr>
<td></td>
<td>Procedure</td>
<td>Procedure</td>
</tr>
<tr>
<td></td>
<td>Design and Analyses</td>
<td>Design and Analyses</td>
</tr>
<tr>
<td></td>
<td>Results</td>
<td>Results</td>
</tr>
<tr>
<td></td>
<td>Discussion</td>
<td>Discussion</td>
</tr>
<tr>
<td></td>
<td>Age-Related Differences in Basal Dopamine</td>
<td>Age-Related Differences in Basal Dopamine</td>
</tr>
<tr>
<td></td>
<td>Ontogenetic Differences in Cocaine-Induced Dopamine</td>
<td>Ontogenetic Differences in Cocaine-Induced Dopamine</td>
</tr>
<tr>
<td></td>
<td>Extraction Fraction</td>
<td>Extraction Fraction</td>
</tr>
<tr>
<td></td>
<td>Comparison of Conventional and Quantitative Microdialysis</td>
<td>Comparison of Conventional and Quantitative Microdialysis</td>
</tr>
<tr>
<td></td>
<td>Conventional vs. Quantitative Microdialysis</td>
<td>Conventional vs. Quantitative Microdialysis</td>
</tr>
</tbody>
</table>
Chapter Four: Concluding Remarks and Implications

References

Appendices

Appendix A: Cocaine Place Conditioning-Figure
Appendix B: Normal Dopamine Concentration Gradient-Figure
Appendix C: Gradual Dopamine Concentration Gradient-Figure
Appendix D: Steep Dopamine Concentration Gradient-Figure
Appendix E: Basal Dopamine Levels-Figure
Appendix F: Dopamine and Repeated Cocaine Pretreatment for PND 35-Figure
Appendix G: Dopamine and Repeated Cocaine Pretreatment for PND 45-Figure
Appendix H: Dopamine and Repeated Cocaine Pretreatment for PND 60-Figure
Appendix I: Percent Change in Dopamine for PND 35-Figure
Appendix J: Percent Change in Dopamine for PND 45-Figure
Appendix K: Percent Change in Dopamine for PND 60-Figure
Appendix L: Age-Related Differences in Cocaine-Induced Dopamine-Figure
Appendix M: Basal Extraction Fraction-Figure
Appendix N: Cocaine-Induced Extraction Fraction-Figure
Appendix O: Extraction Fraction and Dopamine for PND 35-Figure
Appendix P: Extraction Fraction and Dopamine for PND 45-Figure
Appendix R: Extraction Fraction and Dopamine for PND 60-Figure
Appendix S: Conventional and Quantitative Microdialysis for PND 35-Figure
Appendix Q: Conventional and Quantitative Microdialysis for PND 45-Figure
Appendix T: Conventional and Quantitative Microdialysis for PND 60-Figure
List of Figures

Figure 1. Cocaine Place Conditioning 46
Figure 2. Normal Dopamine Concentration Gradient 47
Figure 3. Gradual Dopamine Concentration Gradient 48
Figure 4. Steep Dopamine Concentration Gradient 49
Figure 5. Basal Dopamine Levels 50
Figure 6. Dopamine and Repeated Cocaine Pretreatment for PND 35 51
Figure 7. Dopamine and Repeated Cocaine Pretreatment for PND 45 52
Figure 8. Dopamine and Repeated Cocaine Pretreatment for PND 60 53
Figure 9. Percent Change in Dopamine for PND 35 54
Figure 10. Percent Change in Dopamine for PND 45 55
Figure 11. Percent Change in Dopamine for PND 60 56
Figure 12. Age-Related Differences in Cocaine-Induced Dopamine 57
Figure 13. Basal Extraction Fraction 58
Figure 14. Cocaine-Induced Extraction Fraction 59
Figure 15. Extraction Fraction and Dopamine for PND 35 60
Figure 16. Extraction Fraction and Dopamine for PND 45 61
Figure 17. Extraction Fraction and Dopamine for PND 60 62
Figure 18. Conventional and Quantitative Microdialysis for PND 35 63
Figure 19. Conventional and Quantitative Microdialysis for PND 45 64
Figure 20. Conventional and Quantitative Microdialysis for PND 60 65
In humans, adolescent exposure to illicit drugs predicts the onset of adult drug abuse and suggests that early drug use potentiates adolescent vulnerability to drug addiction. In experiment 1, it was hypothesized that adolescent rats would show a CPP for a low cocaine dose if in fact adolescents are more vulnerable to cocaine’s rewarding effects. Place preferences were measured in early adolescent [postnatal day (PND) 35], late adolescent (PND 45) and young adult (PND 60) rats by injecting either 0, 5 or 20 mg/kg cocaine and conditioning them to environmental cues in a 2-chamber place conditioning apparatus. Significant cocaine preferences were found for all ages at the high dose. Interestingly, PND 35’s were the only age group to have a CPP at the low dose suggesting that PND 35 rats are more sensitive than late adolescent and young adult rats to cocaine’s rewarding effects. In Experiment 2, it was hypothesized that age-related differences in cocaine CPP may be mediated by differences in the mesolimbic dopaminergic (DA) system throughout development. Extracellular DA levels in the nucleus accumbens septi (NAcc) of early adolescent, late adolescent and adult rats were measured via quantitative microdialysis. PND 35, PND 45 and PND 60 rats were injected daily with either 5 mg/kg/ip or saline for 4 days, surgically implanted with a microdialysis probe aimed at the NAcc. Rats were perfused with either 0, 1, 10 or 40 nM DA and the extracellular DA concentration was measured. Our results show that adolescents differ from adults in basal DA with PND 35 rats having low basal DA (0.4
nM), PND 45 rats having high basal DA (1.8 nM) and PND 60 rats having intermediate basal DA (1.3 nM). PND 45 cocaine treated rats showed a 58% decrease in basal DA. All cocaine treated rats, regardless of age, showed a significant increase in DA over baseline in response to a cocaine challenge. Additionally, there were age-related differences in the extraction fraction (Ed), an indirect measure of DA reuptake, with PND 45 and PND 60’s showing a decrease in basal Ed, an effect absent in PND 35’s. Together these findings suggest that there are substantial ontogenetic differences in extracellular DA and DA reuptake and that these differences may provide an explanation for adolescent vulnerability to addiction. Future research should investigate DA supply and degradation processes in naïve and cocaine treated adolescent rats and vulnerability to addiction.
Chapter One

Introduction

For decades, scientists have searched for a biological mediator(s) of drug dependency. A substantial amount of this research has focused on one particular pathway in the brain, the mesolimbic DA system. This system originates in the ventral tegmental area (VTA) and projects to several brain structures including the NAcc, amygdala, hippocampus, septum, olfactory bulb, bed nucleus of the stria terminalis and the prefrontal cortex (Dahlstrom and Fuxe, 1964). Although substance abuse research has focused on the mesolimbic system, a considerable amount of evidence indicates a much broader range of stimuli affects this pathway. In general, the mesolimbic system is activated by motivationally significant stimuli (Blackburn et al., 1992). Any motivationally significant stimulus, whether it is positive/hedonic or negative/anhedonic, are capable of activating the mesolimbic system (Blackburn et al., 1992). Specifically, increases in DA in the NAcc of a rodent, as measured by \textit{in vivo} microdialysis, have been reported in response to many motivationally significant stimuli such as food (Martel and Fantino, 1996), sexual activity (Meisel et al., 1993), novelty (Rebec et al., 1997), aversive stimuli such as shock (Morrow et al., 1995) as well as drugs of abuse (Moghaddam and Bunney, 1989; Koob and Weiss, 1992). Thus, drugs of abuse are only one type of stimuli that can activate the mesolimbic system. More research should be done to clarify behavioral responses regulated by the mesolimbic system. Once these have been clarified, the involvement of the mesolimbic system in substance abuse and dependency may more likely be understood.
An important issue in studies involving the mesolimbic system research has been the functional and structural heterogeneity of the NAcc. The NAcc is anatomically divided into two distinct compartments: core and shell. The core has projections to the dorsolateral ventral pallidal subterritory, an area similar in anatomy to the striatopallidal subregion of the caudate putamen while the shell has projections to the ventromedial portion of the ventral pallidum (Zahm and Heimer, 1990). Increases in extracellular DA levels in the shell of the NAcc have been found in response to motivationally significant stimuli such as drugs of abuse (Pontieri et al., 1995), food (Tanda and Di Chiara, 1998), aversive stimuli (Kalivas and Duffy, 1995), and novel stimuli (Rebec et al., 1997). Motivation in these studies have been defined by increases in the number of lever presses for a reinforcing stimulus, increases in the amount of time spent in the environment paired with a reinforcing stimulus (i.e., CPP), and increases in the amount of stimulus intake (i.e., greater food intake, greater water intake, greater drug self-administration). Together, these findings indicate that the mesolimbic system and specifically the NAcc shell are activated by motivationally significant stimuli.

Theories of drug addiction. In the quest to identify the mediator(s) of addiction, several theories have been proposed. One of the earliest theories of addiction focused on the rewarding effects of drugs (Crow, 1970; Rolls et al., 1974; Fibiger and Phillips, 1974). According to the reward theory of addiction, enhanced DA activity in the mesolimbic system is pleasurable and rewarding. The majority of research during this era found rats will voluntarily electrically stimulate the mesolimbic system and will further change their response rates for intracranial self-stimulation (ICSS) with the administration of dopaminergic agonists/antagonists suggesting that DA is important in
reward. However, the reward theory of addiction has weaknesses in that it only provides an explanation for the early stages of drug use and poorly addresses the occurrence of chronic drug use. With certain drugs, like cocaine, initial administrations are the most pleasurable while later uses have a diminished rewarding effect. Additionally, addicts repeatedly take drugs and use higher drug doses to achieve the same subjective state as in the first use. Therefore reward could not be the sole factor driving drug addiction. An alternative theory, the anhedonia hypothesis (Salamone et al, 1997) provides a better explanation of chronic drug use. This theory suggests that addicts chronically use drugs to avoid the negative affect associated with drug use. Although drug use enhances dopaminergic activity of the mesolimbic system, over time drug use induces changes in synaptic transmission causing either sensitization (cocaine, amphetamine) or habituation (alcohol) of the system depending on the type of drug administered. It is suggested by the anhedonia hypothesis that the addict uses drugs to avoid these physiological and psychological effects, providing a better explanation for chronic drug use than the reward hypothesis. A limitation of the anhedonia hypothesis is that it poorly explains the early stages of drug use. The incompleteness of these two theories led to the development of current theories of drug use. Robinson and Berridge (1993) proposed the incentive salience theory of addiction. Incentive salience is described as “a psychological process that transforms the perception of stimuli, imbuing them with salience, making them attractive, 'wanted', incentive stimuli.” Importantly, Robinson and Berridge stated “the mesolimbic system’s function is to attribute 'incentive salience' to the perception and mental representation of events associated with activation of the system.” Moreover, the function of the mesolimbic system is not simply reward or aversion, but also includes
perception of salient stimuli. The incentive salience theory can be applied to many of the 
motivationally arousing stimuli mentioned above (food, sex, novelty, aversive stimuli and 
drugs of abuse). Di Chiara (1999) proposed that drug addiction is manifested by a 
generalized facilitation of associative learning. In this theory, behavioral control is lost 
and drug-related cues acquire a motivationally relevant valence that reliably induces 
drug-seeking behavior. Both the incentive salience and associative learning theories of 
drug addiction imply that the environment and drug-related cues play an integral role in 
repeated drug use and drug-seeking behaviors. Possible mechanisms underlying chronic 
drug use and the negative effects of withdrawal are discussed in the allostasis theory of 
addiction (Koob and Le Moal, 2001). The allostasis model of addiction states that during 
chronic drug use, homeostatic processes are dysregulated and fail to return to a normal 
range. This allostatic state drives subsequent drug use and in turn causes a downward 
spiral of dysregulation. In response to the proposal of these newer theories of addiction, 
much research has focused on the ability of salient stimuli to induce dopaminergic 
activity in the mesolimbic system and specifically the NAcc.

Drugs of abuse and the mesolimbic dopamine system. Drugs of abuse have 
several similarities in common with the salient stimuli discussed above. Rats exhibit 
place conditioning in response to ethanol (Risinger et al., 2001) heroin (Hand et al., 
1989), morphine (Higgins et al., 1992) amphetamine (Lett, 1989; Meyer et al., 2002), 
methylphenidate (Meririnne et al., 2001) and cocaine (Shippenberg and Heidbreder, 
1995; Horan et al., 2000). Rats will also self-administer drugs of abuse such as heroin 
(Higgins et al., 1994) cocaine (Pudiak and Bozarth, 2002) and ethanol (Tomkins et al., 
2002). Drug use is mediated by the mesolimbic system and specifically the NAcc.
Lesioning DA neurons in the mesolimbic system with 6-hydroxydopamine (6-OHDA) attenuates drug-induced behavior. For example, rats trained to self-administer nicotine were found to no longer lever press for the drug after infusion of 6-OHDA into the NAcc (Corrigall et al., 1992). Lesioning mesolimbic DA neurons results in attenuation of heroin self-administration behavior (Gerrits and Van Ree, 1996). Drug-induced behavior can also be affected by administration of DA agonists/antagonists. Place Preference studies reported CPP after administration of SKF38393, a DA D1 agonist (White et al., 1991). However, administration of haloperidol, a DA antagonist significantly decreased CPP scores relative to control rats (Adams et al., 2001). Drugs (amphetamine, alcohol, cocaine) also increase DA in the NAcc as measured by *in vivo* microdialysis (Moghaddam et al., 1989).

*Expectancy and drugs of abuse.* Another topic of interest has been the effect of expectancies on drug use. Expectancy is defined as the anticipation or predictability of an event (Goldman, 2002; Schultz et al., 1997). Prediction of future events facilitates both humans and animals in their adaptation to the environment. Anticipation of dangerous future events can increase the survival rate of an animal by allowing it more preparatory time. In addition, humans can benefit from expectancies. The knowledge of how and when an event takes place can greatly improve the choices a person makes in the event of adaptive situations. Expectancies are even evident in the CPP paradigm. As an animal is placed in a CPP apparatus, the animal typically spends more time in the chamber it ‘expects’ to induce a drug effect. Even DA neurons in the VTA have been reported to predict cocaine administration. Rodents previously treated with repeated cocaine were shown to have increased DA neuron firing in the VTA just prior to cocaine
Cocaine and the mesolimbic dopamine system. Cocaine, like other drugs of abuse, acts on the mesolimbic system and specifically the NAcc to produce its associated rewarding properties. Cocaine disrupts normal DA transmission by altering DA degradation processes such as DA reuptake. In normal functioning DA reuptake, excess extracellular DA in the NAcc binds to the DA transporter (DAT) to be transported back into the presynaptic terminal (Hitri et al., 1994). However, cocaine binds to the DAT in place of DA causing the transporter to be ineffective. Thus, excess extracellular DA is unable to bind to DAT’s, reuptake is inhibited and excess DA remains in the extracellular fluid of the NAcc. Inhibition of DA reuptake enhances basal accumbal DA and produces the rewarding effects associated with cocaine administration.

The rewarding properties of cocaine can be measured through place conditioning. Cocaine CPP is most effectively established for 20 mg/kg in 4 conditioning trials with the number of trials and doses ranging from 2-6 and 5-30 mg/kg cocaine respectively (Tzschentke, 1998). A CPP effect can even be produced with one administration of cocaine; however most CPP experiments employ repeated cocaine administration to better mimic human patterns of abuse. Rats will self-administer cocaine, however microinjections of DA antagonists into the NAcc attenuates self-administration behavior.
(Koob, 1992). Cocaine has also been shown to elevate basal accumbal DA as measured by in vivo microdialysis to approximately 300% of baseline (Chefer and Shippenberg, 2002; Thompson et al., 2000; Camp et al., 1994; Maisonneuve and Kreek, 1994; Parsons and Justice, 1993; Kalivas and Duffy, 1993; Weiss et al., 1992; Hurd et al., 1989). Doses of cocaine ranged from 2-20 mg/kg depending on the method of administration. Hence, the mesolimbic system is the primary pathway involved in expressing cocaine’s rewarding properties.

**Drugs of abuse and adolescence.** In humans, repeated exposure to drugs during adolescence predicts the onset and severity of substance abuse. Cocaine use during early adolescence is associated with rapid escalation from casual to daily substance abuse (Estroff et al., 1989). Adolescents who repeatedly use drugs also show greater substance consumption as an adult (Taioli and Wynder, 1991; Chen and Millar, 1998). A higher rate of substance dependence is found in those initiating use during adolescence. For example, adolescents exhibit a rapid escalation from initiation to dependence (Anthony and Petronis, 1995; Clark et al. 1998) and more difficulty quitting (Khuder et al., 1999; Chen and Millar, 1998). Even when substance consumption is controlled for, adolescents show a higher prevalence of dependence than adults (Kandel and Chen, 2000) suggesting that the increased risk of adolescent substance abuse is not a consequence of greater total consumption for early users; but relates to accelerated progression of dependence. As a result of these findings, clinical and experimental research has focused on identifying the behavioral and neurochemical mechanisms underlying adolescent vulnerability to addiction. Possibly the combination of adolescent neural developmental and drug-
induced synaptic change may likely potentiate the onset and severity of adult drug dependency.
Chapter Two

Experiment One

Early Adolescents are More Sensitive to the Rewarding Effects of Cocaine than Late Adolescent and Young Adult Rats

In humans, adolescent exposure to illicit drugs predicts the onset of adult drug abuse and suggests that repeated drug use potentiates adolescent vulnerability to drug addiction. It was hypothesized that adolescent rats would show a CPP for a low cocaine dose if in fact adolescents are more vulnerable to cocaine’s rewarding effects. Place preferences were measured in early adolescent (PND 35), late adolescent (PND 45) and young adult (PND 60) rats by injecting either 0, 5 or 20 mg/kg cocaine and conditioning them to environmental cues in a 2-chamber place conditioning apparatus. Significant cocaine preferences were found for all ages at the high dose. Interestingly, PND 35’s were the only age group to have a CPP at the low dose. Together these findings suggest PND 35 rats are more sensitive than late adolescent and young adult rats to cocaine’s rewarding effects. Future research should investigate ontogenetic differences in extracellular DA and how they relate to the development of addiction.

Introduction

In humans, adolescent exposure to illicit drugs predicts the onset of adult drug abuse and suggests that repeated drug use potentiates adolescent vulnerability to drug addiction (Estroff et al., 1989; Anthony and Petronis, 1995; Clark et al. 1998). Adolescent vulnerability to drug addiction may be mediated by age-related differences in the behavioral response to drugs of abuse. To test this hypothesis, animal models of
adolescent drug use have been developed to investigate the impact of drugs on adolescent behavior and brain functioning. Specifically, the place conditioning paradigm provides a measure of drug reward by assessing an animal’s ability to associate drug-induced effects with environmental cues. The amount of time spent in an environment is measured both before and after drug conditioning and a CPP is demonstrated if the rat spends a greater amount of time in the drug-paired environment. Given that there is increased experimentation with drugs and potentiated vulnerability to drug addiction during adolescence (Khuder et al., 1999; Chen and Millar, 1998; Kandel and Chen, 2000), it has been hypothesized that adolescents would find drugs more rewarding than younger and older rats and that the expression of cocaine place conditioning would be greatest in adolescents. In adults, a place preference is most effectively established for a relatively high dose, 20 mg/kg cocaine, in 4 conditioning trials (Spyraki et al., 1982; Bardo et al., 1986; Calcagnetti and Schechter, 1993; Hemby et al., 1994; Durazzo et al., 1994; Kaddis et al., 1995).

Whether adolescents express facilitated, inhibited or similar cocaine CPP to adults is not as clear. Cocaine CPP has been shown in pre-adolescent (Laviola et al., 1992; Pruitt et al., 1995) and early adolescent rodents (Laviola et al., 1992); however, adult comparisons were not included in these experiments. Additionally, the later study showed that early adolescents did not show a CPP at 5 mg/kg cocaine with one drug-pairing. Interestingly, a CPP was expressed at this age after increasing the number of drug-pairings to 4, suggesting that adolescent CPP models will produce variable results that are directly influenced by the type of experimental design employed. Others have found comparable CPP’s in adolescent and adults rats (Campbell et al, 2000) but
neglected to include a baseline comparison which is particularly important for developmental experiments given age-related differences in novelty-induced exploration (Stansfield and Kirstein, 2005).

Although several studies have investigated place conditioning in adolescence, these findings are difficult to interpret due to inconsistency across experiments in the inclusion of an adult comparison, choice of statistical design, species (Schramm-Sapyta et al., 2004), age and drug dose. In view of the fact that adolescents are more vulnerable to developing drug addictions, it may not be that adolescents express the greatest CPP at a particular drug dose, but that they are more physiologically sensitive to the rewarding effects of cocaine. If in fact adolescents are more sensitive to cocaine, then it would be expected that adolescents would demonstrate a CPP for a low cocaine dose (5 mg/kg) that which is typically not rewarding for adults. Therefore the adolescent CPP literature is in need of an experiment that incorporates adult comparisons, a baseline test, a late adolescent age group, and both low/high cocaine doses. The aim of the present study was to investigate cocaine CPP in early adolescent (PND 35), late adolescent (PND 45) and young adults (PND 60). Our results suggest there are age-related differences in cocaine place conditioning with PND 35’s expressing a greater sensitivity to cocaine’s rewarding effects.

Method and Materials

Subjects. Ninety male Sprague-Dawley rats, offspring of breeding pairs (Harlan Laboratories, IN), were used in the present study. The day of birth was designated as PND 0 and litters were sexed and culled to 10 pups per litter on PND 1. Pups remained housed with their respective dams in a temperature and humidity-controlled vivarium on
a 12:12-hr light/dark cycle (lights on from 0700 h and 1900 h). On PND 21, pups were weaned and housed in groups of three. As in humans, the adolescent period for rodents begins with sexual maturation (Odell, 1990). Although the exact age range of adolescence is still controversial (Odell 1990; Spear, 2000; Tirelli et al., 2003), for the purposes of the present study adolescence was operationalized as PND 34-46. Rats were trained and tested at three separate ages: PND 30-35 (early adolescent), PND 40-45 (late adolescent) or PND 55-60 (young adults). To eliminate the potential confound of litter effects, no more than one pup per litter was used for any given condition and remaining pups were used for other ongoing lab experiments. In all respects, maintenance and treatment of the rats were within the guidelines for animal care as approved by the University of South Florida’s Institutional Animal Care and Use Committee and the National Institutes of Health.

Apparatus. The conditioning apparatus was a single runway comprised of black Plexiglas (Rohm and Haas Company, Philadelphia, Pennsylvania) that was divided into two equal sized sections: each (21 x 24.5 x 20.5 cm) with visual and tactile cues of either black and white horizontal striped (1 inch thick) walls with a grey sandpaper floor or black and white vertical striped walls (1 inch thick) with a wire-mesh floor. The chambers were separated by a removable Plexiglas door. A 2-chamber apparatus, rather than a 3-chamber, was used to eliminate age-related differences in novelty-induced exploration (Stansfield and Kirstein, 2005) that may likely be induced by a less familiar central choice chamber which is typically incorporated in the 3-chamber CPP paradigm.

Procedure. The procedure consisted of four phases: handling (days one and two), baseline (day three), drug conditioning (days four-seven) and a CPP test (day eight). On
days one and two, rats were wheeled on a cart into the room where conditioning would take place and gently handled for 3 minutes. Handling occurred twice a day so that the rats would become used to experimenter handling (Maldonado and Kirstein, 2005). On day three, a biased design was used to determine baseline chamber preferences. Naïve-rats were placed in the center of the two CPP chambers in a dimly lit room and given free access to the entire apparatus for fifteen minutes. Time (sec) spent in each chamber was recorded. A camera was suspended above the CPP apparatus to record behavior. The camera signal was digitized and sent to a computer (Dell OptiPlex GX110) for analysis. Once data were received, movement was analyzed by distinguishing the tracked object (e.g., Sprague-Dawley rat) from the black background (Ethovision video tracking system, Noldus, Netherlands). The chamber in which each animal spent the least amount of time was designated as the least preferred (LP) chamber. Starting on the morning of day four, rats were injected with saline intraperitoneally and confined to the preferred (P) chamber for fifteen minutes. At least four hours after the morning injection, rats were injected with 5 or 20 mg/kg/ip cocaine and confined to the LP chamber for fifteen minutes. Control rats received saline injections in both chambers. Conditioning occurred twice a day over four consecutive days for a total of four LP and four P chamber exposures. The apparatus was cleaned with Quatricide (Pharmacal Research Laboratories Incorporated) and ethanol prior to each trial to remove odors. On day eight, the conditioned effects of cocaine were tested. Rats were tested drug-free in the same manner as at baseline (day three).

**Design and analyses.** Given that others have shown similar CPP responses between adolescents and adults (Campbell et al, 2000; Schramm-Sapyta et al., 2004), but
human data has suggested adolescent vulnerability to the rewarding properties of drugs
(Estroff et al., 1989; Taioli and Wynder, 1991; Chen and Millar, 1998; Anthony and
Petronis, 1995; Clark et al., 1998), it was our aim to investigate whether there are age-
related differences in place conditioning for a low dose of cocaine. Planned comparisons
(Bonferroni) were used to test for age and drug effects (Keppel, 1991). An adjusted
alpha value, based on the number of comparisons, was used to control for familywise
error. Comparisons were determined significant at the 0.01 alpha level. Baseline and test
measures were compared for each condition and the difference between the 2 measures
provided a place conditioning score (sec in the LP chamber at test – sec in the LP
chamber at baseline) and was used as the dependent measure. A CPP was defined as a
drug group spending significantly more time in the least preferred chamber than age-
matched saline controls.

Results
Appendix A illustrates cocaine CPP varied with Dose and Age. All three ages
demonstrated a CPP for 20 mg/kg cocaine [PND 35: t(3.91), p < 0.01; PND 45: t(3.14), p
< 0.01; PND 60: t(4.06), p < 0.01]. These findings are consistent with previous adult
CPP findings in that adult rats generally express a CPP for 20 mg/kg cocaine (Spyraki et
al., 1982; Bardo et al., 1986; Calcagnotti and Schechter, 1993; Hemby et al., 1994;
Durazzo et al., 1994; Kaddis et al., 1995). Appendix A also illustrates that PND 35’s
show a unique sensitivity to cocaine’s rewarding effects. PND 35 was the only age to
have a CPP for 5 mg/kg cocaine [t(3.94), p < 0.01]. Additionally, PND 35’s
demonstrated greater CPP than PND 45 [t(2.69), p < .01] and PND 60 [t(2.98), p < .01].
There were no differences between PND 45 and PND 60 for the 5 mg/kg condition \([t(0.11), p > .01]\). These results show that PND 35’s demonstrate a CPP for a low dose of cocaine and that early adolescents are more sensitive than late adolescent and adult rats to the rewarding properties of cocaine.

**Discussion**

These results are the first to show a significant age difference between adolescent and adult rats for a low dose of cocaine using the CPP paradigm. There are several physiological and behavioral mechanisms that may induce greater cocaine sensitivity for PND 35 rats. For example, cocaine produces its rewarding effects by blocking the DAT in the NAcc and consequently increasing extracellular levels of DA (Cooper et al., 2003). It is known that adolescents and adults have similar DAT densities in the NAcc (Tarazi et al., 1998b); however it is not known if the function of the DAT differs between age groups. It may be that DA reuptake rates differ between PND 35, PND 45 and PND 60 resulting in variable extracellular DA levels as a function of age after cocaine administration. Another possibility is that naïve PND 35’s differ in their basal extracellular DA levels. If young adolescent rats have a different tonic level of DA in the NAcc, these rats may be hyperresponsive to rewarding stimuli. It is known that changes in extracellular DA in the NAcc are associated with drug reward (Pontieri et al, 1995). Together, these results suggest that the greater cocaine sensitivity of PND 35 rats may be mediated by ontogenetic differences in extracellular DA.

Age-related differences in cocaine CPP may reflect divergent stimulus associations causing the rewarding properties of cocaine to be expressed differently as a function of age. For example, it has been suggested that drug addiction is manifested by
a generalized facilitation of associative learning (Di Chiara, 1999). PND 35’s may demonstrate facilitated associative learning and as a consequence, easily relate cocaine’s rewarding effects to drug-related cues present in the CPP apparatus. Additionally, the salience of drug-related cues may be expressed differently as a function of age. Robinson and Berridge (1993) discussed the role of the mesolimbic system and specifically the NAcc in the salience of drug-related cues. Possibly the transitioning of the mesolimbic system from preadolescence to adulthood results in aberrations in the salience of contextual cues thus mediating PND 35’s enhanced vulnerability to the rewarding effects of cocaine.

Another possibility is that PND 35’s develop stronger cocaine expectancies than older rats. CPP paradigms measure the rewarding value associated with a drug as well as drug expectancy. After repeated pairings of cocaine with specific contextual cues, rats that spend more time in the drug-paired chamber can be viewed as anticipating or expecting cocaine administration. Essentially these rats are thought to be seeking out the environment associated with the administration of cocaine. Therefore, the results of the present experiment should be extended to suggest that PND 35’s have greater cocaine expectancies and can easily associate contextual cues with cocaine’s rewarding effects. It would be interesting to determine if the present results extend to natural reinforcers such as food, water, sex and even aversive stimuli such as shock or predatory odors.

There are some caveats that should be addressed. Other adolescent behavioral studies have demonstrated hyposensitivity (Adriani and Laviola, 2003; Laviola et al, 1995; Spear and Brick, 1979) to psychostimulants. The present study does not provide evidence for adolescent hyposensitivity; however there are several methodological
differences in the present study that should be noted. The majority of the adolescent hyporesponsivity findings used locomotor activity as a measure of drug sensitivity while the present study used the CPP paradigm. Although both locomotor and place conditioning paradigms are valid measures of drug sensitivity, it is known that these two behavioral measures are associated with the activity of two separate DA pathways, the nigrostriatal and mesolimbic DA systems. Albeit psychostimulants act on both of these pathways, drug-induced behavior is associated with different dopaminergic responses in the terminal areas of these 2 pathways (Cadoni and Di Chiara, 1999). Further, drug-induced locomotor activity does not predict expression of CPP (Martin-Iverson and Reimer, 1996; Hemby et al, 1992). Therefore differences in psychostimulant-induced behavior would be expected when comparing locomotor activity to place conditioning. Others have shown a hyposensitivity to amphetamine place conditioning (Adriani and Laviola, 2003); however care should be taken when comparing amphetamine and cocaine behavioral results given that these 2 drugs work via different neurochemical mechanisms with amphetamine facilitating DA release and cocaine blocking DA reuptake. Additionally, the amphetamine CPP findings are in response to one drug-pairing while the present study incorporated 4 drug-pairings suggesting that ontogenetic differences to the rewarding properties of psychostimulants may vary depending on the extent of drug-pretreatment. The number of drug injections is a likely factor producing conflicting results between the amphetamine CPP and present findings since it has been previously shown that four drug-pairing produced a cocaine CPP in early adolescent mice while one drug-pairing failed to have the same effect (Laviola et al., 1992). Finally, the present CPP paradigm may be more sensitive than others (Campbell et al, 2000; Schramm-
Sapyta et al, 2004) in revealing ontogenetic differences in cocaine-place conditioning in that a baseline comparison was included to control for any age-related differences in novelty-induced exploration (Stansfield and Kirstein, 2005) upon first exposure to the CPP apparatus.

In summary, our results demonstrate that there are age-related differences in cocaine place conditioning with PND 35’s expressing unique sensitivity to cocaine’s rewarding effects. This increased sensitivity may be mediated by cocaine’s effects on the developing mesolimbic system. These neurobehavioral factors appear to vary as a function of age and likely potentiate adolescent vulnerability to drug addiction. Further research should focus on the ontogeny of reward-related associative learning and the involvement of DA in these processes.
In humans, adolescent exposure to illicit drugs predicts the onset of adult drug abuse and suggests that repeated drug use potentiates adolescent vulnerability to drug addiction. Our lab has previously shown that adolescents are more sensitive to the rewarding properties of cocaine with PND 35 rats demonstrating a CPP for a low dose of cocaine (5 mg/kg). It was hypothesized that age-related differences in cocaine CPP may be mediated by differences in the mesolimbic dopaminergic (DA) system throughout development. Extracellular DA levels in the NAcc of both adolescent and adult rats were measured via quantitative microdialysis. Early adolescent (PND 35), late adolescent (PND 45) and adult (PND 60) rats were injected daily with either 5 mg/kg/ip or saline for 4 days and surgically implanted with a microdialysis probe aimed at the NAcc. Rats were perfused with 0, 1, 10 or 40 nM DA and the extracellular DA concentration was measured. Results show that adolescents differ from adults in basal DA with PND 35 rats having low (0.4 nM), PND 45 rats having high (1.8 nM) and PND 60 rats having intermediate (1.3 nM) basal DA. PND 45 cocaine treated rats showed a 58% decrease in basal DA. All cocaine treated rats, regardless of age, showed a significant increase in DA over baseline in response to a cocaine challenge. Additionally, there were age-related differences in the extraction fraction (Ed), an indirect measure of DA reuptake, with PND 45 and PND 60’s showing a decrease in basal Ed, an effect absent for PND 35. Together these findings demonstrate ontogenetic differences in extracellular DA and DA reuptake.
Future research should investigate how these differences impact DA-dependent processes, such as addiction, attention, and learning, in adolescent and adult rats.

*Introduction*

Current neurochemical research in adolescence has focused on the development of the mesolimbic system, a dopaminergic (DA) pathway that originates in the VTA and terminates in the NAcc (Dahlstrom and Fuxe, 1967). Widespread physiological changes occur throughout adolescence including heightened neuronal activity for humans (Chugani et al., 1987), and overproduction and pruning of several receptors in monkeys and rats (Lidow et al., 1991) including changes in DA receptors (Teicher et al., 1995; Tarazi et al., 1998a; Tarazi et al., 1999). In male rats, DA receptors peak in density at PND 40 followed by receptor pruning into young adulthood (PND 60). Studies using gamma-butyrolactone autoreceptor models demonstrate that DA autoreceptors in the NAcc decrease in sensitivity with maturation (Andersen et al., 1997). DAT density in the NAcc rapidly increases until PND 35 (Tarazi et al., 1998b); but stabilizes thereafter. Interestingly, it has been suggested that there are age-related differences in DA degradation processes such as a lack of DAT upregulation in the striatum after repeated cocaine treatment (Collins and Izenwasser, 2002). Additionally, age related differences in enzymatic degradation of DA have been shown in the striatum (Nakano and Mizuno, 1996) and the NAcc (Philpot and Kirstein, 2004). Together, these findings suggest that the mesolimbic system undergoes marked change in DA synthesis, metabolism and receptors during adolescence and drug use during this stage of development may likely alter subsequent normal DA functioning. To our knowledge, no one has quantified basal
extracellular DA levels in the adolescent rat. It would be interesting to determine if adolescents had a hypo- or hypersensitive DA system to aid in the understanding of adolescent neurocircuitry and associated DA-dependent behaviors.

Conventional in vivo microdialysis has been used to measure DA concentrations in various brain regions. However, recovery of DA by the microdialysis probe, or any analyte of interest, is influenced by degradation processes occurring in the brain such as reuptake and metabolism (Shippenberg and Thompson, 1997; Justice, 1993; Smith and Justice, 1994). Analyte degradation processes, primarily reuptake, directly influence the rate at which an analyte is recovered by changing analyte concentration gradients (Justice, 1993; Shippenberg and Thompson, 1997; Olson and Justice, 1993; Smith and Justice, 1994). For example, cocaine blockade of DA reuptake decreases DA recovery while facilitation of DA reuptake increases DA recovery (Smith and Justice, 1994). The effects of DA reuptake on DA recovery by the probe during conventional microdialysis are illustrated in Appendices B-D. Appendix B illustrates how DA concentration gradients in the NAcc are affected by the presence of a microdialysis probe. The NAcc contains DAT’s located on mesolimbic DA terminals. Extracellular DA is reuptaken by the DAT (1) and recycled into the terminal button. The probe mimics the DAT by recovering extracellular DA via the process of diffusion (2). After DA is recovered, the area immediately surrounding the probe is devoid of DA and must be replenished. By the processes of diffusion, DA from undisturbed areas in the NAcc diffuses into the depleted sampling region (3). This flux of DA from surrounding areas demonstrates the extracellular DA concentration gradient in the NAcc of a dialysis rat. Appendix C illustrates the changes in DA recovery when DA reuptake is inhibited. When cocaine is
present, it blocks the DAT, inhibits DA reuptake (1) and excess DA remains in the extracellular fluid (2). As DA is recovered by the probe (3) and the sampling region is depleted of DA, excess DA in the extracellular fluid replenishes the sampling region (4) thus decreasing the need for DA to diffuse from surrounding areas. This decreased flux of DA in the extracellular fluid produces a gradual concentration gradient (5) and less movement of DA to the probe. Decreased reuptake and gradual concentration gradients limit the amount of DA available to be recovered by the probe and as a result underestimate true extracellular DA (see step 3; Olson and Justice, 1993). On the other hand, facilitated reuptake overestimates true extracellular DA. Appendix D illustrates the changes in DA recovery when DA reuptake is facilitated. If DA reuptake is facilitated either by increased density of DAT’s or by faster reuptake rates (1), DA is rapidly removed from the extracellular fluid (2). As DA is recovered by the probe (3) and the sampling region is depleted of DA, DA from undisturbed areas diffuses into the depleted sampling region (4). However, enough DA must diffuse to replenish both the sampling region around the probe in addition to areas devoid of DA from facilitated reuptake. As a consequence, an increased flux of DA in the extracellular fluid produces a steep concentration gradient (5) and facilitated movement of DA to the probe. Increased reuptake and steep concentration gradients enhance the amount of DA available to be recovered by the probe and as a result overestimate true extracellular DA (see step 3; Justice, 1993; Shippenberg and Thompson, 1997). The fact that DA degradation and DA recovery are dependent on each other suggests that any change in DA degradation across experimental groups will confound analyses of basal extracellular DA levels. Therefore, conventional microdialysis should only be used to determine analyte concentrations if
there are no suspected differences in degradation processes across manipulation groups. As noted above, it has previously been shown that there are age-related differences in DA degradation. Therefore it is imperative that a measure of DA recovery is included when measuring basal extracellular DA levels in adolescent rats.

Quantitative microdialysis in adults has been used to simultaneously measure extracellular DA and DA recovery in the NAcc (Parsons et al., 1991a; Parsons et al, 1991b; Olson and Justice, 1993, Smith and Justice, 1994; Shippenberg and Thompson, 1997). In quantitative microdialysis, various concentrations of DA are perfused through the probe in order to measure the point of “no net flux” for DA. When the DA concentration in the perfusate is greater than the DA concentration in the brain, DA diffuses from the probe and into the brain. On the other hand, when the DA concentration in the brain is greater than the DA concentration in the probe, DA diffuses from the brain and into the probe. Therefore, when in vivo DA concentrations are allowed to equilibrate, the brain and probe DA concentrations are equal and there is no net flux across the dialysis membrane. Any change in the tissue concentration of DA thereafter, for example when cocaine is administered, would be a direct consequence of cocaine and not influenced by the presence of the probe. In quantitative microdialysis, extracellular DA levels are calculated by the equation (DA_{in} – DA_{out}) where DA_{in} is the DA concentration in the perfusate and DA_{out} is the DA concentration in the sample. The net difference is averaged and plotted against DA_{in} and a linear regression line is formed for each sample. The x-intercept represents the extracellular DA concentration while the slope of the regression line represents a measure of DA recovery, or the extraction fraction (Ed). Ed has also been used as an indirect measure of DA reuptake because the
Ed is influenced by changes in DA reuptake (see above; Appendices B-D; Olson and Justice, 1993, Smith and Justice, 1994; Shippenberg and Thompson, 1997).

Taken together, development of the mesolimbic system during adolescence and the dependency of DA recovery on DA degradation processes, underlie the importance of using quantitative microdialysis to measure extracellular DA levels in adolescent rats. The aim of the present study was to measure basal and cocaine-induced extracellular DA levels in the NAcc of early adolescent (PND 35), late adolescent (PND 45) and young adult (PND 60) rats. Our results suggest that there are ontogenetic differences in basal/cocaine-induced DA and Ed in the NAcc.

Method and Materials

Subjects. Ninety-three male Sprague-Dawley rats (Harlan Laboratories, IN) bred in our vivarium were used in the present study. Breeding and housing procedures are identical to that stated above in Experiment 1. Rats used were divided into early adolescent (PND 35), late adolescent (PND 45) or young adults (PND 60). At the time of surgery, rats weighed between 200-340 g (PND 35, M = 110 g; PND 45, M = 225 g; PND 60, M = 299 g).

Pre-exposure and surgical procedures. Experiments began at PND 29, PND 39 or PND 54. The experiment consisted of 4 phases: handling (days 1-2), pretreatment (days 3-6), surgery (afternoon of day 6), and dialysis (day 7). All rats were handled daily in 3 minute sessions in order to decrease the stress of being handled by the experimenter during injections (Maldonado & Kirstein, 2005). For the next 4 consecutive days, rats received daily intraperitoneal injections of 5mg/kg cocaine or saline (0.9% NaCl). The 5mg/kg cocaine dose was chosen according to the results of experiment 1 that showed
ontogenetic differences in CPP. At least four hours following the last injection, rats were
anesthetized using a ketamine/ xylazine cocktail (1.0 and 0.15 mg/kg/ip). An incision
was made over the skull and the rat was mounted on a stereotaxic instrument for surgery.
Two holes for skull screws and one for the guide cannula were drilled in the skull. The
guide cannula was lowered into the brain using appropriate age-defined coordinates to a
site just above the NAcc (Philpot et al, 2001). The guide cannula was affixed to the skull
with cranioplast and the probe (2 mm membrane, 320 ODS, 30kDa MW cutoff,
Bioanalytical Systems Inc., IN) was immediately lowered into the NAcc. Rats were
singly housed in the dialysis testing environment overnight and allowed at least 18 h for
recovery.

Quantitative microdialysis procedures & neurochemical analyses. On the
evening prior to dialysis, probes were perfused continuously with artificial cerebrospinal
fluid (145 mM NaCl, 2.4 mM KCl, 1.0 mM MgCl, 0.2 mM ascorbate, pH = 7.2) for at
least twelve h prior to the start of sampling at a flow rate of 0.5 µL/min. The following
morning, DA solutions were prepared fresh from a 1 µM stock solution in artificial
cerebrospinal fluid to 1, 10 or 40 nM concentrations. Brain microdialysis probes were
connected through the use of a liquid switch to a 500 µL Hamilton gastight syringe and a
Bioanalytical syringe pump. The flow rate was increased to 2 µL/min and the perfusion
medium was then changed to 0, 1, 10 or 40 nM DA. After an equilibration period of 1.5
h, dialysates were collected by an automated fraction collector at 10-min intervals into
refrigerated (4°C) microcentrifuge tubes containing 2.0 µl of hydrochloric acid to prevent
enzymatic breakdown. Three baseline dialysate samples were taken from the NAcc after
which animals received an injection of 5 mg/kg/ip cocaine or saline. Sampling continued
for an additional 2 h. Dialysate samples (12.0 µl) were either run immediately or quickly stored at -80° C until analyzed. Brains were removed, frozen and cut for histological verification of probe placement in the NAcc. Analyses of dialysate samples were performed by high performance liquid chromatography with electrochemical detection (HPLC-EC) set to oxidize DA at 700 mV (Bioanalytical Systems, IN). A digital detector (Epsilon, Bioanalytical Systems, IN) was used with a radial flow carbon working electrode, referenced to an Ag/AgCl electrode. DA was eluted with a mobile phase consisting of 75 mM sodium phosphate, 1.4 mM octane sulfonic acid, 1 mM EDTA and 10% v/v acetonitrile with a pH = 2.9 and set at a flow rate of 60 µl/min. Dialysate samples (6 µl) were injected onto a C-18 microbore column, 100 x 1 µm, 3 mm ODS for peak separation (Bioanalytical Systems, IN). The HPLC was calibrated with a standard curve consisting of 100 to 0.1 nM DA standards. The range of detection was 1-10 nA and the average retention time for DA was 6 minutes. Peaks were verified by spiking one sample per rat with a DA standard. Data were recorded and quantified by Chromgraph on a Dell Dimension 2100.

*Design and analyses.* Samples were analyzed by the equation DAin - DAout = net DA, where DAin was the amount of DA perfused through the brain and DAout was the amount of DA obtained in the dialysate (Olson & Justice, 1993). The mean net DA was analyzed by linear regression and solved for basal extracellular DA. The slope of each regression line is equal to the recovery of DA and yields an indirect measure of DA reuptake, Ed. A three-way between subjects design was used to analyze age, drug and time course effects \{[Age (3): PND 35, PND 45, PND 60] x [Treatment (2): saline, cocaine] x [Time (2): Basal DA, Stimulated DA]\}. Paired t-tests were used to isolate age
effects. A wilcoxon test was used to compare percent cocaine-induced DA increases to baseline (median percent DA increase versus 100%). All statistical analyses were determined significant at the 0.05 alpha level.

Results

Overall, there are significant age and treatment differences for basal and cocaine-induced DA. A significant Age x Dose x Time interaction \([F(2, 60) = 6.91, p < 0.05]\) was found.

**Basal dopamine.** Appendix E shows age-related differences in basal DA for both naïve/cocaine treated rats \([\text{Age x Dose interaction } [F(2, 12) = 14.01, p < 0.05]]\). For naïve rats, PND 35 rats had the lowest (0.4 nM), PND 45 rats had the greatest, and PND 60 rats had intermediate (1.3 nM) basal DA. In cocaine treated rats, basal DA levels decreased by 58% for PND 45 while there were no changes in basal DA for PND 35 and PND 60 rats. These data suggest that basal DA varies as a function of both age in both naïve and cocaine pretreated rats.

**Cocaine-induced dopamine.** The time course effects (nM) of cocaine-induced DA for PND 35, PND 45, and PND 60 rats can be seen in Appendices F-H. However, because there were basal DA differences across age, basal DA was normalized to 100% and changes in DA concentrations were expressed as percent change from baseline. Time course effects for percent change in DA for PND 35, PND 45, and PND 60 can be seen in Appendices I-K. All three age groups demonstrated similar increases in percent DA. A wilcoxon test indicates that all three age groups show a significant increase in cocaine-induced DA over baseline (100%). Cocaine increased DA to 318 %, 247 %, and 314 % over basal levels for PND 35, PND 45, and PND 60 respectively. Interestingly, DA peaks and returns to baseline at a different rate for each age \([\text{Age: } F(2, 48) = 4.43, p\]
< 0.05] with PND 35’s peaking quickly at 10 min post injection and PND 45 and PND 60 rats peaking at 20 min post injection (Appendix L). The rate at which cocaine-induced DA returns to baseline differs across age with PND 35 quickly returning to baseline, PND 45 gradually returning to baseline, and PND 60 stabilizing at a higher value than at baseline. These data suggest that extracellular DA remains elevated for variable amounts of time with younger rats exhibiting a rapid decay and adults exhibiting prolonged elevations in cocaine-induced DA.

Extraction fraction. Ed, an indirect measure of DA reuptake, varied by age, dose and time [Age x Time, F(2, 60) = 3.44, p< 0.05; Dose x Time, F(1, 60) = 7.84, p 0.05]. PND 45 (t(20.47), p < 0.05] and PND 60 [t(12.41), p < 0.05] cocaine treated rats show decreased basal Ed after cocaine pretreatment (Appendix M). Decreased basal Ed in response to administration of cocaine has been previously shown in adults (Olson and Justice, 1993; Smith and Justice, 1994; Chefer et al., 2002). There were no changes in basal Ed for cocaine treated PND 35 rats. These data suggest that the magnitude of cocaine blockade of the DAT increases as the rat matures.

Basal Ed values were normalized to 100% and changes in Ed post challenge injection were compared across age (Appendix N). Cocaine-induced Ed varied with age and dose [Age x Dose: F(2, 48) = 4.79, p < 0.05]. In cocaine treated rats, both the PND 35 (t(3.22), p < 0.05] and PND 60 (t(4.58), p < 0.05] rats show a decrease in Ed after a cocaine challenge, as expected because cocaine blocks the DAT. Ed does not decrease in cocaine challenged PND 45’s, however, this may be influenced by a floor effect because basal DA and basal Ed levels have already decreased 58% and 25%, respectively. Adults show the greatest decrease in Ed in comparison to younger rats suggesting a greater
magnitude of cocaine-induced DAT blockade for adult rats. The time course effects of percent DA and percent Ed were compared at each age and are illustrated in Appendices O-Q. Ed follows the same temporal pattern as DA with Ed decreasing when DA peaks. This inverse relationship is supported by the fact that cocaine blocks the DAT, increases extracellular DA and decreases recovery of DA via a more gradual concentration gradient (Smith and Justice, 1994).

Comparison of conventional and quantitative microdialysis. Rats only perfused with aCSF provided a conventional microdialysis group so that conventional and quantitative microdialysis could be compared. DAout from the aCSF group was compared to extracellular values obtained via quantitative microdialysis (Appendices R-T). Extracellular DA varied as a function of microdialysis method [Age x Time x Method: F(2, 124) = 8.32, p < 0.05]. Stimulated DA for PND 60 was underestimated by conventional microdialysis [Appendix T; Age x Time: F(1, 44) = 4.77, p < 0.05]. Underestimation of extracellular DA in the NAcc has been previously shown in the adult quantitative microdialysis literature (Olson and Justice, 1993). Interestingly, the opposite was found for PND 45 rats in that extracellular DA was overestimated by conventional microdialysis [Appendix S; Dose: F(1, 44) = 3.94, p < 0.05]. There were no differences in DA between the 2 microdialysis methods for PND 35 rats (Appendix R). Therefore, DA concentrations appear to vary depending on the type of microdialysis method employed. Further, as conventional microdialysis neglects to control for DAT-probe interactions, these results provide additional evidence suggesting ontogenetic differences in the functioning of the DAT.
Discussion

**Age-related differences in basal dopamine.** The present study is the first to quantify basal extracellular DA levels in the NAcc of adolescent rats. There are differences in basal DA not only between adolescents and adults, but also within adolescence (Appendix E). Additionally, PND 45 rats show the greatest drug-induced plasticity in that they are the only age to show a decrease in basal DA after repeated cocaine pretreatment. A decrease in basal DA after administration of a DA agonist is suggestive of change in one or more dopaminergic degradation processes such as upregulation of the DAT via increased transporter density and increased rate of DA reuptake. Other DA degradation processes, such as increased DA metabolism or facilitated negative feedback via supersensitive DA autoreceptors, could also be involved. Cocaine-induced changes in DA degradation processes are likely contributors to the decreased basal DA for PND 45 as ontogenetic differences in DA metabolism (Philpot and Kirstein, 2004) and DA autoreceptors (Andersen et al., 1997) have been shown in the NAcc. Although cocaine blockade of the DAT primarily affects DA degradation processes (i.e. reuptake, metabolism), changes in DA supply can alter basal DA as well by decreased DA synthesis or decreased tonic release of DA. All of the aforementioned synaptic changes would mediate decreased basal DA after repeated administration of cocaine in late adolescent rats.

Differences in basal extracellular DA in adolescent rats suggest fluctuations and instability of DA neuronal activity in the mesolimbic system throughout development. Human adolescent brains undergo a physiological shift in primary brain activity from the limbic system during adolescence to more involvement of cortical areas in adulthood.
(Lewis et al, 1997). Together, the limbic mediated adolescent brain and fluctuating DA levels in the NAcc would support the hypothesis that adolescence is a time of transitional neuronal activity in the mesolimbic system. These data support studies comparing DA activity in the NAcc and PFC of adolescent rats. Spear (2000) eloquently discusses the inverse relationship between DA activity in the NAcc and PFC with early adolescent rats (PND 30) showing greater PFC and less NAcc DA activity than older adolescent rats (PND 40). Similarly, rats that express high DA levels also have heightened basal firing patterns in the mesolimbic system (Grace et al., 1995). These heightened basal concentrations, or tonic DA release, are regulated by glutamatergic afferents from the PFC, hippocampus and amygdala (Grace, 2000; O’Donnell et al, 1999). Fluctuating concentrations of extracellular DA in the NAcc throughout adolescence as shown by the findings of the present study may be mediated by differences in glutamatergic cortical regulation of the mesolimbic system throughout development.

**Ontogenetic differences in cocaine-induced dopamine.** Cocaine-induced DA peaks faster for early adolescent rats than for the other 2 ages. DA peaks at 10 min for PND 35 while PND 45 and PND 60 show a delayed DA peak at 20 min post cocaine injection (Appendix L). Interestingly, we have previously shown that early adolescents demonstrate a CPP for 5 mg/kg cocaine, while PND 45 and PND 60’s do not (Appendix A). A quick onset of cocaine-induced DA and prompt return to baseline values for early adolescent rats results in elevations in DA that are closely associated with the presentation of drug-related cues. Rather, for late adolescent and adult rats, DA is cleared less efficiently and elevations in DA are not closely time locked to drug cue presentation. Thus, associations between drugs and drug-related cues would be stronger
in rats exhibiting quick onset of cocaine-induced DA. The present data supports expectancy theory (Montague et al., 1996; Schultz et al., 1997) which suggests changes in the concentration or onset of mesolimbic DA provides information on the magnitude of reward and the consistency of reward presentation. Others have shown VTA DA neurons fire in response to reward related cues presented immediately prior to obtaining a reward (Carrelli and Ijames, 2000) and can be related to the quicker onset of cocaine-induced DA for PND 35 rats in the present study. For example, CPP conditioning trials are commonly 15 min. During place conditioning, DA levels have already peaked and are starting to return to baseline for early adolescent rats while DA levels have not yet peaked in the late adolescent & adult rats. As a result, rats that have peak DA levels while still inside the CPP chamber will associate drug effects with the chamber cues while other rats may not make a strong connection between the drug and chamber cues because DA is quite possibly peaking after completion of the conditioning trial. The quick onset of DA observed for PND 35 rats in the present study may be the neurochemical mechanism mediating low dose cocaine CPP in early adolescent rats. As all three age groups in the present study had similar percent increases in cocaine-induced DA, it may be the temporal changes in DA, and not absolute DA levels per se, that mediates adolescent vulnerability to drug addiction.

**Extraction fraction and dopamine reuptake.** The present data demonstrate ontogenetic differences in basal and cocaine inhibition of DA reuptake (Appendices M-N). The findings in adults are similar to previous reports that both show decreases in basal Ed and a lack of change in DA levels after repeated cocaine pretreatment (Chefer et al, 2002). The lack of change in basal Ed and small but significant decrease in cocaine-
induced Ed for cocaine pretreated PND 35’s demonstrates less DAT inhibition. PND 45 rats illustrate a transitional stage between minimal cocaine induced effects on DA reuptake in early adolescent rats and a substantial inhibition of DA reuptake for adults. The Ed data provides additional evidence for the suggested differences in DA clearance rate as seen in the cocaine-induced DA findings above. PND 35 rats clear DA quickly via facilitated DA reuptake while the oldest rats demonstrate the greatest cocaine-induced DA effect via prolonged elevations of extracellular DA and substantial inhibition of DA reuptake. Together, these data suggest age-related differences in DAT functioning with more efficient DA degradation in early adolescent rats relative to older ages.

Conventional vs. quantitative microdialysis. Adult data in the present study is similar to previous work that compared dialysate and extracellular levels in adult male Sprague-Dawley rats (Appendices R-T). Olson and Justice (1993) found that animals perfused with aCSF had lower DA levels than measured through quantitative microdialysis, suggesting that basal extracellular DA was underestimated using conventional microdialysis. Interestingly, the age differences found in the present study suggest that basal extracellular DA concentrations are dependent on the type of microdialysis technique employed. Although both microdialysis techniques show significant age-related differences, dialysate levels in late adolescents are overestimated (Appendix S) while adult levels are underestimated (Appendix T). Therefore, conventional microdialysis may suggest much larger differences between age, thus creating false positives. Interpretation of conventional microdialysis studies should be carefully examined when applied to developmental work. These theoretical concerns directly relate to degradation processes that are still developing throughout adolescence.
Age-related differences in estimated dialysate levels as measured via conventional microdialysis may likely be dependent on ontogenetic differences in DA concentration gradients in the NAcc.

In conclusion, there are ontogenetic differences in basal/cocaine-induced DA and DA reuptake in the NAcc. Early adolescents have less and late adolescents have more basal DA than adults in the NAcc. All age groups demonstrated similar percent increases in cocaine-induced DA. DA reuptake, as measured by Ed, varied as a function of age with early adolescent rats showing facilitated DA reuptake relative to older rats. Fluctuations and changes in the activity of DA in the NAcc may play a role in adolescent vulnerability to drug addiction. It has been previously shown by our lab that early adolescent rats who demonstrate the quickest onset and decay of cocaine-induced DA will express a CPP for a low dose of cocaine (5 mg/kg), that which adults do not find rewarding. Together these findings suggest that early adolescent rats are vulnerable to developing a drug addiction after the repeated administration of cocaine. Implications could be used to treat adolescent drug addiction as well as provide insight into how the adolescent brain responds to dopaminergic drugs.
Chapter Four

Concluding Remarks and Implications

In conclusion, the present studies illustrate that there are ontogenetic differences in the rewarding properties of cocaine, basal DA and DA reuptake. Early adolescent rats demonstrate a CPP for a low dose of cocaine suggesting an enhanced sensitivity to the rewarding properties of repeated cocaine. We also show a quick onset of cocaine-induced DA and facilitated DA reuptake for early adolescent rats which may allow cocaine-induced DA to be closely associated with the presentation of drug-related cues. For late adolescent and adult rats, DA may be cleared less efficiently causing cocaine-induced increases in DA to not be closely time locked to drug cue presentation. Thus, associations between drugs and drug-related cues would be stronger in rats exhibiting quick onset of cocaine-induced DA and facilitated clearing of excess DA from the synapse. The present data supports expectancy theory (Montague et al., 1996; Schultz et al., 1997) which suggests changes in the concentration or onset of mesolimbic DA provide information on the magnitude of reward and the consistency of reward presentation. Therefore, the overall activity of the mesolimbic system, and not absolute DA levels per se, may be the factor mediating adolescent vulnerability to drug addiction as all three age groups in the present study had similar percent increases in cocaine-induced DA.

The present findings suggest that human adolescents who repeatedly take cocaine may be particularly vulnerable to cue elicited cocaine craving if in fact they exhibit the same quick onset of cocaine-induced DA as demonstrated in the present study. It would be interesting to find if there were age-differences in brain activity during drug cue
presentation for adolescent and adult humans. The present findings may also provide insight for the treatment of adolescent and adult cocaine addicts. Clinical and experimental research should continue to focus on the development of the adolescent brain so to better understand how to treat adolescent substance abuse.
References


Appendices
Appendix A: Cocaine Place Conditioning

All ages had a CPP for the high dose, 20 mg/kg cocaine. PND 35’s were the only age to have a CPP for 5 mg/kg cocaine. Each bar represents mean and SEM.
Appendix B: Normal Dopamine Concentration Gradient
Appendix C: Gradual Dopamine Concentration Gradient

Undisturbed DA

Block reuptake

Cocaine

Probe

decreased recovery

5 gradual DA concentration
Appendix D: Steep Dopamine Concentration Gradient

1 Facilitated reuptake
2 Increased recovery
3 Probe
4 steep DA concentration
5 Undisturbed DA
For naïve rats, PND 35 rats had the lowest (0.4 nM), PND 45 rats had the greatest (1.8 nM), and PND 60 rats had intermediate (1.3 nM) basal DA. In cocaine treated rats, basal DA levels decreased by 58% for PND 45 while there were no changes in basal DA for PND 35 and PND 60 rats. Each bar represents mean and SEM.
The time course effects (nM) of cocaine-induced DA for PND 35. The perpendicular line denotes the cocaine injection. Each data point represents the x-intercept of a linear regression.
Appendix G: Dopamine and Repeated Cocaine Pretreatment for PND 45

The time course effects (nM) of cocaine-induced DA for PND 45. The perpendicular line denotes the cocaine injection. Each data point represents the x-intercept of a linear regression.
Appendix H: Dopamine and Repeated Cocaine Pretreament for PND 60

The time course effects (nM) of cocaine-induced DA for PND 60. The perpendicular line denotes the cocaine injection. Each data point represents the x-intercept of a linear regression.
The time course effects for percent change in baseline for PND 35. The perpendicular line denotes the cocaine injection.
Appendix J: Percent Change in Dopamine for PND 45

The time course effects for percent change in baseline for PND 45. The perpendicular line denotes the cocaine injection.
Appendix K: Percent Change in Dopamine for PND 60

The time course effects for percent change in baseline for PND 60. The perpendicular line denotes the cocaine injection.
Appendix L: Age-Related Differences in Cocaine-Induced Dopamine

A comparison of the time course effects for percent change in baseline across age. Cocaine increased DA to 318 %, 247 %, and 314 % over basal levels for PND 35, PND 45, and PND 60 respectively. DA peaks and returns to baseline at a different rate for each age with PND 35’s peaking at 10 min and PND 45 and PND 60 rats peaking at 20 min post injection. The rate at which cocaine-induced DA returns to baseline differs across age with PND 35 quickly returning to baseline, PND 45 gradually returning to baseline, and PND 60 stabilizing at a higher value than at baseline. The perpendicular line denotes the cocaine injection.
Appendix M: Basal Extraction Fraction

PND 45 and PND 60 cocaine treated rats show decreased basal Ed after cocaine pretreatment. There were no changes in basal Ed for cocaine treated PND 35 rats. Each bar represents mean and SEM.
Appendix N: Cocaine-Induced Extraction Fraction

In cocaine treated rats, both the PND 35 and PND 60 rats show a decrease in Ed after a cocaine challenge. Ed does not decrease in cocaine challenged PND 45’s. Each bar represents mean and SEM.
The time course effects of percent DA and percent Ed were compared for PND 35 rats. The perpendicular line denotes the cocaine injection.
The time course effects of percent DA and percent Ed were compared for PND 45 rats. The perpendicular line denotes the cocaine injection.
Appendix Q: Extraction Fraction and Dopamine for PND 60

The time course effects of percent DA and percent Ed were compared for PND 60 rats. The perpendicular line denotes the cocaine injection.
Appendix R: Conventional and Quantitative Microdialysis for PND 35

There were no differences in DA between the 2 microdialysis methods for PND 35 rats. Each bar represents mean and SEM.
Extracellular DA was overestimated by conventional microdialysis for PND 45 rats. Each bar represents mean and SEM.
Cocaine-induced DA for PND 60 was underestimated by conventional microdialysis. Each bar represents mean and SEM.