2007

Enduring changes in reward mechanisms after developmental exposure to cocaine: The role of the D2 receptor

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Enduring Changes in Reward Mechanisms After Developmental Exposure To Cocaine: The Role Of The D2 Receptor

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
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College of Arts and Sciences
University of South Florida

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Date of Approval:
October 30, 2007

Keywords: adolescence, development, dopamine, D2R antagonist, conditioned place preference, reward, novelty preference, cocaine

As a child
I sought your
Guidance and strength
To help me find happiness in life.

Now as an Adult
I walk
Beside you
And find happiness in sharing
Your understanding
Your love and
Your friendship

Acknowledgements

I would like to thank Dr. Cheryl Kirstein for being such an amazing and wonderful mentor; you were always there to support me, to back me up and give me advice. Everyone needs someone like that in his or her lives.

Dr. Shimizu, Dr. Cimino, Dr. Salomon and Dr. Bickford: Thank you for serving on my dissertation committee and providing me with insightful feedback.

Dr. Rex Philpot: Thank you for everything you have done for me over the last 6 years. You supported me when no one else could and always provided me with the honest (although sometimes brutal) truth! Don't change; everyone should appreciate your helpful truthiness!

Everyone in the Kirstein lab has provided me with support and feedback throughout the years, no matter how emotional and stressed we were: thank you!!!!

Toni Maldonado: we may be as different as chalk and cheese but we sure do enjoy each other's company! I will never forget the fun we had in the lab, especially those infamous water fights!

My parents: you were always there to support me. I know it has been a tough 6 years but no matter what you always believed in me and stood behind me. I should be telling YOU how proud I am! I love you both very much!

My dogs and cat: who have no idea why I spent the last 6 years playing with rats but who always greeted me with a warm wag of the tail and a cold nose to the face (maybe not the cat!).

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ABSTRACT

During adolescent brain maturation, there are likely sensitive periods where environmental conditions, including drug exposure, may influence development by modifying neuronal connections. Altering neuronal function may produce different phenotypes than expected under normal conditions that may influence subsequent responding to drugs of abuse after the brain is fully mature. Experiment one investigated the relationship between novelty preference and cocaine place preference in adolescent and adult rats. High responding adolescent rats displaying greater free choice novelty exploration (but not forced novelty locomotion) expressed decreased cocaine place conditioning compared to low responding rats. No relationship was found in adult rats. Experiment two evaluated novelty-induced behaviors in adulthood after adolescent cocaine exposure. Repeated cocaine administration produced greater stress and anxiogenic behavioral responses to novelty in adult rats. Repeated alcohol administration produced less-inhibited novelty-induced behaviors in adulthood. Experiment three and four evaluated the consequence of developmental cocaine exposure on the rewarding efficacy of cocaine in adolescence and adulthood. Additionally, the interaction of D2 receptors and the rewarding efficacy of cocaine were investigated. After developmental cocaine exposure, adolescent and adult rats demonstrate decreased rewarding efficacy to cocaine. Importantly, blockade of the D2 receptor prevents cocaine-induced neurochemical changes, potentially regulating the behavioral and neurochemical alterations that occur after repeated drug use that increases the likelihood of dependence. Together, these data implicate both short and long-term

behavioral adaptations that occur after developmental cocaine exposure that may result in a predisposition to develop adulthood drug dependence.

Chapter One

Introduction

Adolescence is a period when the brain is undergoing many complex changes that can exert long-term influences on decision making and cognitive processes (for review, see (Spear 2000)). It is also a period of experimentation, and Estroff (Estroff, Schwartz et al. 1989) has reported that illicit drug use can begin as early as age 12, with peak periods of initiation between ages 15 and 19. The mean age of illicit drug initiation in adults categorized as having a substance use disorder is 16 years old, with initiation rare after age 20 (Anthony 1991). In fact, initiation rates are so high that more than half (54%) of high school seniors have had at least one experience with an illicit compound (Johnston LD 2002). During the 1990's, there was a steady rise in the frequency of cocaine use in teenagers, by 2003, 4.3% of eighth graders, 5.7% of tenth graders, and 8.2% of high school seniors reported frequent use of cocaine (Johnston LD 2002). The fact that initiation of cocaine use is so dramatic during the adolescent period is particularly disconcerting given that the escalation of cocaine use appears more rapidly among teenagers than adult users, suggesting a greater addictive potential during adolescence than in adulthood (Estroff, Schwartz et al. 1989). Generally, adults who initiate drug use during adolescence are more likely to have higher lifetime rates of drug use and progress to dependency more rapidly than those who began drug use in adulthood (Helzer JE 1991; Kandel, Yamaguchi et al. 1992; Clark DB 1998). Moreover, adolescents demonstrate a more abrupt progression of illicit drug use and development of substance use disorders than adults (Warner, Kessler et al. 1995), suggesting that this ontogenetic period renders the adolescent more vulnerable to addiction.

Development of the central nervous system (CNS) during adolescence may play a key role in the increased likelihood to initiate drug use (for review, see (Spear 2000). Moreover, disruption of development of the CNS may result in subsequent long-term increases in the probability of drug use and dependence. During adolescence, critical neural structures involved in

substance abuse are regulated primarily by the limbic system which is associated with emotional and impulsive behaviors (for review, see (Spear 2000; Chambers RA 2003). Adolescence is a critical period of transition from a more emotional regulation of the structures that mediate substance abuse to a more mature cortical regulatory mechanism (Spear 2000). During adolescence, the primary dopaminergic (DAergic) projections to the nucleus accumbens septi (NAcc) extend from the ventral tegmental area (VTA), and are predominately modulated by the amygdala (Oades and Halliday 1987). However in adulthood, this previously amygdaloid-modulated system receives projections from the medial prefrontal cortex (mPFC) this developmental transition is critical in the functional nature of the system (Cunningham, Bhattacharyya et al. 2002). The development of this system allows for a transition from more emotionally directed behavior to more contextually regulated behavior. Because adolescents lack sufficient cortical regulation (provided by the mPFC), their behavior tends to be more impulsive and guided by emotion than adults, increasing the chances of risky behaviors (e.g. initiating drug use) (Campbell, Lytle et al. 1969; Chambers RA 2003). Additionally, repeated administration of cocaine during this period may cause a functional change in accumbal dopamine (DA) levels by altering amygdalar modulation of accumbal DA release and/or altering the functional role/development of the mPFC input; consequently, leading to an increased risk of dependency during adulthood. These ontogenetic changes, with the fact that adolescence is a key period of drug initiation, together, make a powerful argument for treating adolescence as a key time period for investigating the development of drug addiction.

Theories of Addiction

Anhedonia Hypothesis: Over the years, many different theories have been proposed to explain the mysteries of drug addiction. One of the initial beliefs about addiction was that early in the process, drug use was maintained due to subjective euphoric effects and with subsequent repeated exposure; homeostatic neuroadaptations lead to tolerance and dependency. Further, following these compensatory changes, withdrawal becomes extremely unpleasant, and often the individual would reestablish drug use again to avoid the negative symptoms associated with withdrawal.

This theory has been known by a variety of names such as: pleasure-pain, hedonic homeostasis, hedonic dysregulation, positive-negative reinforcement and reward allostasis (Solomon 1980; Koob, Caine et al. 1997; Koob and Le Moal 1997; Koob and Le Moal 2001). The basic principle of this theory is that a drug user initiates drug use to get the positive highs and after the neuroadaptations, to avoid the negative lows associated with withdrawal. The dependence on the drug to feel "normal" is presumed to sustain regular and addictive use. This theory has limitations in that it fails to explain drug relapse. Drug addicts often relapse into drug-taking again, even after they have been abstinent and free from the effects of withdrawal. Also, the absence of withdrawal symptoms does not protect against future relapse, as so many drug rehabilitation survivors can confirm. To summarize, conditioned feelings of withdrawal do not seem to be sufficiently strong enough or reliable enough to serve as the principle explanation of relapse (Robinson and Berridge 1993).

Aberrant Learning Theory: Another more recent theory of addiction that has gained a considerable amount of attention investigates the role of learning in the transition to addiction. For example, cues that predict the availability of rewards can powerfully activate brain reward circuitry [e.g. NAcc] in both non-human animals (Schultz, Dayan et al. 1997) and humans (Knutson, Adams et al. 2001), sometimes even better than the reward itself. Animals that are trained in the conditioned place preference paradigm (CPP) will spend more time in the environment which was previously paired with the drug (Tzschentke 2000) and less time in the unpaired chamber. Also, rats that were differentially trained to lever press for either cocaine and an auditory stimulus or water and a different auditory stimulus, showed discrete populations of accumbal neurons that were selectively activated by cocaine-associated stimuli but not water-associated stimuli (Carelli and Ijames 2001). Rats were able to discriminate between the auditory stimuli cues for cocaine and water and therefore were anticipating and/or expecting the reward, as evidenced by the activation of neurons in the NAcc. This learning theory ascertains that the change from recreational use to addiction involves a transition from behavior originally controlled by explicit and cognitively guided expectations produced by the memory of drug pleasure to compulsive drug use.

However, this fails to explain why drug cues become overpowering. Humans exhibit many habits in every day life, but there is a noticeable difference in this type of behavior as compared to the compulsive actions of drug addicts. This is a very insightful theory; however it fails to explain why compulsive behaviors become dominant over everyday activities, which leads to the next theory of addiction.

Incentive-Sensitization Theory: One contemporary theory of addition, labeled incentive-sensitization, focuses on how drug cues trigger excessive incentive motivation for drugs, leading to compulsive drug seeking, drug taking and relapse (Robinson and Berridge 1993). The main idea being that drugs of abuse change specific connections and circuits in brain systems, specifically accumbal-related areas, that mediate motivational functioning and learning, the emphasis of incentive salience. As a consequence, these neural circuits may become enduringly hypersensitive (or sensitized) to specific drug effects and to drug-associated stimuli (Schultz, Dayan et al. 1997). This drug-induced change is called neural sensitization (Berridge and Robinson 1998). Berridge and Robinson (Berridge and Robinson 1998) have proposed that this sensitized system leads psychologically to excessive attribution of incentive salience to drug-cues causing craving for drugs. The incentive-sensitization view suggests that addiction is a disorder of incentive motivation due to drug-induced sensitization of neural systems that mediate stimulus salience; therefore drug craving and use can be triggered by the presence of drug cues whose enhanced salience increases the likelihood of addictive behaviors (Robinson and Berridge 1993). This theory is appropriate for explaining the occurrence of findings such as the effects of novel and aversive stimuli increasing accumbal DA levels (Bradberry, Gruen et al. 1991; Imperato, Angelucci et al. 1992).

In summary, all three of these theories contribute much insight to aid in the understanding of drug addiction. However, just one theory cannot seem to explain addiction in its entirety, but possibly a combination of them can give a more accurate representation of what is occurring along the complex path to addiction.

The frequency of substance use disorders is elevated in adults diagnosed with several psychological disorders (Reiger DA 1990; Anthony 1991; Helzer JE 1991; Bucholz 1999; Blanco, Moreyra et al. 2001). Adolescents with similar disorders are also more likely to be diagnosed with substance use disorders (Swadi 1999; Zeitlin 1999; Shaffer, Forman et al. 2000). The fact that these mental disorders and adolescence are associated with substance use disorders suggests that common brain mechanisms may trigger drug susceptibility and potentially, addiction. These biological/neurochemical substrates might manifest into a behavioral trait or traits present in adolescents. Defective impulse control is a behavioral trait that characterizes psychiatric and substance use disorder groups (Swadi 1999; Moeller, Barratt et al. 2001; Rogers and Robbins 2001). Adolescence is marked by high levels of risk taking behavior relative to individuals of other ages. Human adolescents exhibit a disproportional amount of reckless behavior, sensation seeking and risk taking (Arnett 1999; Trimpop RM 1999). Not only is novelty seeking and high risk behaviors during adolescence present in humans, but also non-human animals (Douglas, Varlinskaya et al. 2003; Stansfield, Philpot et al. 2004). Adolescent mice engage in greater risk taking during exploration of a plus-maze (Macri S 2002) and exhibit hyperactivity on several behavioral measures (Adriani, Chiarotti et al. 1998; Adriani and Laviola 2000). Furthermore, studies have demonstrated a strong correlation between novelty preference and impulsive reactivity with both the rewarding efficacy of psychomotor stimulants and self-administration rates in animals (Hooks, Colvin et al. 1992; Klebaur, Bevins et al. 2001). High sensation seeking (HS) rats show higher rates of amphetamine and cocaine-induced locomotor activity and will self-administer these drugs more readily than low sensation seeking (LS) rats (Hooks, Jones et al. 1991). Moreover, HS rats seem to participate in far greater risk taking behaviors and show much higher behavioral and neurochemical responses in reaction to environmental stressors or pharmacological challenges than LS rats (Bevins RA 1997; Klebaur, Bevins et al. 2001). Taken together, these data suggest a relationship between sensation-seeking and novelty-seeking/impulsivity, making it more likely that adolescent's will become involved in risky behaviors

which may include drug use, initiation and increased vulnerability to the rewarding properties of these drugs.

Conditioned Place Preference

CPP is a behavioral paradigm used to measure the motivational and rewarding/ aversive properties of a variety of stimuli including: water, food, sucrose, access to conspecifics, novelty, access to copulation and drugs of abuse (i.e. cocaine, alcohol, nicotine, LSD) (for review, see (Bardo and Bevins 2000). In this procedure, a stimulus (for example: cocaine) is paired to an environment with distinct visual and tactile cues and at a different time point, a neutral stimuli (i.e. saline injection) is paired with a different environment with different visual and tactile cues. After several pairings (typically 4-8 total exposure to both chambers), the animals are allowed to freely explore both environments and time spent in the drug-paired chamber is compared to the saline-paired chamber. If the animal spends significantly more time in the drug paired chamber compared to the saline paired chamber, it is determined to be a CPP, and the drug is considered to be appetitive, however, if the animal spends significantly less time in the drug paired chamber compared to the saline paired chamber, this is considered a conditioned place aversion (CPA) and the drug is considered aversive.

Many studies have confirmed the CPP-inducing effects of amphetamine and cocaine in rats and mice. Most of these studies have used adult rats or mice; however, cocaine-induced CPP has been demonstrated in 10-, 17-, 35- and 45-day old rats (Pruitt, Bolanos et al. 1995; Badanich, Adler et al. 2006) and 21-day old mice (Laviola, Dell'Omo et al. 1992) and amphetamine-induced CPP has been demonstrated in 14-day old mice (Laviola, Dell'Omo et al. 1994). Reward measured by CPP has also been demonstrated for the psychostimulants and DA reuptake blockers (-)-amphetamine (Timar, Gyarmati et al. 1996), cocaethylene (Schechter 1995), Methamphetamine (Cunningham and Noble 1992), GBR12783 (Le Pen, Duterte-Boucher et al. 1996), nomifensine (Martin-Iverson, Ortmann et al. 1985), methylphenidate (Clark DB 1998; Sellings, McQuade et al. 2006) and bupropion (Ortmann 1985). The above studies demonstrate that drugs that cause an increase in extracellular DA produce CPP, however, the attempts to

determine DA receptor subtypes responsible for mediating the DAergic effect have yielded inconsistent findings.

Researchers have determined that drugs that yield increased extracellular DA are rewarding, however, to elucidate DA's role in this behavior, drug conditioning that decreases extracellular DA needs to be evaluated. A conditioned place aversion (CPA) has been demonstrated for the D1 antagonists SCH 23390, SCH 39166 and A-69024 (Shippenberg and Herz 1987; Shippenberg, Bals-Kubik et al. 1991; Cervo and Samanin 1995; Funada and Shippenberg 1996), a high dose of the atypical neuroleptic Olanzapine (Meil and Schechter 1997) and the DA release inhibitor CGS 10746B (Calcagnetti and Schechter 1991). Taken together, these results suggest that elevated extracellular DA is rewarding, whereas decreased extracellular DA is aversive, further implicating DAergic mechanisms in initiation and possibly maintenance, of drug dependence.

Mesolimbic DA Pathway and Reward

Ventral Tegmental Area (VTA): The mesolimbic system begins in the ventral tegmental area (VTA) and projects through the medial forebrain bundle to the amygdala, lateral septum, bed nucleus of the stria terminalis, hippocampus, and the NAcc (Oades and Halliday 1987). The VTA is subdivided into two compartments, which are determined by the localization of cell bodies in the VTA and their projection areas. Thus, the paranigral DA neurons project to the NAcc and are associated with reward and locomotor activity (Le Moal and Simon 1991) and the parabrachial DA neurons project to cortical structures that are involved with the modulation of cognitive functions (Williams and Goldman-Rakic 1998). Electrical self-stimulation of the VTA has generally shown an increase in DA release and metabolism in the NAcc and medial prefrontal cortex (mPFC) (Fiorino, Coury et al. 1993). The VTA is important as extracellular DA regulates neuronal release in downstream targets via activation of DA autoreceptors. Thus, simulation of DAergic neurons in the VTA causes an increase of extracellular DA, which subsequently activates the D2 autoreceptor, which inhibits firing in the VTA and downstream accumbal and cortical targets.

Different drugs of abuse have effects on DA along the mesolimbic pathway; however, not all drugs have the same effect on different regions. For example, animals will self administer

ethanol directly into the VTA (Rodd ZA 1998) but interestingly enough, animals will self-administer cocaine into NAcc (McBride, Murphy et al. 1999), but not the VTA (De La Garza, Callahan et al. 1998). This shows that although the mesolimbic pathway mediates the rewarding effects of certain drugs, their primary action occurs at different points of the pathway, and possibly by different mechanisms/pathways (e.g. reuptake inhibition vs. stimulation of pre- or postsynaptic receptors). Even though the NAcc has been of primary interest in examining the DAergic modulation of acute administration of DAergic agonists (e.g. cocaine and amphetamine), the VTA is important when investigating long term changes in accumbal neuron sensitivity after chronic DAergic agonist exposure as it has been demonstrated that VTA autoreceptors sensitivity changes after repeated exposure to psychostimulants (Henry, Hu et al. 1998).

NAcc: The NAcc is located in the basal forebrain, rostral to the preoptic area and immediately adjacent to the septum and is innervated by DA-secreting terminal boutons from neurons of the VTA (Skagerberg, Lindvall et al. 1984). The accumbens contains two functionally distinct subcompartments: the shell and core (for review see, (Rodd ZA 1998; Kelley 2004)). The shell is strongly interconnected with the hypothalamus and VTA and is important in regulating ingestive behaviors (Rodd ZA 1998). Reciprocal DAergic innervations from the VTA to the accumbens shell modulate motivational salience and contribute to establishing learned associations between motivational events and concurrent environmental perceptions (Bassareo and Di Chiara 1999). In contrast, the core is anatomically associated with the anterior cingulate and orbitofrontal cortex and appears to be a primary site that mediates the expression of learned behaviors in response to stimuli predicting motivationally relevant events (Kelley 2004). The involvement of the core in expressing adaptive behavior depends not on DAergic afferents, but rather, on glutamatergic afferents from the PFC (Di Ciano, Cardinal et al. 2001).

The output from the NAcc is projected to the ventral pallidum, which has been postulated to be responsible for motor execution of goal directed behaviors. It has been hypothesized that the NAcc serves as an interface between limbic and motor systems (Nauta, Smith et al. 1978). Importantly, stimulation of DA receptors in the NAcc will reinforce behavior [e.g. animals will lever press for electrical stimulation of the NAcc (Olds and Fobes 1981). Animals will also lever press

for direct infusions of DA and amphetamines directly into the NAcc, and some evidence has shown that animals will directly self-administer cocaine into the shell, but not the core of the NAcc (McKinzie, Rodd-Henricks et al. 1999). DA levels in the NAcc can be measured by *in vivo* microdialysis, a technique that samples extracellular cerebral spinal fluid. Many studies have found that administration (either self-administration or experimenter administration) of cocaine and amphetamine increases the levels of extracellular DA in the NAcc (Hoebel, Monaco et al. 1983). As mentioned earlier, the NAcc not only mediates reward, but other salient (e.g. aversion) stimuli as well (Salamone 1992). For example, footshock and tail pinch increase accumbal DA release in rats as measured by *in vivo* microdialysis. Moreover, presentation of a cue previously paired with drug administration increases accumbal DA, as does exposure to a novel stimulus or novel environment (De Leonibus, Verheij et al. 2006), indicative that the NAcc not only mediates rewarding stimuli, but also aversive and salient stimuli.

Extensive research has demonstrated complex mechanisms regulating not only the accumbens in reward, but also other aversive and attentional stimuli and extracellular DA; suggesting the possibility that drug use may not be maintained just because it is rewarding, but because it is conditioned.

DA: There are several neurotransmitters that have a considerable effect on brain activity. One that seems to be of major interest in regards to the effects of drugs of abuse including cocaine is DA. DA is synthesized from tyrosine and is broken down into 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Lindvall and Bjorklund 1974). DA acts via G protein-coupled receptors in a typical neuromodulatory fashion (Missale, Nash et al. 1998). These neuromodulatory actions are characterized by large temporal and spatial dimensions (Greengard 2001), that surpass the immediate surroundings of the synapse to include distant somatodendritic and presynaptic receptors following diffusion of the transmitter through the extracellular space (Gonon 1997). DA neurons are characterized by two patterns of activity: a tonic single-spike mode, and a phasic, bursting mode (Grace 2000). Tonic firing is reflected by steady state levels of extracellular DA and is responsible for basal DAergic concentrations, whereas phasic, burst firing is characterized by more rapid changes in extracellular DA that may be triggered by

rewarding, aversive or salient stimuli (Schultz 1998; Bradberry and Rubino 2004). Once released, DA diffuses throughout the extracellular fluid from which it is slowly cleared either by reuptake (e.g. dopamine transporter [DAT] or metabolism (e.g. COMT or MAO). Many researchers have concluded that DA plays an important role in mediating the reward value of food, drink, sex, drugs of abuse, and brain stimulation (for review, see (Bardo 1998). Although the exact mechanisms are still unknown, it is believed that drugs of abuse seize the reward circuitry that mediates responding to natural reinforcers such as food (Hernandez and Hoebel 1988) and sex (Damsma, Pfaus et al. 1992). The most compelling evidence that supports this theory is that animals self-administer chemicals that mimic DA (i.e., direct DA receptor agonists) or increase extracellular DA (i.e., indirect agonists) directly into the brain (e.g. NAcc). Moreover, in operant procedures, the response contingent delivery of DA agonists directly into the NAcc can serve as a reinforcer for that response. Hoebel et al. demonstrated that rats self-administer D-amphetamine, which increases extracellular DA, within the NAcc (Hoebel, Monaco et al. 1983). In addition, Carlezon has demonstrated that rats self-administer DA reuptake blockers and that rats acquire and maintain self-administration of direct DA receptor agonists into the NAcc (Carlezon, Devine et al. 1995; McBride, Murphy et al. 1999). Using place-preference procedures, the rewarding effects of direct and indirect DA agonists (e.g. amphetamine and cocaine) have been shown. Importantly, animals do not self-administer DA antagonists that decrease extracellular DA, and decrease responding for DAergic agonists when co-administered with DA antagonists (Bari and Pierce 2005). It has been hypothesized that long term elevations of mesolimbic DA by chronic cocaine exposure results in neuroadaptations within the mesolimbic DA system that manifest behaviorally as a transition from casual drug use to dependency (Chao and Nestler 2004). Two critical targets for these long-term adaptations are the DAT and D2 receptors, which mediate function and number of DAT in DAergic neurons (Mayfield and Zahniser 2001).

D2 & DAT: Dopamine receptors have been classified into two categories, D1-like (i.e. D1 and D5) and D2-like (i.e. D2, D3 and D4 receptors). Although D1-like receptors are located exclusively postsynaptically, D2-like are located postsynaptically and presynaptically, where they serve as autoreceptors, modulating membrane excitability, DA synthesis, DA release and

membrane transporter density (Wolf and Roth 1987; Santiago and Westerink 1991). D1 and D2 receptors are G-protein coupled and differ in their activity on the signal transduction pathway, 3′,5′-cyclic adenosine monophosphate (cAMP) (Birnbaumer and Brown 1990). Activation of D1 receptors stimulates adenylate cyclase via Gs [which stimulates protein kinase A (PKA)], while activation of D2 receptors inhibits adenylate cyclase via Gi (i.e. inhibition of PKA) (Kebabian and Calne 1979; Birnbaumer and Brown 1990). In contrast, activation of D1 and D2 receptors exert similar influences on the protein kinase C (PKC) pathway where stimulation of the D1 (Giambalvo and Wagner 1994) or D2 receptor results in a reduction of PKC levels (Iannazzo, Sathananthan et al. 1997). Although stimulation of D1 receptors following cocaine administration is critical in the rewarding effects of the drug, it is likely that long-term adaptations to chronic cocaine exposure are mediated by the stimulation of D2 autoreceptors which are involved in regulating synaptic DA levels (Wolf and Roth 1987; Santiago and Westerink 1991) and therefore, D2 receptors represent an important area of investigation in the development of addiction.

One of the presynaptic mechanisms regulated by the presynaptic autoreceptor are the DAT (Mayfield and Zahniser 2001). The DAT are the target of some drugs of abuse (e.g. amphetamine and cocaine), and may mediate the rewarding and reinforcing aspects of these drugs. Stimulants like cocaine and methylphenidate competitively inhibit DAT resulting in increased synaptic concentration of DA released from axon varicosities and dendrites, prolonged interaction of DA with both its postsynaptic and presynaptic receptors and behavioral activation.

Several studies have demonstrated that repeated cocaine administration results in increased DAT in the NAcc (Daws, Callaghan et al. 2002). DAT serve to remove DA from the synaptic cleft into the presynaptic terminal and therefore are important in regulating synaptic DA levels. Altering DAT densities results in modification of DA transmission by affecting DA reuptake: with increased DAT resulting in decreased synaptic DA, and decreased DAT number resulting in elevated synaptic DA levels (Zhang, Coffey et al. 1997). To demonstrate the importance of DAT and drug addiction, drug reward and reinforcement were investigated in DAT knockout mice. Contrary to expectations, DAT knockout mice still self-administer cocaine and exhibit conditioned place preference for cocaine (Hall, Li et al. 2002), which led to the

reevaluation of the DA hypothesis. However, complete deletion of DAT causes adaptive changes in DA homeostasis, including alterations in DA synthesis, storage, extracellular levels, and receptor expression and functions (Caron 1996). These adaptive changes may significantly alter normal reward pathways. Recently a knockin mouse line was generated carrying a functional DAT that is insensitive to cocaine (Chen, Tilley et al. 2006). In these mice, cocaine suppressed locomotor activity, did not elevate extracellular DA in the NAcc, and did not produce a conditioned place preference.

Importantly, changes in PKC activity have been demonstrated to alter DAT density in rats (Kitayama, Dohi et al. 1994) and humans (Vaughan, Huff et al. 1997). The activation of intracellular signaling proteins, specifically PKC, regulates the surface expression of DAT (Mayfield and Zahniser 2001). Inhibition of PKC by D2 receptor activation prevents the internalization of DAT, maximizing the number of active transporters on the membrane surface, and attenuating synaptic DA levels (Pristupa, McConkey et al. 1998). Conversely, increased PKC activity in the absence of D2 receptor stimulation results in increased DAT internalization, fewer active transporters and consequently greater synaptic DA levels (Huff, Chio et al. 1998). *In vivo* studies have demonstrated that repeated cocaine-induced increases in striatal uptake were attenuated by pretreatment with pimozide, a D2-antagonist (Parsons, Schad et al. 1993). Moreover, acute or chronic exposure to a D2 receptor antagonist decreases DA transport into striatal tissue *in vitro* and local administration of a D2 antagonist reduces DA uptake *in vivo* (Meiergerd, Patterson et al. 1993; Rothblat and Schneider 1997). Clearance of DA *in vivo* has been shown to decrease in the striatum, NAcc and PFC following administration of a selective D2- but not D1- antagonist (Cass and Gerhardt 1994). Meiergerd (Meiergerd, Patterson et al. 1993) demonstrated that DA uptake velocity is increased after agonist activation of D2 receptors, and is subsequently blocked by a selective D2 receptor antagonist. Taken together, these modifications in DAT number by D2 receptor mediated PKC activity indicate the critical role of D2 receptor/ DAT interactions in the regulation of synaptic DA levels and implicate D2 receptor activity as a potential target for the manifestation of long term adaptations in the mesolimbic DA system that manifest behaviorally as addiction following repeated cocaine.

Cocaine & Mesolimbic DA system

When cocaine is administered, it reaches all areas of the brain, but readily binds to specific areas within the reward pathway (i.e., NAcc and VTA). In a normally functioning individual, DA is released from the presynaptic cell into the synaptic cleft where it either binds to the postsynaptic cell or reuptaken into the presynaptic cell by DAT. When cocaine is administered, it binds with high-affinity to the DAT, which in turn, inhibits reuptake into the presynaptic cell, therefore increasing the amount of DA present in the synaptic cleft. Acute doses of cocaine have been shown to increase accumbal DA levels from 200-1170% for 80 to 100 minutes depending upon the dose (Kuczenski, Segal et al. 1991; Camp, Browman et al. 1994; Strecker, Eberle et al. 1995; Reith, Li et al. 1997). As shown from previous research, acute administration of cocaine, regardless of dose but following a dose response curve, produces significant and long lasting increases in extracellular levels of DA in the mesolimbic DA system. Similar findings have been shown in preadolescent and adolescent animals (Philpot and Kirstein 1998; Badanich, Adler et al. 2006).

Repeated administration of psychostimulants results in behavioral sensitization or reverse tolerance in an enhanced behavioral response to a subsequent drug challenge (Vanderschuren and Kalivas 2000). Consequently, rats who have repeatedly administered cocaine over at least 7 days, will show an elevated locomotor reaction in response to the drug which prevails up to seven days after cessation of the drug (Cass and Zahniser 1993). Sensitization not only occurs behaviorally, but neurochemically. Repeated drug exposure produces changes and adaptations at a cellular level which in turn alters the functioning of the entire pathway in which those neurons work (Kleven, Woolverton et al. 1988). These changes lead to the complex processes of tolerance, dependence and of course, sensitization (Wise 1980; Koob and Le Moal 1997). Sensitization is characteristic of repeated intermittent cocaine administration, whereas tolerance (defined as a smaller effect from a given dose of drug after previous exposure to that drug) occurs after continuous infusion of cocaine (Post 1980). Rats injected once a day with cocaine show enhanced inhibition of DA uptake, whereas rats getting a continuous infusion of cocaine show attenuated inhibition of DA uptake by cocaine (Izenwasser

and Cox 1992). Also, there seems to be different degrees of sensitization, such that longer times between cocaine injections produce greater sensitization (Post 1980). Sensitization, tolerance and dependence also result in functional adaptations such as increased cAMP pathway activity, increased calcium regulatory element binding protein (CREB) and also increased changes in immediate early genes (e.g. FosB) (Nestler and Aghajanian 1997).

Repeated administration of cocaine also produces significant changes in DA during withdrawal. *In vivo* microdialysis studies in the NAcc have shown that once self-administration of cocaine has ended, basal DA levels decrease significantly during this withdrawal period (Parsons, Smith et al. 1991). Taken together, these studies in adult animals show that repeated cocaine administration results in complicated changes in the DA mesolimbic pathway that continue long after drug use has stopped, and processes such as these may be implicated in craving and relapse.

Several researchers have demonstrated the importance of D2 receptors and DAT in the mediation of cocaine reward. D2 antagonists block the ability of cocaine to support a CPP (Adams, Careri et al. 2001). Additionally, D2 antagonists administered systemically not only decrease cocaine self-administration, but reduce the breakpoint to self-administer cocaine (Roberts, Loh et al. 1989; Barrett, Miller et al. 2004). The NAcc shell may mediate these effects as direct infusions of a D2 antagonist also decrease cocaine self-administration (Bari and Pierce 2005). These studies demonstrate that blockade of the D2 receptor decreases the rewarding and reinforcing efficacy of cocaine. Additionally, some researchers have been unable to establish a CPP in DAT knockout mice (Sora, Hall et al. 2001) whereas Sora et al. has demonstrated a CPP in DAT knockout (Sora, Wichems et al. 1998). Moreover, inhibition of DAT reduces cocaine self-administration (Lindsey, Wilcox et al. 2004) and decreased DAT binding has been associated with decreased cocaine self-administration (Wee, Carroll et al. 2006), indicating a role of DAT in the mediation of reward and reinforcement of cocaine. Importantly, chronic cocaine administration has been shown to upregulate DAT (Daws, Callaghan et al. 2002), and mice exhibiting an overexpression of DAT find cocaine more rewarding than wild-type mice (Donovan, Miner et al. 1999) implicating the importance of DAT density in the rewarding efficacy of cocaine.

Mesolimbic DA pathway and Behavior during adolescence

Adolescence is an important developmental period. It is also the period of initiation and maintenance of drug use and potentially drug addiction. Sexual maturation in the male rat encompasses postnatal days (PND) 30 through 55; this is the indicator to denote adolescence (Odell 1990) and the reason for selecting these ages to investigate. Very few models of adolescent drug addiction in animals have been developed to examine the remarkable differences between adolescents and adults.

Novelty seeking and high-risk behaviors seem to be highly associated with adolescence. Along this unique stage of development, distinct social, behavioral and neurochemical changes emerge, to assist with the important life events that will occur. For example, learning and acquiring skills necessary to permit survival away from parental caretakers (Spear 2000). This phenomenon being evolutionary adaptive as a means to avoid inbreeding (Schlegel A 1991).

In order for a successful transition from childhood to adulthood, an important aspect to gaining independence is when adolescents shift their social orientations from adults to peers (Steinberg 1989) and typically spend a significant amount of time interacting with their peers as opposed to adults. Adolescence is also marked by high levels of risk taking behavior relative to individuals of other ages. Human adolescents as a group exhibit a disproportional amount of reckless behavior, sensation seeking and risk taking (Trimpop RM 1999). Risk taking in adolescents poses some negative consequences such as accidents, pregnancy, AIDS, suicides and drug dependence (Irwin 1989). Although risk taking may be hazardous, it can also be beneficial. Risk taking and exploratory type behaviors allow an individual to explore adult behavior and may also serve (as mentioned above) as a protective evolutionary factor. Adolescent increase in risk taking and novelty seeking may trigger adolescent departure from the parental units by giving incentive to explore novel areas away from home and thus avoiding inbreeding via dispersal of the offspring during sexual development (Schlegel A 1991).

Similar to humans, adolescent rats are behaviorally different from younger and older rats. Periadolescent rats have been reported to be more hyperactive and inattentive (Spear and Brake 1983), exhibit greater novelty-preference (Stansfield and Kirstein 2006) and have reduced

responsiveness to some of the effects of alcohol (Silveri and Spear 1998), amphetamine (Bolanos, Glatt et al. 1998), and cocaine (Laviola, Wood et al. 1995). In the CPP paradigm, adolescent rats show a preference for nicotine, whereas the adult rats did not (Vastola, Douglas et al. 2002). Also, it has been demonstrated that adolescent rats showed a preference for moderate doses of alcohol and cocaine, whereas the adults had no preference (Philpot, Badanich et al. 2003; Badanich, Adler et al. 2006). Many behavioral alterations that are age-specific seen in human adolescents are observed in adolescent rats from PND 30 to PND 42, making adolescent animal models very useful to evaluate neurochemical and behavioral changes due to drug use during this important stage of development.

Impact of cocaine during adolescence

Few studies have examined the DAergic neuroadaptations that take place after repeated exposure to cocaine during adolescence, not only a developmental period during which drug use initiation is widespread, but also a critical period for the remodeling of the mesolimbic and mesocortical brain regions and their neuronal DA projections (for review, see (Spear 2000)). Rosenberg & Lewis (Rosenberg and Lewis 1995) were among those researchers who saw a common developmental pattern in the overproduction and subsequent pruning of synaptic connections during the period preceding adulthood. The D1 and D2 receptors have been of major focus for years in regards to overproduction and pruning as these receptors increase in density in the first few weeks of life (Hartley and Seeman 1983). Subsequently, Teicher et al have demonstrated receptor overproduction and elimination in both the striatum and prefrontal cortex (Teicher, Andersen et al. 1995; Andersen, Thompson et al. 2000). In addition, alterations in receptor binding and sensitivity in various neurotransmitter systems have been reported during adolescence (Trauth, Seidler et al. 1999) along with changes in the myelination of neurons (Hamano, Iwasaki et al. 1996). Importantly, DAT are overproduced and pruned during adolescence as the striatum transitions to its adult state in rats (Moll, Mehnert et al. 2000) and humans (Haycock, Becker et al. 2003). As DAT density increases during adolescence, enhanced reuptake reduces the extracellular levels of DA (Andersen and Gazzara 1993) and a subsequent upregulation of postsynaptic receptors, and their second messenger systems take place

(Andersen 2002). These developmental differences may not only predispose adolescents to be more vulnerable to the rewarding effects of drugs of abuse, but may leave them more vulnerable to addiction after drug exposure due to interference with the normal synaptic pruning that takes place in the transition from adolescence to adulthood.

Conclusion

During brain maturation, there are likely sensitive periods (i.e. adolescence) where environmental conditions, including drug exposure, may influence development by modifying neuronal connections and subsequently altering function. Aberrant levels of stimulation by drug exposure may produce different phenotypes than expected under normal developmental conditions that may influence subsequent responding to drugs of abuse after the brain is fully mature. More specifically, given that repeated cocaine in the adult rat yields increased DAT densities following cocaine exposure, and D2 autoreceptors have been implicated in this process, it is hypothesized that as adolescent rats have greater DAT and D2 receptors than adults, that artificially elevating DA levels (i.e. cocaine) will interfere with the normal pruning of these connections, thereby changing responsivity to rewarding stimuli in adolescence and adulthood. Elucidating the mechanisms by which addictive drug exposure (e.g. cocaine) during adolescence renders the adult more vulnerable to drug abuse is of utmost importance in a society that has a striking percentage of adolescents who experiment with cocaine.

Chapter Two

Does Novelty Preference Behavior Correlate With The Rewarding Efficacy Of Cocaine In Adolescent And Adult Rats?

Abstract

Adolescence is a time of high-risk behavior and increased exploration. This developmental period is marked by a greater probability to initiate drug use and is associated with an increased risk to develop addiction and adulthood dependency. Human adolescents are predisposed toward an increased likelihood of risk taking behaviors (Zuckerman 1986), including drug use or initiation. The aim of this study was investigate the relationship between differences in response to forced and free choice novelty and the susceptibility to the rewarding effects of the drug in the adolescent and adult rat. The present findings demonstrate that adolescent animals displaying greater free choice novelty exploration expressed decreased cocaine place conditioning compared to animals demonstrating decreased free choice novelty exploration; suggesting that LR adolescent rats demonstrate an increased rewarding efficacy to cocaine compared to HR adolescent rats. No differences were detected between forced novelty exposure and cocaine place conditioning. No differences were detected between forced novelty exposure and cocaine place conditioning in adult rats. Additionally, no relationship was found in adult rats between free choice novelty exploration and cocaine place conditioning. It seems a dissociation exists between forced novelty exposure and free choice novelty exploration in adolescent rats, suggesting that stress-induced locomotion and novelty-seeking behavior are different biobehavioral phenomena and might be activated by different neural and hormonal substrates. Future studies need to evaluate the neurochemical differences between individual behavioral traits during development that predispose them to initiate and maintain drug use.

Introduction

Adolescence is a period when the brain is undergoing many complex changes that can exert long-term influences on decision making and cognitive processes (for review, see (Spear 2000). It is also a period of experimentation, and Estroff (Estroff, Schwartz et al. 1989) has reported that illicit drug use can begin as early as age 12, with peak periods of initiation between ages 15 and 19. The mean age of illicit drug initiation in adults categorized as having a substance use disorder is 16 years old, with initiation rare after age 20 (Anthony 1991). In fact, initiation rates are so high that more than half (54%) of high school seniors have had at least one experience with an illicit compound (Johnston LD 2002). During the 1990's, there was a steady rise in the frequency of cocaine use in teenagers, by 2003, 4.3% of eighth graders, 5.7% of tenth graders, and 8.2% of high school seniors reported frequent use of cocaine (Johnston LD 2002). The fact that initiation of cocaine use is so dramatic during the adolescent period is particularly disconcerting given that the escalation of cocaine use appears more rapidly among teenagers than adult users, suggesting a greater addictive potential during adolescence than in adulthood (Estroff, Schwartz et al. 1989). Generally, adults who initiate drug use during adolescence are more likely to have higher lifetime rates of drug use and progress to dependency more rapidly than those who began drug use in adulthood (Helzer JE 1991; Kandel, Yamaguchi et al. 1992; Clark DB 1998). Moreover, adolescents demonstrate a more abrupt progression of illicit drug use and development of substance use disorders than adults (Warner, Kessler et al. 1995), suggesting this ontogenetic period renders the adolescent more vulnerable to addiction.

The frequency of substance use disorders is elevated in adults diagnosed with several psychological disorders (Regier, Farmer et al. 1990; Anthony 1991; Helzer JE 1991; Bucholz 1999; Blanco, Moreyra et al. 2001). Adolescents with similar disorders are also more likely to be diagnosed with substance use disorders (Swadi 1999; Zeitlin 1999; Shaffer, Forman et al. 2000). The fact that these mental disorders and adolescence are associated with substance use disorders suggests common brain mechanisms may trigger drug susceptibility and potentially, addiction. These biological/neurochemical substrates might manifest into a behavioral trait or traits present in adolescents. Defective impulse control is a behavioral trait that characterizes

psychiatric and substance use disorder groups (Swadi 1999; Moeller, Barratt et al. 2001; Rogers and Robbins 2001). Adolescence is marked by high levels of risk taking behavior relative to individuals of other ages. Human adolescents exhibit a disproportional amount of reckless behavior, sensation seeking and risk taking (Arnett 1999; Trimpop RM 1999). Novelty seeking and high-risk behaviors during adolescence are not only present in humans, but also non-human animals (Adriani, Chiarotti et al. 1998; Spear 2000; Douglas, Varlinskaya et al. 2003; Stansfield, Philpot et al. 2004; Stansfield and Kirstein 2006). Importantly, studies have demonstrated a strong correlation between novelty preference and impulsive reactivity with both the rewarding efficacy of psychomotor stimulants and self-administration rates in animals (Hooks, Colvin et al. 1992; Klebaur, Bevins et al. 2001). Researchers utilize two novelty preference paradigms: forced novelty exposure and free choice novelty exploration. Forced novelty exposure measures stress induced locomotor activity in a novel open field whereas free choice novelty exploration measures either frequency to approach a novel object or total time spent with a novel object in a familiarized environment (Stansfield and Kirstein 2006). High responder (HR) adult rats to forced novelty show enhanced sensitivity to drug stimulant effects, higher rates of amphetamine and cocaine-induced locomotor activity and will self-administer these drugs more readily than low responder (LR) rats (Piazza, Deminiere et al. 1989; Hooks, Jones et al. 1991; Cools, Ellenbroek et al. 1997). Moreover, HR rats seem to participate in far greater risk taking behaviors and show much higher behavioral and neurochemical responses in reaction to environmental stressors or pharmacological challenges than LR rats (Bevins RA 1997; Klebaur, Bevins et al. 2001). Pelloux et al. demonstrated that HR to forced novelty exposure consumed less oral amphetamine compared to LR (Pelloux, Costentin et al. 2004). Additionally, Pelloux and colleagues demonstrated that novelty preference is positively correlated with consumption of a low concentration morphine solution (Pelloux, Costentin et al. 2006). Taken together, these data suggest a relationship between novelty-seeking and drug use, making it more likely that adolescent's will become involved in risky behaviors which may include drug use, initiation and increased vulnerability to the rewarding properties of these drugs.

Few studies thus far have examined the relationship between individual differences in novelty preference and the propensity to find drugs of abuse rewarding [using the conditioned place preference (CPP) paradigm] in both adolescent and adult animals which may subsequently render the animal more vulnerable to drug dependence. CPP is a behavioral paradigm used to measure the motivational and rewarding/ aversive properties of a variety of stimuli including: water, food, sucrose, access to conspecifics, novelty, access to copulation and drugs of abuse (i.e. cocaine, alcohol, nicotine, LSD) (for review, see (Bardo and Bevins 2000)). In this procedure, a stimulus (for example: cocaine) is paired with an environment with distinct visual and tactile cues and at a different time point, a neutral stimulus (i.e. saline injection) is paired with a different environment with different visual and tactile cues. After several pairings (typically 4-8 total exposures to both chambers), the animals are allowed to freely explore both environments and time spent in the drug-paired chamber is compared to the saline-paired chamber. Bardo et al. demonstrated that HR adult rats to forced novelty exposure show greater amphetamine induced CPP compared to LR adult rats (Bevins RA 1997). Moreover, forced novelty exposure was positively correlated with oral consumption of amphetamine. In addition, the magnitude of morphine place conditioning is positively correlated with free choice novelty exploration but not forced novelty exposure (Chambers RA 2003).

The aim of this study was to investigate the relationship between individual differences in response to forced and free choice novelty and the susceptibility to the rewarding effect of the drug in the adolescent and adult rat. For this purpose, the current study compares both adolescent and adult reactivity to novelty using both forced novelty exposure and free choice novelty exploration with subsequent evaluation of cocaine place preference.

Methods

Forty male Sprague-Dawley (Harlan Laboratories, Indianapolis, IN) rats, offspring of established breeding pairs in the laboratory (University of South Florida, Tampa) were postnatal day (PND) 30 (μ=134 grams) at the beginning of the study. No more than one male per litter per age was used in a given condition. Pups 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 h light: dark cycle (07:00 h/19:00 h) until PND 21, on PND 21 pups were weaned and male littermates were group housed throughout the entire experiment. Animals were experimentally naive until the beginning of the study (PND 30). The care and use of animals was in accordance with local standards set by the Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals (Health 1989).

Procedure: Beginning on PND 30 or 61, animals were tested on a black plastic circular platform (116 cm diameter) standing 70 cm from the ground, with a white plastic barrier (48 cm height) enclosing the arena (100 cm diameter). A video camera was suspended directly over the table and recorded the animal's behavior using a Noldus Behavioral Tracking System (Noldus, Netherlands).

Over a period of four consecutive days, each rat (PND 30-33 or 61-64) was placed in the open field in one of four randomly selected zones and allowed to freely explore the novel environment for five minutes. This procedure was performed twice a day for a total of 8 habituation trials. Immediately following the 8th trial, animals were removed for 1 minute while a single novel object (approximately 16 cm high) was attached to the center of the table (trial 9). Rats were placed in a random zone and allowed to explore the familiar environment and novel object for five minutes. Forced novelty exposure (i.e. total distance moved (TDM) in an inescapable novel environment) and free choice novelty exploration (i.e. frequency to approach the novel object in a familiarized environment) were measured.

Animals were trained in the CPP paradigm from either PND 34-42 or 64-72 and tested on PND 43 or 73. Animals were trained using a two-chambered apparatus made of clear Plexiglas with a clear Plexiglas cover. Two compartments (21 cm wide x 18 cm long x 21 cm high) separated by a removable wall were used for conditioning. The two chambers provided distinct visual (vertical or horizontal black and white bands) and tactile (wire or sandpaper flooring) cues to establish an association when paired with either saline or cocaine (10.0mg/kg, i.p.). This study utilized a biased design. A video-based tracking system (EthoVision, Noldus Information Technologies) was used to record and quantify the data.

Animals were tested in the CPP apparatus for 15 minutes (wall removed for free access to both chambers) 24 hours prior to the first training session to determine initial preferences for either the horizontal or the vertical striped chamber. In a biased design, the two chambers were designated post-hoc as preferred or aversive, based on the compartment that the animal spent the most and least time in, respectively. Following baseline recording, the animals were trained over a period of 8 days. Each day (between 0900 and 1100 hr) the animals received either saline or cocaine and were confined to the preferred or aversive chamber, respectively, for 15 minutes. For all animals, the order of chamber exposure was alternated daily. Animals were tested approximately 16-18 hours after their last training session. Animals were placed in the apparatus with the wall removed and tested for 15 minutes to determine the conditioned effects of repeated drug exposure. Preference was assessed using a difference score derived by subtracting the total time spent in the initially preferred chamber from the total time spent in the initially aversive chamber on test. Before each trial and test period, the apparatus was cleaned with 70% EtOH to remove any lingering odor cues. All floors were washed with soap and water and left to dry for 24 hours before subsequent use.

Data Analyses: Data analyses were performed with Graphpad Prism (Graphpad, CA). The data were expressed as the means +/- SEM, and the significance level was set at p=0.05. A correlation was used to analyze the relationship between novelty measures and drug sensitivity as measured by CPP. In addition, four t-tests were used to assess differences between LR/HR rats and cocaine place conditioning. A significance level of .05 was used for all analyses.

Results

The present findings reveal no correlation between forced novelty exposure and cocaine place conditioning ($r=-0.2681$, $p>0.05$, see Figure 1), in addition, no cocaine place conditioning differences between LR and HR adolescent rats were found [$t(9)=1.296$, $p>0.05$, see Figure 2]. Interestingly, free choice novelty exploration correlated with cocaine place conditioning ($r=-0.5059$, $p<0.05$, see Figure 3). Adolescent animals displaying greater free choice novelty exploration expressed decreased cocaine place conditioning compared to animals demonstrating decreased free choice novelty exploration [$t(8)=2.256$, $p<0.05$, see Figure 4); suggesting that LR

adolescent rats demonstrate an increased rewarding efficacy to cocaine compared to HR adolescent rats.

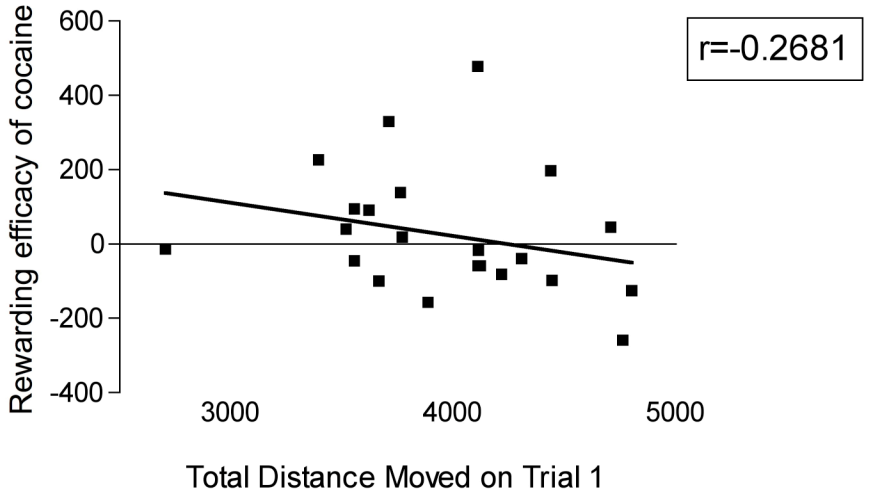

Figure 1: Adolescent animals that demonstrate greater forced novelty-induced locomotor activity demonstrated a reduced but not significant cocaine place preference compared to animals showing decreased novelty-induced locomotor activity.

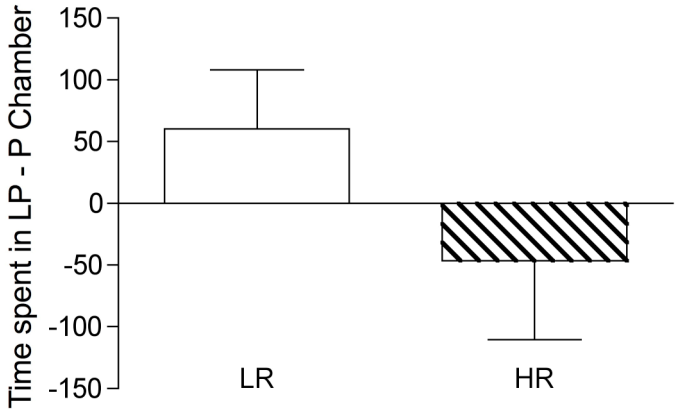

Figure 2: Adolescent animals that demonstrate greater forced novelty-induced locomotor activity demonstrated a reduced but not significant cocaine place preference compared to animals showing decreased novelty-induced locomotor activity.

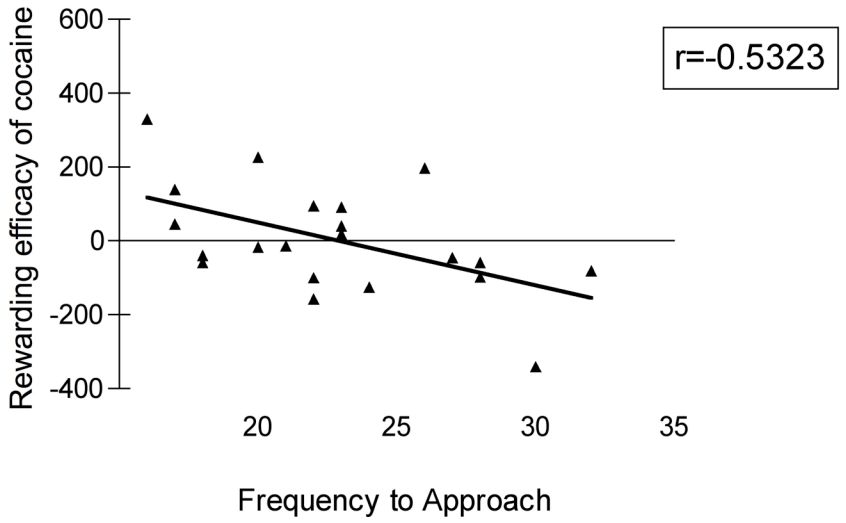

Figure 3: Adolescent animals that demonstrate greater free choice novelty-induced exploration demonstrated a significantly reduced rewarding efficacy to cocaine compared to animals showing decreased novelty-induced exploration.

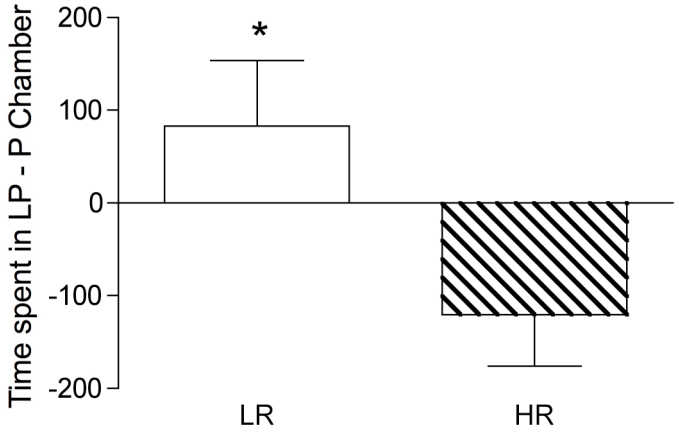

Figure 4: Adolescent animals that demonstrate greater free choice novelty-induced exploration demonstrated a significantly reduced rewarding efficacy to cocaine compared to animals showing decreased novelty-induced exploration

No correlation was detected between forced novelty exposure and cocaine place conditioning in adult rats ($r=-0.280$, $p>0.05$, see Figure 5); in addition, no cocaine place conditioning differences between LR and HR adult rats were found [$t(8)=0.8518$, $p>0.05$, see Figure 6]. Moreover, no correlation was detected in adult rats between free choice novelty exploration and cocaine place conditioning ($r=0.0079$, $p>0.05$, see Figure 7); in addition, no

cocaine place conditioning differences between LR and HR adult rats were found [t(11)=0.1971, p>0.05, see Figure 8].

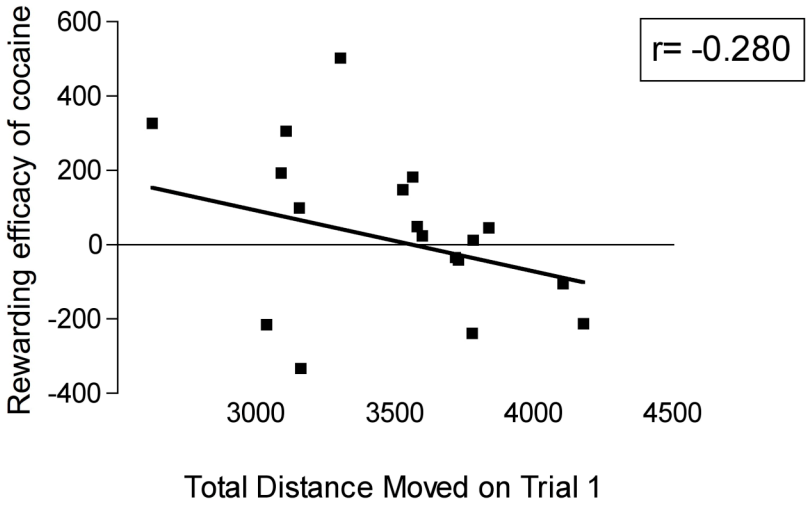

Figure 5: Adult animals that demonstrate greater forced novelty-induced locomotor activity demonstrated a reduced but not significant cocaine place preference compared to animals showing decreased novelty-induced locomotor activity.

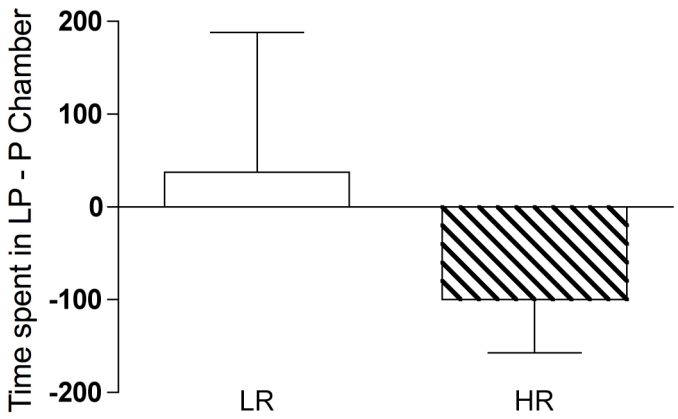

Figure 6: Adult animals that demonstrate greater forced novelty-induced locomotor activity demonstrated a reduced but not significant cocaine place preference compared to animals showing decreased novelty-induced locomotor activity.

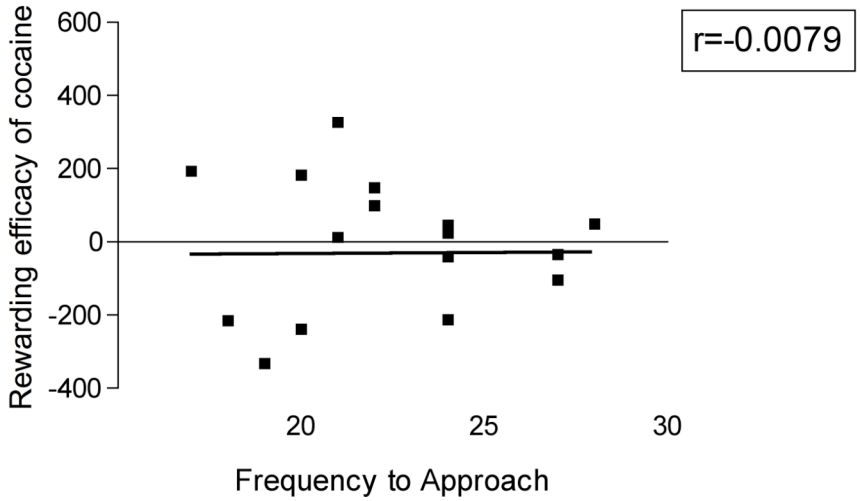

Figure 7: No relationship was found in adult rats between free choice novelty-induced exploration and the rewarding efficacy of cocaine.

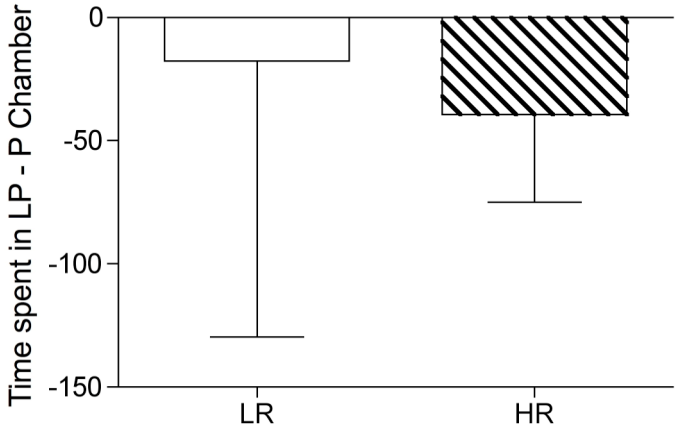

Figure 8: No relationship was found in adult rats between free choice novelty-induced exploration and the rewarding efficacy of cocaine.

Discussion

Previous work in adult animals has demonstrated that a preference for novelty is indicative of a facilitated acquisition of drug use (Bevins RA 1997). Adolescent animals and humans who prefer novelty are more likely to use/ abuse drugs and individuals who initiate use in adolescence will progress to dependency more rapidly than those who began drug use in adulthood (Helzer JE 1991; Kandel, Yamaguchi et al. 1992; Clark DB 1998). The aim of the

present study was to investigate the relationship between individual differences in response to novelty [i.e. forced novelty exposure and free choice novelty exploration] and the expression of cocaine place preference [i.e. rewarding efficacy of cocaine] in the adolescent and adult rat.

The present data provide evidence that LR adolescent (but not adult) rats to free-choice novelty exploration exhibit greater cocaine place conditioning; suggesting that these animals exhibit an increased rewarding efficacy to cocaine compared to HR adolescent rats. These data suggest the possibility that HR adolescent rats based on free choice novelty exploration are less responsive to cocaine place preference than LR rats. No differences between forced novelty exposure and cocaine place conditioning were detected between HR/LR adolescent or adult rats. These results are in agreement with several other researchers (Erb and Parker 1994; Kosten and Miserendino 1998) who did not find differences between adult HR and LR to forced novelty exposure with amphetamine place conditioning, and in fact, demonstrated that the magnitude of place conditioning tended to be lower in HR rats than LR adult rats (Erb and Parker 1994; Gong, Neill et al. 1996), as seen with the current study.

It seems that a dissociation exist between forced novelty exposure and free choice novelty exploration in adolescent rats, suggesting that stress-induced locomotion and novelty-seeking behavior are different biobehavioral phenomena and are likely activated by different neural and hormonal substrates. Interestingly, the relationship between free choice novelty exploration and cocaine place conditioning differs between adolescent and adult rats suggesting individual differences in free choice novelty exploration may be an important behavioral characteristic that predisposes adolescents to engage in cocaine use and demonstrate increased vulnerability to drug dependence.

These findings with place conditioning differ from studies that examine acquisition of self-administration. Researchers have demonstrated that HR adult rats to forced novelty exposure will self-administer psychostimulants more readily than LR adult rats (Piazza, Deminiere et al. 1989) and also demonstrate increased free choice nicotine consumption (Klebaur, Bevins et al. 2001; Abreu-Villaca, Queiroz-Gomes Fdo et al. 2006). However, Bardo et al. recently reported that responses to forced novelty exposure weakly predict responding for amphetamine (Cain, Saucier

et al. 2005). The first possibility for these discrepancies could be due to methodological differences (see (Kosten and Miserendino 1998) for review). In brief, acquisition of self-administration typically uses low doses of drug whereas place-conditioning studies use higher doses. Moreover, place-conditioning procedures use fewer trials than self-administration paradigms, so possible differences between HR/LR learning rates or habituation may confound the findings. In addition, the place conditioning methods employed in the present study may not have been sensitive enough to detect differences between LR and HR rats whereas self-administration procedures allow for greater sensitivity of individual differences between animals. In addition, due to higher locomotor activity in HR rats, they may visit both chambers of the CPP box more during test compared to LR rats, which could interfere with their expression of place preference (Gong, Neill et al. 1996). If the facilitated acquisition of self-administration of psychostimulants is due to greater locomotor activity expressed by HR rats and not due to the enhanced rewarding efficacy of the drug, the implications suggest that the neural mechanisms for psychostimulant reward and locomotor activity are distinguishable. Some evidence suggests that reward and locomotor systems are discrete. Several researchers (Robinson and Berridge 1993) have argued that the reward system is mediated by the mesolimbic pathway which projects from the ventral tegmental area to the nucleus accumbens whereas the locomotor system is mediated by the nigrostriatal pathway which projects from the substantia nigra to the striatum (Oades and Halliday 1987). Hemby et al. reported an increase in locomotor activity but no place conditioning from intra-accumbal cocaine (Hemby, Jones et al. 1992). In addition, intra-accumbal injections of neurotensin block the locomotor effect but not self-administration of cocaine (Robledo, Maldonado et al. 1993). If the mechanisms by which psychostimulants induce hyperactivity are separable from those by which they produce place conditioning, HR rats might show an increased response to the locomotor activating effects of these drugs, but not to the rewarding attributes.

The present data provide evidence that LR adolescent rats to free-choice novelty exploration exhibited greater cocaine place conditioning than HR adolescent rats. These data provide useful information about behavioral differences in adolescent rats in response to cocaine that could provide a neurochemical mechanism to investigate. Failure of these findings to

support self-administration studies suggests that caution be used in generalizing between these paradigms believed to measure similar processes. Place conditioning studies measure the rewarding efficacy of stimuli whereas self-administration studies measure the reinforcing efficacy of stimuli (for review (Bardo and Bevins 2000), see). Animals that demonstrate facilitated acquisition of psychostimulant self-administration (i.e. HR rats) may, in fact, be less responsive to the rewarding efficacy of these drugs and need to self-administer higher doses to obtain similar behavioral and neurochemical effects that LR would obtain at lower doses. Bardo et al. demonstrated that even though forced novelty exposure activity weakly predicted responding for amphetamine, free choice novelty exploration improved this predictive model (Cain, Saucier et al. 2005). Future studies need to evaluate the neurochemical differences between individual behavioral traits in the adolescent that may predispose them to initiate and maintain drug use.

Chapter 3

Chronic Cocaine Or Ethanol Exposure During Adolescence Alters Novelty-Related Behaviors In Adulthood

Abstract

Adolescence is a time of high-risk behavior and increased exploration. This developmental period is marked by a greater probability to initiate drug use and is associated with an increased risk to develop addiction and adulthood dependency and drug use at this time is associated with an increased risk. Human adolescents are predisposed toward an increased likelihood of risk taking behaviors (Zuckerman, 1986), including drug use or initiation. In the present study, adolescent animals were exposed to twenty days of either saline (0.9% sodium chloride), cocaine (20 mg/kg) or ethanol (1 g/kg) i.p. followed by a fifteen-day washout period. All animals were tested as adults on several behavioral measures including locomotor activity induced by a novel environment, time spent in the center of an open field, novelty preference and novel object exploration. Animals exposed to cocaine during adolescence and tested as adults exhibited a greater locomotor response in a novel environment, spent less time in the center of the novel open field and spent less time with a novel object, results that are indicative of a stress or anxiogenic response to novelty or a novel situation. Adolescent animals chronically administered ethanol and tested as adults, unlike cocaine-exposed were not different from controls in a novel environment, indicated by locomotor activity or time spent with a novel object. However, ethanol-exposed animals approached the novel object more, suggesting that exposure to ethanol during development may result in less-inhibited behaviors during adulthood. The differences in adult behavioral responses after drug exposure during adolescence are likely due to differences in the mechanisms of action of the drugs and subsequent reward and/or stress responsivity. Future studies are needed to determine the neural substrates of these long lasting drug-induced changes.

Introduction

Adolescence is a developmental time period that is characterized by the occurrence of high-risk behavior and increased exploration. This ontogenic period is unique as the brain is undergoing many changes that can have a lasting impact on behavior and cognitive processing (for review see (Spear, 2000). Drug use initiation rates are higher during the adolescent period than in any other developmental period. In general, adults who initiate drug use during adolescence are more likely to have higher lifetime rates of drug use and progress to dependency more rapidly than those who began drug use in adulthood (Clark DB, 1998; Helzer JE, 1991; Kandel, Yamaguchi, & Chen, 1992).

Novelty reactivity/ preference is a behavioral trait studied in human and animal models used as a predictor of drug use and potential dependence. A strong relationship between the rewarding aspects of psychomotor stimulants, self-administration rates and novelty preference has been established in animals (Hooks, Colvin, Juncos, & Justice, 1992; Klebaur, Bevins, Segar, & Bardo, 2001). Rats classified as high responders (HR) to novelty [i.e. exhibit greater locomotor activity in a novel environment] exhibit higher rates of amphetamine and cocaine-induced locomotor activity and self-administer these drugs more readily than low responders (LR) to novelty rats [i.e. exhibit decreased locomotor activity in a novel environment] (Hooks, Jones, Smith, Neill, & Justice, 1991). HR rats engage in greater risk taking behaviors and demonstrate higher behavioral and neurochemical alterations in response to environmental stressors or pharmacological challenges (Bevins RA, 1997; Klebaur et al., 2001). Moreover, dopaminergic responsivity differs between adolescent and adult HR or LR rats (Stansfield & Kirstein, 2005). Overall, these data indicate an association between novelty-seeking and risk-taking behaviors, indicating that high novelty seeking individuals will be more likely to engage in risky behaviors that can have considerable long term consequences, such as initiating drug use.

The central nervous system is still developing during adolescence and insults (e.g. chronic drug use) to the brain during this period may play an important role in the increased likelihood to maintain drug use during adulthood (for review, see (Spear, 2000). In adult animals, repeated drug exposure produces changes and adaptations at a cellular level that alters the

functioning of the entire neural pathway (Kleven, Woolverton, Schuster, & Seiden, 1988). These changes result in the development of complex adaptation such as tolerance, dependence and sensitization (Koob & Le Moal, 1997; Wise, 1980). Chronic cocaine exposure results in functional adaptations such as increased cAMP pathway activity, increased cAMP regulatory element binding protein (CREB) and increased changes in immediate early genes (e.g. FosB) (Nestler & Aghajanian, 1997). In addition, chronic ethanol exposure has been implicated with changes in various postreceptor events of the cAMP signal transduction cascade (i.e., Gs protein, protein kinase A, and CREB) (for review, see (Uddin & Singh, 2006) Rats injected once a day with cocaine show increased inhibition of dopamine (DA) uptake (Izenwasser & Cox, 1992), whereas rats receiving a continuous infusion of cocaine exhibit attenuated inhibition of DA uptake by cocaine, suggesting changes in duration of drug exposure subsequently induce differential neural changes. (Izenwasser & Cox, 1992). Moreover, repeated administration of cocaine produced significant changes in DA during withdrawal. *In vivo* microdialysis studies in the NAcc have shown that once self-administration of cocaine has ended, basal DA levels decrease significantly during this withdrawal period (Parsons, Smith, & Justice, 1991). . Taken together, these studies in adult animals show that repeated cocaine and ethanol administration results in complex changes in the DA mesolimbic pathway and molecular and cellular changes in the brain that continue long after drug use has stopped. These changes could subsequently impact behavioral phenotypes and lead to a greater vulnerability to drug dependency.

Enduring changes in sustained attention and anhedonia after chronic adolescent ethanol exposure have recently been reported (Slawecki, 2006). Additionally, adolescent ethanol consumption impairs tone conditioning in both male and female rats whereas adult administration had no long term effects (Smith et al., 2006). These studies are among the first to identify behavioral deficits in adulthood resulting from chronic ethanol exposure in adolescence. To examine long lasting effects of chronic drug exposure during adolescence on novelty induced behavior in adulthood, the present study assessed responses to a novel context or novel object in a familiar environment. Novelty reactivity was assessed using locomotor activity in the novel environment (i.e. total distance moved on trial 1), total time spent in center of the open field,

novelty preference (i.e. time spent with the novel object) and novel object exploration (i.e. total number of approaches to the novel object). The purpose of this study was to determine long-term behavioral effects of adolescent exposure to ethanol or cocaine following withdrawal into adulthood. The purpose of the present study was to determine long lasting behavioral differences in adult animals after repeated ethanol or cocaine administration during adolescence.

Methods

Forty male Sprague-Dawley (Harlan Laboratories, Indianapolis, IN) rats, offspring of established breeding pairs in the laboratory (University of South Florida, Tampa) were postnatal day (PND) 30 (μ=134 grams) at the beginning of the study. No more than one male per litter per age was used in a given condition. Pups 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 h light: dark cycle (07:00 h/19:00 h) until PND 21, on PND 21 pups were weaned and male littermates were group housed throughout the entire experiment. Animals were experimentally naive until the beginning of the study (PND 30). The care and use of animals was in accordance with local standards set by the Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals (Health, 1989).

Drug Pretreatment: Four experimental groups were included in this study. Beginning on PND 30, animals were injected once per day with either saline [0.9% sodium chloride, i.p., n=9] cocaine hydrochloride [20.0 mg/kg, i.p., n=10] or ethanol [1.0 g/kg, i.p., n=9] in their homecages from PND 30 to 50. To insure injection handling had no effect on saline controls, a naïve control group [n=9] was included that remained uninjected for those 20 days. Following 20 days of drug exposure, animals were withdrawn into adulthood (PND 51-69) when they were tested for novelty preference.

Procedure: Beginning on PND 66, animals were tested on a black plastic circular platform (116 cm diameter) standing 70 cm from the ground, with a white plastic barrier (48 cm height) enclosing the arena (100 cm diameter). A video camera was suspended directly over the table and recorded the animal's behavior using a Noldus Behavioral Tracking System (Noldus, Netherlands).

Over a period of four consecutive days, each rat (PND 66-69) was placed in the open field in one of four randomly selected zones and allowed to freely explore the novel environment for five minutes. This procedure was performed twice a day for a total of 8 habituation trials. Immediately following the 8th trial, animals were removed for 1 minute while a single novel object (approximately 16 cm high) was attached to the center of the table (trial 9). Rats were placed in a random zone and allowed to explore the familiar environment and novel object for five minutes. Locomotor activity induced by a novel environment (i.e. total distance moved (TDM) on trial 1), time spent in the center of an open field, novelty preference (i.e. time spent in proximity of the novel object) and novel object exploration (i.e. frequency to approach the novel object) were measured. Novelty preference was defined as time spent within 10.16 cm of the object on trial 9.

Data Analyses: Data analyses were performed with Graphpad Prism (Graphpad, CA). The data were expressed as the means +/- SEM, and the significance level was set at P=0.05. T-tests revealed that naïve and saline pretreated animals did not differ on all measures of activity [$t(13)=0.876$, $p>0.05$ and $t(15)=0.707$, $p>0.05$, respectively] therefore, naïve and saline animals were grouped for all subsequent analyses. Locomotor activity induced by a novel environment (i.e. TDM on trial 1) was analyzed using two-way repeated measures ANOVA with subsequent PLSD post hoc analyses to determine differences across time points and drug conditions. Moreover, three separate one-way ANOVA were performed on time spent in the center of an open field, novelty preference (i.e. time spent with the novel object) and novel object exploration (i.e. frequency to approach a novel object) to assess the effects of adolescent drug exposure. Subsequent post hoc analyses (Dunnett's) were used to isolate differences between drug conditions.

Results

Cocaine pretreatment: The present findings demonstrate that animals pretreated with cocaine during adolescence exhibited significantly greater locomotor activity induced by a novel environment (i.e. TDM) during the first minute of exposure to the novel environment than did naïve/saline pretreated animals [$F(4,30)=16.71$, $p<0.05$] and spent significantly less time in the center of the open field in the first minute than did naïve/saline animals [$F(2,34)= 6.498$, $p<0.05$,

$d_D(2,34)=1.85$]. No differences across locomotor activity were detected following the first minute of exposure. Therefore, chronic exposure to cocaine during adolescence increases novelty-induced locomotor activity immediately following exposure to a novel environment and decreases time spent in the center compared to the periphery of the open field in adulthood (see figure 9 & 10).

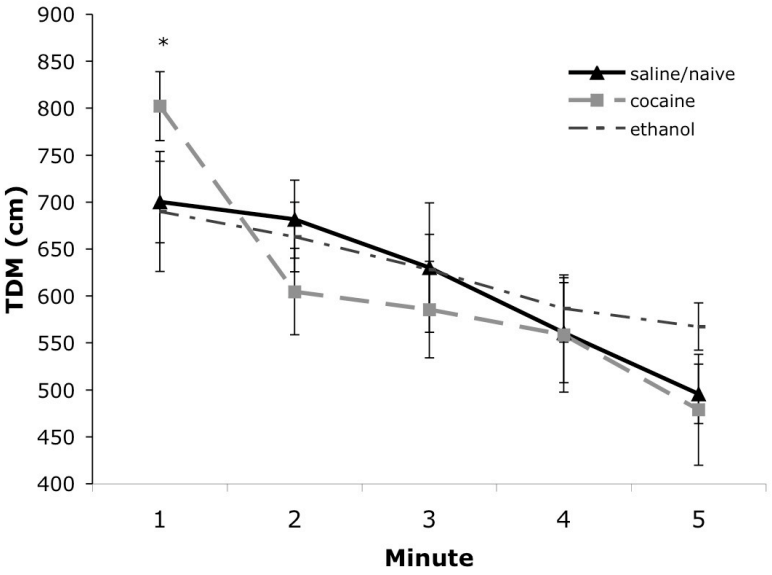

Figure 9: Adolescent animals pretreated with cocaine (grey square) moved significantly more during the first minute of the first exposure to the novel environment as adults than did naïve/saline (black triangle) or ethanol pretreated animals (dashed line). * = differs from naïve/saline or ethanol.

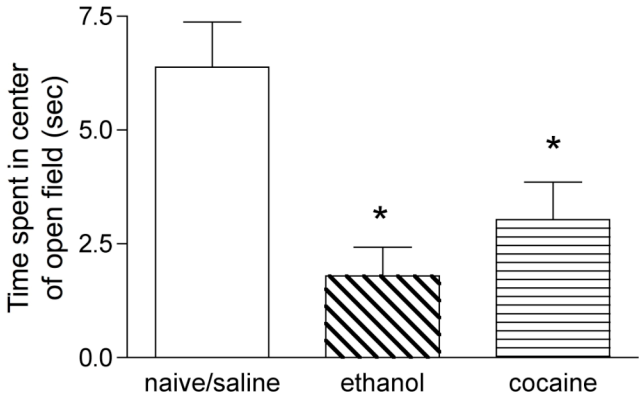

Figure 10: Adolescent animals pretreated with ethanol (hatched lines) or cocaine (horizontal lines) spent significantly less time in the center of the open field as adults compared to naïve/saline animals. *= differs from naïve/saine.

When tested for novelty preference, animals pretreated with cocaine during adolescence spent significantly less time with the novel object (i.e. decreased novelty preference) compared to naïve/saline or ethanol pretreated adult animals [$F(2,31)=3.306$, $p<0.05$], [$d_D(2,31)=21.95$] but did not differ in novel object exploration (i.e. frequency to approach the novel object) compared to saline/naïve or ethanol pretreated rats. Therefore, chronic cocaine during adolescence results in adult animals who spend less time interacting with a novel stimulus compared to naïve/saline or ethanol pretreated adolescents (see figure 11). Because cocaine exerts anorexic effects that might affect activity measures, weights were analyzed across pretreatment conditions. Results indicate that chronic cocaine exposure during adolescence did not significantly alter growth and therefore growth restriction is an unlikely cause of the observed differences in novelty reactivity [$t(38)= 0.2936$, $p>0.05$], (see figure 12).

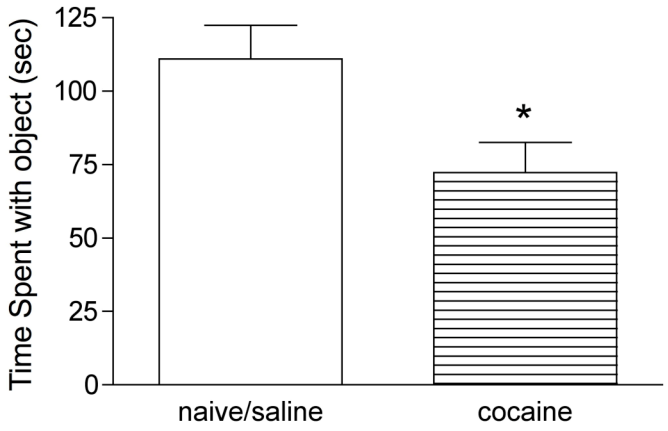

Figure 11: Adolescent animals pretreated with cocaine (horizontal lines) spent significantly less time with the novel object on trial 9 as adults compared to naïve/saline (white bar) or ethanol pretreated animals (hatched lines). * = differs from naïve/saline or ethanol.

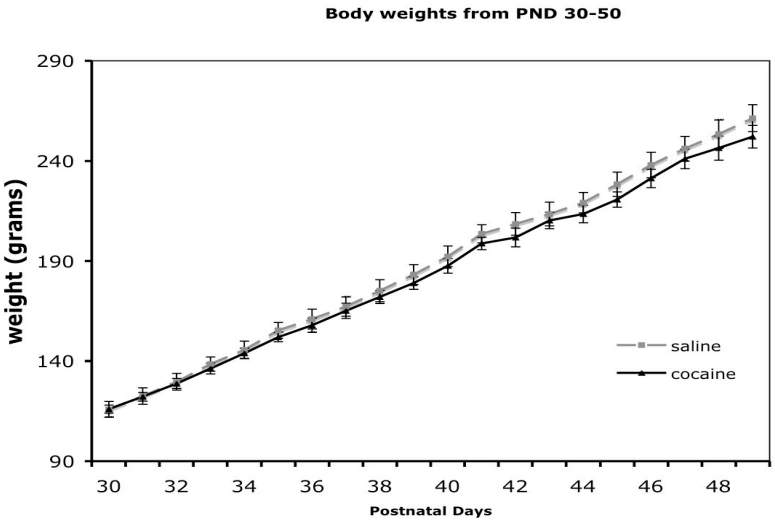

Figure 12: Adolescent exposure to cocaine compared to saline does not significantly restrict growth due to anorexic effects of high doses of cocaine.

Ethanol pretreatment: Animals exposed to chronic ethanol during adolescence spent significantly less time in the center of the open field on trial 1 [$F(2,34)= 6.498$, $p<0.05$] and exhibited greater novel object exploration than did naïve/saline animals or those exposed to cocaine during adolescence [$F(2,30)=3.775$, $p<0.05$] [$d_D(2,30)=3.825$] and compared to naïve/saline pretreated animals. Additionally, alcohol pretreated animals did not differ in locomotor activity induced by a novel environment, novelty preference or total distance moved on test compared to saline/naïve or cocaine pretreated rats. Chronic ethanol exposure during adolescence increases the tendency of animals to engage in more exploratory or novelty seeking behaviors (see figure 10 & 13).

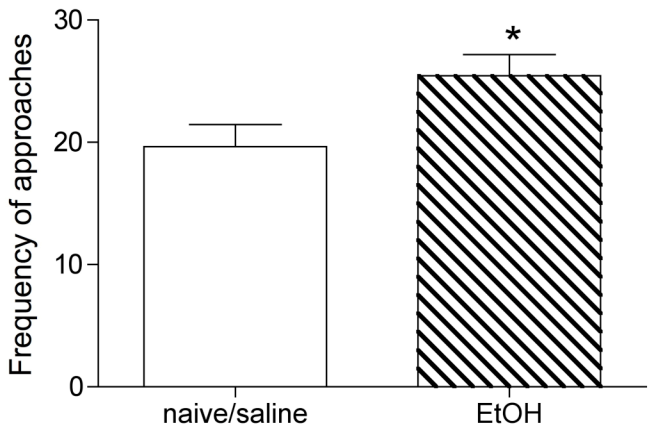

Figure 13: Pretreatment with ethanol (hatched lines) results in significantly more approaches to a novel object during adulthood than naïve/saline (white bar) or cocaine (horizontal lines) pretreated animals. * = differs from naïve/saline or cocaine.

High & low novelty preference: To assess the effects of adolescent drug exposure on the phenotypic expression of novelty reactivity in adulthood, a median split was performed on all animals and the distribution of phenotypes assessed for each treatment. Interestingly, adolescent animals pretreated with cocaine had fewer LR for both novelty preference and novel object exploration (37% and 30%, respectively) than HR (63% and 70%, respectively). In contrast, adolescent cocaine pretreated animals had fewer LR for locomotor activity induced by a novel environment compared to HR (33% and 66%, respectively). This demonstrates that in both the novelty-preference and novel object exploration behavioral measures, repeated cocaine during adolescence produces a predisposition towards LR in adulthood, whereas animals exhibit a tendency towards being a HR when measured on novel environment locomotor activity (see table 1).

	Locomotor Activity Induced by a Novel Environment	
	LR	HR
Naïve	44%	56%
Saline	50%	50%
Cocaine	33%	66%
Ethanol	44%	56%

	Novel Object Exploration	
	LR	HR
Naïve	44%	56%
Saline	56%	44%
Cocaine	45%	55%
Ethanol	34%	66%

	Novelty Preference	
	LR	HR
Naïve	50%	50%
Saline	50%	50%
Cocaine	63%	37%
Ethanol	49%	51%

	Anxiety induced by a Novel Environment	
	LR	HR
Naïve	45%	55%
Saline	45%	55%
Cocaine	70%	30%
Ethanol	77%	33%

Table 1: Adolescent exposure to cocaine predisposes a greater percentage of adult animals to be considered LR measured by novelty-preference and novel object exploration.

Discussion

Previous work in humans has demonstrated that individuals who abuse drugs during adolescence are more likely to be dependent on drugs in adulthood (Clark DB, 1998). In addition, novelty preference has been demonstrated to be indicative of a facilitated acquisition of drug use (Klebaur et al., 2001). The aim of the present study was to examine chronic drug exposure (e.g. cocaine or ethanol) during adolescence on the subsequent novelty-induced activity (e.g. TDM on trial 1 and time spent in the center of the open field) and novelty preference (e.g. time spent and approaches) in adulthood.

The present data provide evidence for long-term behavioral changes that endure after chronic drug administration during adolescence. Repeated exposure to cocaine during adolescence modifies the novelty-induced behavioral phenotype in adulthood. Both the novelty-preference and novel object exploration behavioral measures following repeated cocaine during adolescence produces a predisposition towards LR in adulthood, whereas animals exhibit a tendency towards being a HR when measured on novel environment locomotor activity; suggesting that animals are more at risk to engage in drug use in adulthood after adolescent drug exposure due to an alteration in the behavioral phenotype that increases the vulnerability to engage in drug use. Importantly, adult animals exposed to cocaine during adolescence, exhibited greater locomotor activity induced by a novel environment during the first minute of exposure, decreased time spent in the center of a novel environment and decreased novelty preference, which may be indicative of increased stress or anxiety or enhanced neophobia in adulthood after adolescent cocaine. Van den Buuse et. al (van den Buuse, Van Acker, Fluttert, & De Kloet, 2001) have demonstrated that exposure to the novelty of an open field causes an increase in blood pressure, heart rate, body temperature and exploratory locomotor activity, results indicate that an increase in locomotor activity in a novel environment is stressful or anxiogenic. Cocaine has been shown to produce anxiety in human and animal models, either during cocaine administration or during withdrawal. Increased aversion for the illuminated area of the mouse black and white test box model after cocaine exposure was demonstrated by Costall and colleagues (Costall B, 1989), in addition to increased defensive withdrawal in rats after cocaine exposure (Yang, Gorman, Dunn, & Goeders, 1992) and a decrease in the number of entries into and time spent in the open arms of an elevated plus maze in mice (Yang et al., 1992). Moreover, following withdrawal from repeated cocaine, animals demonstrated an increase in anxiogenic responses in the elevated plus-maze (Sarnyai et al., 1995) and enhanced startle-induced ultrasonic distress vocalizations (Barros HM, 1996). These data are somewhat counterintuitive as cocaine use and abstinence can induce anxiety in humans and anxiogenic responses in animals and therefore may decrease the appetitive value or motivation for the drug. Some researchers have speculated that the controlled activation of the hypothalamic-pituitary-

adrenal (HPA) axis may serve as an arousing stimulus to the animal, very much like novelty seeking behaviors (Goeders, 2002). Importantly, these studies only investigated the short-term effects of withdrawal after drug exposure whereas the current study examined at a longer withdrawal period. Future studies should investigate additional long-term behavioral changes including anxiety related behavioral measures after cessation of chronic cocaine exposure.

The present study also established that adults who were chronically treated with ethanol during adolescence spent less time in the center of the open field during the first minute of trial 1 and had significantly greater novel object exploration, however, these animals did not exhibit a general increase in locomotor activity while the novel object was present. These results suggest that exposure to ethanol during development may result in less-inhibited behaviors during adulthood, and not just a general nonspecific increase in locomotor activity. In humans, several researchers have effectively established a relationship between novelty and/or impulsive behaviors and alcoholism (Dom G, 2006). However, it can be difficult to establish whether high responders to novelty precede alcohol use or are the result of chronic alcohol use.

These data are interesting as it seems that depending on the mechanism of action of the drug, a different set of behavioral responses are revealed. This is likely due, in part, to differences in the neurotransmitter systems affected. For example, cocaine is a strong catecholamine reuptake inhibitor and has been shown to alter responses in the HPA axis (Kuhn & Francis, 1997). Alternatively, ethanol not only affects DA, but also impacts GABA and long-term ethanol use in adults causes an overall inhibition of the CNS. Long-term exposure to drugs of abuse during adolescence may permanently alter neurocircuitry, making animals more vulnerable to drug use or relapse in adulthood possibly due to behavioral characteristics that facilitate this action.

The present data demonstrate that adolescent animals exposed to drugs of abuse exhibit differential behavioral reactivity in response to novelty as adults, however, the current study only examined a moderate washout period (i.e. 16 days); it is speculated that these behavioral effects are lasting and will endure throughout adulthood, however, future studies are needed to determine if this is the case. Importantly, not only have differences been observed between male

and female rats in a novel object conditioned place preference paradigm (Douglas, Varlinskaya, & Spear, 2003); LR and HR male and female rats differ in the acquisition of sucrose-reinforced responding (Klebaur et al., 2001), stressing the importance that future studies should address differences between male and female animals (possibly due to estrous) and their responsivity to novelty and drugs of abuse.

Novelty preference and risk taking behaviors have been associated with both an increased propensity to self-administer drugs of abuse and increase drug intake (Bevins RA, 1997; Hooks et al., 1991). The current study demonstrates that chronic adolescent exposure to alcohol may increase responding to novelty as measured by novel object exploration, which subsequently may render the animal more likely to engage in continued drug use [i.e. relapse (see figure 5)]. However, these ethanol-pretreated adolescents also spent less time in the center of an open field on trial 1 compared to naïve or saline pretreated animals, suggesting that these animals may be more anxious in the novel environment. Young animals exposed to stress (i.e. maternal separation) exhibited greater ethanol intake as adults as well as exhibiting greater stress responses (Huot, Thrivikraman, Meaney, & Plotsky, 2001; Ploj, Roman, & Nylander, 2003), suggesting that the reinforcing efficacy of ethanol increases in animals more reactive to stress. Interestingly, chronic adolescent exposure to cocaine produced increased locomotor activity in a novel environment, which based on previous studies suggests that this behavioral characteristic would predispose the animal to drug self-administration (Bevins RA, 1997; Hooks et al., 1991). Conversely, cocaine pretreated animals demonstrated decreased time spent in the center of the open field on trial 1 and decreased novelty preference, it is possible this is an anxiogenic response in these animals compared to naïve or saline pretreated animals and may facilitate drug use. An increase in cocaine self-administration has been observed in stressed or anxious animals (Covington & Miczek, 2005; Marquardt, Ortiz-Lemos, Lucion, & Barros, 2004), and chronic cocaine causes an increase in anxiety (Hayase, Yamamoto, & Yamamoto, 2005; Rogerio & Takahashi, 1992; Wood & Lal, 1987) providing an explanation for why adolescents exposed to cocaine (who subsequently may be more stressed or anxious) may be more likely to engage in

continued drug use. Future studies need to isolate the rewarding efficacy of drugs of abuse in LR and HR animals to novelty.

It is important to mention the difficulty in interpreting the current data as predictive of adolescent specific addiction in the absence of data collected from animals that were exposed to drug in adulthood. Future studies need to address this possibility. Regardless, the differences in behavioral reactivity in adulthood could have implications in the susceptibility to relapse. Some addiction theories state that during drug administration, strong connections between drug cues and the drug experience are strengthened, (possibly modulated by DA) consequently, increasing the likelihood that an individual will relapse when exposed to these drug cues at a later point (Robinson & Berridge, 1993). Moreover, this could be amplified if drug use occurs during adolescence as the brain is still developing. The transition from adolescence to adulthood is a critical developmental period involving the maturation of the mesocorticolimbic circuitry, where not only the development of this system, but the alteration of this system due to pharmacological insult may produce alterations in response to stress and subsequent increased novelty-seeking, and risk taking behaviors which could result in drug use initiation or relapse (Chambers RA, 2003; Douglas et al., 2003; Spear, 2000).

Chapter Four

Enduring Changes In Reward Mechanisms After Developmental Exposure To Cocaine.

Abstract

Adolescence is a time of high-risk behavior and increased exploration. This developmental period is marked by a greater probability to initiate drug use and is associated with an increased risk to develop adulthood dependency. During brain maturation, there are likely sensitive periods (i.e. adolescence) where environmental conditions, including drug exposure, may influence development by modifying neuronal connections and subsequently altering function. Aberrant levels of stimulation by drug exposure may produce different phenotypes than expected under normal developmental conditions that may influence subsequent responding to drugs of abuse after the brain is fully mature. The aim of the present study was to investigate the consequences of repeated developmental cocaine exposure on the subsequent rewarding efficacy of cocaine in adolescence and adulthood. The present findings reveal that after developmental exposure to cocaine, adolescent and adult rats exhibit decreased rewarding efficacy to both a moderate and a high dose of cocaine. Additionally, pretreatment with cocaine seems to render both adolescent and adult rats behaviorally sensitized to cocaine compared to saline pretreated controls. The present data provide evidence for short and long-term behavioral adaptations that occur after developmental cocaine exposure. Developmental exposure to cocaine decreases place conditioning in both the adolescent and adult rat, indicating developmental cocaine exposure changes the rewarding efficacy of cocaine. Future studies need to determine the neurochemical substrates altered by developmental exposure to cocaine.

Introduction

Adolescence is a stage of life when the brain is undergoing many complex changes that can exert long-term influences on decision making and cognitive processes (for review, see (Spear, 2000). It is also a period of experimentation, and Estroff (Estroff et al., 1989) has reported that illicit drug use can begin as early as age 12, with peak periods of initiation between ages 15 and 19. The mean age of illicit drug initiation in adults categorized as having a substance use disorder is 16 years old, with initiation rare after age 20 (Anthony, 1991). Initiation rates are so high that more than half (54%) of high school seniors have had at least one experience with an illicit compound (Johnston LD, 2002). During the 1990's, there was a steady rise in the frequency of cocaine use in teenagers, by 2003, 4.3% of eighth graders, 5.7% of tenth graders, and 8.2% of high school seniors reported frequent use of cocaine (Johnston LD, 2002). The fact that initiation of cocaine use is so dramatic during the adolescent period is particularly disconcerting given that the escalation of cocaine use appears more rapidly among teenagers than adult users, suggesting a greater addictive potential during adolescence than in adulthood (Estroff et al., 1989). Generally, adults who initiate drug use during adolescence are more likely to have higher lifetime rates of drug use and progress to dependency more rapidly than those who began drug use in adulthood (Clark DB, 1998; Helzer JE, 1991; Kandel et al., 1992). Moreover, adolescents demonstrate a more abrupt progression of illicit drug use and development of substance use disorders than adults (Warner et al., 1995), suggesting that this ontogenetic period renders the adolescent more vulnerable to addiction.

Repeated administration of psychostimulants can result in behavioral sensitization expressed as an enhanced behavioral response to a subsequent drug challenge (Vanderschuren and Kalivas, 2000). Consequently, rats who have repeatedly administered cocaine over several days, will show an elevated locomotor reaction in response to the drug which prevails after cessation of the drug (Cass and Zahniser, 1993). Sensitization not only occurs behaviorally, but neurochemically. Repeated drug exposure produces changes and adaptations at a cellular level which in turn alters the functioning of the entire pathway in which those neurons work (Kleven et al., 1988). These changes lead to the complex processes of tolerance, dependence and of course, sensitization

(Koob and Le Moal, 1997; Wise, 1980). Repeated exposure to psychostimulants has been shown to induce long-lasting changes in dopamine (DA) neurons including decreases in DA levels and its metabolites, decrease in tyrosine hydroxylase activity, decrease in DA metabolism, either an increase or decrease in stimulated release, morphological degeneration of nerve terminals and decreases in Vmax for [3^{H}]DA (Bassareo and Di Chiara, 1999; Borison and Diamond, 1979; McCabe et al., 1987) (Kalivas and Duffy, 1988; Kalivas et al., 1988; Karoum et al., 1990; Peris et al., 1990). Changes in D2 receptor binding site densities have also been reported; Goeders and colleagues reported decreased D2 binding sites in the striatum and an increase in the nucleus accumbens septi (NAcc) immediately following 15 days of cocaine injections (Goeders and Kuhar, 1987). Additionally, an increase in NAcc D2 binding sites has been reported 24 hours but not one week following 8 days of cocaine injections (Peris et al., 1990). Sensitization, tolerance and dependence also result in functional molecular adaptations such as increased cAMP pathway activity, increased cAMP regulatory element binding protein (CREB) and also increased changes in immediate early genes (e.g. FosB) (Nestler and Aghajanian, 1997). Repeated administration of cocaine also produces significant changes in DA during withdrawal. *In vivo* microdialysis studies in the NAcc have shown that once self-administration of cocaine has ended, basal DA levels decrease significantly during this withdrawal period (Parsons et al., 1991). Taken together, these studies in adult animals show that repeated cocaine administration results in complicated changes in the DA mesolimbic pathway that continue long after drug use has stopped, and processes such as these may be implicated in craving and relapse. Similar to humans, adolescent rats are behaviorally different from younger and older rats. Periadolescent rats have been reported to be more hyperactive and inattentive (Spear and Brake, 1983), exhibit greater novelty-preference (Stansfield and Kirstein, 2006) and either reduced responsiveness to some of the effects of alcohol (Silveri and Spear, 1998), amphetamine (Bolanos et al., 1998), and cocaine (Laviola et al., 1995). Recently, several researchers have reported hypersensitivity to the locomotor activating effects of cocaine (Caster et al., 2005; Frantz et al., 2007), decreased sensitization and decreased activity overall compared to adults (Frantz et al., 2007). In the conditioned place preference (CPP) paradigm, adolescent rats show a preference for nicotine,

whereas the adult rats did not (Vastola et al., 2002). Also, it has been demonstrated that adolescent rats showed a preference for moderate doses of alcohol and cocaine, whereas the adults had no preference (Badanich et al., 2006; Philpot et al., 2003). Many behavioral alterations that are age-specific seen in human adolescents are observed in adolescent rats, making these animal models very useful in the evaluation of neurochemical and behavioral changes caused by drug exposure during this important stage of development. During brain maturation, there are likely sensitive periods (i.e. adolescence) where environmental conditions, including drug exposure, may influence development by modifying neuronal connections and subsequently altering function. Aberrant levels of stimulation by drug exposure may produce different phenotypes than expected under normal developmental conditions that may influence subsequent responding to drugs of abuse after the brain is fully mature. Elucidating the mechanisms by which addictive drug exposure (e.g. cocaine) during adolescence renders the adult more vulnerable to continued use is of utmost importance in a society that has a striking percentage of adolescents who experiment with cocaine.
The aim of the present study was to investigate the consequences of repeated developmental cocaine exposure on the subsequent rewarding efficacy of cocaine in adolescence and adulthood.

Methods

One hundred sixty-four male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN), offspring of established breeding pairs in the laboratory (University of South Florida, Tampa) were postnatal day (PND) 30 (μ=134 grams) at the beginning of the study. No more than one male per litter per age was used in a given condition. Pups 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 h light: dark cycle (07:00 h/19:00 h) until PND 21, on PND 21, pups were weaned and male littermates were group housed throughout the entire experiment. Animals were experimentally naive until the beginning of the study (PND 30). The

care and use of animals was in accordance with local standards set by the Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals (Health, 1989).

Procedure: Groups were designated as: S-S (saline pretreatment-saline CPP), S-C (saline pretreatment-cocaine CPP), C-S (cocaine pretreatment-saline CPP) or C-C (cocaine pretreatment-cocaine CPP). Beginning on PND 30, animals were injected once per day with either saline [0.9% sodium chloride, i.p.] or cocaine hydrochloride [10 mg/kg or 20 mg/kg, i.p.] in their homecages from PND 30 to 40. Following 10 days of drug exposure, animals either immediately began behavioral training (i.e. CPP) to determine their place preference for a moderate (i.e. 10 mg/kg, i.p.) or high (i.e. 20 mg/kg, i.p.) dose of cocaine during adolescence (PND 41-50) or were withdrawn into adulthood (PND 60-69) when they began the same behavioral testing. For behavioral studies, rats were placed in a place conditioning apparatus (either in the large test chamber [21 cm wide x 36 long x 21 cm high] or in one compartment [21 cm wide x 18 cm long x 21 cm high]) for each session which took place for 15 minutes once daily for an 8 day period (either PND 41-50 or PND 60-69). To determine if cocaine pretreatment during the adolescent period produced a sensitized behavioral response to cocaine, an additional group of animals were tested for locomotor activity in response to a low dose of cocaine (i.e. 5 mg/kg, i.p.) after developmental saline or cocaine pretreatment (10 mg/kg, i.p) during adolescence (PND 51) or adulthood (PND 70).

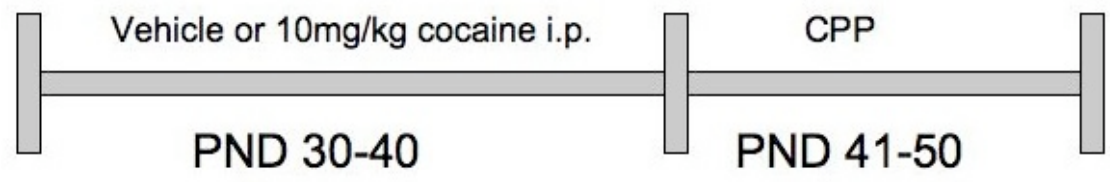

Figure 14: Adolescent rats were pretreated with saline or cocaine and tested for their saline or cocaine preference in adolescence.

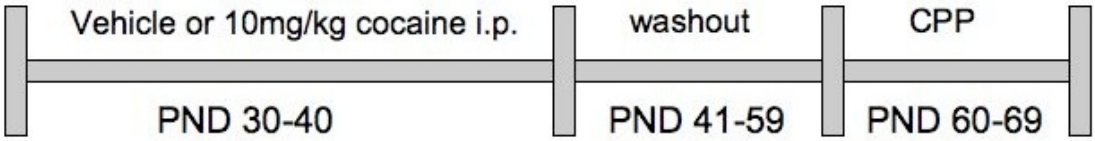

Figure 15: Adolescent rats were pretreated with saline or cocaine and tested for their saline or cocaine preference in adulthood.

Conditioned Place Preference: Animals were trained using a two-chambered apparatus made of clear Plexiglas with a clear Plexiglas cover. Two compartments (21 cm wide x 18 cm long x 21 cm high) separated by a removable wall were used for conditioning. The two chambers provided distinct visual (vertical or horizontal black and white bands) and tactile (wire or sandpaper flooring) cues to establish an association when paired with either saline or cocaine. A video-based tracking system (EthoVision, Noldus Information Technologies) was used to record and quantify the data.

Animals were tested in the CPP apparatus for 15 minutes (wall removed for free access to both chambers) 24 hours prior to the first training session to determine an initial preference for either the horizontal or the vertical striped chamber. In a biased design, the two chambers are designated post-hoc as preferred or aversive, based on the compartment that the animal spends the most and least time in, respectively. Following baseline recording, the animals were trained over a period of 8 days. Each day (between 0900 and 1100 hr) the animals received either saline or cocaine and were confined to the preferred or aversive chamber, respectively, for 15 minutes. For all animals, the order of chamber exposure was alternated daily. Animals were tested approximately 16-18 hours after their last training session. Animals were placed in the apparatus with the wall removed and tested for 15 minutes to determine the conditioned effects of repeated drug exposure. Preference was assessed using a difference score derived by subtracting the total time spent in the initially preferred chamber from the total time spent in the initially aversive chamber on test. Before each trial and test period, the apparatus was cleaned with 70% ethanol to remove any lingering odor cues. Floors were washed with soap and water and air-dried for 24 hours before subsequent use.

Data Analyses: Data analyses were performed with Graphpad Prism (Graphpad, CA). The data were expressed as the means +/- SEM, and the significance level was set at p=0.05. Four separate two-way analyses of variance (ANOVA) were performed on CPP preference scores for both adolescent and adult animals after repeated saline or cocaine to assess the effects of adolescent drug exposure induced by a moderate (i.e. 10mg/kg) or high (i.e. 20mg/kg) dose of cocaine. One-sample t-tests were performed on all preference scores to determine differences from zero to assess cocaine preference. Subsequent post hoc analyses were used to isolate differences between drug conditions. In addition, two two-way repeated ANOVA were performed on locomotor activity across time between saline and cocaine pretreated animals to determine differences in sensitized locomotor activity. Subsequent post hoc analyses were used to isolate differences between drug conditions.

Results

Adolescent CPP: The present findings demonstrate that animals pretreated with saline during development exhibited a significant preference for a moderate dose of cocaine [S-C: $t(7)=3.423$, $p<0.05$] in adolescence, an effect expected as cocaine has been shown to be rewarding at this dose in adult rats. Interestingly, after developmental exposure to cocaine, adolescent animals did not exhibit a preference for a moderate dose of cocaine [C-C: $t(6)=0.057$, $p>0.05$] suggesting that developmental exposure decreases the rewarding efficacy of cocaine during this developmental period. Both control groups did not exhibit a preference for saline [S-S: $t(6)= 0.5807$, $p>0.05$, C-S: $t(6)=0.05700$, $p>0.05$]. The two-way ANOVA revealed an interaction between drug pretreatment and place conditioning during adolescence [$F(1,27)= 4.214$, $p<0.05$]. Post-hoc's revealed that animals place preference for saline (S-S) or cocaine (S-C) differed significantly after saline pretreatment [$t(13)=2.418$, $p<0.05$], demonstrating the rewarding efficacy of cocaine (10mgkg) in adolescent rats. Moreover, adolescent rats pretreated with saline and tested for cocaine place preference (S-C) exhibited a significant difference from animals pretreated with cocaine and tested for saline (C-S) or cocaine (C-C) place preference [$t(13)= 1.979$, $p<0.05$ and $t(15)=3.422$, $p<0.05$, respectively], suggesting that developmental exposure decreases the rewarding efficacy of cocaine during this developmental period. (see Figure 16). These

differences suggest that enduring changes take place after repeated cocaine during development; changing the rewarding efficacy of cocaine in the adolescent animal.

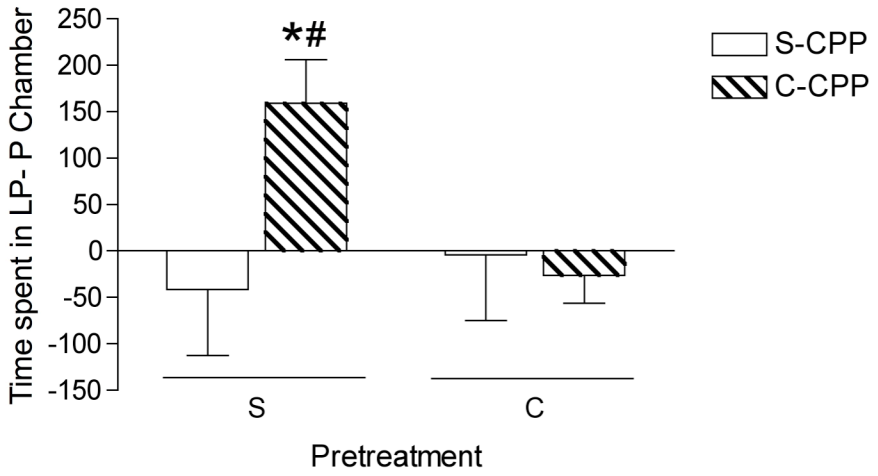

Figure 16: Saline pretreated adolescent rats demonstrated a significant preference for cocaine (i.e. 10mg/kg), whereas cocaine pretreated rats did not demonstrate a preference. Additionally, animals tested for saline (S-S) or cocaine (S-C) preference differed significantly after saline pretreatment. Moreover, saline pretreated adolescent rats tested for cocaine (S-C) preference differed significantly from cocaine-pretreated rats tested for saline (C-S) or cocaine (C-C) preference.
 # = differs from zero
** = differs from all other bars*

Due to the possibility that developmental cocaine exposure changes the dose response curve of the rewarding efficacy of cocaine, a separate group of animals were pretreated with cocaine (i.e.10 mg/kg) and tested for their place preference to a high dose of cocaine (20 mg/kg). Control animals (S-S) did not exhibit a significant saline place preference [$t(6)=0.5807$, $p>0.05$] whereas saline pretreated adolescent animals demonstrated a significant cocaine place preference (S-C) [$t(6)=4.277$, $p<0.05$], demonstrating not only a preference for a moderate but also a high dose of cocaine. Importantly, adolescent rats did not demonstrate a place preference for a high dose of cocaine after developmental exposure [C-C: $t(9)=1.251$, $p>0.05$], an effect seen when tested with a moderate dose of cocaine, suggesting that increasing the dose does not potentiate the rewarding efficacy after developmental exposure. A one-way ANOVA and subsequent post hoc tests (i.e. Tukey's Multiple comparison) revealed significant differences between pretreatment groups (i.e. S-C and C-C) [$F(2,21)=4.605$, $p<0.05$] (see Figure 17). These

data suggest that developmental cocaine exposure renders the adolescent less responsive to the rewarding properties to not only a moderate but also to a high dose of cocaine.

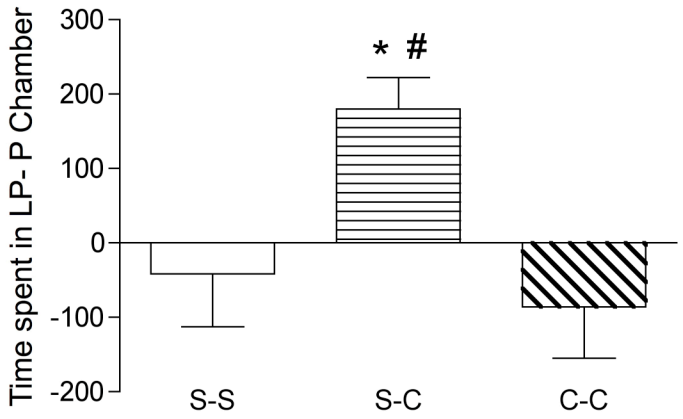

Figure 17: Saline pretreated adolescent rats demonstrated a significant preference for cocaine (i.e. 20mg/kg), whereas cocaine pretreated rats did not demonstrate a preference. Additionally, animals tested for saline (S-S) or cocaine (S-C) preference differed significantly after saline pretreatment. Moreover, saline pretreated adolescent rats tested for cocaine (S-C) preference differed significantly from cocaine-pretreated rats tested for saline (C-S) or cocaine (C-C) preference.
 # = differs from zero
** = differs from all other bars*

Due to the possibility that developmental cocaine exposure sensitizes animals to the neurochemical and behavioral effects of cocaine, saline or cocaine (i.e. 10 mg/kg) pretreated adolescent rats locomotor activity in response to cocaine (i.e. 5 mg/kg) was assessed. Cocaine pretreated rats, when challenged with a low dose of cocaine and placed in an open field, demonstrated greater locomotor activity compared to animals pretreated with saline [$F(1,21)=4.286$, $p<0.05$] [time point 15 minutes: $t(20)=2.069$, $P<0.05$], suggesting that developmental cocaine exposure results in sensitized behavioral responding to subsequent cocaine exposure (see Figure 18). These results suggest that both the cocaine-induced increases in locomotor activity and the lack of place preference for either a moderate or high dose of cocaine after developmental cocaine exposure may be due to dissociation of the locomotor and reward pathways, whereby animals are hyper-responsive to the psychostimulant induced locomotor activating effects but are hypo-responsive to the reward activating properties of the drug.

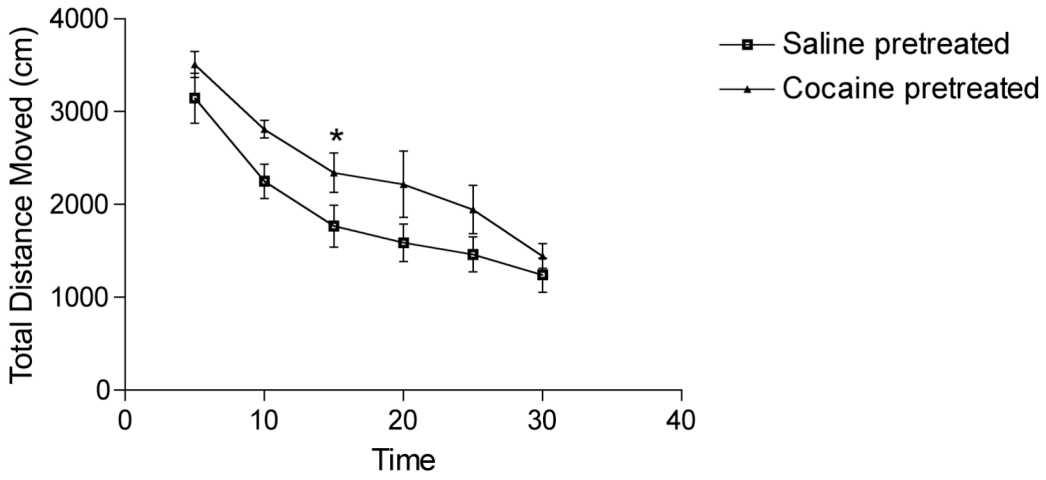

Figure 18: Cocaine pretreated adolescent rats, when challenged with a low dose of cocaine (i.e. 5mg/kg) and placed in an open field, demonstrated greater locomotor activity compared to animals pretreated with saline.
** = differs from saline pretreated*

Adult CPP: The present findings demonstrate that adult animals pretreated with saline during adolescence exhibit a significant place preference for a moderate dose of cocaine [S-C: $t(9)=5.171$, $p<0.05$] an expected effect as cocaine has been shown to be rewarding at this dose in adult rats. As seen above with adolescent rats (see Figure 16), developmental cocaine exposure changed the rewarding efficacy of cocaine lasting into adulthood [C-C: $t(11)=0.4940$, $p>0.05$]. Both control groups did not exhibit a place preference for saline [S-S: $t(10)= 0.7213$, $p>0.05$, C-S: $t(7)=0.4516$, $p>0.05$]. The two-way ANOVA revealed an interaction between drug pretreatment and CPP test during adulthood [$F(1,37)= 9.746$, $p<0.05$]. Post-hoc's revealed that animals tested for saline (S-S) or cocaine (S-C) place preference differed significantly after pretreatment with saline [$t(19)=2.707$, $p<0.05$], demonstrating the rewarding efficacy of cocaine during adulthood. Moreover, saline pretreated adolescent rats tested for cocaine place preference (S-C) were significantly different from cocaine pretreated rats tested for saline (C-S) or cocaine (C-C) place preference [$t(16)= 3.182$, $p<0.05$ and $t(20)=3.203$, $p<0.05$, respectively] (see Figure 19). These differences suggest that enduring changes take place after developmental cocaine exposure; decreasing the rewarding efficacy of cocaine in the adult animal.

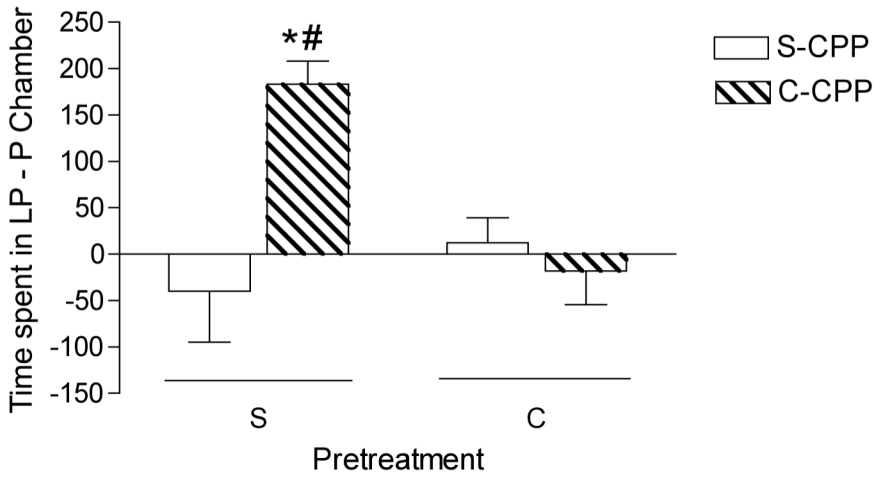

Figure 19: Saline pretreated adolescent rats demonstrated a significant preference for cocaine (i.e. 10mg/kg) in adulthood, whereas cocaine pretreated adolescent rats did not demonstrate a preference in adulthood. Additionally, animals tested for saline (S-S) or cocaine (S-C) preference differed significantly after saline pretreatment in adulthood. Moreover, saline pretreated adolescent rats tested for cocaine (S-C) preference differed significantly from cocaine pretreated rats tested for saline (C-S) or cocaine (C-C) preference in adulthood.
 # = differs from zero
** = differs from all other bars*

Due to the possibility that developmental cocaine exposure changes the dose response curve of the rewarding efficacy of cocaine in adulthood, a separate group of animals were pretreated with cocaine (i.e.10 mg/kg) and tested for their place preference to a high dose of cocaine (20 mg/kg) in adulthood. Control animals (S-S) did not exhibit a significant saline place preference [$t(10)=0.7213$, $p>0.05$] whereas saline pretreated adolescent animals demonstrated a significant cocaine place preference (S-C) in adulthood [$t(9)=2.632$, $p<0.05$], demonstrating not only a place preference for a moderate but also a high dose of cocaine. Importantly, adult rats did not demonstrate a place preference for a high dose of cocaine after developmental exposure [C-C: $t(9)=1.230$, $p>0.05$], an effect seen when tested with a moderate dose of cocaine, suggesting that increasing the dose does not potentiate the rewarding efficacy after developmental exposure. A one-way ANOVA and subsequent post hoc tests (i.e. Tukey's Multiple comparison) revealed significant differences between pretreatment groups (i.e. S-C and C-C) [$F(2,21)=4.605$, $p<0.05$] (see Figure 20). These data suggest that developmental cocaine exposure renders the adult less responsive to the rewarding properties to not only a moderate but also to a high dose of cocaine.

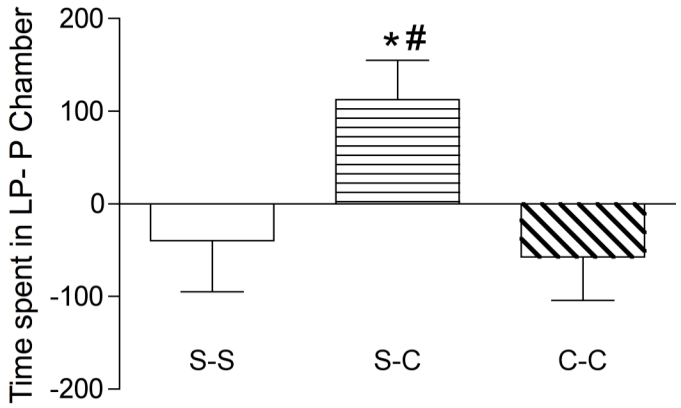

Figure 20: Saline pretreated adolescent rats demonstrated a significant preference for cocaine (i.e. 20mg/kg) in adulthood, whereas cocaine pretreated adolescent rats did not demonstrate a preference in adulthood. Additionally, animals tested for saline (S-S) or cocaine (S-C) preference differed significantly after saline pretreatment in adulthood. Moreover, saline pretreated adolescent rats tested for cocaine (S-C) preference differed significantly from cocaine pretreated rats tested for saline (C-S) or cocaine (C-C) preference in adulthood.
 # = differs from zero
** = differs from all other bars*

Due to the possibility that developmental cocaine exposure sensitizes animals to the neurochemical and behavioral effects of cocaine, saline or cocaine (i.e. 10 mg/kg) pretreated adult rats locomotor activity in response to cocaine (i.e. 5 mg/kg) was assessed. Cocaine pretreated adult rats, when challenged with a low dose of cocaine and placed in an open field, demonstrated greater locomotor activity compared to animals pretreated with saline [$F(1,23)=4.286$, $p<0.05$] [time point 15 minutes: $t(11)=2.240$, $P<0.05$], suggesting that developmental cocaine exposure results in sensitized behavioral responding to subsequent cocaine exposure (see Figure 21) in adulthood. These results suggest that both the cocaine-induced increase in locomotor activity and the lack of place preference for either a moderate or high dose of cocaine in adulthood after developmental cocaine exposure may be due to dissociation of the locomotor and reward pathways, whereby animals are hyper-responsive to the psychostimulant induced locomotor activating effects but are hypo-responsive to the reward activating properties of the drug.

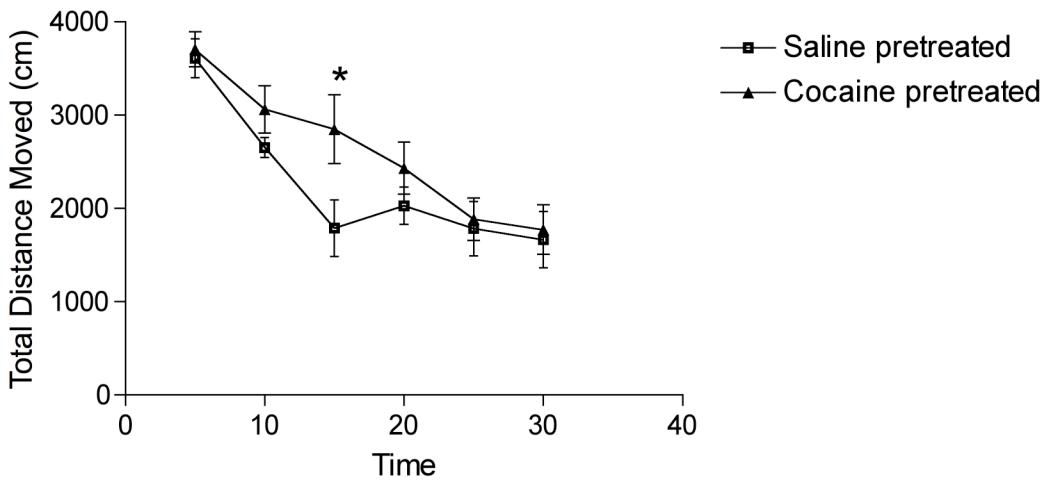

Figure 21: Cocaine pretreated adolescent rats, when challenged with a low dose of cocaine (i.e. 5mg/kg) and placed in an open field in adulthood, demonstrated greater locomotor activity compared to animals pretreated with saline.
** = differs from saline pretreated*

Discussion

Previous work in humans has demonstrated that individuals who abuse drugs during adolescence are more likely to be dependent on drugs in adulthood (Clark DB, 1998). The aim of the present study was to investigate the consequences of repeated developmental cocaine exposure on the subsequent rewarding efficacy of cocaine in both adolescence and adulthood.

The present data provide evidence for short and long-term behavioral adaptations that occur after developmental cocaine exposure. Developmental exposure to cocaine decreases place conditioning in both the adolescent and adult rat, indicating developmental exposure changes the rewarding efficacy of cocaine. A decreased rewarding efficacy of cocaine could be due to an increase in the anxiogenic properties of the drug. Cocaine has been shown to produce anxiety in human and animal models, either during repeated administration or during withdrawal. Increased aversion for the illuminated area of the mouse black and white test box model after cocaine exposure was demonstrated by Costall and colleagues (Costall B, 1989), in addition to increased defensive withdrawal in rats after cocaine exposure (Yang et al., 1992) and a decrease in the number of entries into and time spent in the open arms of an elevated plus maze in mice (Yang et al., 1992). Moreover, following withdrawal from repeated cocaine, animals

demonstrated an increase in anxiogenic responses in the elevated plus-maze (Sarnyai et al., 1995) and enhanced startle-induced ultrasonic distress vocalizations (Barros HM, 1996). These data are somewhat counterintuitive as cocaine use and abstinence can induce anxiety in humans and anxiogenic responses in animals and therefore may decrease the appetitive value or motivation for the drug. Some researchers have speculated that the controlled activation of the hypothalamic-pituitary-adrenal axis may serve as an arousing stimulus to the animal and increase the conditionability and subsequent use of cocaine. Importantly, these studies only investigated the short-term effects of withdrawal after drug exposure whereas the current study examined at a longer withdrawal period. Future studies should investigate additional long-term behavioral changes including anxiety related behavioral measures after cessation of repeated developmental cocaine.

The present data suggest that developmental cocaine exposure produced a sensitized behavioral response to subsequent drug exposure (i.e. increased locomotor activity) compared to saline pretreated controls in both adolescent and adult rats. Repeated administration of psychostimulants can result in behavioral sensitization expressed as an enhanced behavioral response to a subsequent drug challenge (Vanderschuren and Kalivas, 2000). Behavioral sensitization has been demonstrated in both adolescent and adult rats (Karler et al., 1990; Laviola et al., 1995; Robinson and Berridge, 1993), suggesting that repeated cocaine exposure produces long lasting neuronal changes that have enduring effects on behavior and possibly drug dependence.

These results suggest that both the cocaine-induced increase in locomotor activity and the lack of cocaine place conditioning for either a moderate or high dose of cocaine in adolescence or adulthood after developmental exposure may be due to a hyper-responsive nigrostriatal system and a hypo-responsive mesolimbic system. It seems a dissociation exists between locomotor activation and cocaine place conditioning, suggesting that behavioral sensitization and reward mechanisms are different biobehavioral phenomena and might be activated by different neural and hormonal substrates. Several researchers (Robinson and Berridge, 1993) have argued that the reward system is mediated by the mesolimbic pathway

which projects from the ventral tegmental area to the nucleus accumbens whereas behavioral sensitization and locomotor activation is mediated by the nigrostriatal pathway which projects from the substantia nigra to the striatum (Oades and Halliday, 1987). Hemby et al. reported an increase in locomotor activity but no place conditioning from intra-accumbal cocaine (Hemby et al., 1992). In addition, intra-accumbal injections of neurotensin block the locomotor effect but not self-administration of cocaine (Robledo et al., 1993). If the mechanisms by which psychostimulants induce hyperactivity are separable from those by which they produce place conditioning, developmental cocaine exposure may increase responding to the locomotor activating effects of these drugs, but decrease responding to the rewarding attributes.

Repeated psychostimulant administration during adolescence has been reported to change the rewarding efficacy of cocaine in adulthood. Methylphenidate exposure in adolescence decreases the rewarding efficacy of cocaine in adulthood (Andersen et al., 2002; Carlezon et al., 2003). In addition, periadolescent nicotine exposure reduces cocaine reward in adult mice (Kelley and Middaugh, 1999). Other researchers have reported that developmental exposure to methylphenidate facilitates acquisition of i.v. cocaine self administration in adulthood (Brandon et al., 2001). An increase in self-administration may be due to a decrease in the rewarding efficacy of stimuli whereby a higher rate of responding is necessary to maintain similar physiological states as control animals. In addition, developmental exposure to methylphenidate also decreases the rewarding efficacy of natural reinforcers (i.e. sucrose) and sexual behaviors (Bolanos et al., 2003). These behavioral data suggest that repeated psychostimulant exposure in adolescence modifies the responsivity to stimuli in adulthood, signified by decreased sensitivity to reward. Self-reports from human addicts suggest that the rewarding efficacy of stimuli, including drugs of abuse, decrease after repeated use. The belief that decreased rewarding efficacy of drugs will reduce the likelihood to continue drug use is contradictory to self-reports of drugs addicts who continue to engage in drug use despite little to no pleasure after use.

During brain maturation, there are likely sensitive periods (i.e. adolescence) where environmental conditions, including drug exposure, may influence development by modifying neuronal connections and subsequently altering function. Aberrant levels of stimulation by drug

exposure may produce different phenotypes than expected under normal developmental conditions that may influence subsequent responding to drugs of abuse after the brain is fully mature.

Neuroadaptations after developmental cocaine exposure may cause depressive-like signs such as anhedonia (decreased ability to experience reward), dysphoria (feelings of unwellness) or despair (feelings of giving up). Exposure to methylphenidate in adolescence produces an increased expression of the transcription factor, CREB (cAMP response element binding protein) within the NAcc in adulthood. An increase in intra-accumbal CREB activity has been linked with decreased cocaine reward and increased cocaine aversion in place conditioning studies and the development of depressive-like behaviors in the forced swim test (Pliakas et al., 2001). Activation of CREB also induces an increase in dynorphin activity at the kappa opioid receptor, which has been linked with dysphoria and anhedonia (Carlezon et al., 1998). Repeated cocaine has also been shown to increase CREB (Brenhouse et al., 2007) and immobility in the forced swim test (Barron et al., 2005; Magalhaes et al., 2004) in the adult rat. These data suggest that individuals may engage in drug use due to a hypo-responsive reward system and by doing so may alleviate the symptoms of anhedonia and dysphoria. Elucidating the mechanisms by which addictive drug exposure during adolescence renders the adult more vulnerable to continued drug use is of utmost importance in a society that has a striking percentage of adolescents who experiment with cocaine.

It is important to mention the difficulty in interpreting the current data as predictive of adolescent specific addiction in the absence of data collected from animals that were exposed to drug in adulthood. Future studies need to address this possibility. Regardless, the changes in the rewarding efficacy of cocaine in adulthood have implications for the susceptibility to maintain drug use.

Chapter Five

Enduring Changes In Reward Mechanisms After Developmental Exposure To Cocaine: The Role Of The D2 Receptor

Abstract

Adolescence is a developmental period marked by greater probability to initiate drug use and is associated with an increased risk to develop adulthood dependency. During brain maturation, there are likely sensitive periods (i.e. adolescence) where environmental conditions, including drug exposure, may influence development by modifying neuronal connections and subsequently altering function. Aberrant levels of stimulation by drug exposure may produce different phenotypes than expected under normal developmental conditions that may influence subsequent responding to drugs of abuse after the brain is fully mature. More specifically, adult rats demonstrate increased dopamine transporter densities following cocaine exposure. Due to the fact that D2 autoreceptors have been implicated in this process, it is hypothesized that as adolescent rats have greater dopamine transporters and D2 receptors than adults, artificially elevating dopamine levels by inhibiting reuptake will interfere with the normal pruning of these connections, thereby changing responsivity to rewarding stimuli in adolescence and adulthood. The aim of the present study was to investigate the consequences of repeated developmental cocaine exposure on the subsequent rewarding efficacy of cocaine in adolescence and adulthood. Additionally, co-administration of cocaine with a D2 antagonist during adolescence was investigated to determine the interaction of D2 receptors and the rewarding efficacy of cocaine in both adolescence and adulthood. After developmental exposure to cocaine, adolescent and adult rats exhibit decreased rewarding efficacy to both a moderate and a high dose of cocaine. Most significantly, blockade of the D2 receptor prevents cocaine-induced neurochemical changes, potentially regulating the behavioral and neurochemical alterations that occur after repeated drug use that could increase the likelihood of abuse and dependence.

Introduction

Adolescence is a period when the brain is undergoing many complex changes that can exert long-term influences on decision making and cognitive processes (for review, see (Spear, 2000). It is also a period of experimentation, and Estroff (Estroff et al., 1989) has reported that illicit drug use can begin as early as age 12, with peak periods of initiation between ages 15 and 19. The mean age of illicit drug initiation in adults categorized as having a substance use disorder is 16 years old, with initiation rare after age 20 (Anthony, 1991). In fact, initiation rates are so high that more than half (54%) of high school seniors have had at least one experience with an illicit compound (Johnston LD, 2002). During the 1990's, there was a steady rise in the frequency of cocaine use in teenagers, by 2003, 4.3% of eighth graders, 5.7% of tenth graders, and 8.2% of high school seniors reported frequent use of cocaine (Johnston LD, 2002). The fact that initiation of cocaine use is so dramatic during the adolescent period is particularly disconcerting given that the escalation of cocaine use appears more rapidly among teenagers than adult users, suggesting a greater addictive potential during adolescence than in adulthood (Estroff et al., 1989). Generally, adults who initiate drug use during adolescence are more likely to have higher lifetime rates of drug use and progress to dependency more rapidly than those who began drug use in adulthood (Helzer JE, 1991; Kandel et al., 1992; Clark DB, 1998). Moreover, adolescents demonstrate a more abrupt progression of illicit drug use and development of substance use disorders than adults (Warner et al., 1995), suggesting that this ontogenetic period renders the adolescent more vulnerable to addiction.

Repeated administration of psychostimulants can result in behavioral sensitization expressed as an enhanced behavioral response to a subsequent drug challenge (Vanderschuren and Kalivas, 2000). Consequently, rats who have repeatedly administered cocaine over several days, will show an elevated locomotor reactivity in response to the drug which prevails after cessation of the drug (Cass and Zahniser, 1993). Sensitization not only occurs behaviorally, but neurochemically. Repeated drug exposure produces changes and adaptations at a cellular level which in turn alters the functioning of the entire pathway in which those neurons work (Kleven et al., 1988). These changes lead to the complex processes of tolerance, dependence and

sensitization (Wise, 1980; Koob and Le Moal, 1997). Repeated exposure to psychostimulants has been shown to induce long-lasting changes in dopamine (DA) neurons including decreases in DA levels and its metabolites, decrease in tyrosine hydroxylase activity, decrease in DA metabolism, either an increase or decrease in stimulated release, morphological degeneration of nerve terminals and decreases in Vmax for [3^{H}]DA (Borison and Diamond, 1979; McCabe et al., 1987):(Kalivas and Duffy, 1988; Kalivas et al., 1988; Karoum et al., 1990; Peris et al., 1990). One important presynaptic mechanism regulated by the D2 autoreceptor are the dopamine transporters (DAT) (Mayfield and Zahniser, 2001). The DATs are the target of some drugs of abuse (e.g. amphetamine and cocaine), and may mediate the rewarding and reinforcing aspects of these drugs. Several studies have demonstrated that repeated cocaine administration results in increased DAT in the nucleus acumens septi (NAcc) (Daws et al., 2002). Importantly, changes in protein kinase C (PKC) activity have been demonstrated to alter DAT density in rats (Kitayama et al., 1994) and humans (Vaughan et al., 1997). The activation of intracellular signaling proteins, specifically PKC, regulates the surface expression of DAT (Mayfield and Zahniser, 2001). Inhibition of PKC by D2 receptor activation prevents the internalization of DAT, maximizing the number of active transporters on the membrane surface, and attenuating synaptic DA levels (Pristupa et al., 1998). Conversely, increased PKC activity in the absence of D2 receptor stimulation results in increased DAT internalization, fewer active transporters and consequently greater synaptic DA levels (Huff et al., 1998). Acute or chronic exposure to a D2 receptor antagonist decreases DA transport into striatal tissue *in vitro* and local administration of a D2 antagonist reduces DA uptake *in vivo* (Meiergerd et al., 1993; Rothblat and Schneider, 1997). Clearance of DA *in vivo* has been shown to decrease in the striatum, NAcc and prefrontal cortex following administration of a selective D2- but not D1- antagonist (Cass and Gerhardt, 1994). Taken together, these modifications in DAT number by D2 receptor mediated PKC activity indicate the critical role of D2 receptor/ DAT interactions in the regulation of synaptic DA levels and implicate D2 receptor activity as a potential target for the manifestation of long term adaptations in the mesolimbic DA system that manifest behaviorally as dependency following repeated cocaine.

Similar to humans, adolescent rats are behaviorally different from younger and older rats. Periadolescent rats have been reported to be more hyperactive and inattentive (Spear and Brake, 1983), exhibit greater novelty-preference (Stansfield and Kirstein, 2006) and have reduced responsiveness to some of the effects of alcohol (Silveri and Spear, 1998), amphetamine (Bolanos et al., 1998), and cocaine (Laviola et al., 1995). In the conditioned place preference (CPP) paradigm, adolescent rats show a preference for nicotine, whereas the adult rats did not (Vastola et al., 2002). Also, it has been demonstrated that adolescent rats showed a preference for moderate doses of alcohol and cocaine, whereas the adults had no preference (Philpot et al., 2003; Badanich et al., 2006). Many behavioral alterations that are age-specific seen in human adolescents are observed in adolescent rats, making these animal models very useful in the evaluation of neurochemical and behavioral changes due to drug use during this important stage of development. During adolescence, environmental conditions, including drug exposure, may influence brain development and function. Few studies have examined the DAergic neuroadaptations that take place after repeated exposure to cocaine during adolescence, not only a developmental period during which drug use initiation is widespread, but also a critical period for the remodeling of the mesolimbic and mesocortical brain regions and their neuronal DA projections (for review, see (Spear, 2000)). Rosenberg & Lewis (Rosenberg and Lewis, 1995) were among those researchers who saw a common developmental pattern in the overproduction and subsequent pruning of synaptic connections during the period preceding adulthood. The D1 and D2 receptors have been of major focus for years in regards to overproduction and pruning as these receptors increase in density in the first few weeks of life (Hartley and Seeman, 1983). Subsequently, Teicher et al have demonstrated receptor overproduction and elimination in both the striatum and prefrontal cortex (Teicher et al., 1995; Andersen et al., 2000). In addition, alterations in receptor binding and sensitivity in various neurotransmitter systems have been reported during adolescence (Trauth et al., 1999) along with changes in the myelination of neurons (Hamano et al., 1996). Importantly, DAT are overproduced and pruned during adolescence as the striatum transitions to adult state in rats (Moll et al., 2000) and humans (Haycock et al., 2003). As DAT density increases during adolescence, enhanced reuptake

reduces the extracellular levels of DA (Andersen and Gazzara, 1993) and a subsequent upregulation of postsynaptic receptors, and their second messenger systems take place (Andersen, 2002). These developmental differences may not only predispose adolescents to be more vulnerable to the rewarding effects of drugs of abuse, but may leave them more vulnerable to dependence after drug exposure due to interference with the normal synaptic pruning that takes place in the transition from adolescence to adulthood. Given that repeated cocaine in the adult rat yields increases DAT densities and D2 autoreceptors have been implicated in this process, it is hypothesized that as adolescent rats have greater DAT and D2 receptors than adults, artificially elevating DA levels (i.e. cocaine) will interfere with the normal pruning of these connections, thereby changing responsivity to rewarding stimuli in adolescence and adulthood. By virtue of developmental elevations in dopaminergic regulatory mechanisms (D2 & DAT) it is hypothesized adolescents will exhibit unique adaptations following repeated exposure to cocaine that render them more vulnerable to cocaine use in adulthood. The aim of the present study was to investigate the consequences of developmental cocaine exposure with or without concurrent blockade of the D2 receptor on the subsequent rewarding efficacy of cocaine in both adolescence and adulthood.

Methods

One hundred sixty-two male Sprague-Dawley (Harlan Laboratories, Indianapolis, IN) rats, offspring of established breeding pairs in the laboratory (University of South Florida, Tampa) were postnatal day (PND) 30 (μ=134 grams) at the beginning of the study. No more than one male per litter per age was used in any given condition. Pups 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 h light: dark cycle (07:00 h/19:00 h) until PND 21, on PND 21 pups were weaned and male littermates were group housed throughout the entire experiment. Animals were experimentally naive until the beginning of the study (PND 30). The care and use of animals was in accordance with local standards set by the Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals (Health, 1989).

Procedure: Beginning on PND 30, rats were injected once per day with either vehicle (tartaric acid, i.p.) or pimozide (1.0 mg/kg, i.p) followed 3 hours later with either saline [0.9% sodium chloride, i.p.] or cocaine hydrochloride [10 mg/kg, i.p.] (Parsons et al., 1993) in their homecages from PND 30 to 40. Groups are referred to as VS-S (vehicle-saline pretreated: saline CPP), PS-S (pimozide-saline pretreated: saline CPP), VS-C (vehicle-saline pretreated: cocaine CPP), PS-C (pimozide-saline pretreated: cocaine CPP), VC-C (vehicle-cocaine pretreated: cocaine CPP), PC-C (pimozide-cocaine pretreated: cocaine CPP). Following 10 days of drug exposure (i.e. vehicle or pimozide followed by saline or cocaine), animals either immediately began behavioral training (i.e. CPP) to determine their place preference for cocaine (i.e. 10 mg/kg, i.p.) during adolescence (i.e. PND 41-50) or were withdrawn into adulthood (i.e. PND 60-69) [see Figure 2] when they began the same behavioral testing. For behavioral studies, rats were placed in a CPP apparatus (either in the large test chamber [21 cm wide x 36 long x 21 cm high] or in one compartment [21 cm wide x 18 cm long x 21 cm high]) for each session which took place for 15 minutes once daily for an 8 day period (either PND 41-50 or PND 60-69).

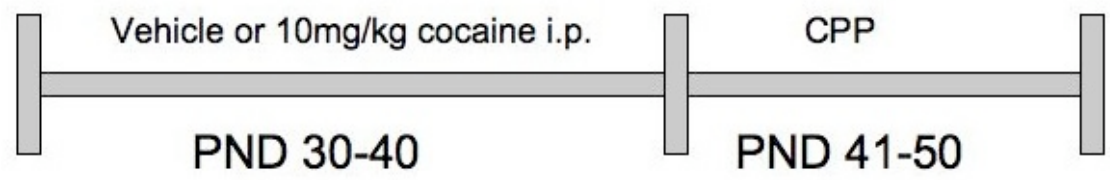

Figure 22: Adolescent rats were pretreated with saline or cocaine and tested for their saline or cocaine preference in adolescence.

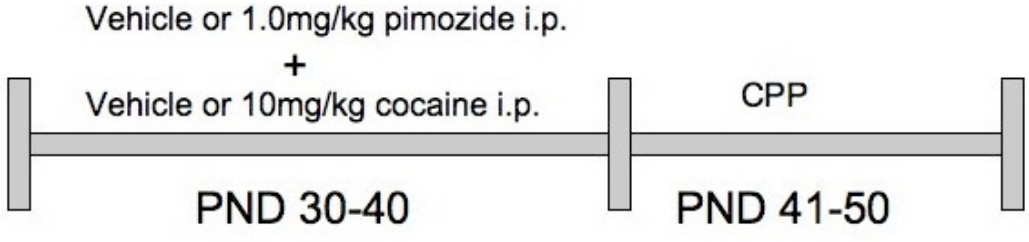

Figure 23: Adolescent rats were pretreated with saline or cocaine and either vehicle or pimozide and tested for their saline or cocaine preference in adolescence.

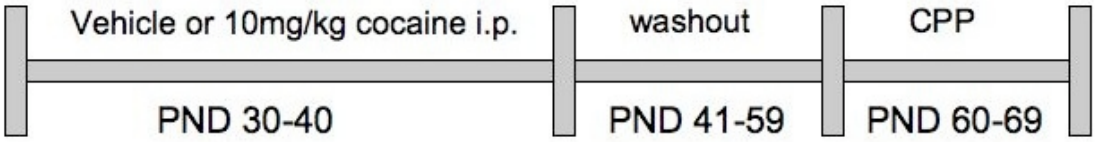

Figure 24: Adolescent rats were pretreated with saline or cocaine and tested for their saline or cocaine preference in adulthood.

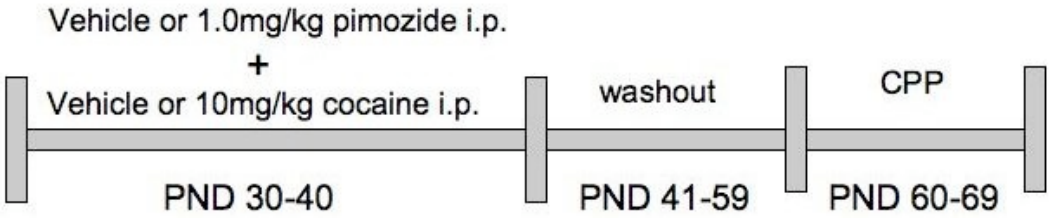

Figure 25: Adolescent rats were pretreated with saline or cocaine and either vehicle or pimozide and tested for their saline or cocaine preference in adulthood.

Conditioned Place Preference: Animals were trained using a two-chambered apparatus made of clear Plexiglas with a clear Plexiglas cover. Two compartments (21 cm wide x 18 cm long x 21 cm high) separated by a removable wall were used for conditioning. The two chambers provided distinct visual (vertical or horizontal black and white bands) and tactile (wire or sandpaper flooring) cues to establish an association when paired with either saline or cocaine. A video-based tracking system (EthoVision, Noldus Information Technologies) was used to record and quantify the data.

Animals were tested in the CPP apparatus for 15 minutes (wall removed for free access to both chambers) 24 hours prior to the first training session, a biased design was used to determine an initial preference for either the horizontal or the vertical striped chamber. In a biased design, the two chambers are designated post-hoc as preferred or aversive, based on the compartment that the animal spends the most and least time in, respectively. Following baseline recording, the animals were trained over a period of 8 days. Each day (between 0900 and 1100 hr) the animals received either saline or cocaine and were confined to the preferred or aversive

chamber, respectively, for 15 minutes. For all animals, the order of chamber exposure was alternated daily. Animals were tested approximately 16-18 hours after their last training session. Animals were placed in the apparatus with the wall removed and tested for 15 minutes to determine the conditioned effects of repeated drug exposure. Preference was assessed using a difference score derived by subtracting the total time spent in the initially preferred chamber from the total time spent in the initially aversive chamber on test. After each trial and test period, the apparatus was cleaned with 70% ethanol to remove any lingering odor cues. Floors were cleaned with soap and water and allowed to air-dry for 24 hours before subsequent use.

Data Analyses: Data analyses were performed with Graphpad Prism (Graphpad, CA). The data were expressed as the means +/- SEM, and the significance level was set at p=0.05. One-sample t-tests were used to assess drug preferences and analyses of variance (ANOVA) were used to determine group differences between conditions. Two separate two-way ANOVA were performed on place preference scores for both adolescent and adult animals after saline or cocaine exposure to assess the effects of developmental drug exposure. Additionally, two separate two-way ANOVA were performed on cocaine place preference scores for adolescent and adult rats after repeated vehicle or pimozide followed by either saline or cocaine exposure to assess the effects of developmental cocaine exposure with concurrent blockade of the D2 receptor on the rewarding efficacy of cocaine in adolescence and adulthood. Additionally, two separate t-tests were performed to assess differences between control drug conditions (i.e. pretreatment with vehicle or pimozide followed with saline and tested for saline place preference). One-sample t-tests were performed on all preference scores to determine differences from zero to assess cocaine preference. Subsequent post hoc analyses were used to isolate differences between drug conditions.

Results

Adolescent CPP: The present findings demonstrate that saline pretreated adolescent rats expressed a significant place preference for cocaine [S-C: $t(7)=3.423$, $p<0.05$], an expected effect as cocaine has been shown to be rewarding at this dose in adult rats. Interestingly, after developmental cocaine exposure, adolescent animals did not exhibit a significant cocaine place

preference [C-C: t(6)=0.057, p>0.05] suggesting that developmental exposure modifies the rewarding efficacy of cocaine during adolescence. Both control groups did not exhibit a saline place preference [S-S: t(6)= 0.5807, p>0.05, C-S: t(6)=0.05700, p>0.05].

A two-way ANOVA revealed an interaction between drug pretreatment (i.e. saline or cocaine) and CPP test (i.e. cocaine) during adolescence [F(1,27)= 4.214, p<0.05]. Post-hoc's revealed that animals place preference for saline (S-S) or cocaine (S-C) differed significantly after saline pretreatment [t(13)=2.418, p<0.05], demonstrating the rewarding efficacy of cocaine (10mgkg) in adolescent rats. Moreover, adolescent rats pretreated with saline and tested for cocaine place preference (S-C) exhibited a significant difference from animals pretreated with cocaine and tested for saline (C-S) or cocaine (C-C) place preference [t(13)= 1.979, p<0.05 and t(15)=3.422, p<0.05, respectively] (see Figure 26), suggesting that developmental exposure alters the rewarding efficacy of cocaine during this developmental period.

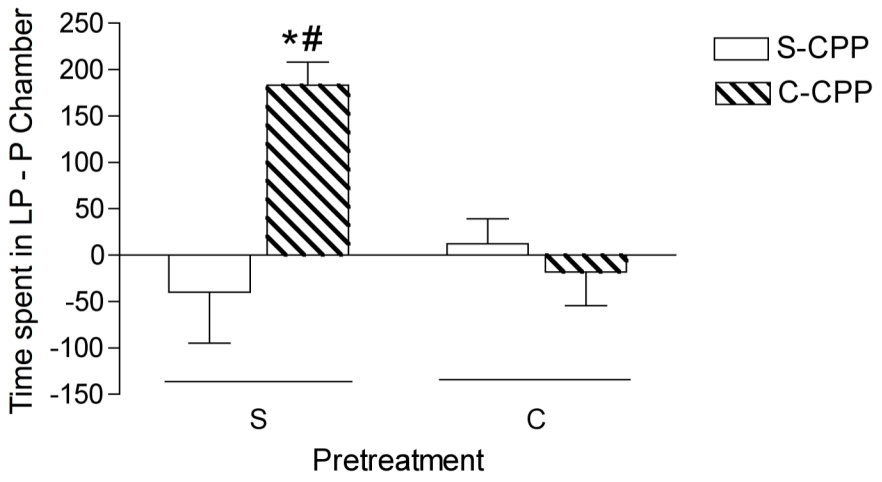

Figure 26: Saline pretreated adolescent rats demonstrated a significant preference for cocaine (i.e. 10mg/kg), whereas cocaine pretreated rats did not demonstrate a preference. Additionally, animals tested for saline (S-S) or cocaine (S-C) preference differed significantly after saline pretreatment. Moreover, saline pretreated adolescent rats tested for cocaine (S-C) preference differed significantly from cocaine-pretreated rats tested for saline (C-S) or cocaine (C-C) preference.
 # = differs from zero
**= differs from all other bars*

These differences suggest enduring changes that occur after developmental cocaine exposure that render the adolescent animal less responsive to the rewarding efficacy of cocaine.

To assess the role of D2 receptors in the mediation of cocaine reward, cocaine place preference was assessed in the adolescent rat after concurrent administration of cocaine and a D2 antagonist.

Adolescence- Developmental Blockade of D2 Receptors: No differences were detected between control conditions (i.e. VS-S and PS-S) [t(15)=0.1430, p>0.05] and control animals did not exhibit a preference for saline [VS-S: t(7)=0.039, p>0.05 and PS-S t(8)=0.1493, p>0.05] (see Figure 27).

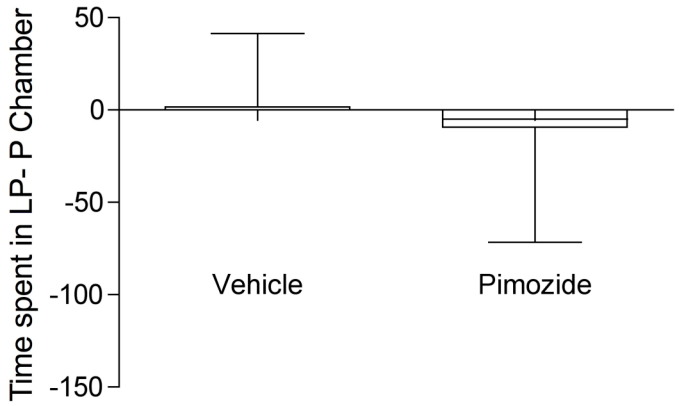

Figure 27: No differences were detected between control conditions and control animals did not exhibit a preference for saline.

Data reveal that rats pretreated with VS or PS demonstrate a significant cocaine place preference [VS-C: t(7)=3.157, p<0.05 and PS-C: t(4)-3.898, p<0.05, respectively] suggesting that pretreatment with vehicle or pimozide alone did not change the rewarding efficacy of cocaine. Importantly, vehicle-cocaine pretreated adolescent animals did not demonstrate a significant cocaine place preference [VC-C: t(5)=2.099, p<0.05], suggesting that the vehicle used in this study did not affect cocaine place preference, and more importantly, replicating previous data in adolescence suggesting that developmental cocaine exposure changes the rewarding efficacy of cocaine. Most significantly, cocaine-pimozide pretreated adolescent animals demonstrated a significant place preference for cocaine [t(5)=3.977, p<0.05.] (see Figure 28), an effect not seen with concurrent administration of vehicle and cocaine alone, suggesting that blockade of the D2 receptor prevents cocaine-induced neurochemical changes, potentially regulating the behavioral

and neurochemical alterations that occur after repeated drug use that could increase the likelihood of abuse and dependence.

A two-way ANOVA revealed an interaction between drug pretreatment (i.e. VS, PS, VC, PC) and CPP test (i.e. 10 mg/kg cocaine) during adolescence [$F(1,25)= 5.072$, $p<0.05$]. Post-hoc's revealed that cocaine preference scores differed between animals pretreated with pimozide-saline (PS-C) and vehicle-cocaine (PC-C) [$t(9)=4.277$, $p<0.05$], in addition, animals pretreated with vehicle-saline (VS-C) and vehicle-cocaine (VC-C) demonstrated different cocaine preference scores on test [$t(12)=3.618$, $p<0.05$] (see Figure 28).

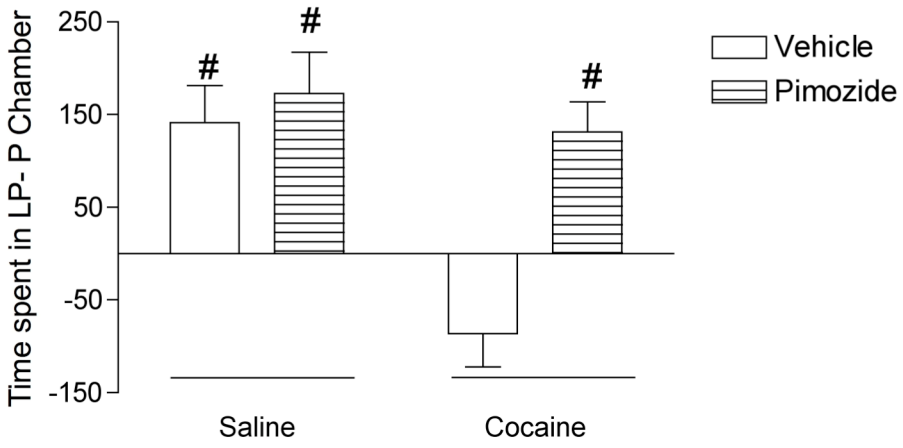

Figure 28: Vehicle-saline and pimozide-saline pretreated adolescent rats demonstrate a significant preference for cocaine. Additionally, vehicle and cocaine pretreated adolescent rats did not demonstrate a significant preference for cocaine. Most significantly, pimozide-cocaine pretreated adolescent animals demonstrate a significant preference for cocaine during adolescence. A two-way ANOVA revealed an interaction between drug pretreatment (i.e. VS, PS, VC, PC) and CPP test (i.e. 10mg/kg cocaine) during adolescence. Post-hoc's revealed that cocaine preference scores differed between animals pretreated with pimozide-saline (PS-C) and vehicle-cocaine (PC-C), in addition, animals pretreated with vehicle-saline (VS-C) and vehicle-cocaine (VC-C) demonstrated different cocaine preference scores on test.
 # = differs from zero
**= differs from all other bars*

Adult CPP: The present findings demonstrate that saline pretreated adolescent rats expressed a significant cocaine place preference in adulthood [S-C: $t(9)=5.171$, $p<0.05$], an expected effect as cocaine has been shown to be rewarding at this dose in adult rats. Developmental cocaine exposure changed the rewarding efficacy of cocaine in adult rats [C-C: $t(11)=0.4940$, $p>0.05$].

Both control groups did not exhibit a saline place preference [S-S: t(10)= 0.7213, p>0.05, C-S: t(7)=0.4516, p>0.05].

A two-way ANOVA revealed an interaction between adolescent drug pretreatment (i.e. saline or cocaine) and CPP test (i.e. cocaine) during adulthood [F(1,37)= 9.746, p<0.05]. Post-hoc's revealed that animals place preference for saline (S-S) or cocaine (S-C) differed significantly after saline pretreatment [t(19)=2.707, p<0.05], demonstrating the rewarding efficacy of cocaine (10 mg/kg) in adult rats. Moreover, adolescent rats pretreated with saline and tested for cocaine place preference (S-C) exhibited a significant difference from animals pretreated with cocaine and tested for saline (C-S) or cocaine (C-C) place preference [t(16)= 3.182, p<0.05 and t(20)=3.203, p<0.05, respectively] (see Figure 29).

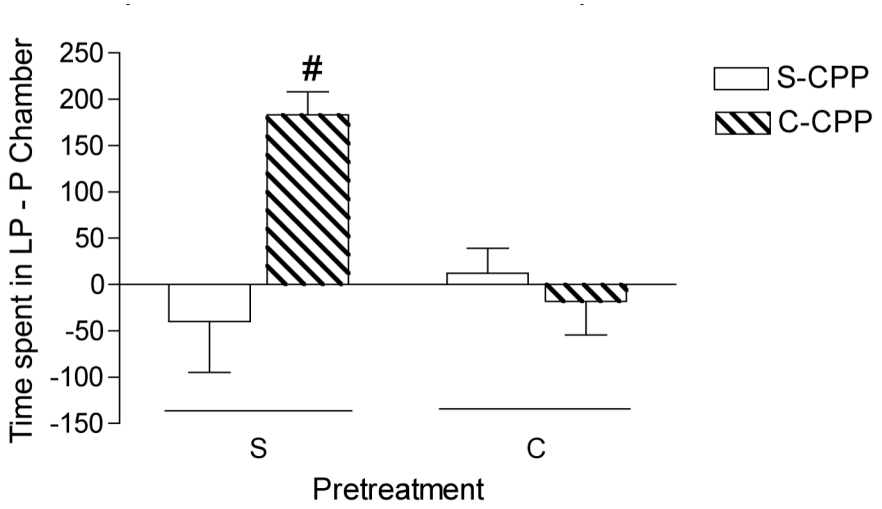

Figure 29: Saline pretreated adolescent rats demonstrated a significant preference for cocaine (i.e. 10mg/kg) in adulthood, whereas cocaine pretreated adolescent rats did not demonstrate a preference in adulthood. Additionally, animals tested for saline (S-S) or cocaine (S-C) preference differed significantly after saline pretreatment in adulthood. Moreover, saline pretreated adolescent rats tested for cocaine (S-C) preference differed significantly from cocaine pretreated rats tested for saline (C-S) or cocaine (C-C) preference in adulthood.
 # = differs from zero
**= differs from all other bars*

These differences suggest enduring changes that occur after developmental cocaine exposure that render the adult animal less responsive to the rewarding efficacy of cocaine. To

assess the role of D2 receptors in the mediation of cocaine reward, cocaine place preference was assessed in the adult rat after concurrent administration of cocaine and a D2 antagonist.

Adulthood- Developmental Blockade of D2 Receptors: No differences were detected between control conditions (i.e. VS-S and PS-S) [t(14)=0.8213, p>0.05] and control animals did not exhibit a preference for saline [VS-S: t(8)=1.225, p>0.05 and PS-S t(7)=2.335, p>0.05] (see Figure 30).

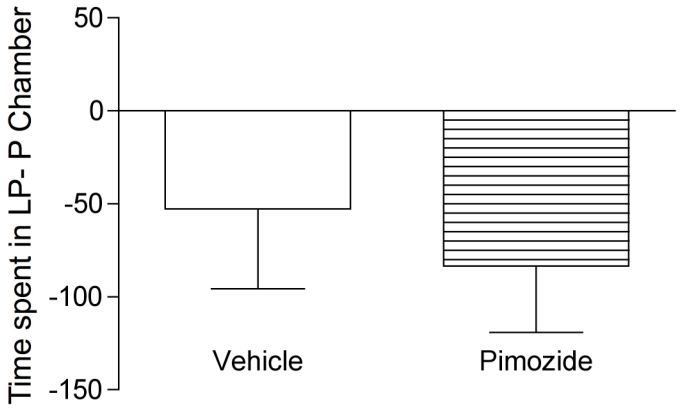

Figure 30: No differences were detected between control conditions and control animals did not exhibit a preference for saline.

Data reveal that adolescent rats pretreated with VS or PS demonstrate a significant cocaine place preference [VS-C: t(8)=2.29, p<0.05 and PS-C: t(4)=2.81, p<0.05, respectively] in adulthood, suggesting that pretreatment with vehicle or pimozide alone did not change the rewarding efficacy of cocaine. Importantly, vehicle-cocaine pretreated adolescent rats did not demonstrate a significant cocaine place preference [VC-C: t(7)=0.524, p>0.05] in adulthood, suggesting that the vehicle used in this study did not affect cocaine place preference, and more importantly, replicating previous data in adolescence suggesting that developmental cocaine changes the rewarding efficacy of cocaine in adulthood. Most significantly, cocaine-pimozide pretreated adolescent rats demonstrated a significant cocaine place preference (see Figure 31), an effect not seen with concurrent administration of vehicle and cocaine alone.

A two-way ANOVA revealed an interaction between drug pretreatment in adolescence (i.e. VS, PS, VC, PC) and CPP test (i.e. 10 mg/kg cocaine) in adulthood [F(1,29)= 5.031, p<0.05]. Post-hoc's revealed that cocaine preference scores differed between animals pretreated with

pimozide-saline (PS-C) and vehicle-cocaine (PC-C) [t(14)=3.257, p<0.05], in addition, rats pretreated with vehicle-saline (VS-C) and vehicle-cocaine (VC-C) demonstrated different cocaine preference scores on test [t(15)=3.145, p<0.05] (see Figure 30). These data suggest that even though enduring changes take place after developmental cocaine exposure, the blockade of the D2 receptor prevents cocaine-induced neurochemical changes, potentially regulating the behavioral and neurochemical alterations that occur after repeated drug use that could increase the likelihood of abuse and dependence.

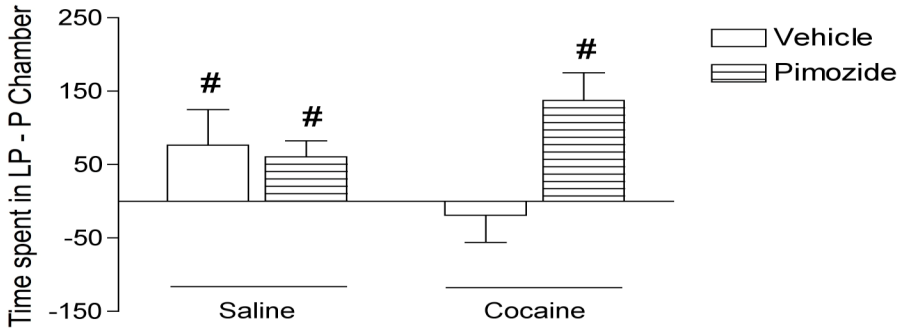

Figure 31: Vehicle-saline and pimozide-saline pretreated adolescent rats demonstrate a significant preference for cocaine in adulthood. Additionally, vehicle and cocaine pretreated adolescent rats did not demonstrate a significant preference for cocaine. Most significantly, pimozide-cocaine pretreated adolescent animals demonstrate a significant preference for cocaine during adulthood. A two-way ANOVA revealed an interaction between adolescent drug pretreatment (i.e. VS, PS, VC, PC) and CPP test (i.e. 10mg/kg cocaine) during adulthood. Post-hoc's revealed that cocaine preference scores differed between animals pretreated with pimozide-saline (PS-C) and vehicle-cocaine (PC-C), in addition, animals pretreated with vehicle-saline (VS-C) and vehicle-cocaine (VC-C) demonstrated different cocaine preference scores on test.
 # = differs from zero
** = differs from all other bars*

Discussion

The present data provide evidence for short and long-term behavioral adaptations that occur after developmental cocaine exposure. Developmental exposure to cocaine decreases place conditioning in both adolescent and adult rats, indicating developmental exposure changes the rewarding efficacy of cocaine. A decreased rewarding efficacy of cocaine could be due to an increase in the anxiogenic properties of the drug. Cocaine has been shown to produce anxiety in human and animal models, either during repeated administration or during withdrawal. Increased

aversion for the illuminated area of the mouse black and white test box model after cocaine exposure was demonstrated by Costall and colleagues (Costall B, 1989), in addition to increased defensive withdrawal in rats after cocaine exposure (Yang et al., 1992) and a decrease in the number of entries into and time spent in the open arms of an elevated plus maze in mice (Yang et al., 1992). Moreover, following withdrawal from repeated cocaine, animals demonstrated an increase in anxiogenic responses in the elevated plus-maze (Sarnyai et al., 1995) and enhanced startle-induced ultrasonic distress vocalizations (Barros HM, 1996). These data are somewhat counterintuitive as cocaine use and abstinence can induce anxiety in humans and anxiogenic responses in animals and therefore may decrease the appetitive value or motivation for the drug. Some researchers have speculated that the controlled activation of the hypothalamic-pituitary-adrenal axis may serve as an arousing stimulus to the animal and increase the conditionability and subsequent use of cocaine. Importantly, these studies only investigated the short-term effects of withdrawal after drug exposure whereas the current study examined at a longer withdrawal period. Future studies should investigate additional long-term behavioral changes including anxiety related behavioral measures after cessation of repeated developmental cocaine.

Repeated psychostimulant administration during adolescence has been reported to change the rewarding efficacy of cocaine in adulthood. Methylphenidate exposure in adolescence decreases the rewarding efficacy of cocaine in adulthood (Andersen et al., 2002; Carlezon et al., 2003). In addition, periadolescent nicotine exposure reduces cocaine reward in adult mice (Kelley and Middaugh, 1999). Other researchers have reported that developmental exposure to methylphenidate facilitates acquisition of i.v. cocaine self administration in adulthood (Brandon et al., 2001). An increase in self-administration may be due to a decrease in the rewarding efficacy of stimuli whereby a higher rate of responding is necessary to maintain similar physiological states as control animals. In addition, developmental exposure to methylphenidate also decreases the rewarding efficacy of natural reinforcers (i.e. sucrose) and sexual behaviors (Bolanos et al., 2003). These behavioral data suggest that repeated psychostimulant exposure in adolescence modifies the responsivity to stimuli in adulthood, signified by decreased sensitivity to reward. Self-reports from human addicts suggest that the rewarding efficacy of stimuli, including

drugs of abuse, decrease after repeated use. The belief that decreased rewarding efficacy of drugs will reduce the likelihood to continue drug use is contradictory to self-reports from drugs addicts who continue to engage in drug use despite little to no pleasure after use.

Neuroadaptations after developmental cocaine exposure may cause depressive-like signs such as anhedonia (decreased ability to experience reward), dysphoria (feelings of unwellness) or despair (feelings of giving up). Exposure to methylphenidate in adolescence produces an increased expression of the transcription factor, CREB (cAMP response element binding protein) within the NAcc in adulthood. An increase in intra-accumbal CREB activity has been linked with decreased cocaine reward and increased cocaine aversion in place conditioning studies and the development of depressive-like behaviors in the forced swim test (Pliakas et al., 2001). Activation of CREB also induces an increase in dynorphin activity at the kappa opioid receptor, which has been linked with dysphoria and anhedonia (Carlezon et al., 1998). Repeated cocaine has also been shown to increase CREB (Brenhouse et al., 2007) and immobility in the forced swim test (Magalhaes et al., 2004; Barron et al., 2005) in the adult rat. These data suggest that individuals may engage in drug use due to a hypo-responsive reward system and by doing so may alleviate the symptoms of anhedonia and dysphoria. Elucidating the mechanisms by which addictive drug exposure during adolescence renders the adult more vulnerable to continued drug use is of utmost importance in a society that has a striking percentage of adolescents who experiment with cocaine.

Importantly, the present data suggest that the blockade of D2 receptors with concurrent developmental cocaine administration prevent the neurochemical changes that have been suggested to occur after adolescent cocaine exposure, behaviorally expressed as decreased cocaine place conditioning that would potentially increase abuse potential. Stimulation of D1 receptors following cocaine administration seems to be critical in the rewarding effects of the drug, although it is likely that long-term adaptations to chronic cocaine exposure are mediated by the stimulation of D2 autoreceptors which are involved in regulating synaptic DA levels (Wolf and Roth, 1987; Santiago and Westerink, 1991). Several researchers have demonstrated the importance of D2 receptors and DAT in the mediation of cocaine reward. D2 antagonists block

the ability of cocaine to support a place preference (Adams et al., 2001). Additionally, D2 antagonists administered systemically not only decrease cocaine self-administration, but reduce the breakpoint to self-administer cocaine (Roberts et al., 1989; Barrett et al., 2004). The NAcc shell may mediate these effects as a direct infusion of a D2 antagonist also decreases cocaine self-administration (Bari and Pierce, 2005). These studies demonstrate that blockade of the D2 receptor decreases the rewarding and reinforcing efficacy of cocaine, however, few studies have examined the consequences of long-term cocaine induced changes that are altered after blockade of D2 receptors. Uhl et al. demonstrated that blockade of D1 or D2 receptors prevents the development of cocaine induced behavioral sensitization (Karler et al., 1990) Importantly, *in vivo* studies have demonstrated that repeated cocaine-induced increases in striatal uptake were attenuated by pretreatment with pimozide, a D2-antagonist (Parsons et al., 1993).

Taken together, these studies in adult animals treated in adolescence, demonstrate that cocaine exposure results in complicated changes in the DA mesolimbic pathway that continue long after drug use has stopped, and processes such as these may be implicated in drug dependence, craving and relapse. These data suggest that blockade of the D2 receptor prevents cocaine-induced neurochemical adaptations, potentially regulating the behavioral and neurochemical alterations that occur after repeated drug use that increase abuse liability. These data implicate D2 receptor activity as a potential target for the manifestation of long term adaptations in the mesolimbic DA system that manifest behaviorally as dependency following developmental cocaine exposure.

Chapter Six

General Discussion

Development of the central nervous system (CNS) during adolescence may play a key role in the increased likelihood to initiate drug use (for review, see (Spear 2000)). Moreover, disrupting the development of the CNS may result in subsequent long-term increases in the probability of drug use and dependence. During adolescence, critical neural structures involved in substance abuse are regulated primarily by the limbic system, which is associated with emotional and impulsive behaviors (for review, see (Spear 2000; Chambers RA 2003)). Adolescence is a critical period of transition from a more emotional regulation of the structures that mediate substance abuse to a more mature cortical regulatory mechanism (Spear 2000). During adolescence, the primary dopaminergic projections to the nucleus accumbens septi (NAcc) extend from the ventral tegmental area (VTA), and are predominately modulated by the amygdala (Oades and Halliday 1987). However in adulthood, this previously amygdaloid-modulated system receives projections from the medial prefrontal cortex (mPFC); this developmental transition is critical in the functional nature of the system (Cunningham, Bhattacharyya et al. 2002). The development of this system allows for a transition from more emotionally directed behavior to more contextually regulated behavior. Because adolescents lack sufficient cortical regulation (input by the mPFC), their behavior tends to be more impulsive and guided by emotion than adults, increasing the chances of risky behaviors (e.g. initiating drug use) (Campbell, Lytle et al. 1969; Chambers RA 2003). Additionally, repeated administration of cocaine during this period may cause a functional change in accumbal dopamine levels by altering amygdalar modulation of accumbal DA release and/or altering the functional role/development of the medial prefrontal cortex input; consequently, leading to an increased risk of dependency during adulthood. These ontogenetic changes, with the fact that adolescence is a key period of drug initiation, together, make a powerful argument for treating adolescence as a key time period for investigating the development of drug addiction.

The frequency of substance use disorders is elevated in adults diagnosed with several psychological disorders (Regier, Farmer et al. 1990; Anthony 1991; Bucholz 1999; Blanco, Moreyra et al. 2001). Adolescents with similar disorders are also more likely to be diagnosed with substance use disorders (Swadi 1999; Zeitlin 1999; Shaffer, Forman et al. 2000). The fact that these mental disorders and adolescence are associated with substance use disorders suggests that common brain mechanisms may trigger drug susceptibility and potentially, addiction. These biological/neurochemical substrates might manifest into a behavioral trait or traits present in adolescents. Defective impulse control is a behavioral trait that characterizes psychiatric and substance use disorder groups (Swadi 1999; Moeller, Barratt et al. 2001; Rogers and Robbins 2001). Adolescence is marked by high levels of risk taking behavior relative to individuals of other ages. Along this unique stage of development, distinct social, behavioral and neurochemical changes emerge, to assist with the important life events that will occur. For example, learning and acquiring skills necessary to permit survival away from parental caretakers (Spear 2000). This phenomenon being evolutionary adaptive as a means to avoid inbreeding (Schlegel A 1991).

In order for a successful transition from childhood to adulthood, an important aspect to gaining independence is when adolescents shift their social orientations from adults to peers (Steinberg 1989) and typically spend a significant amount of time interacting with their peers as opposed to adults. Risk-taking in adolescence poses some negative consequences such as accidents, pregnancy, AIDS, suicide and drug dependence (Irwin 1989; Spear, Kirstein et al. 1989). Although risk-taking may be hazardous, it can also be beneficial. Risk-taking and exploratory type behaviors allow an individual to explore adult behavior and may also serve (as mentioned above) as a protective evolutionary factor. Adolescent increase in risk-taking and novelty seeking may trigger adolescent departure from the parental units by giving incentive to explore novel areas away from home and thus avoiding inbreeding via dispersal of the offspring during sexual development (Schlegel A 1991).

Novelty-seeking behaviors are an innate behavior in both human and non-human adolescents. Importantly, studies have demonstrated a strong correlation between novelty preference and the rewarding efficacy of psychomotor stimulants and self-administration rates in

animals (Hooks, Colvin et al. 1992; Klebaur, Bevins et al. 2001). High novelty seeking rats show higher rates of amphetamine and cocaine-induced locomotor activity and will self-administer these drugs more readily than low novelty seeking rats (Hooks, Colvin et al. 1992). Moreover, high novelty seeking rats seem to participate in far greater risk-taking behaviors and show much higher behavioral and neurochemical responses in reaction to environmental stressors or pharmacological challenges than low novelty seeking rats (Bevins RA 1997; Klebaur, Bevins et al. 2001). Additionally, adolescent animals classified as high responders to novelty based on activity in a novel environment and also by time spent with a novel object in a familiar environment exhibited greater morphine place conditioning in adulthood compared to low responders to novelty (Zheng, Tan et al. 2004). The present data provide evidence that LR adolescent (but not adult) rats to free-choice novelty exploration exhibit greater cocaine place conditioning; suggesting that these animals exhibit an increased rewarding efficacy to cocaine compared to HR adolescent rats. It seems that a dissociation exists between forced novelty exposure and free choice novelty exploration in adolescent rats, suggesting that stress-induced locomotion and novelty-seeking behavior are different biobehavioral phenomena and might be activated by different neural and hormonal substrates. Interestingly, the relationship between free choice novelty exploration and cocaine place conditioning differs between adolescent and adult rats suggesting individual differences in free choice novelty exploration may be an important behavioral characteristic that predisposes adolescents to engage in cocaine use and demonstrate increased vulnerability to drug dependence. Findings with place conditioning differ from studies that examine acquisition of self-administration. Researchers have demonstrated that HR adult rats to forced novelty exposure will self-administer psychostimulants more readily than LR adult rats (Piazza, Deminiere et al. 1989) and also demonstrate increased free choice nicotine consumption (Klebaur, Bevins et al. 2001; Abreu-Villaca, Queiroz-Gomes Fdo et al. 2006). However, Bardo et al. recently reported that responses to forced novelty exposure weakly predict responding for amphetamine (Cain, Saucier et al. 2005).

If the facilitated acquisition of self-administration of psychostimulants is due to greater locomotor activity expressed by HR rats and not due to the enhanced rewarding efficacy of the drug, the implications suggest that the neural mechanisms for psychostimulant reward and locomotor activity are distinguishable. Some evidence suggests that reward and locomotor systems are discrete. Several researchers (Robinson and Berridge 1993) have argued that the reward system is mediated by the mesolimbic pathway which projects from the ventral tegmental area to the nucleus accumbens whereas the locomotor system is mediated by the nigrostriatal pathway which projects from the substantia nigra to the striatum (Oades and Halliday 1987). Hemby et al. reported an increase in locomotor activity but no place conditioning from intra-accumbal cocaine (Hemby, Jones et al. 1992). In addition, intra-accumbal injections of neurotensin block the locomotor effect but not self-administration of cocaine (Robledo, Maldonado et al. 1993). If the mechanisms by which psychostimulants induce hyperactivity are separable from those by which they produce place conditioning, HR rats might show an increased response to the locomotor activating effects of these drugs, but not to the rewarding attributes.

Failure of these findings to support self-administration studies suggests that caution be used in generalizing between these paradigms believed to measure similar processes. Place conditioning studies measure the rewarding efficacy of stimuli whereas self-administration studies measure the reinforcing efficacy of stimuli (for review see, (Bardo and Bevins 2000). Animals that demonstrate facilitated acquisition of psychostimulant self-administration (i.e. HR rats) may, in fact, be less responsive to the rewarding efficacy of these drugs and need to self-administer higher doses to obtain similar behavioral and neurochemical effects that LR would obtain at lower doses.

Few studies have examined the DAergic neuroadaptations that take place after repeated exposure to cocaine during adolescence, not only a developmental period during which drug use initiation is widespread, but also a critical period for the remodeling of the mesolimbic and mesocortical brain regions and their neuronal DA projections (for review, see (Spear 2000)). Rosenberg & Lewis (Rosenberg and Lewis 1995) were among those researchers who saw a common developmental pattern in the overproduction and subsequent pruning of synaptic

connections during the period preceding adulthood. The D1 and D2 receptors have been of major focus for years in regards to overproduction and pruning as these receptors increase in density in the first few weeks of life (Hartley and Seeman 1983). Subsequently, Teicher et al have demonstrated receptor overproduction and elimination in both the striatum and prefrontal cortex (Teicher, Andersen et al. 1995; Andersen, Thompson et al. 2000). In addition, alterations in receptor binding and sensitivity in various neurotransmitter systems have been reported during adolescence (Trauth, Seidler et al. 1999) along with changes in the myelination of neurons (Hamano, Iwasaki et al. 1996). Importantly, DAT are overproduced and pruned during adolescence as the striatum transitions to its adult state in rats (Moll, Mehnert et al. 2000) and humans (Haycock, Becker et al. 2003). As DAT density increases during adolescence, enhanced reuptake reduces the extracellular levels of DA (Andersen and Gazzara 1993) and a subsequent upregulation of postsynaptic receptors, and their second messenger systems take place (Andersen 2002). These developmental differences may not only predispose adolescents to be more vulnerable to the rewarding effects of drugs of abuse, but may leave them more vulnerable to addiction after drug exposure due to interference with the normal synaptic pruning that takes place in the transition from adolescence to adulthood.

The present data provide evidence for long-term behavioral changes that endure after chronic cocaine administration during adolescence. Repeated exposure to cocaine during adolescence modifies the novelty-induced behavioral phenotype in adulthood. Both the novelty-preference and novel object exploration behavioral measures following repeated cocaine during adolescence produces a predisposition towards LR in adulthood, whereas animals exhibit a tendency towards being a HR when measured on novel environment locomotor activity; suggesting that animals are more at risk to engage in drug use in adulthood after adolescent drug exposure due to an alteration in the behavioral phenotype that increases the vulnerability to engage in drug use. Importantly, adult animals exposed to cocaine during adolescence, exhibited greater locomotor activity induced by a novel environment during the first minute of exposure, decreased time spent in the center of a novel environment and decreased novelty preference, which may be indicative of increased stress or anxiety or enhanced neophobia in adulthood after

adolescent cocaine. Van den Buuse et. al (van den Buuse, Van Acker et al. 2001) have demonstrated that exposure to the novelty of an open field causes an increase in blood pressure, heart rate, body temperature and exploratory locomotor activity, results indicate that an increase in locomotor activity in a novel environment is stressful or anxiogenic. Cocaine has been shown to produce anxiety in human and animal models, either during cocaine administration or during withdrawal. Future studies should investigate additional long-term behavioral changes including anxiety related behavioral measures after cessation of chronic cocaine exposure.

Interestingly, chronic adolescent exposure to cocaine produced increased locomotor activity in a novel environment, which based on previous studies suggests that this behavioral characteristic would predispose the animal to drug self-administration (Hooks, Jones et al. 1991; Bevins RA 1997). Conversely, cocaine pretreated animals demonstrated decreased time spent in the center of the open field on trial 1 and decreased novelty preference, it is possible this is an anxiogenic response in these animals compared to naïve or saline pretreated animals and may facilitate drug use. An increase in cocaine self-administration has been observed in stressed or anxious animals (Marquardt, Ortiz-Lemos et al. 2004; Covington and Miczek 2005), and chronic cocaine causes an increase in anxiety (Wood and Lal 1987; Rogerio and Takahashi 1992; Hayase, Yamamoto et al. 2005) providing an explanation for why adolescents exposed to cocaine (who subsequently may be more stressed or anxious) may be more likely to engage in continued drug use.

Repeated psychostimulant administration during adolescence has been reported to change the rewarding efficacy of cocaine in adulthood (as seen in experiment 3). Moreover, methylphenidate exposure in adolescence decreases the rewarding efficacy of cocaine in adulthood (Andersen, Arvanitogiannis et al. 2002; Carlezon, Mague et al. 2003). In addition, periadolescent nicotine exposure reduces cocaine reward in adult mice (Kelley and Middaugh 1999). Other researchers have reported that developmental exposure to methylphenidate facilitates acquisition of i.v. cocaine self administration in adulthood (Brandon, Marinelli et al. 2001). An increase in self-administration may be due to a decrease in the rewarding efficacy of stimuli whereby a higher rate of responding is necessary to maintain similar physiological states

as control animals. In addition, developmental exposure to methylphenidate also decreases the rewarding efficacy of natural reinforcers (i.e. sucrose) and sexual behaviors (Bolanos, Barrot et al. 2003). These behavioral data suggest that repeated psychostimulant exposure in adolescence modifies the responsivity to stimuli in adulthood, signified by decreased sensitivity to reward. Self-reports from human addicts suggest that the rewarding efficacy of stimuli, including drugs of abuse, decrease after repeated use. The belief that decreased rewarding efficacy of drugs will reduce the likelihood to continue drug use is contradictory to self-reports from drugs addicts who continue to engage in drug use despite little to no pleasure after use.

Neuroadaptations after developmental cocaine exposure may cause depressive-like signs such as anhedonia (decreased ability to experience reward), dysphoria (feelings of unwellness) or despair (feelings of giving up). Exposure to methylphenidate in adolescence produces an increased expression of the transcription factor, CREB (cAMP response element binding protein) within the NAcc in adulthood. An increase in intra-accumbal CREB activity has been linked with decreased cocaine reward and increased cocaine aversion in place conditioning studies and the development of depressive-like behaviors in the forced swim test (Pliakas, Carlson et al. 2001). Activation of CREB also induces an increase in dynorphin activity at the kappa opioid receptor, which has been linked with dysphoria and anhedonia (Carlezon, Thome et al. 1998). Repeated cocaine has also been shown to increase CREB (Brenhouse, Howe et al. 2007) and immobility in the forced swim test (Magalhaes, Summavielle et al. 2004; Barron, White et al. 2005) in the adult rat. These data suggest that individuals may engage in drug use due to a hypo-responsive reward system and by doing so may alleviate the symptoms of anhedonia and dysphoria. Elucidating the mechanisms by which addictive drug exposure during adolescence renders the adult more vulnerable to continued drug use is of utmost importance in a society that has a striking percentage of adolescents who experiment with cocaine.

Stimulation of D1 receptors following cocaine administration seems to be critical in the rewarding effects of the drug, although it is likely that long-term adaptations to chronic cocaine exposure are mediated by the stimulation of D2 autoreceptors which are involved in regulating synaptic DA levels (Wolf and Roth 1987; Santiago and Westerink 1991). Several researchers

have demonstrated the importance of D2 receptors and DAT in the mediation of cocaine reward. D2 antagonists block the ability of cocaine to support a place preference (Adams, Careri et al. 2001). Additionally, D2 antagonists administered systemically not only decrease cocaine self-administration, but reduce the breakpoint to self-administer cocaine (Roberts, Loh et al. 1989; Barrett, Miller et al. 2004). The NAcc shell may mediate these effects as a direct infusion of a D2 antagonist also decreases cocaine self-administration (Bari and Pierce 2005). These studies demonstrate that blockade of the D2 receptor decreases the rewarding and reinforcing efficacy of cocaine, however, few studies have examined the consequences of long-term cocaine induced changes that are altered after blockade of D2 receptors. Uhl et al. demonstrated that blockade of D1 or D2 receptors prevents the development of cocaine induced behavioral sensitization (Karler, Chaudhry et al. 1990) Importantly, *in vivo* studies have demonstrated that repeated cocaine-induced increases in striatal uptake were attenuated by pretreatment with pimozide, a D2-antagonist (Parsons, Schad et al. 1993). Importantly, the present data suggest that the blockade of D2 receptors with concurrent developmental cocaine administration prevent the neurochemical changes that have been suggested to occur after adolescent cocaine exposure, behaviorally expressed as decreased cocaine place conditioning that would potentially increase the likelihood of drug use and dependence.

Taken together, these studies in adult animals demonstrate that developmental cocaine exposure results in complicated changes in the DA mesolimbic pathway that continue long after drug use has stopped, and processes such as these may be implicated in drug dependence, craving and relapse. These data suggest that blockade of the D2 receptor prevents cocaine-induced neurochemical changes, potentially regulating the behavioral and neurochemical alterations that occur after repeated drug use that increases the likelihood of dependence. These data implicate D2 receptor activity as a potential target for the manifestation of long term adaptations in the mesolimbic DA system that manifest behaviorally as dependency following developmental cocaine exposure.

References

Abreu-Villaca, Y., E. Queiroz-Gomes Fdo, et al. (2006). "Individual differences in novelty-seeking behavior but not in anxiety response to a new environment can predict nicotine consumption in adolescent C57BL/6 mice." Behav Brain Res 167(1): 175-82.

Adams, J. U., J. M. Careri, et al. (2001). "Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats." Behav Pharmacol 12(8): 603-11.

Adriani, W., F. Chiarotti, et al. (1998). "Elevated novelty seeking and peculiar d-amphetamine sensitization in periadolescent mice compared with adult mice." Behav Neurosci 112(5): 1152-66.

Adriani, W. and G. Laviola (2000). "A unique hormonal and behavioral hyporesponsivity to both forced novelty and d-amphetamine in periadolescent mice." Neuropharmacology 39(2): 334-46.

Andersen, S. L. (2002). "Changes in the second messenger cyclic AMP during development may underlie motoric symptoms in attention deficit/hyperactivity disorder (ADHD)." Behav Brain Res 130(1-2): 197-201.

Andersen, S. L., A. Arvanitogiannis, et al. (2002). "Altered responsiveness to cocaine in rats exposed to methylphenidate during development." Nat Neurosci 5(1): 13-4.

Andersen, S. L. and R. A. Gazzara (1993). "The ontogeny of apomorphine-induced alterations of neostriatal dopamine release: effects on spontaneous release." J Neurochem 61(6): 2247-55.

Andersen, S. L., A. T. Thompson, et al. (2000). "Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats." Synapse 37(2): 167-9.

Anthony, J. C., Helzer, J.E. (1991). "Syndromes of drug abuse and dependence." Psychiatric Disorders in America: 116-154.

Arnett, J. J. (1999). "Adolescent storm and stress, reconsidered." Am Psychol 54(5): 317-26.

Badanich, K. A., K. J. Adler, et al. (2006). "Adolescents differ from adults in cocaine conditioned place preference and cocaine-induced dopamine in the nucleus accumbens septi." Eur J Pharmacol.

Bardo, M. T. (1998). "Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens." Crit Rev Neurobiol 12(1-2): 37-67.

Bardo, M. T. and R. A. Bevins (2000). "Conditioned place preference: what does it add to our preclinical understanding of drug reward?" Psychopharmacology (Berl) 153(1): 31-43.

Bari, A. A. and R. C. Pierce (2005). "D1-like and D2 dopamine receptor antagonists administered into the shell subregion of the rat nucleus accumbens decrease cocaine, but not food, reinforcement." Neuroscience 135(3): 959-68.

Barrett, A. C., J. R. Miller, et al. (2004). "Effects of dopamine indirect agonists and selective D1-like and D2-like agonists and antagonists on cocaine self-administration and food maintained responding in rats." Neuropharmacology 47 Suppl 1: 256-73.

Barron, S., A. White, et al. (2005). "Adolescent vulnerabilities to chronic alcohol or nicotine exposure: findings from rodent models." Alcohol Clin Exp Res 29(9): 1720-5.

Barros HM, M. K. (1996). "Withdrawal from oral cocaine in rats: ultrasonic vocalizations and tactile startle." Psychopharmacology 125: 379-384.

Bassareo, V. and G. Di Chiara (1999). "Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments." Neuroscience 89(3): 637-41.

Belluzzi, J. D., R. Wang, et al. (2005). "Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats." Neuropsychopharmacology 30(4): 705-12.

Berridge, K. C. and T. E. Robinson (1998). "What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?" Brain Res Brain Res Rev 28(3): 309-69.
Bevins RA, K. J., Bardo MT (1997). "Individual differences in response to novelty, amphetamine-induced activity and drug administration in rats." Behavioral pharmacology 8(2-3): 113-123.
Birnbaumer, L. and A. M. Brown (1990). "G proteins and the mechanism of action of hormones, neurotransmitters, and autocrine and paracrine regulatory factors." Am Rev Respir Dis 141(3 Pt 2): S106-14.
Blanco, C., P. Moreyra, et al. (2001). "Pathological gambling: addiction or compulsion?" Semin Clin Neuropsychiatry 6(3): 167-76.
Bolanos, C. A., M. Barrot, et al. (2003). "Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood." Biol Psychiatry 54(12): 1317-29.
Bolanos, C. A., S. J. Glatt, et al. (1998). "Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis." Brain Res Dev Brain Res 111(1): 25-33.
Borison, R. L. and B. I. Diamond (1979). "Kainic acid animal model predicts therapeutic agents in Huntington's chorea." Trans Am Neurol Assoc 104: 67-9.
Bradberry, C. W., R. J. Gruen, et al. (1991). "Individual differences in behavioral measures: correlations with nucleus accumbens dopamine measured by microdialysis." Pharmacol Biochem Behav 39(4): 877-82.
Bradberry, C. W. and S. R. Rubino (2004). "Phasic alterations in dopamine and serotonin release in striatum and prefrontal cortex in response to cocaine predictive cues in behaving rhesus macaques." Neuropsychopharmacology 29(4): 676-85.
Brandon, C. L., M. Marinelli, et al. (2001). "Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats." Neuropsychopharmacology 25(5): 651-61.
Brenhouse, H. C., M. L. Howe, et al. (2007). "Differential activation of cAMP response element binding protein in discrete nucleus accumbens subregions during early and late cocaine sensitization." Behav Neurosci 121(1): 212-7.
Bucholz, K. K. (1999). "Nosology and epidemiology of addictive disorders and their comorbidity." Psychiatr Clin North Am 22(2): 221-40.
Cain, M. E., D. A. Saucier, et al. (2005). "Novelty seeking and drug use: contribution of an animal model." Exp Clin Psychopharmacol 13(4): 367-75.
Calcagnetti, D. J. and M. D. Schechter (1991). "Conditioned place aversion following the central administration of a novel dopamine release inhibitor CGS 10746B." Pharmacol Biochem Behav 40(2): 255-9.
Camp, D. M., K. E. Browman, et al. (1994). "The effects of methamphetamine and cocaine on motor behavior and extracellular dopamine in the ventral striatum of Lewis versus Fischer 344 rats." Brain Res 668(1-2): 180-93.
Campbell, B. A., L. D. Lytle, et al. (1969). "Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat." Science 166(905): 635-7.
Carelli, R. M. and S. G. Ijames (2001). "Selective activation of accumbens neurons by cocaine-associated stimuli during a water/cocaine multiple schedule." Brain Res 907(1-2): 156-61.
Carlezon, W. A., Jr., D. P. Devine, et al. (1995). "Habit-forming actions of nomifensine in nucleus accumbens." Psychopharmacology (Berl) 122(2): 194-7.
Carlezon, W. A., Jr., S. D. Mague, et al. (2003). "Enduring behavioral effects of early exposure to methylphenidate in rats." Biol Psychiatry 54(12): 1330-7.
Carlezon, W. A., Jr., J. Thome, et al. (1998). "Regulation of cocaine reward by CREB." Science 282(5397): 2272-5.
Caron, M. G. (1996). "Images in neuroscience. A mouse knockout." Am J Psychiatry 153(11): 1387.
Cass, W. A. and G. A. Gerhardt (1994). "Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake." Neurosci Lett 176(2): 259-63.

Cass, W. A. and N. R. Zahniser (1993). "Cocaine levels in striatum and nucleus accumbens: augmentation following challenge injection in rats withdrawn from repeated cocaine administration." Neurosci Lett 152(1-2): 177-80.
Caster, J. M., Q. D. Walker, et al. (2005). "Enhanced behavioral response to repeated-dose cocaine in adolescent rats." Psychopharmacology (Berl) 183(2): 218-25.
Cervo, L. and R. Samanin (1995). "Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference." Brain Res 673(2): 242-50.
Chambers RA, T. J., Potenza MN (2003). "Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability." American journal of psychiatry 160(6): 1041-1052.
Chao, J. and E. J. Nestler (2004). "Molecular neurobiology of drug addiction." Annu Rev Med 55: 113-32.
Chen, R., M. R. Tilley, et al. (2006). "Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter." Proc Natl Acad Sci U S A 103(24): 9333-8.
Clark DB, K. L., Tarter RE (1998). "Adolescent versus adult onset and the development of substance use disorders in males." Drug Alcohol Dependence 49: 115-121.
Cools, A. R., B. A. Ellenbroek, et al. (1997). "Differences in vulnerability and susceptibility to dexamphetamine in Nijmegen high and low responders to novelty: a dose-effect analysis of spatio-temporal programming of behaviour." Psychopharmacology (Berl) 132(2): 181-7.
Costall B, K. M., Naylor RJ, Onaivi ES (1989). "The actions of nicotine and cocaine in a mouse model of anxiety." Pharmacology biochemistry and behavior 33(1): 197-203.
Covington, H. E., 3rd and K. A. Miczek (2005). "Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation." Psychopharmacology (Berl) 183(3): 331-40.
Cunningham, C. L. and D. Noble (1992). "Methamphetamine-induced conditioned place preference or aversion depending on dose and presence of drug." Ann N Y Acad Sci 654: 431-3.
Cunningham, M. G., S. Bhattacharyya, et al. (2002). "Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence." J Comp Neurol 453(2): 116-30.
Damsma, G., J. G. Pfaus, et al. (1992). "Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion." Behav Neurosci 106(1): 181-91.
Daws, L. C., P. D. Callaghan, et al. (2002). "Cocaine increases dopamine uptake and cell surface expression of dopamine transporters." Biochem Biophys Res Commun 290(5): 1545-50.
De La Garza, R., 2nd, P. M. Callahan, et al. (1998). "The discriminative stimulus properties of cocaine: effects of microinfusion of cocaine, a 5-HT1A agonist or antagonist, into the ventral tegmental area." Psychopharmacology (Berl) 137(1): 1-6.
De Leonibus, E., M. M. Verheij, et al. (2006). "Distinct kinds of novelty processing differentially increase extracellular dopamine in different brain regions." Eur J Neurosci 23(5): 1332-40.
Di Ciano, P., R. N. Cardinal, et al. (2001). "Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior." J Neurosci 21(23): 9471-7.
Dom G, H. W., Sabbe B (2006). "Differences in impulsivity and sensation seeking between early- and late-onset alcoholics." Addictive behavior 31(2): 298-308.
Donovan, D. M., L. L. Miner, et al. (1999). "Cocaine reward and MPTP toxicity: alteration by regional variant dopamine transporter overexpression." Brain Res Mol Brain Res 73(1-2): 37-49.
Doremus, T. L., S. C. Brunell, et al. (2003). "Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats." Pharmacol Biochem Behav 75(2): 411-8.
Douglas, L. A., E. I. Varlinskaya, et al. (2003). "Novel-object place conditioning in adolescent and adult male and female rats: effects of social isolation." Physiol Behav 80(2-3): 317-25.

Erb, S. M. and L. A. Parker (1994). "Individual differences in novelty-induced activity do not predict strength of amphetamine-induced place conditioning." Pharmacol Biochem Behav 48(3): 581-6.
Estroff, T. W., R. H. Schwartz, et al. (1989). "Adolescent cocaine abuse. Addictive potential, behavioral and psychiatric effects." Clin Pediatr (Phila) 28(12): 550-5.
Fiorino, D. F., A. Coury, et al. (1993). "Electrical stimulation of reward sites in the ventral tegmental area increases dopamine transmission in the nucleus accumbens of the rat." Behav Brain Res 55(2): 131-41.
Frantz, K. J., L. E. O'Dell, et al. (2007). "Behavioral and neurochemical responses to cocaine in periadolescent and adult rats." Neuropsychopharmacology 32(3): 625-37.
Funada, M. and T. S. Shippenberg (1996). "Differential involvement of D1 and D2 dopamine receptors in the expression of morphine withdrawal signs in rats." Behav Pharmacol 7(5): 448-453.
Gerald, M. S. and J. D. Higley (2002). "Evolutionary underpinnings of excessive alcohol consumption." Addiction 97(4): 415-25.
Giambalvo, C. T. and R. L. Wagner (1994). "Activation of D1 and D2 dopamine receptors inhibits protein kinase C activity in striatal synaptoneurosomes." J Neurochem 63(1): 169-76.
Goeders, N. (2002). "The HPA axis and cocaine reinforcement." Psychoneuroendocrinology 27(13-33).
Goeders, N. E. and M. J. Kuhar (1987). "Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens." Alcohol Drug Res 7(4): 207-16.
Gong, W., D. B. Neill, et al. (1996). "Locomotor response to novelty does not predict cocaine place preference conditioning in rats." Pharmacol Biochem Behav 53(1): 191-6.
Gonon, F. (1997). "Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo." J Neurosci 17(15): 5972-8.
Grace, A. A. (2000). "The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving." Addiction 95 Suppl 2: S119-28.
Greengard, P. (2001). "The neurobiology of dopamine signaling." Biosci Rep 21(3): 247-69.
Hall, F. S., X. F. Li, et al. (2002). "Cocaine mechanisms: enhanced cocaine, fluoxetine and nisoxetine place preferences following monoamine transporter deletions." Neuroscience 115(1): 153-61.
Hamano, K., N. Iwasaki, et al. (1996). "A quantitative analysis of rat central nervous system myelination using the immunohistochemical method for MBP." Brain Res Dev Brain Res 93(1-2): 18-22.
Hartley, E. J. and P. Seeman (1983). "Development of receptors for dopamine and noradrenaline in rat brain." Eur J Pharmacol 91(4): 391-7.
Hayase, T., Y. Yamamoto, et al. (2005). "Persistent anxiogenic effects of a single or repeated doses of cocaine and methamphetamine: interactions with endogenous cannabinoid receptor ligands." Behav Pharmacol 16(5-6): 395-404.
Haycock, J. W., L. Becker, et al. (2003). "Marked disparity between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum." J Neurochem 87(3): 574-85.
Health, N. I. o. (1989). "Guide for the care and use of laboratory animals (DHEW Publication No.86-23)." U.S. Government printing office Washington, DC.
Helzer JE, B. M., McEvoy LT (1991). "Alcohol abuse and dependence, in psychiatric disorders in America: The epidemiologic catchment area study." New York, Free Press: 81-115.
Hemby, S. E., G. H. Jones, et al. (1992). "Conditioned locomotor activity but not conditioned place preference following intra-accumbens infusions of cocaine." Psychopharmacology (Berl) 106(3): 330-6.
Henry, D. J., X. T. Hu, et al. (1998). "Adaptations in the mesoaccumbens dopamine system resulting from repeated administration of dopamine D1 and D2 receptor-selective agonists: relevance to cocaine sensitization." Psychopharmacology (Berl) 140(2): 233-42.

Hernandez, L. and B. G. Hoebel (1988). "Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis." Life Sci 42(18): 1705-12.
Hoebel, B. G., A. P. Monaco, et al. (1983). "Self-injection of amphetamine directly into the brain." Psychopharmacology (Berl) 81(2): 158-63.
Hooks, M. S., A. C. Colvin, et al. (1992). "Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis." Brain Res 587(2): 306-12.
Hooks, M. S., G. H. Jones, et al. (1991). "Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine." Synapse 9(2): 121-8.
Huff, R. M., C. L. Chio, et al. (1998). "Signal transduction pathways modulated by D2-like dopamine receptors." Adv Pharmacol 42: 454-7.
Huot, R. L., K. V. Thrivikraman, et al. (2001). "Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment." Psychopharmacology (Berl) 158(4): 366-73.
Iannazzo, L., S. Sathananthan, et al. (1997). "Modulation of dopamine release from rat striatum by protein kinase C: interaction with presynaptic D2-dopamine-autoreceptors." Br J Pharmacol 122(8): 1561-6.
Imperato, A., L. Angelucci, et al. (1992). "Repeated stressful experiences differently affect limbic dopamine release during and following stress." Brain Res 577(2): 194-9.
Infurna, R. N. and L. P. Spear (1979). "Developmental changes in amphetamine-induced taste aversions." Pharmacol Biochem Behav 11(1): 31-5.
Irwin, C. E., Jr. (1989). "Risk taking behaviors in the adolescent patient: are they impulsive?" Pediatr Ann 18(2): 122, 124, 125 passim.
Izenwasser, S. and B. M. Cox (1992). "Inhibition of dopamine uptake by cocaine and nicotine: tolerance to chronic treatments." Brain Res 573(1): 119-25.
Johnston LD, O. M. P., Bachman JG (2002). "Monitoring the future national results on adolescent drug use: overview of key findings." National Institute of Drug Abuse Publication No. 03-5374.
Kalivas, P. W. and P. Duffy (1988). "Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release." J Neurochem 50(5): 1498-504.
Kalivas, P. W., P. Duffy, et al. (1988). "Behavioral and neurochemical effects of acute and daily cocaine administration in rats." J Pharmacol Exp Ther 245(2): 485-92.
Kandel, D. B., K. Yamaguchi, et al. (1992). "Stages of progression in drug involvement from adolescence to adulthood: further evidence for the gateway theory." J Stud Alcohol 53(5): 447-57.
Karler, R., I. A. Chaudhry, et al. (1990). "Amphetamine behavioral sensitization and the excitatory amino acids." Brain Res 537(1-2): 76-82.
Karoum, F., R. L. Suddath, et al. (1990). "Chronic cocaine and rat brain catecholamines: long-term reduction in hypothalamic and frontal cortex dopamine metabolism." Eur J Pharmacol 186(1): 1-8.
Kebabian, J. W. and D. B. Calne (1979). "Multiple receptors for dopamine." Nature 277(5692): 93-6.
Kelley, A. E. (2004). "Memory and addiction: shared neural circuitry and molecular mechanisms." Neuron 44(1): 161-79.
Kelley, A. E. (2004). "Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning." Neurosci Biobehav Rev 27(8): 765-76.
Kelley, B. M. and L. D. Middaugh (1999). "Periadolescent nicotine exposure reduces cocaine reward in adult mice." J Addict Dis 18(3): 27-39.
Kitayama, S., T. Dohi, et al. (1994). "Phorbol esters alter functions of the expressed dopamine transporter." Eur J Pharmacol 268(2): 115-9.
Klebaur, J. E., R. A. Bevins, et al. (2001). "Individual differences in behavioral responses to novelty and amphetamine self-administration in male and female rats." Behav Pharmacol 12(4): 267-75.

Kleven, M., W. Woolverton, et al. (1988). "Behavioral and neurochemical effects of repeated or continuous exposure to cocaine." NIDA Res Monogr 81: 86-93.
Knutson, B., C. M. Adams, et al. (2001). "Anticipation of increasing monetary reward selectively recruits nucleus accumbens." J Neurosci 21(16): RC159.
Koob, G. F., S. B. Caine, et al. (1997). "Opponent process model and psychostimulant addiction." Pharmacol Biochem Behav 57(3): 513-21.
Koob, G. F. and M. Le Moal (1997). "Drug abuse: hedonic homeostatic dysregulation." Science 278(5335): 52-8.
Koob, G. F. and M. Le Moal (2001). "Drug addiction, dysregulation of reward, and allostasis." Neuropsychopharmacology 24(2): 97-129.
Kosten, T. A. and M. J. Miserendino (1998). "Dissociation of novelty- and cocaine-conditioned locomotor activity from cocaine place conditioning." Pharmacol Biochem Behav 60(4): 785-91.
Kuczenski, R., D. S. Segal, et al. (1991). "Amphetamine, cocaine, and fencamfamine: relationship between locomotor and stereotypy response profiles and caudate and accumbens dopamine dynamics." J Neurosci 11(9): 2703-12.
Kuhn, C. and R. Francis (1997). "Gender difference in cocaine-induced HPA axis activation." Neuropsychopharmacology 16(6): 399-407.
Laviola, G., G. Dell'Omo, et al. (1992). "Ontogeny of cocaine hyperactivity and conditioned place preference in mice." Psychopharmacology (Berl) 107(2-3): 221-8.
Laviola, G., G. Dell'Omo, et al. (1994). "d-amphetamine conditioned place preference in developing mice: relations with changes in activity and stereotypies." Behav Neurosci 108(3): 514-24.
Laviola, G., R. D. Wood, et al. (1995). "Cocaine sensitization in periadolescent and adult rats." J Pharmacol Exp Ther 275(1): 345-57.
Le Moal, M. and H. Simon (1991). "Mesocorticolimbic dopaminergic network: functional and regulatory roles." Physiol Rev 71(1): 155-234.
Le Pen, G., D. Duterte-Boucher, et al. (1996). "Place conditioning with cocaine and the dopamine uptake inhibitor GBR12783." Neuroreport 7(18): 2839-42.
Levin, E. D., A. H. Rezvani, et al. (2003). "Adolescent-onset nicotine self-administration modeled in female rats." Psychopharmacology (Berl) 169(2): 141-9.
Lindsey, K. P., K. M. Wilcox, et al. (2004). "Effects of dopamine transporter inhibitors on cocaine self-administration in rhesus monkeys: relationship to transporter occupancy determined by positron emission tomography neuroimaging." J Pharmacol Exp Ther 309(3): 959-69.
Lindvall, O. and A. Bjorklund (1974). "The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method." Acta Physiol Scand Suppl 412: 1-48.
Logue A.W, P.-C. T. (1985). "The effect of food deprivation on self-control." Behavioural Processes 10: 355-368.
Logue, A. W. (1985). "Conditioned food aversion learning in humans." Ann N Y Acad Sci 443: 316-29.
Macri S, A., W, Chiarotti F, Laviola G (2002). "Risk-taking during exploration of a plus-maze is greater in distant than in juvenile or adult mice." Animal Behaviour 64: 541-546.
Magalhaes, A., T. Summavielle, et al. (2004). "Effects of postnatal cocaine exposure and environmental enrichment on rat behavior in a forced swim test." Ann N Y Acad Sci 1025: 619-29.
Marquardt, A. R., L. Ortiz-Lemos, et al. (2004). "Influence of handling or aversive stimulation during rats' neonatal or adolescence periods on oral cocaine self-administration and cocaine withdrawal." Behav Pharmacol 15(5-6): 403-12.
Martin-Iverson, M. T., R. Ortmann, et al. (1985). "Place preference conditioning with methylphenidate and nomifensine." Brain Res 332(1): 59-67.
Mayfield, R. D. and N. R. Zahniser (2001). "Dopamine D2 receptor regulation of the dopamine transporter expressed in Xenopus laevis oocytes is voltage-independent." Mol Pharmacol 59(1): 113-21.

Mazur, J. E. and D. Coe (1987). "Tests of transitivity in choices between fixed and variable reinforcer delays." J Exp Anal Behav 47(3): 287-97.
McBride, W. J., J. M. Murphy, et al. (1999). "Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies." Behav Brain Res 101(2): 129-52.
McCabe, R. T., G. R. Hanson, et al. (1987). "Methamphetamine-induced reduction in D1 and D2 dopamine receptors as evidenced by autoradiography: comparison with tyrosine hydroxylase activity." Neuroscience 23(1): 253-61.
McKinzie, D. L., Z. A. Rodd-Henricks, et al. (1999). "Cocaine is self-administered into the shell region of the nucleus accumbens in Wistar rats." Ann N Y Acad Sci 877: 788-91.
Meiergerd, S. M., T. A. Patterson, et al. (1993). "D2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo." J Neurochem 61(2): 764-7.
Meil, W. M. and M. D. Schechter (1997). "Olanzapine attenuates the reinforcing effects of cocaine." Eur J Pharmacol 340(1): 17-26.
Missale, C., S. R. Nash, et al. (1998). "Dopamine receptors: from structure to function." Physiol Rev 78(1): 189-225.
Moeller, F. G., E. S. Barratt, et al. (2001). "Psychiatric aspects of impulsivity." Am J Psychiatry 158(11): 1783-93.
Moll, G. H., C. Mehnert, et al. (2000). "Age-associated changes in the densities of presynaptic monoamine transporters in different regions of the rat brain from early juvenile life to late adulthood." Brain Res Dev Brain Res 119(2): 251-7.
Nauta, W. J., G. P. Smith, et al. (1978). "Efferent connections and nigral afferents of the nucleus accumbens septi in the rat." Neuroscience 3(4-5): 385-401.
Nestler, E. J. and G. K. Aghajanian (1997). "Molecular and cellular basis of addiction." Science 278(5335): 58-63.
O'Dell, L. E., A. W. Bruijnzeel, et al. (2004). "Nicotine withdrawal in adolescent and adult rats." Ann N Y Acad Sci 1021: 167-74.
Oades, R. D. and G. M. Halliday (1987). "Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity." Brain Res 434(2): 117-65.
Odell, W. (1990). "Sexual Maturation in the rat." 183-210.
Olds, M. E. and J. L. Fobes (1981). "The central basis of motivation: intracranial self-stimulation studies." Annu Rev Psychol 32: 523-74.
Ortmann, R. (1985). "The conditioned place preference paradigm in rats: effect of bupropion." Life Sci 37(21): 2021-7.
Parsons, L. H., C. A. Schad, et al. (1993). "Co-administration of the D2 antagonist pimozide inhibits up-regulation of dopamine release and uptake induced by repeated cocaine." J Neurochem 60(1): 376-9.
Parsons, L. H., A. D. Smith, et al. (1991). "Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine." Synapse 9(1): 60-5.
Pawlak, C. R. and R. K. Schwarting (2002). "Object preference and nicotine consumption in rats with high vs. low rearing activity in a novel open field." Pharmacol Biochem Behav 73(3): 679-87.
Pelloux, Y., J. Costentin, et al. (2004). "Differential effects of novelty exposure on place preference conditioning to amphetamine and its oral consumption." Psychopharmacology (Berl) 171(3): 277-85.
Pelloux, Y., J. Costentin, et al. (2006). "Novelty preference predicts place preference conditioning to morphine and its oral consumption in rats." Pharmacol Biochem Behav 84(1): 43-50.
Peris, J., S. J. Boyson, et al. (1990). "Persistence of neurochemical changes in dopamine systems after repeated cocaine administration." J Pharmacol Exp Ther 253(1): 38-44.
Perry, J. L., E. B. Larson, et al. (2005). "Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats." Psychopharmacology (Berl) 178(2-3): 193-201.
Philpot, R. M., K. A. Badanich, et al. (2003). "Place conditioning: age-related changes in the rewarding and aversive effects of alcohol." Alcohol Clin Exp Res 27(4): 593-9.

Philpot, R. M. and C. L. Kirstein (1998). "The effects of repeated alcohol exposure on the neurochemistry of the periadolescent nucleus accumbens septi." Neuroreport 9(7): 1359-63.
Piazza, P. V., J. M. Deminiere, et al. (1989). "Factors that predict individual vulnerability to amphetamine self-administration." Science 245(4925): 1511-3.
Pliakas, A. M., R. R. Carlson, et al. (2001). "Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens." J Neurosci 21(18): 7397-403.
Ploj, K., E. Roman, et al. (2003). "Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats." Neuroscience 121(3): 787-99.
Post, R. M. (1980). "Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance." Life Sci 26(16): 1275-82.
Poulos, C. X., A. D. Le, et al. (1995). "Impulsivity predicts individual susceptibility to high levels of alcohol self-administration." Behav Pharmacol 6(8): 810-814.
Powell, S. B., M. A. Geyer, et al. (2004). "The balance between approach and avoidance behaviors in a novel object exploration paradigm in mice." Behav Brain Res 152(2): 341-9.
Pristupa, Z. B., F. McConkey, et al. (1998). "Protein kinase-mediated bidirectional trafficking and functional regulation of the human dopamine transporter." Synapse 30(1): 79-87.
Pruitt, D. L., C. A. Bolanos, et al. (1995). "Effects of dopamine D1 and D2 receptor antagonists on cocaine-induced place preference conditioning in preweanling rats." Eur J Pharmacol 283(1-3): 125-31.
Regier, D. A., M. E. Farmer, et al. (1990). "Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study." Jama 264(19): 2511-8.
Reiger DA, F. M., Rae DS, Lock BZ, Keith SJ, Judd LL, Goodwin FK (1990). "Comorbidity of mental disorders with alcohol and other drugs of abuse." JAMA 264: 2511-2518.
Reith, M. E., M. Y. Li, et al. (1997). "Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers." Psychopharmacology (Berl) 134(3): 309-17.
Roberts, D. C., E. A. Loh, et al. (1989). "Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment." Psychopharmacology (Berl) 97(4): 535-8.
Robinson, T. E. and K. C. Berridge (1993). "The neural basis of drug craving: an incentive-sensitization theory of addiction." Brain Res Brain Res Rev 18(3): 247-91.
Robledo, P., R. Maldonado, et al. (1993). "Neurotensin injected into the nucleus accumbens blocks the psychostimulant effects of cocaine but does not attenuate cocaine self-administration in the rat." Brain Res 622(1-2): 105-12.
Rodd ZA, M. D., Dagon CL, Murphy JM, McBride WJ (1998). "Intracranial self-administration of ethanol into the posterior VTA by Wistar rats." Soc. Neurosci. Abst 24: 1479.
Rogerio, R. and R. N. Takahashi (1992). "Anxiogenic properties of cocaine in the rat evaluated with the elevated plus-maze." Pharmacol Biochem Behav 43(2): 631-3.
Rogers, R. D. and T. W. Robbins (2001). "Investigating the neurocognitive deficits associated with chronic drug misuse." Curr Opin Neurobiol 11(2): 250-7.
Rosenberg, D. R. and D. A. Lewis (1995). "Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: a tyrosine hydroxylase immunohistochemical analysis." J Comp Neurol 358(3): 383-400.
Rothblat, D. S. and J. S. Schneider (1997). "Regionally specific effects of haloperidol and clozapine on dopamine reuptake in the striatum." Neurosci Lett 228(2): 119-22.
Salamone, J. D. (1992). "Complex motor and sensorimotor functions of striatal and accumbens dopamine: involvement in instrumental behavior processes." Psychopharmacology (Berl) 107(2-3): 160-74.

Santiago, M. and B. H. Westerink (1991). "The regulation of dopamine release from nigrostriatal neurons in conscious rats: the role of somatodendritic autoreceptors." Eur J Pharmacol 204(1): 79-85.
Sarnyai, Z., E. Biro, et al. (1995). "Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats." Brain Res 675(1-2): 89-97.
Schechter, M. D. (1995). "Cocaethylene produces discriminative stimulus properties in the rat: effect of cocaine and ethanol coadministration." Pharmacol Biochem Behav 51(2-3): 285-9.
Schlegel A, B. I. H. (1991). "Adolescence: an anthropological inquiry." New York, Free Press.
Schramm-Sapyta, N. L., R. W. Morris, et al. (2006). "Adolescent rats are protected from the conditioned aversive properties of cocaine and lithium chloride." Pharmacol Biochem Behav 84(2): 344-52.
Schultz, W. (1998). "Predictive reward signal of dopamine neurons." J Neurophysiol 80(1): 1-27.
Schultz, W., P. Dayan, et al. (1997). "A neural substrate of prediction and reward." Science 275(5306): 1593-9.
Sellings, L. H., L. E. McQuade, et al. (2006). "Characterization of dopamine-dependent rewarding and locomotor stimulant effects of intravenously-administered methylphenidate in rats." Neuroscience 141(3): 1457-68.
Shaffer, H. J., D. P. Forman, et al. (2000). "Awareness of gambling-related problems, policies and educational programs among high school and college administrators." J Gambl Stud 16(1): 93-101.
Shippenberg, T. S., R. Bals-Kubik, et al. (1991). "Neuroanatomical substrates mediating the aversive effects of D-1 dopamine receptor antagonists." Psychopharmacology (Berl) 103(2): 209-14.
Shippenberg, T. S. and A. Herz (1987). "Place preference conditioning reveals the involvement of D1-dopamine receptors in the motivational properties of mu- and kappa-opioid agonists." Brain Res 436(1): 169-72.
Shram, M. J., D. Funk, et al. (2006). "Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine." Psychopharmacology (Berl) 186(2): 201-8.
Silveri, M. M. and L. P. Spear (1998). "Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny." Alcohol Clin Exp Res 22(3): 670-6.
Skagerberg, G., O. Lindvall, et al. (1984). "Origin, course and termination of the mesohabenular dopamine pathway in the rat." Brain Res 307(1-2): 99-108.
Slawecki, C. J. (2006). "Two-choice reaction time performance in Sprague-Dawley rats exposed to alcohol during adolescence or adulthood." Behav Pharmacol 17(7): 605-14.
Smith, L. N., C. G. McDonald, et al. (2006). "Long-term changes in fear conditioning and anxiety-like behavior following nicotine exposure in adult versus adolescent rats." Pharmacol Biochem Behav.
Solomon, R. L. (1980). "The opponent-process theory of acquired motivation: the costs of pleasure and the benefits of pain." Am Psychol 35(8): 691-712.
Sora, I., F. S. Hall, et al. (2001). "Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference." Proc Natl Acad Sci U S A 98(9): 5300-5.
Sora, I., C. Wichems, et al. (1998). "Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice." Proc Natl Acad Sci U S A 95(13): 7699-704.
Spear, L. P. (2000). "The adolescent brain and age-related behavioral manifestations." Neurosci Biobehav Rev 24(4): 417-63.
Spear, L. P. and S. C. Brake (1983). "Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats." Dev Psychobiol 16(2): 83-109.
Spear, L. P., C. L. Kirstein, et al. (1989). "Cocaine effects on the developing central nervous system: behavioral, psychopharmacological, and neurochemical studies." Ann N Y Acad Sci 562: 290-307.

Stansfield, K. H. and C. L. Kirstein (2005). "Neurochemical effects of cocaine in adolescence compared to adulthood." Brain Res Dev Brain Res 159(2): 119-25.
Stansfield, K. H. and C. L. Kirstein (2006). "Effects of novelty on behavior in the adolescent and adult rat." Dev Psychobiol 48(1): 10-5.
Stansfield, K. H., R. M. Philpot, et al. (2004). "An animal model of sensation seeking: the adolescent rat." Ann N Y Acad Sci 1021: 453-8.
Steinberg, L. (1989). "Pubertal maturation and parent adolescent distance: an evolutionary perspective."
Strecker, R. E., W. F. Eberle, et al. (1995). "Extracellular dopamine and its metabolites in the nucleus accumbens of Fischer and Lewis rats: basal levels and cocaine-induced changes." Life Sci 56(6): PL135-41.
Swadi, H. (1999). "Individual risk factors for adolescent substance use." Drug Alcohol Depend 55(3): 209-24.
Teicher, M. H., S. L. Andersen, et al. (1995). "Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens." Brain Res Dev Brain Res 89(2): 167-72.
Timar, J., Z. Gyarmati, et al. (1996). "Differences in some behavioural effects of deprenyl and amphetamine enantiomers in rats." Physiol Behav 60(2): 581-7.
Trauth, J. A., F. J. Seidler, et al. (1999). "Adolescent nicotine exposure causes persistent upregulation of nicotinic cholinergic receptors in rat brain regions." Brain Res 851(1-2): 9-19.
Trimpop RM, K. J., Kirkaldy B (1999). "Comparing personality constructs of risk-taking behavior." Personality and individual differences 26: 237-254.
Tzschentke, T. (2000). "The medial prefrontal cortex as a part of the brain reward system." Amino Acids 19(1): 211-219.
Uddin, R. K. and S. M. Singh (2006). "Ethanol-responsive genes: identification of transcription factors and their role in metabolomics." Pharmacogenomics J.
van den Buuse, M., S. A. Van Acker, et al. (2001). "Blood pressure, heart rate, and behavioral responses to psychological "novelty" stress in freely moving rats." Psychophysiology 38(3): 490-9.
Vanderschuren, L. J. and P. W. Kalivas (2000). "Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies." Psychopharmacology (Berl) 151(2-3): 99-120.
Varlinskaya, E. I. and L. P. Spear (2004). "Acute ethanol withdrawal (hangover) and social behavior in adolescent and adult male and female Sprague-Dawley rats." Alcohol Clin Exp Res 28(1): 40-50.
Varlinskaya, E. I. and L. P. Spear (2004). "Changes in sensitivity to ethanol-induced social facilitation and social inhibition from early to late adolescence." Ann N Y Acad Sci 1021: 459-61.
Vastola, B. J., L. A. Douglas, et al. (2002). "Nicotine-induced conditioned place preference in adolescent and adult rats." Physiol Behav 77(1): 107-14.
Vaughan, R. A., R. A. Huff, et al. (1997). "Protein kinase C-mediated phosphorylation and functional regulation of dopamine transporters in striatal synaptosomes." J Biol Chem 272(24): 15541-6.
Warner, L. A., R. C. Kessler, et al. (1995). "Prevalence and correlates of drug use and dependence in the United States. Results from the National Comorbidity Survey." Arch Gen Psychiatry 52(3): 219-29.
Wee, S., F. I. Carroll, et al. (2006). "A reduced rate of in vivo dopamine transporter binding is associated with lower relative reinforcing efficacy of stimulants." Neuropsychopharmacology 31(2): 351-62.
Williams, S. M. and P. S. Goldman-Rakic (1998). "Widespread origin of the primate mesofrontal dopamine system." Cereb Cortex 8(4): 321-45.
Wilmouth, C. E. and L. P. Spear (2004). "Adolescent and adult rats' aversion to flavors previously paired with nicotine." Ann N Y Acad Sci 1021: 462-4.

Wise, R. A. (1980). "Action of drugs of abuse on brain reward systems." Pharmacol Biochem Behav 13 Suppl 1: 213-23.
Wolf, M. E. and R. H. Roth (1987). "Dopamine neurons projecting to the medial prefrontal cortex possess release-modulating autoreceptors." Neuropharmacology 26(8): 1053-9.
Wood, D. M. and H. Lal (1987). "Anxiogenic properties of cocaine withdrawal." Life Sci 41(11): 1431-6.
Yang, X. M., A. L. Gorman, et al. (1992). "Anxiogenic effects of acute and chronic cocaine administration: neurochemical and behavioral studies." Pharmacol Biochem Behav 41(3): 643-50.
Zeitlin, H. (1999). "Psychiatric comorbidity with substance misuse in children and teenagers." Drug Alcohol Depend 55(3): 225-34.
Zhang, L., L. L. Coffey, et al. (1997). "Regulation of the functional activity of the human dopamine transporter by protein kinase C." Biochem Pharmacol 53(5): 677-88.
Zheng, X. G., B. P. Tan, et al. (2004). "Novelty-seeking behavior and stress-induced locomotion in rats of juvenile period differentially related to morphine place conditioning in their adulthood." Behav Processes 65(1): 15-23.
Zuckerman, M. (1986). "Sensation seeking and the endogenous deficit theory of drug abuse." NIDA Res Monogr 74: 59-70.

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