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An Evaluation of the Dopaminergic Systems' Response to a Natural Reinforcer: A Comparison Between Cocaine Pretreatment in Adolescent and Adult Rats

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An Evaluation of the Dopaminergic Systems' Response to a Natural Reinforcer: A
Comparison Between Cocaine Pretreatment in Adolescent and Adult Rats

By

Briony Catlow

A thesis submitted in partial fulfillment
of the requirements for the degree of
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ABSTRACT

The long-term consequences of adolescent drug use in shaping a network primed for addiction is an issue of utmost importance. The use of cocaine during adolescent development could alter the normal growth of the reward system and affect the adult mesolimbic system, however, there is scant literature aimed at finding out if animals are more vulnerable to the adverse effects of drugs during adolescence. The present study investigated whether cocaine pretreatment in adolescent and adult rats produced differences in cocaine-induced neurochemical cross-sensitization to a naturally reinforcing substance in adulthood. To evaluate the responsivity of the mesolimbic system after repeated cocaine, sucrose was offered during the dialysis procedure and dialysate was collected. All saline pretreated rats had significant increases in DA levels compared to baseline levels and there were no difference in the age of pretreatment. Rats pretreated with cocaine as adults also had significant

increases in DA levels after sucrose. Interestingly, sucrose intake significantly enhanced DA levels in cocaine pretreated adolescent rats. The results from this experiment clearly show that in rats pretreated with cocaine during adolescence there is an enhance response of the DAergic system in response to a naturally reinforcing substance therefore; cocaine exposure during adolescence results in persistent long term changes in the mesolimbic pathway. Future studies need to ascertain the underlying mechanisms and their role in the process of addiction.

Chapter 1: Background Review

Approximately 50 million Americans have used cocaine at some time, and more than 6 million Americans use cocaine regularly, making cocaine the most abused illicit psychostimulant of the 1990s in the United States (Das, 1993). However, cocaine is not a drug that is exclusively used by adults, in fact, the 2004 Monitoring the Future Study reported that 2.3% of high school seniors had used cocaine in the previous month (Johnson, 2005). Furthermore, the number of 12th graders who have used cocaine at some point in their lifetime increased from 7.7% in 2003 to 8.1% in 2004 (Johnson, 2005). A 2004 survey carried out by the NIDA indicated that 3.4%, 5.4% and 8.1% of 8th, 10th and 12th graders respectively have used cocaine in their lifetime (Johnson, 2005). This increase in drug use is most likely due to higher availability of drugs, more so now than ever before. The problem of drug use in the United States today has driven the government to lead a war on drugs. This attempt to stop the sale and use of cocaine in the United States has not been successful in reducing the number of people using cocaine. The White House Office of National Drug Control Policy (ONDCP) conducted surveys and found that between the years 1995 and 1998 Americans spent \$38 billion on cocaine (Office of National Drug Control Policy, 1999). The United States commits more money into fighting the war on drugs so that now the national drug control budget aimed at preventing drug use and abuse, as well as the sales of illegal drugs, has grown from \$1.5 billion in 1981, to an estimated \$11.7 billion dollars in 2004 (ONDCP, 2003). Over the

past 15 years, more than \$250 billion has been spent to tackle the “war on drugs” (ONDCP, 2003); however, cocaine use continues to be a problem (ONDCP, 2003).

Theories of Addiction

At one time, people under the influence of drugs were viewed as criminals and mentally ill. However this view changed by the end of the nineteenth century as physicians identified drug users as lacking control over drug use, and in need of medical attention. The term addiction (*addicere* “to sentence”) became used to describe problems associated with repeatedly using drugs of abuse and to diagnose excessive drug use. Early theories of addiction focused on physical signs of withdrawal as a key factor in diagnosing a person with a drug addiction. Addiction experts theorized that the addict was driven into drug relapse or the re-initiation of drug use in an attempt to stop the withdrawal syndrome and avoid the negative affects associated with drug use (Wise & Bozarth, 1982). This idea was termed the negative reinforcement model or the anhedonia hypothesis and proposes repeated exposure to drugs results in drug tolerance and withdrawal symptoms appear when drug use ceases. Tolerance refers to a decrease in the effect of a drug after repeated exposure so that the user has to increase the dose to get the effect produced by the original lower dose of the drug. The compensatory reactions to the primary effects of the drug are the withdrawal symptoms which produce an effect opposite of the effect of the drug (Trujillo & Akil, 1991; Zukin, Tempel, & Gardner, 1984). These symptoms are extremely unpleasant and reducing these effects represents negative reinforcement (i.e., negative reinforcement is the reinforcement of behavior that stops an aversive stimulus). This model could well explain the reasons why addicts

continue drug use; however it does not explain why many addicts will voluntarily undergo withdrawal and endure the extremely unpleasant withdrawal symptoms (e.g., 'cold-turkey' with heroin withdrawal) to reduce their drug tolerance so that they can resume drug use at a lower dose in order to maintain the costly habit. Interestingly, this theory failed to identify cocaine as an addictive drug since cocaine (an extremely addictive drug) does not produce a strong physical dependency (i.e., tolerance and withdrawal syndrome). In contrast, other drugs such as antidepressants, inhalers used to treat asthma which contained a β -adrenergic agonist and many drugs used in the treatment of hypertension produce tolerance and withdrawal yet these drugs are not addictive (Hyman & Malenka, 2001). Taken together, the negative reinforcement model is not an adequate model of addiction as it does not explain the willingness of drug users to voluntarily undergo withdrawal, or that drugs used to treat medical conditions produce tolerance and withdrawal yet they are not addictive, and fails to explain drug relapse (see also Robinson & Berridge, 1993).

The next attempt to explain addiction consisted of a positive reinforcement model. This model focused on addictive drugs as positive reinforcers and stated that humans continue to self administer these drugs because of the pleasure and euphoria produced, not the avoidance of an unpleasant withdrawal syndrome (Stewart, De Wit, & Eikelboom, 1984; Wise & Bozarth, 1987; Wise, 1988). The neural correlate to euphoria was identified as dopamine (DA) release in the nucleus accumbens septi (NAcc) since drugs of abuse and natural reinforcers alike produce this effect (White, 1996). However, when Salamone (1996) reported that DA is released in the NAcc in response to aversive

stimuli as well as pleasurable stimuli, an obvious flaw in the theory of positive reinforcement was revealed (Salamone, 1996).

In 1993 Robinson and Berridge proposed that when a potentially addictive drug activates the mesolimbic DA system, this gives incentive salience to the sensory and environmental stimuli present at the time of drug use. Therefore, when a person experiences these stimuli that were associated with prior drug use it triggers drug craving (i.e., the intense impulse to take the drug). This theory was termed the Incentive-Salience Theory of Addiction and is thought explain the transition from casual drug use to compulsive drug seeking, taking and relapse (Robinson & Berridge, 1993; Robinson & Berridge, 2000; Robinson & Berridge, 2001). The neural correlates of this theory involve a lasting sensitization of the mesolimbic DA system to drugs and/or drug-related stimuli (Schultz, 1997; Schultz, Dayan, & Montague, 1997). The sensitivity of the mesolimbic DA system was appropriately termed neural sensitization (Robinson & Berridge, 1993; Kalivas, Sorg, & Hooks, 1993). A highly sensitized mesolimbic system results in stronger associations made between the drug and the drug-related cues which strengthens the motivational salience of the stimuli and leads to craving and drug relapse. Variability in the ability of the system to recover (e.g., plasticity) from the sensitization process could help to explain why some people use and abuse potentially addictive drugs at some point in their lives, however, not all people become addicts.

The Mesolimbic System

Scientists have developed ways of studying drug abuse using animal models. More specifically, drugs of abuse are administered to animals while the behavioral, neuroanatomical and neurochemical effects are studied in the laboratory. It is well established that when drugs of abuse are administered to animals, these drugs act on a particular pathway within in brain (Rolls, Rolls, Kelly, Shaw, Wood & Dale, 1974; Kelly & Iversen, 1976). This pathway has been termed the reward or mesolimbic pathway. The mesolimbic system is in part comprised of DA neurons with in the ventral tegmental area (VTA) which project to the NAcc, septum, olfactory tubercle, amygdala, and piriform cortex (Dahlstrom et al., 1964). The NAcc consists of two subregions: the core and shell (Zahm & Brog, 1992; Zahm & Heimer, 1993; Zahm, 1999). Neuronal activity in the core of the NAcc is most commonly associated with locomotor activity (Boye, Grant, & Clarke, 2001) whereas increases in DA release in the NAcc shell is associated with salient stimuli and motivation (Kalivas & Duffy, 1995; Pontieri, Tanda, & Di Chiara, 1995). The NAcc receives afferent projections from the basolateral amygdala (Brog, Salyapongse, Deutch, & Zahm, 1993; Wright, Beijer, & Groenewegen, 1996; Zahm & Brog, 1992), the prefrontal cortex (PFC) (Brog, Salyapongse, Deutch & Zahm, 1993; Zahm & Brog, 1992; McGeorge & Faull, 1989), and the hippocampus (Brog, Salyapongse, Deutch & Zahm, 1993; Groenewegen, Vermeulen-Van der Zee, te, & Witter, 1987; Zahm & Brog, 1992). When the mesolimbic system is activated, DA neurons in the VTA depolarize, leading to DA release in the NAcc (Hernandez & Hoebel, 1988).

The mesolimbic system is activated by natural reinforcers (e.g., food, water, and the opportunity to mate) and drugs of abuse (e.g., cocaine, AMPH, methamphetamine, MDMA, nicotine and alcohol) and the effects of these substances are mediated in part by the NAcc (Smith & Schneider, 1988; Fibiger, Nomikos, Pfaus, & Damsma, 1992; Mark, Rada, Pothos, & Hoebel, 1992; Mitchell & Gratton, 1994). This mediation is due to DA release in the NAcc and is terminated either by reuptake mechanisms or enzymatic degradation via monoamine oxidase (MAO) or catechol-O-methyltransferase (COMT) (Dyck, 1978; Axelrod, 1971). The reuptake system whose main component is the presynaptic membrane protein DA-transporter (DAT) functions to decrease DA in the extracellular space (Lishajko, 1969; Hitri, Hurd, Wyatt, & Deutsch, 1994). When DA is in the synapse, it binds to the DAT and is transported back into the presynaptic neuron where it is either repackaged into vesicles or broken down by MAO (Karoum, Chrapusta, Brinjak, Hitri, & Wyatt, 1994). When cocaine is administered it disrupts the system because it has a high affinity for the DAT and competitively binds to the DAT (Pothos & Sulzer, 1998). This prevents the reuptake of DA into the presynaptic neuron, resulting in excess DA in the synapse (Koob, 1992a; Koob, 1992b). The excess DA in the synapse activates GABAergic medium spiny neurons which project to the VTA, Ventral Pallidum (VP) and lateral hypothalamus (LH) (Wise & Bozarth, 1984; Wise & Bozarth, 1985). Together, the activation of the mesolimbic pathway results in appetite suppression, euphoria and hyperactivity.

Natural Reinforcers

A 'reinforcer' is defined as an event which increases the probability of a operant or instrumental response upon which it is contingent (Skinner, 1966). Natural reinforcers such as food, water, and sex act on the reward pathway and the effects are mediated in part by DA in the NAcc (Smith & Schneider, 1988; Fibiger, Nomikos, Pfaus, & Damsma, 1992; Mark, Rada, Pothos, & Hoebel, 1992; Mitchell & Gratton, 1994; Everitt & Wolf, 2002). DA is also released into the NAcc when a male rat is presented with a receptive female, and remains elevated throughout the display of sexual behavior (Pfaus, Damsma, Nomikos, Wenkstern, Blaha, & Phillips, 1990; Pfaus & Phillips, 1991; Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Wenkstern, Pfaus, & Fibiger, 1993; Becker, Rudick, & Jenkins, 2001). In an environment previously paired with sexual activity, male (Mehrara & Baum, 1990) and female (Oldenburger, Everitt, & de Jonge, 1992) rats show a conditioned place preference (CPP) for that environment. Microdialysis studies have shown that natural reinforcers like sucrose (Hajnal & Norgren, 2001; Hajnal, Smith, & Norgren, 2004) and water (Guion & Kirstein, 2001) increase DA in the shell region of the NAcc. Furthermore, licking sucrose solutions increases DA in NAcc in a concentration dependent manner and this sucrose-induced increase in DA is greater than water (Hajnal, Smith, & Norgren, 2004). Laboratory animals can be trained to self-administer drugs of abuse and natural reinforcers which act on the mesolimbic pathway. Male rats will lever-press in order to gain access to a female rat in estrus (Korczynski, Beck, & Bialy, 1989). Animals will lever-press for electrical stimulation directly to the NAcc and for natural reinforcers such as food or water, furthermore, this responding is

rapidly extinguished if a DA antagonist (spiroperidol) is administered (Rolls, Rolls, Kelly, Shaw, Wood & Dale, 1974).

In addition, the anticipation alone of a food reward activates accumbal DA. DA is also released in response to cues which signal the opportunity to respond for sucrose (in animals trained in response-dependent sucrose delivery) (Roitman, Stuber, Phillips, Wightman & Carelli, 2004). Increases in accumbal DA have been observed prior to the self-administration of food when lever-pressing for a food reward (Kiyatkin & Gratton, 1994). In addition, when a neutral stimulus such as a light is paired with a natural reinforcer (food), DA neurons in the VTA increase in firing rate before, and during training (Ljungberg, Apicella, & Schultz, 1992) demonstrating the role of this pathway in motivated behavior.

The Effects of Cocaine

Cocaine is a psychostimulant and its effects include the suppression of hunger and fatigue, and inducing euphoria in humans. In rodents, cocaine decreases food consumption, increases motor activity, stimulates operant behaviors, enhances conditioned responding and produces place preference (Mucha, van der, O'Shaughnessy, & Bucenieks, 1982). The main effect of cocaine is that it increases in the amount of available monoamine transmitters at synapses. Specifically, cocaine increases the amount of DA, serotonin (5-HT) and norepinephrine (NE) at synapses by binding to the presynaptic transporter protein and blocking their reuptake (Ritz, Cone, & Kuhar, 1990). However, DA has been shown to be the critical transmitter involved in the acute reinforcing effects of cocaine (Ettenberg, Pettit, Bloom, & Koob, 1982). Studies using

self-administration have generated evidence implicating the mesolimbic DA system with the acute reinforcing effects of cocaine. Ettenberg and colleagues (1982) demonstrated that administering a low dose of a DA receptor antagonist, injected both locally and systemically blocks cocaine self-administration.

The functions of the NAcc have been studied extensively and reveal it to be more than a mere mediator of reward. Mogenson, Jones & Yim (1980) proposed that the NAcc acts as an interface between motivational and motor processes. The NAcc has also been shown to mediate associative learning (Di Chiara, 1999). Furthermore, Schultz, Dayan & Montague (1997) demonstrated that the NAcc is involved in the prediction of drug reward, a phenomena termed “expectancy” (Schultz, Dayan, & Montague, 1997). As was mentioned earlier, natural reinforcers like food act on the mesolimbic pathway and in fact, even stimuli related to food elicit DA increases within the NAcc (Blackburn, Phillips & Fibiger, 1987; Blackburn, Phillips, Jakubovic & Fibiger, 1989). Increases in accumbal DA have been observed prior to the self-administration of food when lever-pressing for a food reward (Kiyatkin & Gratton, 1994). The effects of drug expectancy have been shown to be a critical motivating force in the self-administration of many drugs of abuse.

An Animal Model: Behavioral Sensitization

The acute administration of psychostimulants produces an increase in locomotor activity and stereotyped behavior, behaviors that are thought to be mediated by the mesolimbic and nigrostriatal DA pathways (Fontana, Post, Weiss & Pert, 1993a; Fontana, Post & Pert, 1993b). Repeated exposure to cocaine results in an enhanced

behavioral response to a subsequent drug challenge (Kalivas & Duffy, 1993; Kalivas, Sorg & Hooks, 1993). This effect, termed behavioral sensitization, has been studied extensively in adult animal models and has been implicated in the process of addiction and drug craving (Stewart & Badiani, 1993; Robinson & Berridge, 1993). Behavioral sensitization can be elicited by direct DA agonists (apomorphine, quinpirole) and markedly by psychostimulants such as cocaine and amphetamine (Stewart & Badiani 1993; Post, Lockfeld, Squillac & Contel, 1981) and has been implicated in the transition from casual drug use to addiction (Robinson & Berridge, 1993). It is thought that the long-lasting nature of behavioral sensitization could be attributed to the persistent enhanced responsiveness of neural inputs to NAcc, such as DAergic neurons from the VTA and glutamatergic (Glu) neurons from the PFC and basolateral amygdala (Pierce & Kalivas, 1997; White & Kalivas, 1998; Vanderschuren & Kalivas, 2000).

A significant aspect of drug abuse involves anticipating a drug reward as a result of prior experience with that drug. Several laboratories have shown that behavioral sensitization is not a phenomena produced by the drug alone, as it is influenced by the environment in which drug administration occurred. This effect, termed context-dependent sensitization enhances cocaine-induced locomotor activity compared to animals where the drug was not paired with an environmental context (Fontana, Post, & Pert, 1993; Brown & Fibiger, 1992). However, the results are mixed with data on DA release in contextually conditioned sensitization. Brown and colleagues (1992) found no difference in DA release between context-dependent and context-independent drug treated groups, whereas Fontana and colleagues (1993) found increases in DA levels as a result of the cocaine-paired environment. It is clear that expectancy is accompanied by

DA increases in the NAcc, however, mixed results suggest that in addition to the mesolimbic DAergic system there may be additional processes underlying conditioned drug sensitization.

Animals sensitized to a particular drug show increases in locomotor activity after the administration of a different drug of the same class. This phenomenon is known as cross-sensitization and has been shown with many drugs of abuse and more recently with natural reinforcers (Avena & Hoebel, 2003a; Avena & Hoebel, 2003b; Pierce & Kalivas, 1995; Kalivas & Weber, 1988; Schenk, Snow & Horger, 1991; Greenberg & Segal, 1985). Animals on a high sugar diet show greater behavioral sensitization when administered AMPH (Avena & Hoebel, 2003a) and cocaine (Gosnell, 2005) than animals on control diet. Furthermore, animals who show behavioral sensitization to AMPH exhibit sugar-induced hyperactivity (AMPH-sugar cross-sensitization) (Avena & Hoebel, 2003b). Evidence for cross-sensitization is particularly interesting concerning the efficacy of natural reinforcers in a person who has used or abused cocaine (Majewska, 1996).

Drug Use Initiated in Adolescence

Most research in the area of drug abuse has been conducted on adult animals; however, the development of drug addiction commonly occurs in adolescence as mentioned earlier. There is substantial risk among these individuals of developing a cocaine addiction and mortality rates associated with its use are high (Gold, Semlitz, Dackis, & Extein, 1985; Gold, Galanter & Stimmel, 1986; Miller, Gold & Millman, 1989). Because human drug use is frequently initiated during adolescence, it is important

to understand the effects of cocaine use during the periadolescent period, with specific attention directed towards the potential changes in reinforcement mechanisms as a consequence of adolescent use. Alterations in responsivity of these mechanisms have significant implications not only for continued addiction into adulthood, but also in the efficacy of a variety of reinforcers, given this common neurochemical pathway (Blackburn et al., 1987; Blackburn et al., 1989; Salamone, 1996).

Adolescence Animal Research

In humans, adolescence is a period of transition that ranges from childhood to adulthood. In rats, adolescence is thought to be encompassed by the time period from postnatal day (PND) 20 to PND 55 (Odell et al., 1976; Ojeda et al., 1986). Some authors have further categorized it into three phases: early (prepubescent animals, PND 21 to 34), mid (periadolescent, PND 34-46) and late adolescence (young adult, PND 46-59) (Tirelli, Laviola, & Adriani, 2003).

Adolescence is a critical time period in which brain maturation and development take place. One importance characteristic of the adolescent brain is the extensive over production and pruning of synapses (Huttenlocher, 1984; Rakic et al., 1994). This over production and pruning includes cholinergic, DAergic, serotonergic, GABAergic and adrenergic receptors (Lidow et al., 1991; Lidow et al., 1992). Additionally, the ontogenetic profile of DA receptors expression has been characterized in the rat striatum. There is a constant increase in DA receptor densities throughout development which peak at PND 28-30 (Murrin et al., 1986; Zeng et al., 1988; Murrin et al., 1990; Rao et al.,

1991). Further evidence shows that striatal DA receptors are over-expressed before the onset of puberty, peak at PND 40 and then decrease to adult levels (Teicher, Andersen, & Hostetter, Jr., 1995; Andersen et al., 2000). It has been found that DAT expression is highest during adolescence in human striatum (Meng et al., 1999). However animal studies revealed that DAT expression in NAcc and striatum increases with age peaking at PND 60 (Tarazi et al., 1998; Coulter et al., 1997).

Studies have shown that adolescent rats are unique in baseline behavior and reactivity to psychostimulants compared to animals of other ages. Adolescent animals were shown to be hyperactive compared to younger or older animals. Specifically, adolescents were more active in an open field, hole-poked more and engaged in more play behavior than younger and older rats (Spear & Brake, 1983). Furthermore, adolescent female rats were more active than males; however, this effect was reduced when animals were not handled post-weaning (Bronstein, Wolkoff, & Levine, 1975). A recent study showed that adolescent rats showed more cocaine-induced locomotor activity compared to adult rats (Catlow & Kirstein, 2005). However, adolescent animals have also been shown to respond less to the locomotor activating effects of acute cocaine than juvenile animals (Spear & Brick, 1979) and appeared unresponsive to amphetamine (AMPH) compared to both younger and older animals (Lanier & Isaacson, 1977). The latter finding was based on five-minutes of amphetamine induced locomotor activity, when such an effect can last hours. Handling rats prior to cocaine administration can affect locomotor activity, in fact, adolescent rats showed significantly greater cocaine-induced locomotor activity compared to adults rats when rats were handled prior to drug administration (Maldonado & Kirstein, 2005). More recently, studies have found that

after repeated cocaine, adolescent rats sensitize to the locomotor effects of cocaine to a lesser degree than juvenile (Snyder, Katovic, & Spear, 1998) and adult rats (Laviola, Wood, Kuhn, Francis, & Spear, 1995). Laviola and colleagues (1995) demonstrated not only that periadolescent rats exhibit cocaine-induced behavioral sensitization, but that their sensitization profile is different from adult animals. More specifically, the adult animals exhibited stereotypy a result of cocaine-induced sensitization and the adolescent animals did not. The authors attribute this effect to adolescent hyposensitivity of the DAergic system. In contrast, adolescent mice show an enhanced AMPH-induced locomotor sensitization profile compared to adults (Adriani, Chiarotti, & Laviola, 1998). Furthermore, adolescent rats show prominent sensitization of AMPH-induced striatal DA release where adults do not (Laviola, Pascucci, & Pieretti, 2001). The behavioral data presented above is mixed, in that both hyper- and hyposensitivity are observed as a result of a DA agonist. It is well known that drugs of abuse have a direct effect on the DAergic system (Koob & Nestler, 1997). It is also known that periadolescent rats have less basal DA in the striatum (Andersen & Gazzara, 1993) and the NAcc (Philpot & Kirstein, 1999). Given the above information it is important to consider the behavioral and biological effects that cocaine has on the adolescent animal and how this potent drug influences the development and efficacy of the mesolimbic system.

Purpose of the Study

The long-term consequences of adolescent drug use in shaping a network primed for addiction is an issue of utmost importance. The use of cocaine during adolescent development likely alters the normal growth of this system and affects the adult

mesolimbic system, however, there is scant literature aimed at finding out if animals are more vulnerable to the adverse effects of drugs during adolescence. Thus, the present study investigated whether cocaine pretreatment in adolescent and adult rats produced differences in cocaine-induced neurochemical cross-sensitization in adulthood. The purpose of this study was to determine the effects of cocaine exposure during adolescence on the adult rat mesolimbic system. More specifically, after a period of cocaine pretreatment and withdrawal, basal DA levels in the NAcc were evaluated. The second goal was to investigate whether cocaine pretreatment in adolescent and adult rats produced differences in DAergic responsivity to a natural reinforcer in both groups when tested in adulthood. In this case sucrose or water was offered during the dialysis procedure and DA levels monitored. It was hypothesized that rats pretreated with cocaine as adults would cross-sensitize to sucrose but that adolescent pretreated rats would show a different profile. The rats pretreated with cocaine as adolescents may in fact be less responsive to the natural reinforcer as a result of the irreversible effects of cocaine administration on the developing brain. The results from these experiments outlined in the materials and methods section provide information pertaining to the long-term effects of cocaine use during adolescent development on the functioning and responsivity of the adult mesolimbic system. The neural alterations induced by cocaine during the adolescent period could cause changes in basal DA within NAcc and drive physiological craving and drug seeking behaviors. This could be an important mechanism that ultimately leads to the re-initiation of drug use.

Objectives and Hypotheses

1. Determining if cocaine pretreatment in adolescent and adult rats produces different basal DA levels in adulthood.

Hypothesis 1: Cocaine exposure during adolescence alters normal development of the mesolimbic system leading to decrease in basal tone of DA.

2. Determining if a natural reinforcer (sucrose) produces an increase in accumbal DA and if this effect is diminished in rats pretreated with cocaine as adolescents.

Hypothesis 2: Sucrose produces an increase in DA release in the NAcc (cross-sensitization) in rats pretreated as adults, however, rats pretreated with cocaine as adolescents show a dulled DA responsivity to sucrose.

Chapter 2: Manuscript

Introduction

Very little is known about how drugs of abuse alter normal brain development and change the efficacy of natural reinforcers in adulthood. Many teenagers experiment with drugs, in fact, a 2004 survey carried out by the NIDA indicated that 21.5%, 39.8% and 51.1% of 8th, 10th and 12th graders respectively have used an illicit drug in their lifetime. These statistics translate to an estimated 16 million out of a possible 33.6 million adolescents in the US (U.S Census Bureau, 2004). Given that drug use frequently occurs during adolescence it is important to understand the long term effects of cocaine use (and other drugs of abuse) during the adolescent period because drug use during adolescence may compromise the circuitry primed for addiction. The present study investigated whether cocaine pretreatment in adolescent and adult rats produced differences in basal tone and cocaine-induced neurochemical cross-sensitization to a natural reinforcer in adulthood.

Cocaine is a psychostimulant which acts by blocking the reuptake of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) in the mesolimbic pathway and elsewhere (Miller et al., 1989). Researchers studying the neurobiological mechanisms of drugs of abuse have shown that the mesolimbic pathway (reward system) is activated by cocaine and other drugs of abuse. The mesolimbic system consists of dopaminergic (DAergic) inputs from the ventral tegmental area (VTA) which projects to several brain regions including the nucleus accumbens septi (NAcc), hippocampus, prefrontal cortex

(PFC), amygdala, septum, olfactory bulb and the bed nucleus of the stria terminalis (Dahlstrom & Fuxe, 1964).

DA is also involved in attention and plays a role in operant or instrumental conditioning. An event that increases the probability of an operant or instrumental response upon which it is contingent is called a reinforcer (Skinner, 1966). Natural reinforcers such as food, water, and the opportunity to mate increase activity of the mesolimbic pathway, and these effects are mediated in part by DA in the NAcc (Smith & Schneider, 1988; Fibiger, Nomikos, Pfaus, & Damsma, 1992; Mark, Rada, Pothos, & Hoebel, 1992; Mitchell & Gratton, 1994; Everitt & Wolf, 2002). DA is also released into the NAcc when a male rat is presented with a receptive female, and remains elevated throughout the display of sexual behavior (Pfaus et al., 1990; Pfaus & Phillips, 1991; Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Wenkstern, Pfaus, & Fibiger, 1993; Becker, Rudick, & Jenkins, 2001). Microdialysis studies have shown that natural reinforcers like sucrose (Hajnal & Norgren, 2001; Hajnal, Smith, & Norgren, 2004) and water (Guion & Kirstein, 2001) increase DA in the shell region of the NAcc. Furthermore, licking sucrose solutions increases DA in NAcc in a concentration dependent manner and this sucrose-induced increase in DA is greater than water (Hajnal, Smith, & Norgren, 2004). Laboratory animals can be trained to self-administer drugs of abuse and natural reinforcers, all increasing activity of the mesolimbic pathway. Animals will lever-press for electrical stimulation directly to the NAcc and for natural reinforcers such as food or water, furthermore, this responding is rapidly extinguished if a DA antagonist (spiroperidol) is administered (Rolls et al., 1974).

In addition, the anticipation of a food reward increases accumbal DA. DA is released in response to cues which signal the opportunity to respond for sucrose in animals trained in response-dependent sucrose delivery (Roitman, Stuber, Phillips, Wightman, & Carelli, 2004). Increases in accumbal DA have been observed prior to the self-administration of food when lever-pressing for a food reward (Kiyatkin & Gratton, 1994). In addition, when a neutral stimulus such as a light is paired with a natural reinforcer (food), DA neurons in the VTA increase in firing rate before, and during training (Roitman, Stuber, Phillips, Wightman, & Carelli, 2004; Ljungberg, Apicella, & Schultz, 1992) demonstrating that this pathway is involved in motivated behaviors.

An animal model based on psychostimulant-induced paranoia and psychosis in humans has been developed. The administration of cocaine (and other psychostimulants) produces an increase in locomotor activity and stereotyped behavior, behaviors that are thought to be mediated by the mesolimbic and nigrostriatal DA pathways (Fontana, Post, Weiss, & Pert, 1993). Repeated exposure to cocaine results in an enhanced behavioral response to a subsequent drug challenge (Kalivas & Duffy, 1989). This effect, termed behavioral sensitization, has been studied extensively in adult animal models and has been implicated in the process of addiction and drug craving (Stewart & Badiani, 1993; Robinson & Berridge, 1993). Behavioral sensitization can be generated by direct DA agonists (e.g., apomorphine, quinpirole) and markedly by psychostimulants such as cocaine and amphetamine (AMPH) (Stewart et al., 1993; Post, Lockfeld, Squillace, & Contel, 1981) and has been implicated in the transition from casual drug use to addiction (Robinson & Berridge, 1993). It is thought that the long-lasting nature of behavioral sensitization could be attributed to the persistent enhanced responsiveness of neural

inputs to NAcc, such as DAergic neurons from the VTA and glutamatergic (Glu) neurons from the PFC and basolateral amygdala (Pierce & Kalivas, 1997; White & Kalivas, 1998; Vanderschuren & Kalivas, 2000).

Animals sensitized to a particular drug show increases in locomotor activity after the administration of a different drug of the same class. This phenomenon is known as cross-sensitization and has been shown with many drugs of abuse and more recently with natural reinforcers (Avena & Hoebel, 2003a; Avena & Hoebel, 2003b; Pierce & Kalivas, 1995; Kalivas & Weber, 1988; Schenk, Snow, & Horger, 1991; Greenberg & Segal, 1985). Animals on a high sugar diet show greater behavioral sensitization when administered AMPH (Avena et al., 2003a) and cocaine (Gosnell, 2005) than animals on control diet (e.g., typical rat chow). Furthermore, animals who show behavioral sensitization to AMPH exhibit sugar-induced hyperactivity (AMPH-sugar cross-sensitization) (Avena et al., 2003b). Evidence for cross-sensitization is particularly interesting concerning the efficacy of natural reinforcers in a person who has used or abused cocaine (Majewska, 1996).

To date, most studies investigating the effects of psychostimulants on the mesolimbic system have been undertaken using adult rats. However, the initiation of drug use happens most frequently during adolescence, a time period in which brain maturation and development take place (for review see Spear, 2000). In humans, adolescence is a period of transition that ranges from childhood to adulthood. In rats, adolescence is thought to be encompassed by the time period from approximately postnatal day (PND) 20 to PND 55 (Odell & Swerdloff, 1976; Ojeda, Urbanski, & Ahmed, 1986). Some authors have further categorized it into three phases: early

(prepubescent animals, PND 21 to 34), mid (periadolescent, PND 34-46) and late adolescence (young adult, PND 46-59) (Tirelli, Laviola, & Adriani, 2003).

During the adolescent period neuronal circuits continue to change and develop. One important characteristic of the adolescent brain is the extensive overproduction and pruning of synapses (Huttenlocher, 1984; Rakic, Bourgeois, & Goldman-Rakic, 1994). This overproduction and pruning includes cholinergic, DAergic, serotonergic, GABAergic and adrenergic receptors (Lidow, Goldman-Rakic, & Rakic, 1991; Lidow & Rakic, 1992). Additionally, the ontogenetic profile of DA receptor expression has been characterized in the rat striatum. There is a constant increase in DA receptor densities throughout development which peak at about PND 28-30 (Murrin & Zeng, 1986; Zeng, Hyttel, & Murrin, 1988; Murrin & Zeng, 1990; Rao, Molinoff, & Joyce, 1991). Further evidence shows that striatal DA receptors are overexpressed before the onset of puberty, peak at around PND 40 and then decrease to adult levels (Teicher, Andersen, & Hostetter, Jr., 1995; Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000). It has been found that DA transporter (DAT) expression is highest during adolescence in human striatum (Meng, Ozawa, Itoh, & Takashima, 1999). However, animal studies revealed that DAT expression in NAcc and striatum increases with age peaking at PND 60 (Tarazi, Tomasini, & Baldessarini, 1998; Coulter, Happe, & Murrin, 1997). From the evidence provided above it is clear that developmental changes occur during the adolescent period, however, it is presently unknown how drugs of abuse alter this preprogrammed development.

Investigating both the short and long-term effects of adolescent drug use is an issue of utmost importance because the use of cocaine during adolescence may alter the

mesolimbic pathway. There is scant literature aimed at finding out if animals are more vulnerable to the adverse effects of drugs during adolescence. Therefore, the present study investigated whether cocaine pretreatment in adolescent and adult rats produced differences in basal tone and responsivity of the DAergic system to a natural reinforcer (i.e., sucrose) in adulthood. The rats pretreated with cocaine as adolescents may in fact be less responsive to the natural reinforcer as a result of the lasting effects of cocaine administration on the developing brain.

Materials and Methods

Subjects

Subjects consisted of Sprague-Dawley rats derived from established breeding pairs in the laboratory at the University of South Florida (Tampa, FL). Date of birth was designated as postnatal day (PND) 0. Litters contained 8-10 pups after culling. No more than one pup per litter was placed in a given treatment condition. The pups remained with the dam and littermates until weaning. Animals were weaned on PND 21 and housed with their same sex littermates with free access to food and water and maintained in a temperature and humidity controlled room on a 12-hour light/dark cycle with lights on at 7:00 A.M. Animals were pretreated at one of two ages: adolescent (PND 35-44) or adult (PND 70-79), withdrawn, then challenged as adults (PND 65 and PND 100). All National Institutes for Health (NIH) guidelines for the Care and Use of Laboratory Animals were followed (National Institutes of Health, 1986).

Procedure

Animals were handled for 3 days prior to pretreatment for 5 minutes per day. Drug pretreatment consisted of the administration of 20 mg/kg cocaine or saline (i.p.) once daily for a period of 10 days from PND 35-44 and PND 70-79. Following drug exposure animals were drug free for a period of 21 days from the ages: PND 45-65 and PND 80-100. On PND 58 or 93 (during withdrawal) animals had a guide cannula surgically implanted and were allowed 1 week to recover before microdialysis on either PND 65 or PND 100.

In order to train the rats to drink during the microdialysis procedure, four days before microdialysis, rats were put on a limited access water schedule described as follows. At 1000 hours the rats were transported to the laboratory, and placed in the Ratern apparatus (Bioanalytical Systems, West Lafayette, Indiana) where they were able to access water for a period of 20 minutes. All Ratern bowls had a cotton ball attached to the top, immediately before the presentation of drink, 0.5 ml banana odor was placed on the cotton ball that was taped to the top of the Ratern bowl. After the conditioning to drink in the apparatus, the rats were placed back in their home cages and returned to the colony room. Animals had no access to water in their home cages.

In Vivo microdialysis was performed 20 days after the last injection. All animals were adults (PND 65 or 100) on microdialysis testing day. This held the period of withdrawal constant across ages.

Behavior

Locomotor Activity was recorded on days 1, 5 and 10 of the pretreatment period. On these days rats were injected and placed in the locomotor activity apparatus for a total of 45 minutes. After a 15-minute habituation period, subjects received a single i.p. injection of 20 mg/kg cocaine or saline and were monitored for 30 minutes post-injection. On all other days rats were injected in their home cages and then returned to the colony room.

Apparatus

The locomotor activity apparatus consisted of a circular table with a black Plexiglas surface on which a white Plexiglas circular barrier (diameter = 101 cm, height = 45.72 cm) was placed. A camera connected to analysis software located above the apparatus recorded locomotor activity. The apparatus was located in a room away from the animal colony and the door remained closed during all testing sessions. Activity was recorded and analyzed using Ethovision Video Tracking System created by Noldus. This software tracked and recorded the Total Distance Moved (TDM) (cm) of each animal during the testing session.

For the conditioning phase and the microdialysis procedure, animals were placed in a Ratum System (see above). The Ratum System consists of a large plastic round bottom bowl (14" x 16"). For the conditioning phase a cotton ball with banana odor was attached to the top of the Ratum system. For the microdialysis procedure, the animal was placed inside the apparatus the night before the microdialysis experiment, and microdialysis probe was inserted. Rats were then able to habituate to the environment overnight.

Surgical Procedures and In Vivo Microdialysis

Animals were anesthetized on either PND 58 or 93 using a ketamine/xylazine cocktail (1.0 and 0.15 mg/kg/ip). An incision was made over the skull and the rat was mounted on the stereotaxic instrument for surgery. Four holes were drilled in the skull (three for skull screws and one for the guide cannula). A 21-gauge stainless-steel guide cannula (Plastics One) was inserted aimed at the NAcc shell (Anterior/Posterior: +1.2

mm, Lateral: 0.8 mm, and Ventral: 2.6 mm relative to bregma and the surface of the level skull) and affixed to the skull with cranioplast. Animals were returned to their homecage for a 1 week recovery period (during which conditioning occurred). The day before dialysis, the probe was inserted through the guide cannula and protruded 4 mm from the guide cannula shaft to reach the NAcc shell. Rats were then placed in a BAS Return system bowl overnight for habituation. Inlet tubing was attached to a 2.5 ml Hamilton syringe mounted on a WPI syringe pump (sp3201w) set to a flow rate of 0.1 μ l/min overnight. *In vivo* microdialysis probes with 2 mm membrane tips (o.d. 512; MW cutoff 13 kDA) were perfused continuously with artificial cerebrospinal fluid (136 mM NaCl, 3.7 mM KCl, 1.2 mM CaCl₂, 1.0 mM MgCl₂, 10.0 mM NaHCO₃ at pH=7.4) for twelve hours prior to the start of sampling. On either PND 65 or 100, dialysates were collected at a flow rate of 1.0 μ l/min in ten-minute intervals from the probe outlet silica into tubes containing 2.0 μ l of 0.1 M hydrochloric acid (HCl) and immediately frozen. Following four baseline samples, animals were allowed access to either water or 0.3 M sucrose solution and sampling continued for an additional 120 minutes. Dialysate samples (12 μ l) were stored at -80° C until analyzed.

Neurochemical Analysis

Analysis of dialysate samples was performed with a reverse phase high performance liquid chromatography system (BAS) coupled to an electrochemical detector (HPLC-EC) set to oxidize catecholamines (650 mV). An amperometric detector with a LC-4C carbon working electrode referenced to an Ag/AgCl electrode was used. Neurochemical analyses include the detection of DA. The mobile phase consisted of

0.04 M sodium acetate, 0.01 M citric acid, 0.05 mM sodium octyl sulfate, 20.911 M disodium EDTA, 0.013 M NaCl and 10% v/v methanol (pH 4.5) set at a flow rate of 50 μ l/min. Samples (6 μ l) were injected onto a C-18 microbore column for peak separation. Data were recorded and quantified by Rainin Dynamax Software on a Power Macintosh 8500/120.

Histology

Following probe removal, rats were euthanized via CO₂ inhalation. Brains were removed and frozen in 2-methylbutane (-40° C) and stored at -80° C. Brains were sliced into 40 μ m sections, mounted on slides and stained with cresyl violet. Probe placements were verified histologically for placement in the NAcc shell.

Design and Analysis

DA levels were obtained after collecting Dialysate for a time period of 160 minutes. Baseline (BL) samples were collected during the first 40 minutes of microdialysis. The subsequent samples (2 hours) were all taken after the presentation of the natural reinforcer. In order to assess the increase in DA levels after the presentation of the natural reinforcer, DA levels were converted to Area Under the Curve (AUC) using Prism software.

Separate analyses were performed with total distance moved (TDM), stereotypy, baseline (BL) DA, AUC DA, DA % baseline, body weight and liquid consumption as dependant measures. Locomotor activity was measured using TDM and stereotypy as dependent measures. Both TDM and Stereotypy were analyzed using a 2 (Age: PND 35,

60) X 2 (Drug: saline, cocaine) ANOVA with day (1,5,10) as the repeated measure. BL DA levels were analyzed with a 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) ANOVA. Both DA AUC levels and liquid consumption (ml) were analyzed with a 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) X 2 (Natural reinforcer: water, sucrose) ANOVA. DA % baseline was analyzed using a 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) X 2 (Natural Reinforcer: Water, 0.3M Sucrose) ANOVA with time (0-160 minutes) as a repeated measure. Body weight (g) was measured each day during limited access water and analyzed using a 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) ANOVA with day (4) as a repeated measure. Subsequent analyses were performed to isolate simple effects with appropriate post-hoc analyses. A 5% level of significance was set.

Results

Locomotor Activity

For the analysis of TDM a 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) ANOVA with day (1,5,10) as the repeated measure revealed a main effect of drug [$F(1,28)=49.6$, $p<0.05$], with cocaine treated rats engaging in significantly more TDM than saline injected controls. There was also a main effect of Day [$F(2,56)=4.7$, $p<0.05$], which showed that rats engaged in significantly more TDM on day 1 than days 5 and 10. In addition, there was a significant interaction between Day and Drug [$F(2,56)=4.6$, $p<0.05$], which revealed that cocaine treated rats had significantly higher TDM on day 1 than on days 5, 10 and compared to saline injected rats on all days (see Figure 1A).

A 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) ANOVA with day (1,5,10) as the repeated measure was used to analyze the dependent measure stereotypy. There were significant main effects of Age [$F(1,28)=6.5$, $p<0.05$], Drug [$F(1,28)=373.5$, $p<0.05$] and Day [$F(2,56)=14.4$, $p<0.05$]. However there was also a significant interaction between Day and Drug [$F(2,56)=14.3$, $p<0.05$] indicating stereotypy scores from rats injected with cocaine significantly increased from day 1 to day 5 to day 10, suggesting that behavioral sensitization occurred in rats administered cocaine (see Figure 1B).

DA levels: Area Under the Curve

A 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) ANOVA on baseline DA revealed a significant Age by Drug interaction [$F(1,28)=4.4$, $p<0.05$]. As can be seen in figure 2 rats pretreated with cocaine during adulthood had significantly lower baseline

DA levels than saline controls and adolescent cocaine experienced rats. No differences in BL DA were detected between rats pretreated with saline or cocaine during adolescence.

A 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) X 2 (Natural reinforcer: water, sucrose) ANOVA on AUC DA revealed a significant interaction between Age and Drug [$F(1,24)=4.9, p<0.05$], showing that adolescent rats pretreated with 20 mg/kg of cocaine have significantly higher DA levels in response to a natural reinforcer than those pretreated with saline during either adolescence or adulthood and those pretreated with cocaine as adults ($p<0.05$) (see Figure 3A/B insert). In addition, there was a significant interaction between Age and Natural reinforcer [$F(1,24)=4.3, p<0.05$]. Further analysis by the Fisher's LSD test revealed that rats pretreated during the adolescent period have significantly higher DA levels after drinking sucrose solution compared to rats pretreated during adulthood ($p<0.05$).

DA levels: Percent change from Baseline

A 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) X 2 (natural reinforcer: water, 0.3M sucrose) ANOVA with time (0-160 minutes) as a repeated measure revealed a significant interaction between Age and Natural reinforcer [$F(1,24)=4.8, p<0.05$] with adolescent pretreated rats having significantly higher DA levels in response to sucrose (M=150%) than all other groups. In addition, there was significant interaction of Age, Drug, Natural reinforcer and Time [$F(15,360)=1.8, p<0.05$]. As can be seen in figure 4 drinking water or sucrose resulted in a significant increase from baseline in all rats pretreated as adolescents or adults with saline or cocaine ($p<0.05$). Interestingly, rats

pretreated with cocaine as adolescents had significantly higher DA release as a result of drinking sucrose compared to all other groups ($p<0.05$).

Liquid Consumption

A 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) X 2 (Natural reinforcer: water, sucrose) ANOVA on liquid consumption (ml) revealed a significant main effect of natural reinforcer [$F(1,24)=145.9$, $p<0.05$]. Independent of age or drug, rats consumed significantly more water (Mean = 12.1 ml) than sucrose solution (Mean = 3.6 ml) ($p<0.05$). As shown in Figure 5, there were no differences in the amount of water, or sucrose consumed based on drug pretreatment or age of pretreatment.

Body Weight

A 2 (Age: PND 35, 60) X 2 (Drug: saline, cocaine) ANOVA with day of limited access water treatment as a repeated measure revealed a significant main effect of Age [$F(1,28)=191.4$, $p<0.05$] with the adult pretreated rats weighing significantly more (M=387.1g) than the adolescent pretreated rats (M=294g) ($p<0.05$). In addition, a main effect of day of limited access water treatment was present with body weight decreasing from day 1 (M=346.1g), to day 4 (M=337.9g) (2.4%) ($p<0.05$). There was a significant interaction between Day limited access water treatment, Age and Drug [$F(3,84)=4.5$, $p<0.05$] with cocaine pretreated adults weighing significantly more than saline pretreated adults by day 4 ($p<0.05$) (see Figure 6).

Discussion

The neuroplasticity involved in response to the administration of drugs of abuse has been a big concern for scientists for decades. One real issue that we are now confronting is that these studies have been done in adult animals without examining the long term effects that drugs of abuse impose on neural development. In society, a large percentage of adolescents initiate drug use, impacting their brains resulting in changes which have unrevealed long term effects. The present study shows that those changes are extended to the point where they alter the response of the reward system to a naturally reinforcing stimulus (as oppose to the drug).

The present study demonstrates that basal DA levels in the shell region of the NAcc are affected differentially as a function of age by repeated cocaine use. Specifically, rats pretreated with cocaine as adults had lower baseline DA levels than saline pretreated rats. This finding is consistent with other studies in the adult literature (Parsons, Smith & Justice, 1991; Segal & Kuczenski, 1992; Maisonneuve, Ho & Kreek, 1995). It has been postulated that a decrease in tyrosine hydroxylase levels within the NAcc leads to decreased basal DA levels, and this results in a postsynaptic downregulation of the DAergic system (Parsons, Smith & Justice 1991; Segal & Kuczenski, 1992; Maisonneuve, Ho & Kreek, 1995). It is interesting that rats pretreated during adolescence with the same dose of cocaine for the same length of time did not have a reduction in basal levels. This finding suggests that rats which are treated with cocaine during adolescence are able to recover from the effect of cocaine, demonstrating neuronal plasticity.

In all conditions there was a significant increase in DA in the NAcc after drinking sucrose solution or water. These results are consistent with other studies which have shown that sucrose (Hajnal, Smith, & Norgren, 2004) and water (Guion & Kirstein, 2001) both increase DA in the shell region of the NAcc. Therefore, the finding that DA levels increased in response to both water and sucrose in saline pretreated rats was anticipated. Based on previous findings that adult animals on a high sugar diet showed greater behavioral sensitization when administered AMPH (Avena et al., 2003a) and cocaine (Gosnell, 2005) than animals on a control diet. It was expected that the cocaine pretreated adults would show a cross-sensitized response and thus have higher DA levels in response to sucrose than saline pretreated rats, however, this was not the case. DA levels did significantly increase in response to sucrose in the cocaine pretreated adults but this increase was not different from saline pretreated adults, thus there was no enhanced sensitivity of the DAergic system to sucrose. This is not surprising because human studies have shown that cocaine addicts have a diminished response to stimuli that activate the mesolimbic system in control subjects (Majewska, 1996).

Interestingly, sucrose intake significantly enhanced DA levels in cocaine pretreated adolescent rats. Other reports have also found that the effects of cocaine during adolescence are long lasting. For example, Brandon and colleagues administered 15 mg/kg of cocaine for 5 days and found that adolescent rats which were pretreated with cocaine showed persistent behavioral sensitization 2 months after cocaine cessation (Brandon, Marinelli, Baker & White, 2001). In addition, the administration of a high dose methylphenidate during adolescence, which like cocaine blocks the DAT (Kuczenski & Segal, 1997), caused cross-sensitization with cocaine (Brandon, Marinelli,

Baker & White, 2001). Adolescent exposure to methylphenidate alters DAergic neurons in the VTA, an effect that is dependent on the length of withdrawal. In early withdrawal from methylphenidate, there was an increase in the excitability of VTA neurons, whereas in late withdrawal when the rats were young adults there was a decrease in DAergic activity (Brandon, Marinelli & White, 2003). These data suggest that cocaine use or a non-clinical high dose of methylphenidate during adolescence has long lasting effects on the reward system. Furthermore, the present findings demonstrate that this long lasting sensitivity of the mesolimbic system after cocaine use during adolescence extends to palatable foods. This effect seems likely to be driven by the plastic nature of the developing adolescent brain.

During adolescence, brain maturation and development take place and are characterized by extensive overproduction and subsequent pruning of synapses (Huttenlocher, 1984; Rakic et al., 1994). Striatal DA receptor densities peak at PND 40 then decrease to adult levels (Teicher, Andersen, & Hostetter, Jr., 1995; Andersen et al., 2000). DAT expression in NAcc and striatum increases with age, peaking at PND 60 (Tarazi et al., 1998; Coulter et al., 1997). Due to increasing numbers of DATs during adolescence, higher reuptake decreases basal DA in striatum (Andersen & Gazzara, 1993) and NAcc (Philpot & Kirstein, 1999) and upregulates cyclic-AMP signaling (Andersen, 2002). The profile of the adolescent mesolimbic system, with lower basal DA levels, increased DA receptors and second messenger systems, could be overstimulated in the presence of cocaine. Persistent postsynaptic stimulation hyperpolarizes GABAergic neurons and allows for more release of DA into the extracellular space which could explain cocaine-induced hyperactivity observed in adolescent rats (Catlow & Kirstein,

2005; Maldonado & Kirstein, 2005). During adolescence, the PFC sends out glutamatergic projections to VTA and limbic areas (for Review see Lewis, 1997). It is plausible that repeated cocaine during adolescence overstimulates the mesolimbic system causing more dendritic arborizations of glutamatergic afferents to the VTA. The plasticity-induced change during this time in development, in combination with repeated cocaine, may not have been down-regulated as a result of drug withdrawal and instead remained robust, as there was no effect of repeated cocaine during adolescence on adult basal DA levels. This might explain why there is a stronger response to sucrose in the adolescents pretreated with cocaine. There could be more glutamatergic connections from the PFC to activate the VTA and stimulate the release of DA in the NAcc. Repeated cocaine during adolescence could result in a circuitry primed for vulnerability to addiction.

The DA response exhibited by cocaine pretreated adults can be explained by the theory of neural sensitization. Repeated cocaine results in plastic changes including reduction of D2-R on Gabaergic efferents (Bowers, McFarland, Lake, Peterson, Lapish, Gregory, Lanier & Kalivas, 2004) and arborizations of the PFC afferents (Carlezon & Nestler, 2002). During cocaine abstinence a downregulation of messages in the mesolimbic system occurs, resulting in lower basal DA, as is seen in the adult cocaine pretreated condition presented in this study. When the system is artificially stimulated by cocaine, DA levels in the NAcc are elevated due to less inhibitory feedback on the VTA from a reduction in D2-R on Gabaergic neurons and the amplification and activation of silent synapses of PFC inputs to VTA, causing sustained stimulation of DA in the NAcc (Carlezon & Nestler, 2002; Bowers, McFarland, Lake, Peterson, Lapish, Gregory, Lanier

& Kalivas, 2004). This is not the case with sucrose as the effects of sucrose on the reward system are more subtle and activate the mesolimbic system indirectly. The activation of the mesolimbic DA system in response to naturally rewarding stimuli provides incentive salience information to the neural correlates of reward-related stimuli and drives wanting or craving (Koob, 1992c; Berridge & Robinson, 1998).

In the present study, the amount of natural reinforcer consumed varied but was unaffected by drug or age of pretreatment. Results from this study show that rats consumed more water than sucrose. It is possible that rats consumed less sucrose due to neophobia and the expectation of drinking water. It is also possible that, because sucrose is a palatable food, the consumption of sucrose increases activity in the mesolimbic system from the hypothalamus therefore increasing the inhibition on the mesolimbic system, triggering the signal to stop drinking more rapidly (Puig de Parada, Paez, Parada, Hernandez, Molina, Murzi & Contreras, 1997). The NAcc has direct connections to the lateral hypothalamus (Heimer, Zahm, Churchill, Kalivas & C. Wohltmann, 1991; Kirouac & Ganguly, 1995; Zahm & Brog, 1992). Since DA levels were relatively similar it appears that rats drank an amount of liquid until the appropriate amount of inhibition on the DAergic system was received.

Neuronal developmental is an important factor to consider when investigating the long term effects of drug abuse. Brain circuits involved with emotion, judgment, and inhibitory control develop during the adolescent period which could increase the risk for substance abuse during development (Chambers, Taylor & Potenza, 2004). It is well known that drugs of abuse alter neurotransmitter systems and that the development and fine tuning of these systems occur during adolescence. The results from this experiment

clearly show that in rats pretreated with cocaine during adolescence there is an enhance response of the DAergic system in response to a naturally reinforcing substance therefore; cocaine exposure during adolescence results in persistent long term changes in the mesolimbic pathway. Future studies need to ascertain the underlying mechanisms and their role in the process of addiction.

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Appendicies

Appendix A

Figure 1A

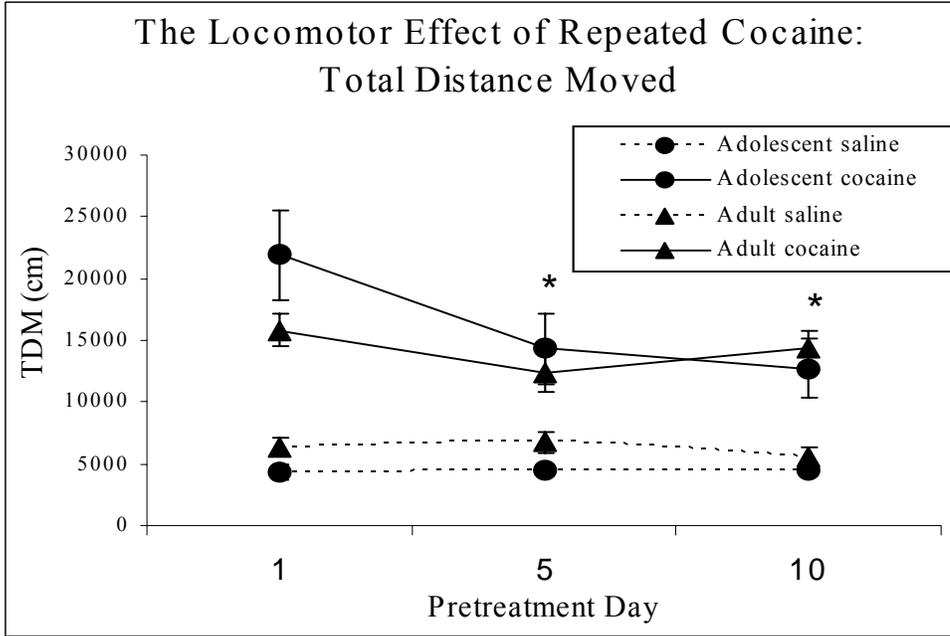
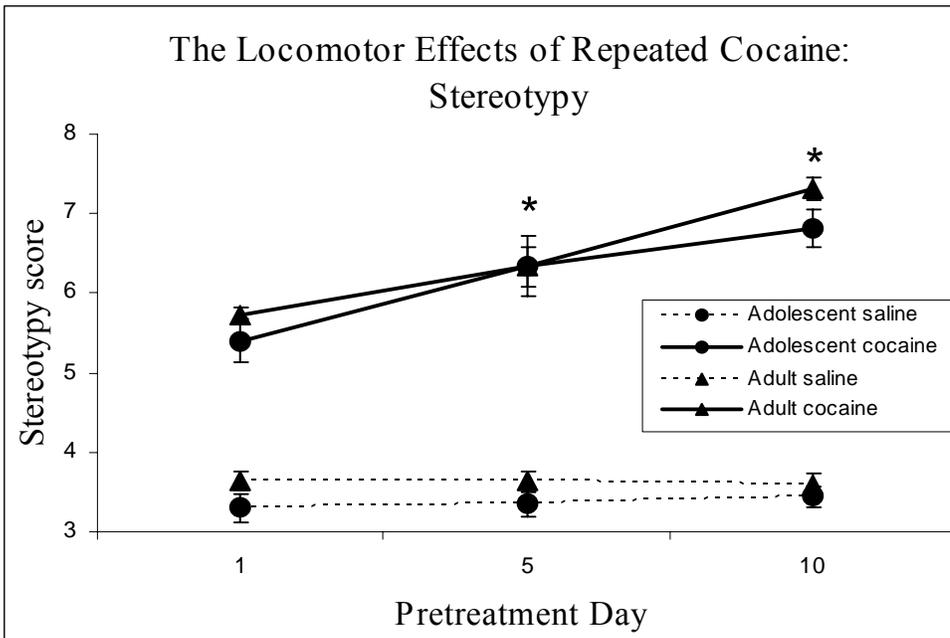
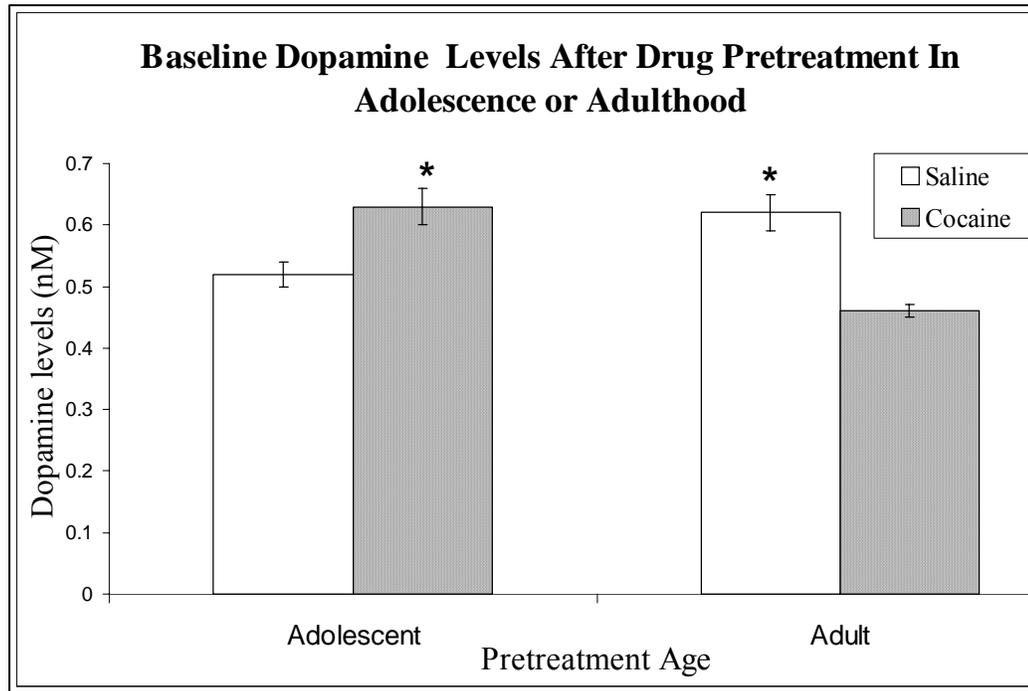


Figure 1B



Appendix B

Figure 2



Appendix C

Figure 3A

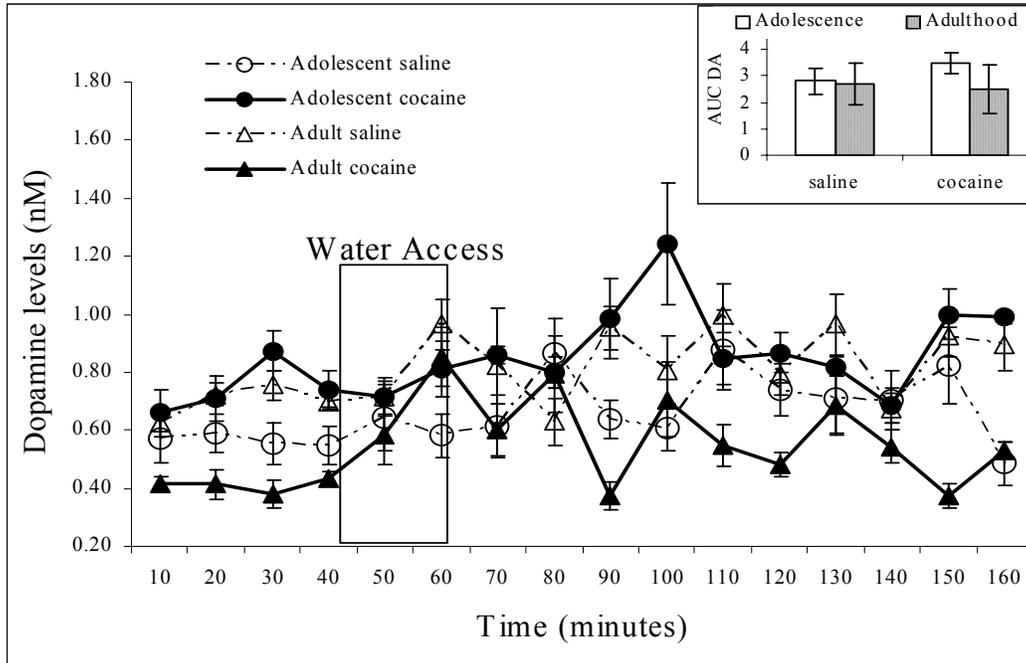
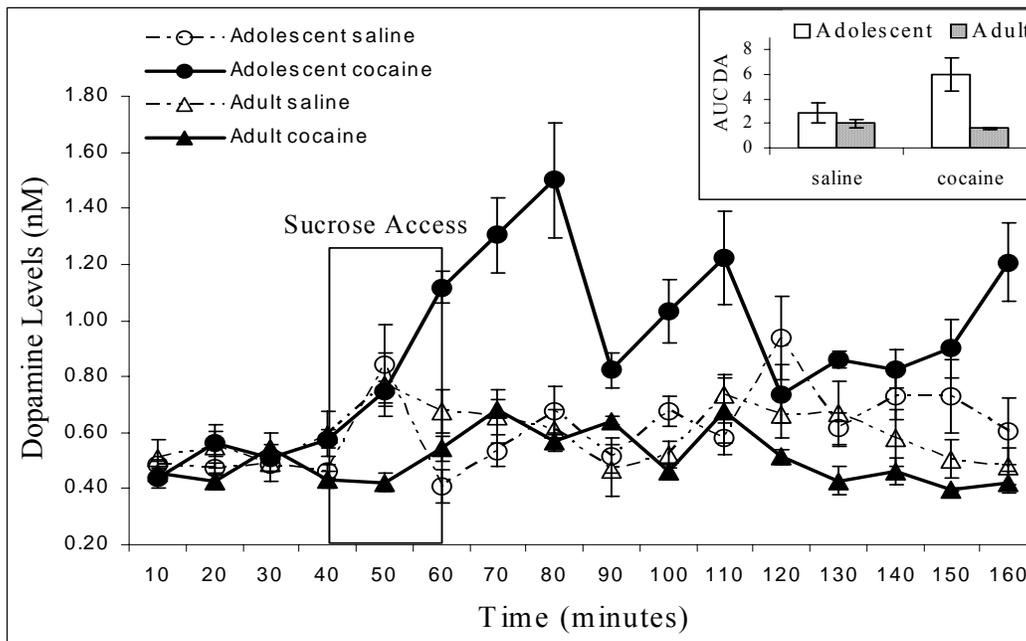


Figure 3B



Appendix D

Figure 4A

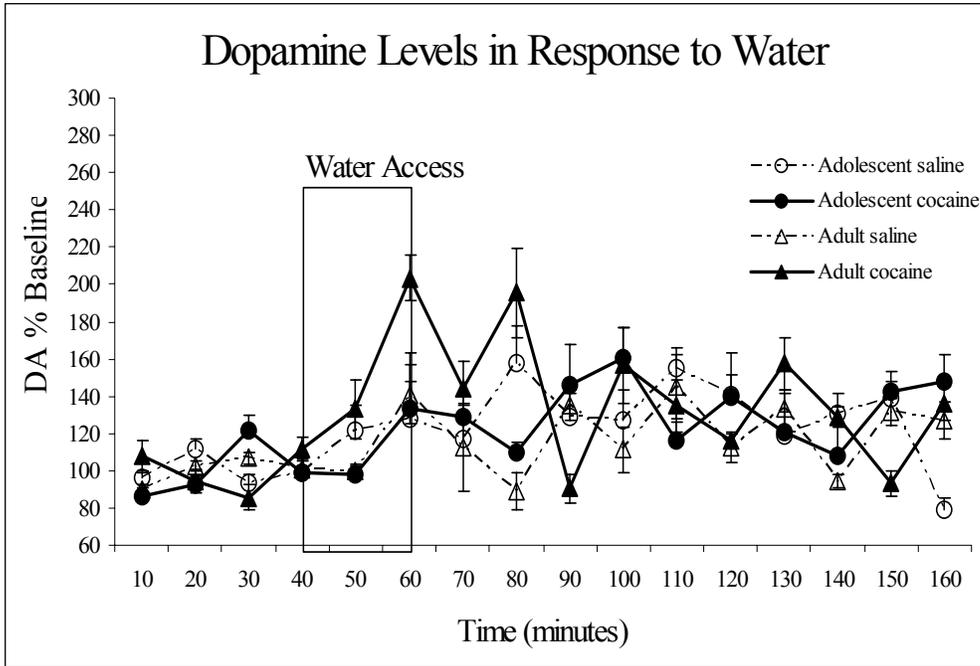


Figure 4B

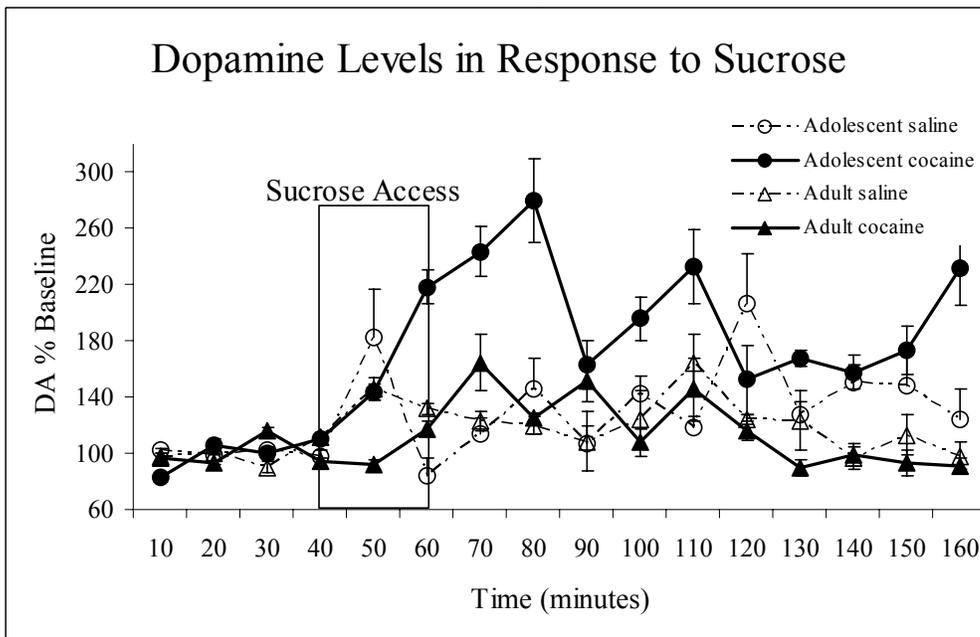
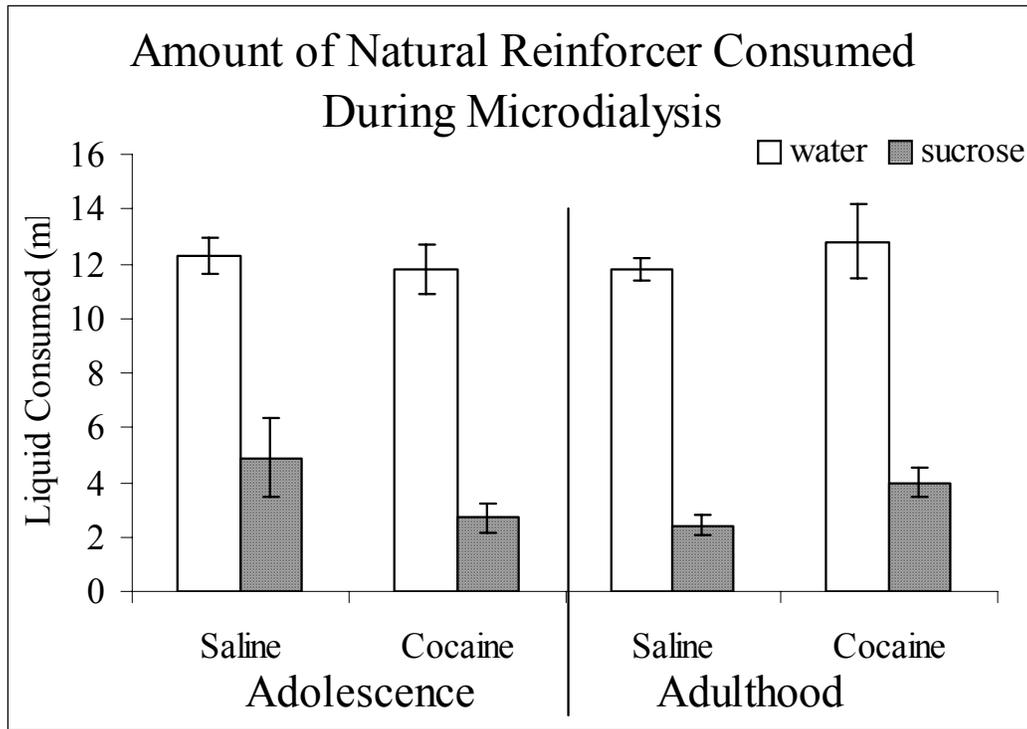
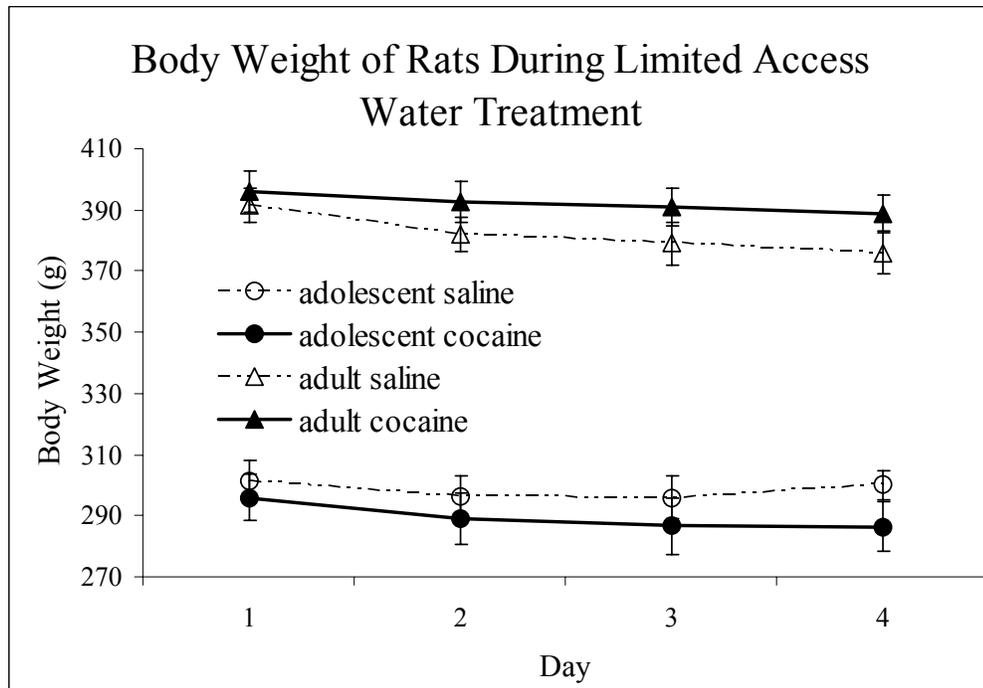


Figure 5



Appendix F

Figure 6



Appendix G

Figure Legends

Figure 1 illustrates the locomotor activity of rats during the pretreatment period. Rats received 20mg/kg of cocaine or saline for 10 days from ages PND 35-44 or PND 70-79. Locomotor activity was recorded on day 1, day 5 and day 10. A) Shows that the total distance moved (cm) in cocaine treated rats was significantly higher on day 1 than on days 5 and 10. B) Average stereotypy scores from rats injected with cocaine significantly increased from day 1 to day 5 to day 10, suggesting that behavioral sensitization occurred in rats administered cocaine.

* indicates significant difference from cocaine pretreated rats on day 1.

Figure 2 illustrates baseline DA (nM) levels after drug pretreatment during either adolescence (PND 35-44) or adulthood (PND 70-79) with either saline or 20mg/kg of cocaine. Rats pretreated with cocaine during adulthood had significantly lower baseline DA levels than saline controls. In addition, cocaine pretreated adults had significantly lower baseline DA levels than rats pretreated with cocaine during adolescence.

* indicates significant difference from adult cocaine pretreated rats.

Note: 8 rats were used in each condition.

Figure 3 shows DA levels [nM] in response to a natural reinforcer. A) DA levels in response to water and B) DA levels in response to 0.3 M sucrose. Insert graphs show DA levels (normalized by AUC analysis) during the presentation of water (A) or 0.3 M

Sucrose (B). Adolescent rats pretreated with 20 mg/kg of cocaine have significantly higher DA levels in response to a natural reinforcer than those pretreated with saline during either adolescence or adulthood and those pretreated with cocaine as adults ($p<0.05$). Furthermore rats pretreated during the adolescent period have significantly higher DA levels after drinking sucrose solution compared to rats pretreated during adulthood ($p<0.05$). Note: 4 rats were used in each condition.

Figure 4 shows DA levels in response to a natural reinforcer presented by percent change from baseline. A) illustrates the B) demonstrates that rats pretreated with cocaine during adolescence have a significant increase in DA compared to all other conditions ($p<0.05$).

Figure 5 illustrates the amount of liquid that was consumed by rats for 20 minutes during microdialysis. Rats consumed significantly more water (Mean = 12.1 ml) than sucrose solution (Mean = 3.6 ml) ($p<0.05$). There were no differences in the amount of water, or sucrose consumed based on drug pretreatment or age of pretreatment.

Figure 6 shows the body weight (g) of rats over the four days of limited access water. No differences in body weight were detected as a result of drug pretreatment and rats pretreated as adults weighed significantly more (M=387.1g) than the adolescent pretreated rats (M=294g) ($p<0.05$).