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Neurochemical Analysis Of Cocaine In Adolescence And Adulthood

By

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A thesis submitted in partial fullfillment
of the requirements for the degree of
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Neurochemical Analysis Of Cocaine In Adolescence And Adulthood

Kirstie H. Stansfield

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 dependency in adulthood. Human adolescents are predisposed toward an increased likelihood of risk taking behaviors (Zuckerman M, 1986), including drug use or initiation. The purpose of this study was to examine differences in developmental risk taking behaviors and neurochemical responsivity to cocaine based on these behavioral characteristics. Adolescent and adult animals were exposed to a novel stimulus in a familiar environment to assess impulsivity, novelty preference and exploratory behaviors, subsequently, *in vivo* microdialysis was performed to assess dopaminergic responsivity to cocaine. Adolescent animals had greater novelty-induced locomotor activity, greater novelty preference, were more impulsive and showed higher exploratory behaviors compared to adult animals.

Furthermore, the results demonstrate neurochemical differences between adolescent and adult animals in novel environment exploratory behavior, novel object preference, novelty-induced impulsivity and novelty-induced exploration. These data support the notion that adolescents may be predisposed toward sensation seeking and consequently are more likely to engage in risk taking behaviors, such as drug use initiation.

Chapter One

Introduction

Adolescence is a time of high risk behavior and increased exploration. It is a period when the brain is undergoing many complex changes that can exert long-term influences on cognitive processes. Adolescence is marked by a greater probability to initiate drug use and initiation during this time is associated with an increased risk to develop addiction and dependency in adulthood. Specifically, Estroff (Estroff TW, Schwartz RH & Hoffmann NG, 1989) has reported that most illicit drug use begins at approximately age 12, with peak periods of initiation between ages 15 and 19. 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, 2000). During the 1990's, there was a steady rise in the frequency of drug use in teenagers, by 2001, 4.3% of eighth graders, 5.7% of tenth graders, and 8.2% of high school seniors, reported a long-term use of cocaine (Johnston LD, 2000). 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 TW, Schwartz RH & Hoffmann NG, 1989).

Generally, adults who initiated 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, Kirisci L Tarter RE, 1998). Development of the central nervous system (CNS) during adolescence may play a key role in the increased likelihood to initiate drug use. Moreover, disruption of the development of the CNS may result in subsequent long term increases in the probability of drug use and dependence. During adolescence, critical structures involved in substance abuse are regulated primarily by the limbic system which is associated with emotional and impulsive behaviors. However, adolescence is a period of transition from a more emotional regulation of critical structures that mediate substance abuse to a more mature cortical regulatory mechanism (Spear LP, 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. However by adulthood, these previously amygdala modulated regulatory actions are replaced by those projecting from the medial prefrontal cortex (mPFC) indicating some developmental transition in the functional nature of the system.

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 initiating drug use. Chronic administration of drugs

(e.g. cocaine) during this period may cause a functional change in accumbal DA efflux by altering amygdalar modulation of accumbal DA release and/or altering the functional role of the mPFC input; consequently, leading to an increased risk of dependency during adulthood. Together, these implications make a powerful argument for treating adolescence as a key time period for researching 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 is that early in the process, drug use is 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 will 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 (Koob GF & Le Moal M, 1997; Koob GF & Le Moal M, 2001; Koob GF, Caine SB Parsons L Markou A & Weiss F, 1997; Solomon RL, 1977). 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 prolonged 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 TE & Berridge KC, 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 animals (Schultz W, Dayan P & Montague PR, 1997a) and humans (Knutson B, Adams CM Fong GW & Hommer D, 2001), sometimes even better than the reward itself (Schultz W, 1998). 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 TM, 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 NAcc neurons that were selectively activated by cocaine-associated stimuli but not water-associated stimuli (Carelli RM, Ijames SG, 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 us 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 TE & Berridge KC, 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 W, Dayan P & Montague PR, 1997c). This drug-induced change is called neural sensitization (Berridge KC & Robinson TE, 1998). Robinson and Berridge (Berridge KC & Robinson TE, 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 TE & Berridge KC, 1993). This theory is appropriate for explaining the occurrence of findings such as the effects of novel and aversive stimuli on accumbal dopamine (DA) levels (see DA and salience section below).

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 us a more accurate representation of what is occurring along the complex path to addiction.

Dopamine

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 O & Bjorklund A, 1974). 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 (Bardo MT, 1998).

Natural Reinforcers: Early in the 1970's, intracranial self-stimulation (ICSS) was studied extensively in relation to its effect on catecholamines, including DA. Historically, Olds and Milner have shown that an animal will lever-press for ICSS (Olds J & Milner P, 1954), and several studies indicated that DA systems are critically involved in this process (Crow TJ, 1972; German DC & Bowden DM, 1974). Microdialysis and voltammetry studies in rats have shown significant increases in DA in the NAcc during drinking, feeding and sexual behaviors (Di Chiara G, 1995; Wilson C, Nomikos GG Collu M & Fibiger HC, 1995). Additionally, in operant responding for juice reinforcement in monkeys electrophysiology techniques have shown activation of neurons in the NAcc (Bowman EM, Aigner AT Richmond ABJ, 1996). Not only will animals respond for these natural reinforcers, there is also evidence for increased neuronal firing in the VTA. Studies have also shown that drinking (induced by restricted access); salt intake (induced by sodium depletion); or eating (induced by food deprivation) will trigger the release of DA in the NAcc (Blander DS, Mark GP Hernandez L and Hoebel BG, 1988; Chang VC, Mark GP Hernandex L & Hoebel BG, 1988). Sexual behavior, additionally, causes the release of DA in the NAcc (Damsma G, Pfaus JG Wenkstern D Phillps AG & Fibiger HC, 1992) (Becker JB, Rudick CN Jenkins WJ, 2001)whereby sexual contact with a rat of the opposite sex triggers an increase in DA levels.

Laboratory animals will also self-administer DA reuptake blockers such as buproprion, mazindol, and nomifensine (Corwin RL, Woolverton WL Schuster CR & Johanson CZE, 1987; Wilson MC & Schuster CR, 1976;

Winger G & Woods JH, 1985) as well as piperazine, a highly selective DA reuptake blocker (Van Der Zee P, Koger HS Gootjes J & Hespe WZ, 1980). Along the same lines, animals will also self-administer direct DA agonists such as apomorphine and piribedil (Yokel RA & Wise RA, 1978). Moreover, DA blockade decreases responding for reinforcers. Animals trained to self-administer a saccharin solution, decreased their appetitive responding after a DA antagonist, haloperidol, was administered (Royalle DR & Klemm WR, 1981). Even responding for naturally reinforcing stimuli such as food, water and sex can be altered by the administration of either a DA agonist or antagonist, demonstrating that the DA system is critically involved. As seen from previous research, natural reinforcers have a profound influence on reward behavior and these types of reinforcers also generate an increase activity in the mesolimbic DA pathway and in accumbal DA levels.

Drug Use: Drugs of abuse also have a profound effect on the mesolimbic DA system. It has been shown that opiates (Esposito RU & Kornetsky C, 1978), amphetamines (Olds ME, 1978), marijuana (Gardner EL, Paredes W Smith D Donner A Milling C Cohen D & Morrison D, 1988), dissociate anesthetics, barbiturates, benzodiazepines and alcohol (Wise RA, 1980) all increase DA in the NAcc. A number of laboratories have shown that cocaine produces a strong enhancement of extracellular DA in the neostriatal and NAcc terminal projection areas of this reward-related DA system (Di Chiara G, Imperato A, 1988; Hernandez L & Hoebel BTG, 1988; Hurd YL, Weiss F Koob G & Ungerstedt U, 1989). As of today, many researchers have found this

phenomenon using *in vivo* microdialysis which allows sampling from the brain of freely moving animals. Not only does cocaine administration increase DA levels, but DA antagonists block the rewarding efficacy of cocaine (Koob GF, Caine SB Parsons L Markou A & Weiss F, 1997). Given that all these drugs have an impact on DA levels; it is important to consider how and by what mechanisms DA plays a role in mediating reward.

Aversive Stimuli: Not only do natural reinforcers have an influence on accumbal DA, but stimulus salience, (e.g. novel and/or aversive stimuli) also raise fundamental questions. Stressors such as footshock and restraint have been shown to activate the mesolimbic DA system. Previous research has shown that 15 minutes of restraint stress increases the content of DA metabolites in the shell but not the core of the NAcc (Deutch AY, Bourdelais AJ & Zahm DS, 1993). Also, Kalivas & Duffy (Kalivas PW & Duffy P, 1995) confirmed that mild stress induced elevations of extracellular DA for a period of at least 20 minutes in the shell of the NAcc. Animals exposed to aversive (shock) conditioning exhibited elevated DA activity in the NAcc, VTA and mPFC (Morrow BA, Taylor JR & Roth RH, 1995).

The fact that aversive stimuli increase DA levels has implications in favor of the incentive-salience theory. Specifically, not only positive hedonic stimuli can activate the mesolimbic DA system, but negative stimuli also have an effect on this system, therefore the attribution of incentive salience.

Latent Inhibition (LI): DA is not only implicated in reward, but appears to play an important role in attentional processes. LI is a procedure designed to

measure attention by exposing subjects to a stimulus repeatedly without consequence (preexposure) and later using this stimulus as a conditioned stimulus (CS) in a classical conditioning paradigm (Killcross AS & Robbins TW, 1993). This procedure results in a delayed attainment of conditioned responding compared to subjects that had no preexposure to the stimulus. The preexposure to a stimulus interferes with the ability to later learn an association. Accumbal DA plays an important role in LI, and attentional processes. Administration of DAergic agonists prevent LI, meaning that subjects learn the association in the conditioning paradigm even when that stimulus has been preexposed (Solomon PR & Staton DM, 1982). Therefore, facilitating attention to the stimulus and not allowing for generalization. However, administration of DAergic antagonists aid LI, for example, subjects take longer to learn the associations in the contingency (Weiner I & Gal G, 1996), due to decreased attention to that stimulus. DA and its relationship to LI provides insight that the NAcc plays not just a role in reward but in the regulation of attentional processes, and subsequently, a role in drug use maintenance through increased attention to environmental factors and/or cues that surround drug use.

Novelty: The personality trait, sensation seeking, has long been associated with the increased risk of drug abuse in humans (Fulker D, Eysenck SBG & Zuckerman M, 1980). In rodent models, novelty preference is used as an indicator of sensation seeking given that rats are inherently neophobic. Animals are considered high responders (HR) to novelty (or novelty

preference) when they spend more time in a novel environment compared to the time they spend in a familiar environment. Animals are considered to be low responders (LR) to novelty (or novelty aversion) when they spend more time in the familiar environment compared to the new or novel environment (Dellu F, Piazza PV Mayo W Le Moal M &Simon H, 1996). When exposed to a novel environment, HR rats have high rates of locomotor activity whereas the LR rats show low rates of activity. Rats placed in a novel environment express a surge in catecholamine activity in the NAcc (shell) and mPFC (Rebec GV, Grabner CP Johnson M Pierce RC & Bardo MT, 1997). Novelty HR's show greater increases in extracellular DA in the NAcc than LR when exposed to an environmental (tail pinch) or a pharmacological (cocaine) challenge (Hooks MS, Colvin AC Juncos JL & Justice JB Jr, 1992; Rouge-Pont F, Piazza PV Kharouby M Le Moal M & Simon H, 1993). Typically, there is a robust sensitization that occurs with repeated cocaine administration (Kalivas PW & Duffy P, 1995). A less robust sensitization occurs when the drug is administered to animals in a novel test environment compared to those given the same dose in the home cage (Badiani A, Browman KE Robinson TE, 1995). HR rats show higher rates of amphetamine and cocaine-induced locomotor activity and will self-administer these drugs more readily than LR rats (Hooks MS, Jones GH Smith AD Neill DB & Justice JB Jr., 1991). 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, Klebaur JE & Bardo MT., 1997). This could be comparable to humans in so much that people labeled as high sensation-seekers, may be more likely to become involved in risky behaviors such as reckless driving, sky diving or drug use (Zuckerman M, 1990).

Mesolimbic DA System and Brain Structures

*Ventral Tegmental Area (VTA)**:*** The mesolimbic system begins in the VTA and projects through the medial forebrain bundle to the amygdala, lateral septum, bed nucleus of the stria terminalis, hippocampus, and the NAcc (Oades RD & Halliday GM, 1987). DA neurons in the VTA are the cells of origin of the mesolimbic/mesocortical DA pathway and provide DAergic innervations of the NAcc (Oades RD & Halliday GM, 1987). Electrical self-stimulation of this area has generally shown an increase in DA release and metabolism in the NAcc and medial prefrontal cortex (mPFC) (Fiorino DF, Coury A Fibiger HC & Phillips AG, 1993). You et. al (You ZB, Chen YG & Wise RA, 2001), have shown that lateral hypothalamic self-stimulation increases dendritic release of DA and accumulation of its metabolites in the VTA. 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, Mckinzie DL Dagon CL Murphy JM & McBride WJ, 1998) but interestingly enough, animals will self-administer cocaine into the nucleus accumbens (McBride WJ, Murphy JM & Ikemoto S, 1999), but not the VTA (De La Garza R, Callahan PM & Cunningham KA, 1998). This showing that

although the rewarding effects of certain drugs are mediated by the mesolimbic pathway, 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).

Nucleus Accumbens (NAcc): The NAcc is located in the basal forebrain, rostral to the preoptic area and immediately adjacent to the septum. It is innervated by DA-secreting terminal boutons from neurons of the VTA (Skagerberg G, Lindvall O & Bjorklund A, 1984). This is an area that seems to play a very important role in the physiology of reward and reinforcement in relation to drugs of abuse, including cocaine. Stimulation of the DA receptors in the NAcc will reinforce behavior [e.g. animals will lever press for electrical stimulation of the NAcc (Olds ME & Fobes JK, 1981)]. Animals will also lever press for direct infusions of DA and amphetamines directly into the NAcc (Hobel BG, Monaco AP Hernandez L Ausili EF Stanley BG & Lenard L, 1983). As mentioned previously, DA levels in the NAcc can be measured by *in vivo* microdialysis, which samples extracellular cerebral spinal fluid (CSF). Many studies have found that administration (either self-administration or experimenter administration) of cocaine and amphetamine increase the levels of extracellular DA in the NAcc (Hoebel BG & Hernandez L, 1989). As mentioned earlier, the NAcc not only mediates reward, but other salient (e.g. aversion) stimuli as well (Salamone JD, 1992). From the multitude of research performed in the NAcc, it is evident that there are complex mechanisms regulating not only reward, but other aversive and attentional stimuli in

relation to DA levels, suggesting the possibility that use of drugs of abuse may not be maintained just because they are rewarding, but because they are salient or conditioned.

Cocaine and the 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). As previously discussed, the NAcc and VTA consist of DA synapses. 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 a dopamine transporter protein (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 dose (Camp DM, Browman KE & Robinson TE, 1994; Kuczenski R, Segal DS & Aizenstein ML, 1991; Reith ME, Li MY & Yan QS, 1997; Strecker RE, Eberle WF & Ashby CR Jr, 1995). 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 animals (Philpot RM & Kirstein CL, 1998).

Repeated administration of psychostimulants results in behavioral sensitization or reverse tolerance in an enhanced behavioral response to a subsequent drug challenge (Vanderschuren LJ & Kalivas PW, 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 WA & Zahniser NR, 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 M, Woolverton W Schuster C & Seiden, 1988). These changes lead to the complex processes of tolerance, dependence and of course, sensitization (Koob GF & Le Moal M, 1997; Wise RA, 1980). Sensitization is characteristic of repeated intermittent cocaine administration, where in 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 RM, 1980). Rats injected once a day with cocaine show enhanced inhibition of DA uptake (Izenwasser S & Cox BM, 1992), whereas rats getting a continuous infusion of cocaine show attenuated inhibition of DA uptake by cocaine (Izenwasser S & Cox BM, 1992). Also, there seems to be different degrees of sensitization, such that longer times between cocaine injections produce greater sensitization (Post RM, 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 (FosB) (Nestler EJ & Aghajanian GK, 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 LH, Smith AD & Justice JB Jr, 1991). Taken together, these studies in adult animals show that repeated cocaine administration results in complicated changes in the DA mesolimbic pathway that continues long after drug use has stopped, and may be implicated in craving and relapse.

Expectancy: Another puzzling aspect of drug use deals with the issue of drug expectancy-induced changes. Cues that were previously paired with a reward initiate neurochemical and behavioral responses like those present during the actual reward. CPP studies have shown that an animal will spend more time in the chamber in which it 'expects' to receive a reward than the one it never received a reward in previously, suggesting an anticipatory or expectancy effect. In addition to expectancy-induced behavioral changes there are also expectancy-induced neurochemical changes. Cocaine and alcohol *in vivo* microdialysis studies have shown an expectancy effect with accumbal DA levels increasing significantly when the animal "expect" to receive an injection of the drug, but actually receives a saline injection(Philpot RM & Kirstein CL, 1998). DA neurons and subsequent behaviors seem to be activated by conditioned, reward-predicting stimuli that enable the animal to learn and

eventually expect a reward based on previous performance. Expectancies may be an evolutionary adaptation that allows an animal to predict future events, allowing extra time for preparatory behaviors and possibly increasing the likelihood of escape from dangerous situations.

Drug expectancies may play an important role in human drug addiction, since stimuli associated with drug taking behavior in humans have the ability to elicit strong drug 'craving' feelings which repeatedly leads to drug relapse (O'Brien CP, Childress AR McLellan AT & Ehrman R, 1992). Non-human primate studies have shown via physiological recordings, activation of VTA, NAcc and ventral striatum neurons in response to anticipation of rewarding stimuli such as water or fruit juice (Schultz W, Dayan P & Montague PR, 1997b). Recent studies have been able to replicate these non-human primate studies of reward prediction to human brain reward activation. Berns et al. (Berns GS, McClure SM Pagnoni G & Montague PR, 2001) have shown activation of brain reward regions in response to temporal predictability of rewards such as water and juice.

Mesolimbic System 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 WD, 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. Many neurobehavioral 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 in their ability to evaluate neurochemical and behavioral changes due to drug use during this important stage of development.

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 LP, 2000). This phenomenon being evolutionary adaptive as a means to avoid inbreeding (Schlegel A & Barry III H, 1991).

Human Social Interaction: 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 L, 1989) and typically spend a significant amount of time interacting with their peers as opposed to adults. Human adolescents as a group exhibit a disproportional amount of reckless behavior, sensation-seeking and risk taking (Trimpop RM, Kerr JH & Kirkcaldy B, 1999). Risk taking in adolescents poses some negative consequences such as suicides, accidents, AIDS, pregnancy and drug dependence (Irwin Jr.CE, 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. Adolescents 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 (Schlegel A & Barry III H, 1991).

Similar to humans, periadolescent rats are behaviorally and pharmacologically different from younger and adult rats. Periadolescent rats have been reported to be more hyperactive and inattentive (Spear LP & Brake SC, 1983) and have reduced responsiveness to some of the effects of alcohol (Silveri MM & Spear LP, 1998), amphetamine (Bolanos CA, Glatts J and Jackson D, 1998), and cocaine (Laviola G, Wood RD Kuhn C Francis R & Spear LP, 1995). In the CPP paradigm, adolescent rats show a preference for nicotine, whereas the adult rats did not (Vastola BJ, Douglas LA Vaarlinskaya EI & Spear LP, 2002). Also, Philpot et al (Philpot RM, Badanich KA & Kirstein CL, 2003) demonstrated that adolescent rats showed a preference for moderate doses of alcohol, whereas the adults had no preference at that dose.

Neurochemical Changes: There are also dramatic changes in the adolescent brain, both circuitry and neurochemistry. The mesolimbic and mesocortical brain regions and their DA projections undergo substantial remodeling during the adolescent period, for review see (Spear LP, 2000). Rosenberg & Lewis (Rosenberg DR & Lewis DA, 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. D1 and D2 receptors increase in density in the first few weeks of life (Hartley EJ & Seeman P, 1983). Subsequently, Teicher et al (Andersen SL, Thompson AT Rutstein M Hostetter JC & Teicher MH, 2000; Teicher MH, Andersen SL & Hostetter JC Jr., 1995) have demonstrated receptor overproduction and elimination in both the striatum and prefrontal cortex, but have failed to show evidence that the NAcc follows the same overproduction and pruning construct (Andersen SL, Thompson AT Rutstein M Hostetter JC & Teicher MH, 2000). In addition, alterations in receptor binding and sensitivity in various neurotransmitter systems have been reported during adolescence (Trauth JA, Seidler FJ McCook EC & Slotkin TA, 1999) along with changes in myelination of neurons (Hamano K, Iwasaki N Takeya T & Takita H, 1996).

Adolescents, whether human or non-human animals, exhibit many behavioral, social and neurochemical adaptations that enable them to develop successfully, however, these adaptations can have negative implications when these normal developmental behaviors result in persistent deviant actions such as drug abuse. The present studies are designed to look at the relationship between novelty preference and DA responsiveness to cocaine among adolescent and adult animals.

Chapter Two

Experiment One

Impulsivity in the Adolescent Rat

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 dependency in adulthood. Human adolescents are predisposed toward an increased likelihood of risk taking behaviors (Zuckerman M, 1986), including drug use or initiation. The purpose of this study was to examine differences in developmental risk taking behaviors. Adolescent and adult animals were exposed to a novel stimulus in a familiar environment to assess impulsivity, novelty preference and exploratory behaviors. Adolescent animals had greater novelty-induced locomotor activity, greater novelty preference, were more impulsive and showed higher exploratory behaviors compared to adult animals. These data support the notion that adolescents may be predisposed toward sensation seeking and consequently are more likely to engage in risk taking behaviors, such as drug use initiation.

Introduction

Adolescence is a time of high risk behavior and increased exploration. It is also a period when the brain is undergoing many complex changes that can exert long-term influences on decision making and cognitive processes (Spear LP, 2000). Adolescence is also marked by a greater probability to initiate drug use and is associated with an increased risk to develop addiction and dependency in adulthood. Estroff (Estroff TW, Schwartz RH & Hoffmann NG, 1989) has reported that illicit drug use can begin at approximately age 12, with peak periods of initiation between ages 15 and 19. In fact, more than half (54%) of high school seniors have had at least one experience with an illicit compound (Wallace JM Jr., 2003). The fact that illicit drug use is so dramatic during the adolescent period is of particular concern given that the escalation of use appears more rapidly among teenagers than adult users, suggesting a greater abuse potential during adolescence than in adulthood (Estroff TW, Schwartz RH & Hoffmann NG, 1989). Individuals who initiate use prior to ages 11-14 are more likely to progress to addiction as adults (DeWit DJ, Adlaf EM Offord DR Ogborne AC, 2000).

Several researchers (Trimpop RM, Kerr JH & Kirkcaldy B, 1999) (Arnett, JJ., 1999) have shown a relative predisposition toward sensation-

seeking in human adolescents, a factor that Zuckerman associates with increased likelihood of risk taking behaviors (Zuckerman M, 1984) (Zuckerman M, 1986), including drug use or initiation (Bardo MT, Donohew RL Harrington NG, 1996; Bates ME, Labouvie EW White HR, 1986; Forsyth G, Hunleby JD, 1987). Measures of sensation-seeking are highly associated with impulsivity (Eysenck SB & Eysenck HJ, 1977; Shedler J, Block J, 1990), indicating this as a valid measure of risk taking behavior probability, specifically, drug use initiation (Hansell S and White HR, 1991). Similar to humans, adolescent rats have been shown to exhibit greater responding to novelty compared to adult rats (Douglas L, Varlinskaya E Spear L, 2003). Furthermore, numerous studies have indicated that there is a strong correlation between novelty preference and impulsive reactivity with both the rewarding efficacy of psychomotor stimulants and self-administration rates in animals (Hooks MS, Colvin AC Juncos JL & Justice JB Jr, 1992) (Klebaur JE, Bevins RA Segar TM Bardo MT, 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 MS, Jones GH Smith AD Neill DB & Justice JB Jr., 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, Klebaur JE & Bardo MT., 1997) (Klebaur JE, Bevins RA Segar TM Bardo MT, 2001). Interestingly, adolescents who have been diagnosed with attention-

deficit/hyperactivity disorder (ADHD) are at a greater risk for substance use than an adolescent not suffering from this disorder (Biederman J, Wilens TE Mick E Faraone SV Spencer TJ, 1998; Molina B, Pelham W, 2003). This is important as one of the key features of ADHD is impulsivity, suggesting this trait may play a role in individuals with ADHD being predisposed to substance abuse. Taken together, these data suggest a strong relationship between sensation-seeking and novelty-seeking/impulsivity, making it more likely that adolescents will become involved in risky behaviors including drug use and initiation.

Several approaches have been used to divide animals into high or low drug abuse profiles. Some researchers have used exposure to a novel environment to induce locomotor increases as a predictor of drug abuse liability (Kabbaj M, Devine DP Savage VR and Akil H., 2000). Recent work has shown that novelty preference is a reliable measure that can be used to divide animals into high responders (HR) and low responders (LR) (Stansfield KH, Philpot RM & Kirstein CL, 2004). To examine differences between adolescent and young adult animals, the present study examined behavioral responses to a novel context or novel object in a familiar environment. The purpose of this study was to determine an effective procedure for characterization of individual and developmental differences in novelty induced locomotion and impulsivity (i.e., decreased latency to approach a novel object).

Methods

Animals

Fifty Sprague-Dawley (Harlan Laboratories) rats postnatal day (PND) 34 (μ=134 g) and PND 59 (μ=293 g) at the time of testing were used as subjects in these experiments. 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, following which pups were weaned and group housed. 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 (National Institutes of Health, 1986).

Procedure

Animals were tested on a black plastic circular platform (216 cm in diameter) standing 70 cm from the ground, with a white plastic barrier enclosing the arena (216 cm). A video camera was suspended directly over the table and recorded the animal's behavior using a Noldus Behavioral Tracking System.

Over a period of four consecutive days, each rat (PND 31-34 and 56-59) was placed on 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 6.5 in. high) was attached to the center of the table. Rats were placed in a random zone and allowed to explore the familiar environment for five minutes. Both time spent in proximity of the novel object, and activity induced by the presence of the novel object were measured. Novelty preference was defined as time spent within 10.16 cm of the object on test. Novelty-induced locomotion and total distance moved (TDM) were measured on all trials. Impulsivity was operationalized as latency to approach the novel object.

RESULTS

Novelty

The present findings demonstrate that adolescent animals exhibited significantly greater TDM on the first trial as compared to adult animals, $t(1,46)=2.100$, $p<0.05$ (appendix A). Both adolescent and adult animals exhibited a significant reduction in TDM from trial 1 to trial 8, $t(1,42)= 3.533$, $p< 0.001$, $t(1,49)= 3.006$, $p<0.05$, respectively (appendix B). Importantly, activity in the presence of the novel object on test did not differ across age, $t(1,48)=0.3005$, $p>0.05$ (appendix C).

Additionally, adolescent animals spent more time with the novel object, $t(1,43)= 2.082$, $p<0.05$ as compared with young adult animals (appendix D).

Impulsive & explorative behavior

There was a significant effect of age on latency to approach the novel object, $t(1,44)=2.449$, $p<0.05$ (appendix E), and frequency approaching the novel object, $t(1,43)=2.370$, $p<0.05$ (appendix F). Adolescents approached the object more rapidly and returned to the object more frequently on test.

Discussion

The present study utilized a novel paradigm to assess impulsivity and novelty preference in adolescent and adult animals. The results indicate a developmental difference between impulsive and novelty preference behaviors in adolescent versus adult animals. The present results replicate and extend present findings of enhanced novelty responding in adolescent animals using a Conditioned Place Preference paradigm (Douglas L, Varlinskaya E Spear L, 2003). Adolescent animals are more active in a novel context than adult counterparts, while activity induced by a localized novel stimulus was similar across age. Importantly, the present study showed from trial 1 to trial 8, there was a significant reduction in total distance moved in both age groups, with no differences detected between ages on trial 8. Notably, adolescent rats habituated significantly faster to the novel environment than did adult animals. TDM in younger animals was significantly higher on the first trial compared to all other trials, however, with adult rats, only trial 1 differed from the last trial in TDM. Also, adolescent rats spent more than twice as much time interacting with a novel object placed in a familiarized environment compared to older rats (i.e., novelty preference) which supports previously published data using a

different paradigm (Douglas L, Varlinskaya E Spear L, 2003). In the present study, the behavior was recorded by a computerized tracking system, suggesting that this effect is robust. Together, these results indicate that adolescent animals are highly reactive to a novel environment, stressing the importance of habituating animals when performing developmental research.

The second part of the study examined impulsive and exploratory-type behaviors across adolescent and adult animals. Adolescent animals exhibited a significantly lower latency to approach the novel object when placed in the habituated environment than did the adult animals. This would suggest that adolescents engage in more risk-taking behaviors more frequently than older animals, because the shorter the latency to approach, the less time an animal has to evaluate whether the novel object is a threat, a behavior that would be considered risky. Not only were adolescent animals more impulsive, they also approached the novel object more frequently, suggesting they are more likely to explore something unfamiliar in their environment and subsequently spent more time, on average, with the novel object after approach. Taken together, these data reveal that adolescent animals express greater novelty induced reactivity along with a greater preference for novelty. Interestingly, adolescent animals exhibited a significant reduction in the number of approaches to the novel object on test from minutes 1 to 2, whereas adult animal's number of approaches remained relatively constant over the entire trial, suggesting that adults do not habituate. This observation suggests that, like TDM across trials, adolescent animals habituate faster within a given trial than do adult animals.

Furthermore, adolescents are more impulsive and engage in more exploratory behaviors. These data support the notion that adolescents may be predisposed toward sensation seeking (Arnett, JJ., 1999) and consequently are more likely to engage in risk taking behaviors (Zuckerman M, 1986), such as drug use initiation.

Chapter Three

Experiment Two

Neurochemical Effects of Cocaine in Adolescence Compared to Adulthood

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 dependency in adulthood. Human adolescents are predisposed toward an increased likelihood of risk taking behaviors (Zuckerman M, 1986), including drug use or initiation. The purpose of this study was to characterize adolescent versus adult developmental differences classified as high-responding or low-responding based on several behavioral measures and subsequently to examine the neurochemical responsivity to a systemic challenge of cocaine. The results demonstrate neurochemical differences between adolescent and adult animals in novel environment exploratory behavior, novel object preference, novelty-induced impulsivity and novelty-induced exploration. The data demonstrate that adolescent animals' exhibit a greater behavioral activation compared to their adult counterparts, in addition, the paradigm shows the simplicity of separation based on individual variability within each behavioral measure. These results illustrate that a response to a pharmacological challenge of cocaine exhibits a complex interaction with age and behavioral characteristics.

Introduction

Adolescence is a time of high risk behavior and increased exploration. It is a period when the brain is undergoing many complex changes that can exert long-term influences on decision making and cognitive processes. Adolescence is also marked by a greater probability to initiate drug use and initiation during this time is associated with an increased risk to develop addiction and dependency in adulthood. Specifically, Estroff (Estroff TW, Schwartz RH & Hoffmann NG, 1989) has reported that most illicit drug use begins at approximately age 12, with peak periods of initiation between ages 15 and 19. 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, 2000). During the 1990's, there was a steady rise in the frequency of drug use in teenagers, by 2003, 4.3% of eighth graders, 5.7% of tenth graders, and 8.2% of high school seniors, reported a long-term use of cocaine (Johnston LD, 2000). 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 TW, Schwartz RH & Hoffmann NG, 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, Kirisci L Tarter RE, 1998). Development of the central nervous system (CNS) during adolescence may play a key role in the increased likelihood to initiate drug use. Moreover, disruption of the development of the CNS may result in subsequent long term increases in the probability of drug use and dependence. During adolescence, critical structures involved in substance abuse are regulated primarily by the limbic system which is associated with emotional and impulsive behaviors. However, 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 LP, 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 RD & Halliday GM, 1987). However by adulthood, these previously amygdala-modulated regulatory actions are replaced by projections from the medial prefrontal cortex (mPFC) indicating some developmental transition in the functional nature of the system. 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). Additionally, chronic administration of an agonist (e.g. cocaine) during this period may cause a functional change in accumbal

dopamine (DA) efflux by altering amygdalar modulation of accumbal DA release and/or altering the functional role of the mPFC input; consequently, leading to an increased risk of dependency during adulthood. Together, these implications make a powerful argument for treating adolescence as a key time period for researching the development of drug addiction.

It is clear that adolescence is an important developmental period. Despite this, very few models of adolescent drug addiction in animals have been developed to examine the remarkable differences between adolescents and adults. Many neurobehavioral age-specific alterations that are seen in human adolescents are also observed in adolescent rats from around postnatal days (PND) 30 to PND 42 (Odell WD, 1990). Adolescent animal models need to evaluate neurochemical and behavioral changes due to drug use during this important stage of development.

Novelty seeking and high risk behaviors seem to be highly associated with adolescence (Douglas L, Varlinskaya E Spear L, 2003; Stansfield KH, Philpot RM & Kirstein CL, 2004) (Fulker D, Eysenck SBG & Zuckerman M, 1980). 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 LP, 2000). This phenomenon being evolutionary adaptive as a means to avoid inbreeding (Schlegel A & Barry III H, 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 L, 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, Kerr JH & Kirkcaldy B, 1999). Risk taking in adolescents poses some negative consequences such as suicides, accidents, AIDS, pregnancy and drug dependence(Irwin Jr.CE, 1989). Although risk taking may be hazardous, it can also be beneficial.

Similar to humans, adolescent rats are behaviorally and pharmacologically different from younger and older adult rats. Adolescent rats have been reported to be more hyperactive and inattentive (Maldonado AM & Kirstein CL, 2005) (Spear LP & Brake SC, 1983) and have reduced responsiveness to some of the sedating effects of alcohol (Silveri MM & Spear LP, 1998), amphetamine (Bolanos CA, Glatts J and Jackson D, 1998), and cocaine (Laviola G, Wood RD Kuhn C Francis R & Spear LP, 1995). In the conditioned place preference (CPP) paradigm, adolescent rats show a preference for nicotine, whereas the adult rats did not (Vastola BJ, Douglas LA Vaarlinskaya EI & Spear LP, 2002). Also, Philpot et al (Philpot RM, Badanich KA & Kirstein CL, 2003) demonstrated that adolescent rats showed a preference for moderate doses of alcohol, whereas the adults had a conditioned place aversion.

There are also dramatic changes in the adolescent brain, both circuitry and neurochemistry. The mesolimbic and mesocortical brain regions and their DA projections undergo substantial remodeling during the adolescent period (Spear LP, 2000). Rosenberg & Lewis (Rosenberg DR & Lewis DA, 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. D1 and D2 receptors increase in density in the first few weeks of life (Hartley EJ & Seeman P, 1983). Subsequently, Teicher et al (Teicher MH, Andersen SL & Hostetter JC Jr., 1995) have demonstrated receptor overproduction and elimination in both the striatum and prefrontal cortex, but have failed to show evidence that the NAcc follows the same overproduction and pruning construct (Andersen SL, Thompson AT Rutstein M Hostetter JC & Teicher MH, 2000). In addition, alterations in receptor binding and sensitivity in various neurotransmitter systems have been reported during adolescence (Trauth JA, Seidler FJ McCook EC & Slotkin TA, 1999) along with changes in myelination of neurons (Hamano K, Iwasaki N Takeya T & Takita H, 1996).

Adolescent animals, both human and non-human, exhibit many behavioral, social and neurochemical adaptations that enable them to develop successfully. However, these adaptations can have negative implications when these normal developmental behaviors emerge as persistent deviant actions that result in drug abuse. The present study examined the relationship between

novel environment exploratory behavior, novel object preference, novelty-induced exploration and novelty-induced impulsivity in relation to DA responsiveness to cocaine among adolescent and adult animals.

Methods

Behavioral testing

To isolate high responding (HR) versus low responding (LR) rats based on several measures of behavioral sensitivity, fifty Sprague-Dawley (Harlan) rats postnatal day (PND) 34 (μ=134g) and PND 59 (μ=293.13g) at the time of testing were used as subjects in these experiments. 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, following which pups were weaned and group housed.

Animals were tested in a dimly lit room on a black plastic circular platform (216 cm in diameter) standing 70 cm from the ground, with a white plastic barrier enclosing the arena (216 cm). A video camera was suspended directly over the table and recorded the animal's behavior using a Noldus Behavioral Tracking System (See experiment one).

In all respects, maintenance and treatment of the animals were in accordance with the guidelines established by the NIH (NIH, Guide for the care and use of laboratory animals, 2005).

Surgery

Animals were anesthetized on either PND 34 or 59 using a ketamine/ xylazine cocktail (1.0 and 0.15 mg/kg/ip respectively). An incision was made over the skull and the rat was mounted on a stereotaxic instrument for surgery. Three holes were drilled in the skull (two for skull screws and one for the guide cannula). The guide cannula was lowered to the NAcc shell (Philpot RM, McQuown S & Kirstein CL, 2001) and affixed to the skull with cranioplast. Probes were immediately lowered following surgery into the anesthetized rat aimed at the NAcc.

In vivo microdialysis Apparatus

Animals were singly housed with ad lib food and water in a BAS Raturn System bowl for recovery overnight. The Raturn system consisted of a large round bottom bowl (14" by 16"). The animals were tethered via a locking collar clamp and a counter balanced arm through which dialysis tubing was threaded. An optical switch mechanism signaled rotation of the bowl in the opposite direction of the animal's movement enabling the animal to move about freely.

In Vivo Microdialysis

The probe inlet tubing was attached to a 2 ml Hamilton syringe mounted on a BAS syringe pump set to a flow rate of 0.5 µl/min. *In vivo* microdialysis probes with 2 mm membrane tips (BAS) were perfused continuously with artificial cerebrospinal fluid (145 mM NaCl, 2.4 mM KCL, 1.0 mM MgCl, 0.2 mM ascorbate, pH=7.4) for twelve hours prior to the start

of sampling. On either PND 35 or 60, dialysates were collected at a flow rate of 0.5 ul/min at ten-minute intervals from the probe outlet tubing into refrigerated microcentrifuge tubes containing 2.0 µl of 0.25M hydrochloric acid (HCl). Following the collection of six baseline samples, animals received an injection of 0.9% saline mg/kg/ip. After the control injections, sampling continued at ten-minute intervals for 120 minutes after which an injection of cocaine (cocaine HCL was obtained from Sigma and dissolved in 0.9% saline) was administered (20 mg/kg/ip). Sampling continued at ten-minute intervals for an additional 120 minutes. Dialysate samples (12.5 µl) were either run immediately on an HPLC-EC or stored at -80°C until analyzed at a later date.

Neurochemical Analyses

Analysis of dialysate samples was performed with a reverse phase high performance liquid chromatography system (BAS) coupled with electrochemical detection (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 to identify chemicals. Neurochemical analyses included the detection of DA and its major metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). 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 60 µl/min. Samples (6µl) were injected onto a C-18 microbore column for peak separation. Data were recorded and quantified by Rainin Dynamax Software on a Power Macintosh 7500/100.

Histology

Following probe removals, rats were euthanized via CO^2 inhalation. Brains were rapidly removed and frozen in 2 methylbutane and stored at -80°C. Brains were sliced into 40µm sections, which were mounted on slides and stained with Cresyl Violet. Probe placements were verified for placement in the NAcc shell. Any animals whose probes were not verified in the NAcc shell were examined but excluded from statistical analysis.

Design and Analysis

Basal DA values were converted to Area Under the Curve (AUC) to determine DA levels after the saline and cocaine injections. Cocaine induced DA levels were divided from the control levels (saline) in order to determine an individual animals responsivity to cocaine. Behavioral data was then used to separate animals into HR or LR based on the mean split of all animals in the experiment. Subsequent Student's t-tests were performed to isolate differences between groups. In addition, DA turnover was assessed by a DOPAC/DA ratio and performing the same statistical analyses as described above.

Results

Animals were separated based on several behavioral measures including novel environment exploratory behavior, novel object preference, novelty-induced exploration and novelty-induced impulsivity. As seen in appendices G-J, behavior was clearly defined and easily differentiated as to being classified as a high responding or a low responding animal.

Interestingly, basal DA was significantly lower in adolescent animals compared to adult animals [$t(1,17)=2.057$, $p<0.05$, appendix K]. Overall, no cocaine induced dopaminergic differences were detected when collapsing across age [$t(1,17)=0.2403$, $p>0.05$, appendix L] and an analysis of DA turnover (DOPAC/DA AUC) revealed no differences in cocaine induced dopaminergic activity in adolescent animals compared to adult animals, [$t(1,8)= 0.04$, $p>0.05$, appendix M], for this reason an AUC statistical analysis was performed on each animals basal levels of DA.

The present findings indicate that adult animals who exhibited a greater novel environment exploratory behavior (total distance moved on trial 1) had greater DAergic responsivity to cocaine that the LR counterparts [$t(1,9)= 2.347$, $p<0.05$, appendix N]. No differences were detected between low and high responding adolescent animals.

The present findings indicate that more impulsive adolescent animals have a greater cocaine induced DAergic response compared to less impulsive animals [$t(1,7)= 3.581$, $p<0.05$, appendix O].

No differences in DAergic activity in adult animals was detected based on the impulsivity measure [$t(1,9)= 0.178$, $p>0.05$, appendix O].

Interestingly, no differences were detected between adolescent animals DA responsivity in relationship to the time spent with the novel object. However, adult animals who spent less time with the novel object on test exhibited greater DAergic responsivity to a cocaine challenge [$t(1,9)= 2.444$, $p<0.05$, appendix P]. Nevertheless, adolescent animals who approached the

novel object more frequently exhibited a greater dopaminergic responsivity to a cocaine challenge versus adolescents who approached the novel stimuli less during the test, [$t(1,7)= 3.581$, $p<0.05$, appendix Q]. In comparison, adult animals who approached the novel stimuli more frequently exhibited a smaller dopaminergic responsivity to cocaine, [$t(1,9)= 2.734$, $p<0.05$, appendix Q].

Discussion

Previous work in adult animals has shown that a preference for novelty is indicative of a facilitated acquisition of drug abuse (Klebaur JE, Bevins RA Segar TM Bardo MT, 2001). Research looking at adolescents has found that adolescent animals and humans who prefer novelty are more likely to use/abuse drugs (Spear LP, 2000; Zuckerman M, 1986). The present study's goal was to determine if differences existed in neurochemical activity in relationship to the novelty- seeking profiles of adolescent and adult animals. Several researchers have shown that adult animals have higher basal DA in response to a novel environment (TDM on trial 1) and also a greater DAergic response to cocaine and amphetamine that exhibited high novel environment exploratory behavior (Bradberry CW, Gruen RJ Berridge CW & Roth RH, 1991; Hooks MS, Colvin AC Juncos JL & Justice JB Jr, 1992; Hooks MS, Jones GH Smith AD Neill DB & Justice JB Jr., 1991; Rouge-Pont F, Piazza PV Kharouby M Le Moal M & Simon H, 1993). These studies show that HR adult rats classified by novel environment exploratory behavior exhibit a greater DAergic response to a pharmacological challenge of cocaine compared to their LR counterparts.

The present data support and extend these previous findings. Interestingly, adolescent animals demonstrated a different behavioral and neurochemical pattern than adult animals. HR adolescent animals who approached the novel object faster during test exhibited a significantly greater DAergic response to a subsequent cocaine challenge compared to their LR counterparts. This is interesting as HR adolescent animals DA levels were significantly higher over LR while adult animals LR and HR had comparable cocaine-induced increases in DA. Adolescent LR and HR did exhibit equal cocaine-induced DA when divided based on time spent with novel object. However, on this behavioral measure, LR adults (i.e., adult animals that spent less time with the object) had significantly increased cocaine-induced DA when compared to the adult animals that spent more time with the object, perhaps indicative of a more responsive mesolimbic pathway (i.e. attention due to neophobia) that was not apparent in adolescent animals. Similarly, adults which approached the object less during test (i.e., LR) had greater cocaine-induced DA during challenge compared to HR adults while the opposite was true for adolescent animals (i.e., LR had a significantly increased cocaine-induced DA than HR adolescent animals). During adolescence, behaviors associated with higher novelty-induced exploration and impulsivity is related to a greater DAergic response to cocaine, whereas adult animals that exhibit the same behavioral profile demonstrate a reduced DAergic response compared to their LR counterparts. This suggests that DA is involved with sensory gaiting which may explain why initiation of drug use during adolescence may

lead to enhanced incentive salience (i.e. attention) of environmental cues surrounding use.

A pharmacological challenge of cocaine acts as an indirect agonist blocking reuptake of DA via the dopamine transporter (DAT) and does not increase the amount of DA being synthesized, therefore, the extracellular DA in response to cocaine demonstrates a measure of basal DA tone. However, it is known that cocaine decreases firing of VTA neurons, potentially due to actions at the D2 autoreceptors. Therefore, this increase DA in adolescence may represent decreased sensitivity or number of autoreceptors (Chen YC, Choi JK Andersen SL Rosen BR Jenkins BG, 2004) or perhaps an immaturity of other feedback regulatory systems (Jones EA, Want JQ McGinty JF, 2001). These findings further suggest that adolescent animals who exhibit greater novelty-induced exploration (i.e. frequency of approaches) and novelty-induced impulsivity (i.e. latency to approach) have an elevated DAergic tone. In contrast, adult animals who have greater novelty-induced exploration (frequency of approaches), novel object preference (time spent with object) and behavioral activation in response to a novel object (TDM on test) have a lower DAergic tone supporting the maturation of inhibitory control in the regulation of DA in the NAcc. The transition from adolescence to adulthood involves several critical developmental changes in brain pathways involving attention, decision making, emotional regulation and behavioral activation and inhibition. Specifically, the corticolimbic circuitry consisting of the PFC and the subsequent interaction with the amygdala (AMY) and NAcc with

innervations mediated via DAergic and glutamatergic (GLUergic) projections. Since the AMY is involved with contextual conditioning and emotional regulation, it can be viewed as an activational system as opposed to the PFC, which is involved with behaviors involved in the cognitive processes of decision making, planning, impulse control, self-monitoring and forward thinking, it can be characterized as a behavioral inhibitory system. Cunningham (Cunningham MG, Bhattacharyya S & Benes FM, 2002) demonstrated an increase in amygdalo-prefrontal fiber innervation during adolescence, suggesting that the connectivity between emotional learning (AMY), and executive decision making (PFC) regulating regions is still being developed. Campbell (Campbell BA, Lytle LD & Fibiger HC, 1969) has demonstrated that the activational system develops before the inhibitory system matures, which subsequently leads to a period during adolescence characterized by high novelty-seeking and risk taking behaviors.

As the present data demonstrate, adolescent animals' exhibit a greater behavioral activation compared to their adult counterparts, in addition, this newly established paradigm is an effective means by which separation based on individual variability within each behavioral measure can be achieved. Additionally, these results illustrate that a response to a pharmacological challenge of cocaine exhibits a complex interaction with age and behavioral measures such as impulsivity and novelty preference. The transition from adolescence to adulthood is a critical developmental period involving the maturation of the corticolimbic circuitry, where the development of the

activational system (with little inhibitory control) produces increased novelty-seeking, and risk taking behaviors corresponding to changes in DA levels in the NAcc. The transition into adulthood is associated with the development of an inhibitory behavioral system that competes with the activational system and is manifested by a reduction in risk taking behaviors and a subsequent alteration of DA production in the NAcc.

The present findings support the notion that adolescent animals show different behavioral and neurochemical profiles than adults that may possibly be driven by the initial development of the AMY regulated activational system and the delay in the development of the PFC inhibitory system. The interval between the development of the activational and inhibitory system may account for the novelty-seeking and novelty-induced impulsivity that distinguishes the adolescent period which is marked by increased risk-taking behaviors such as drug use initiation.

Summary

Taken together, there is a complex interaction between adolescent and adult behavioral and neurochemical characteristics that must be considered when designing developmental research experiments modeled on adult paradigms. Adolescent animals exhibit greater novel environment exploratory behavior, novel object exploration, novelty-induced exploration and impulsivity in relationship to adult animals. This is a clear developmental difference between adolescence and adulthood that is behaviorally observable with the purpose of isolating novelty-seeking and risk-taking behaviors. Using the present paradigm, not only are these behaviors between adolescent and adult animals reliably identified, this approach also serves as a good means to distinguish between high responding and low responding animals based on these behavioral categories. In addition, adolescent and adult animals demonstrate unique neurochemical profiles possibly due to the continuing development of certain brain structures. The robust findings demonstrated in the area examined in the present study (ie. the mesolimbic projection area, the NAcc) are regulated by both the AMY and the PFC. The AMY is associated with emotional learning and is developed during adolescence, however, a region critically involved with executive decision making (PFC) is still being developed, which suggests that while adolescents have an activational system,

they lack inhibitory control therefore are more likely to engage in risky behaviors such as drug use initiation. Future studies should examine the dynamic interaction and ontogeny of these structures and their role in the development of addiction.

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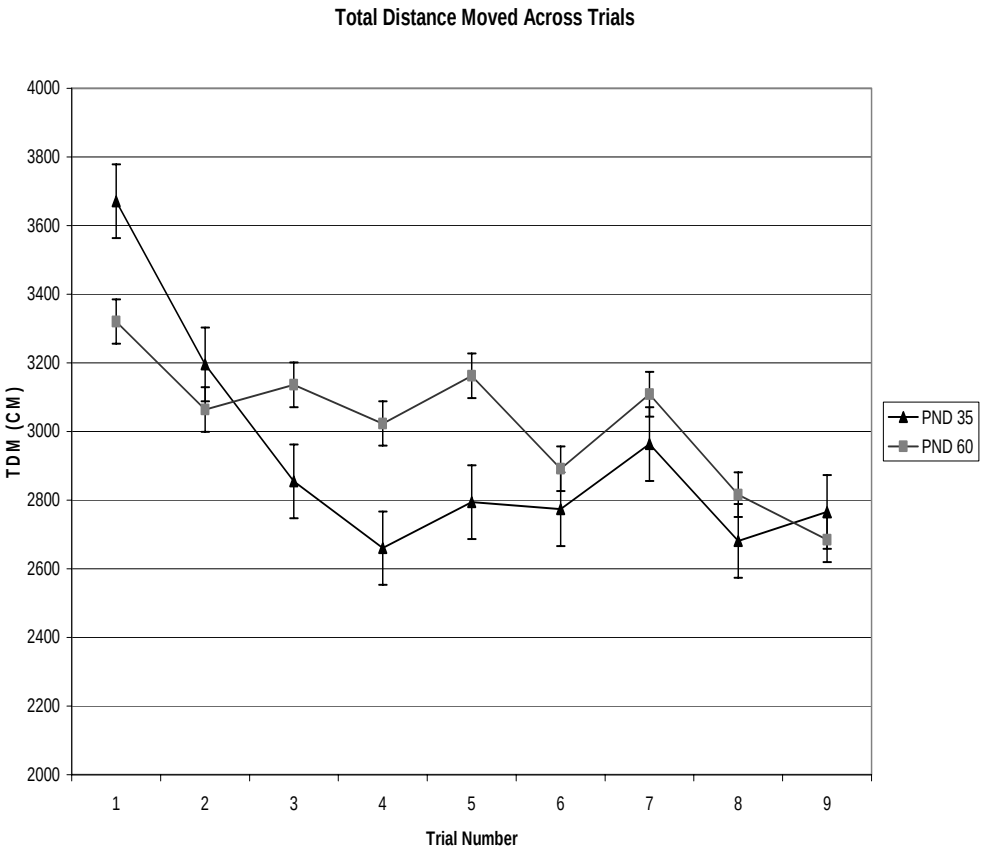
Appendices

Appendix A: Total Distance Moved on Trial 1

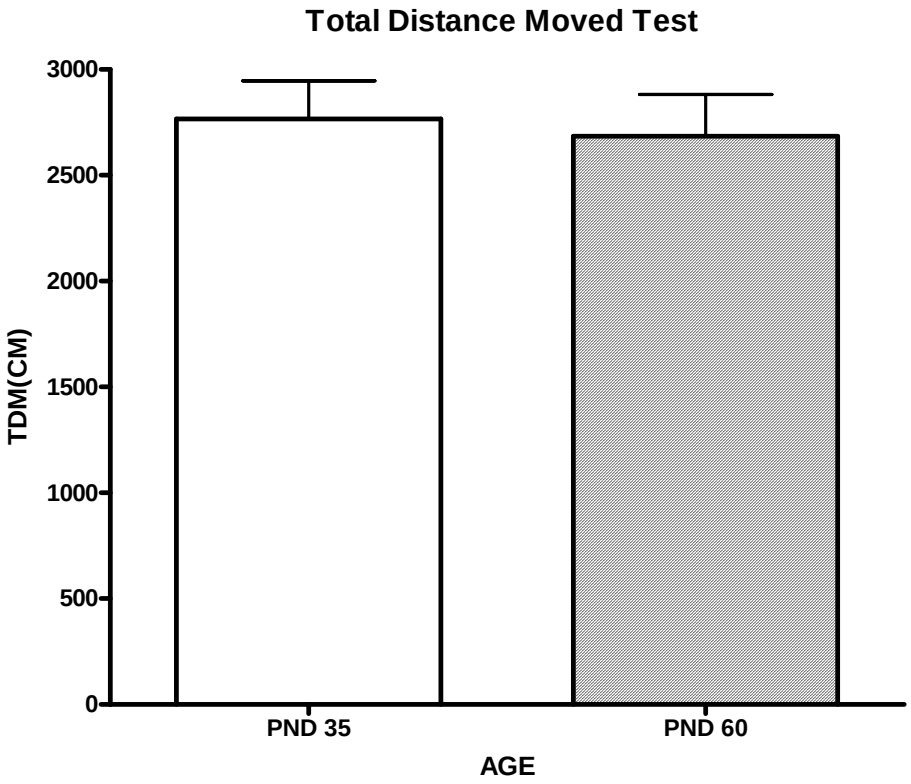
Adolescent animals (white bar) moved significantly more during the first exposure to the novel environment than did adult animals (black lines).

Appendix B: Total Distance Moved Across Trials



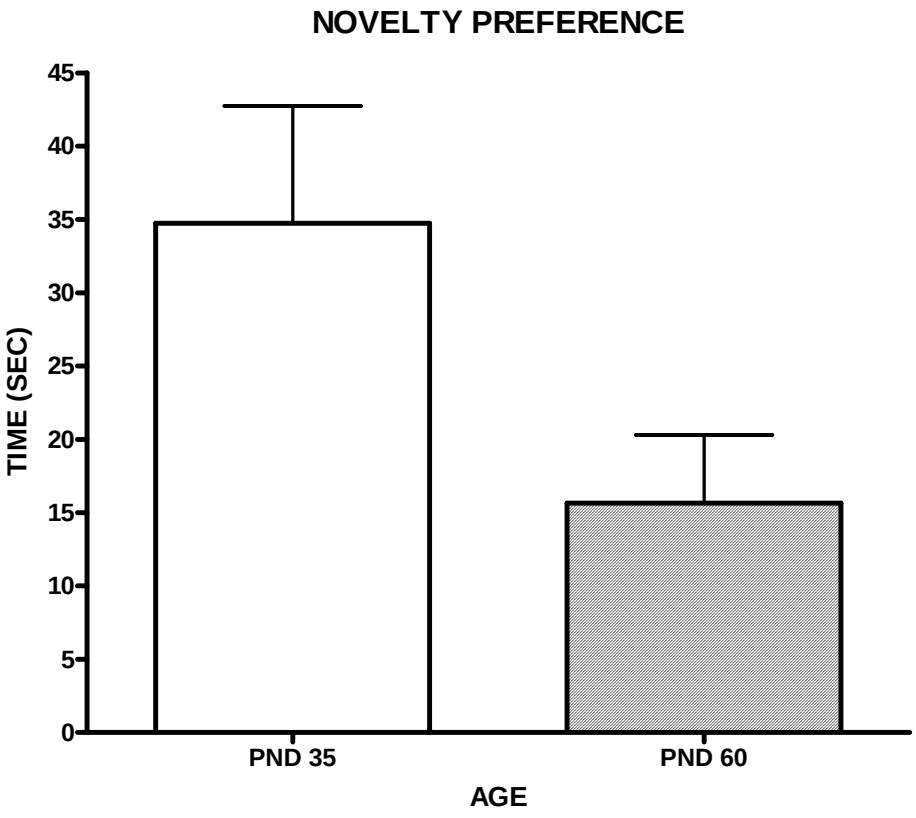
Adolescent animals (black triangles) habituated to the novel environment after 4 trials while activity levels in adults (grey squares) remain relatively stable across trials.

Appendix C: Total Distance Moved on Test



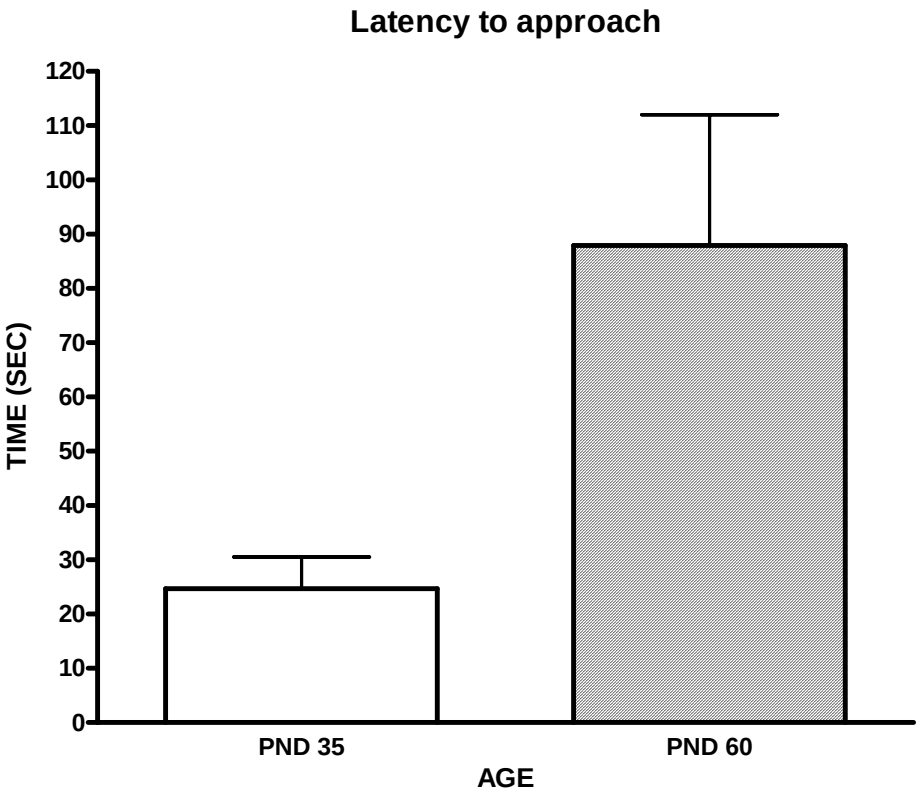
During testing with the novel object, adolescent (white bar) and adult (black lines) animals traveled similar amounts.

Appendix D: Novelty Preference



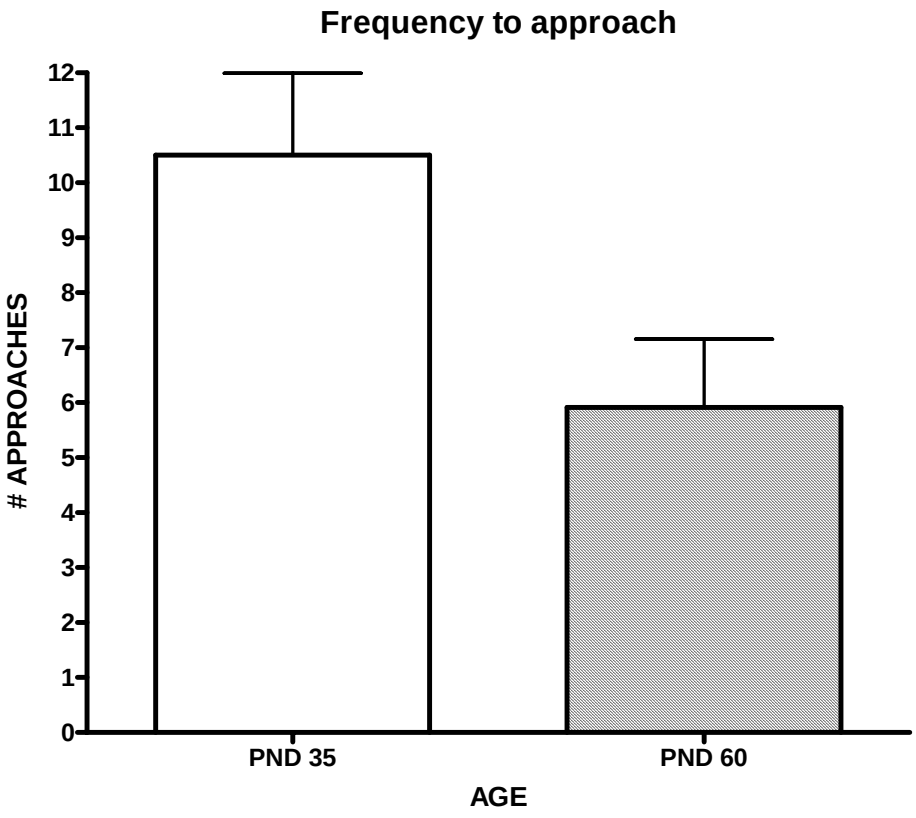
During test, adolescent animals (white bar) spent significantly more time interacting with the novel object than did adults (black lines).

Appendix E: Latency to Approach



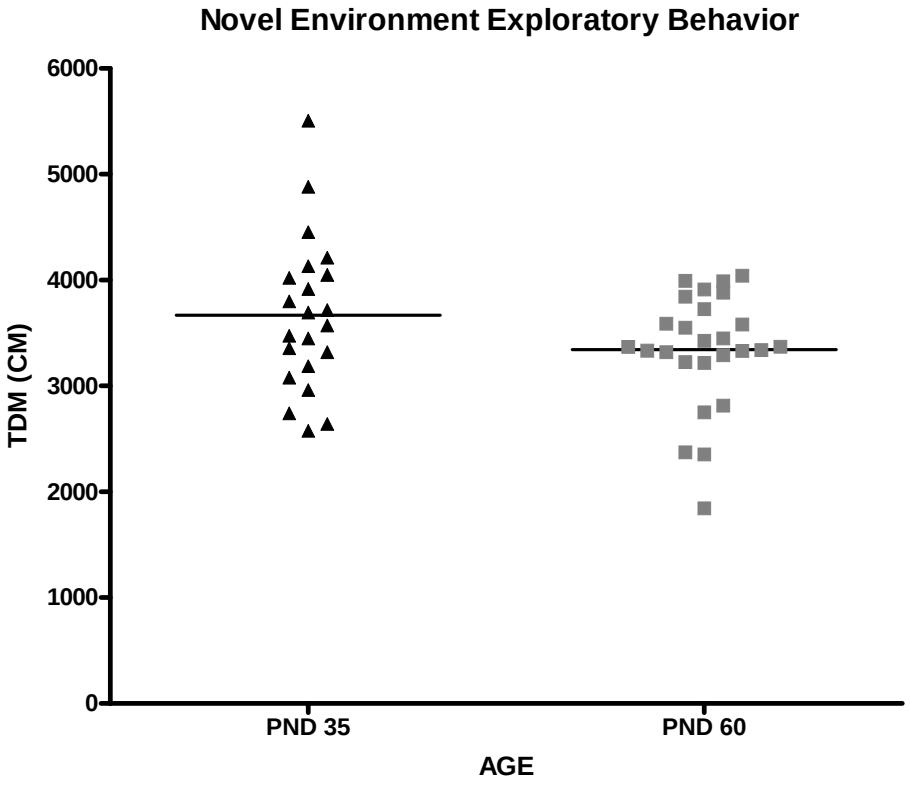
During test, adolescent animals (white bar) approached the novel object significantly faster than did adults (black lines).

Appendix F: Frequency to Approach



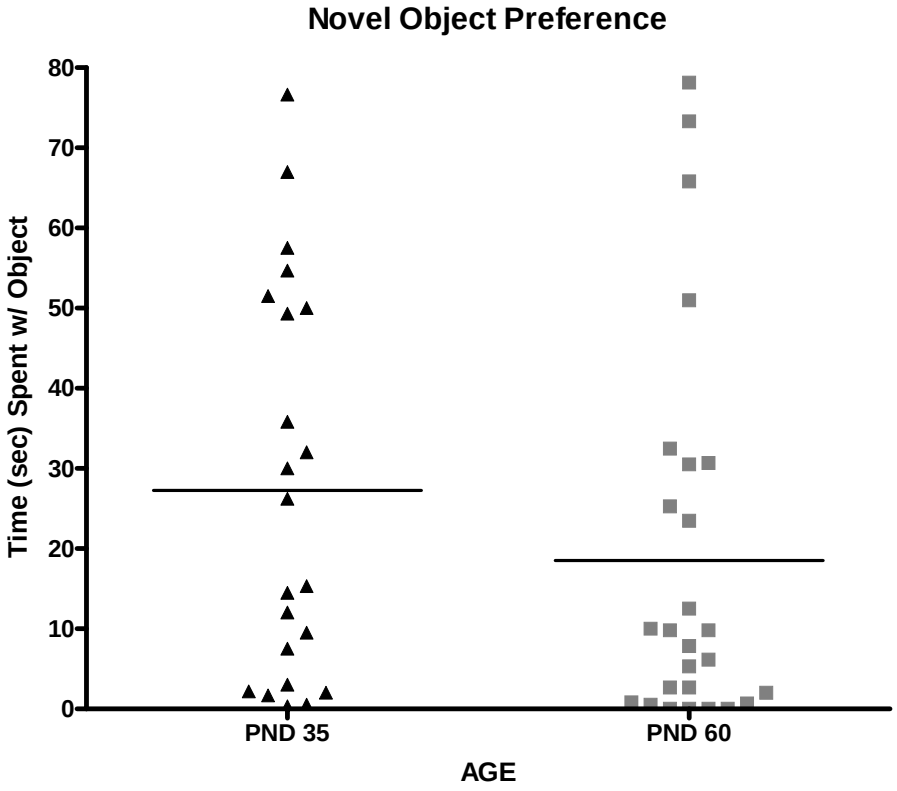
Adolescent animals (white bar) approached the novel object significantly more times than did the adults (black lines).

Appendix G: Novel Environment Exploratory Behavior



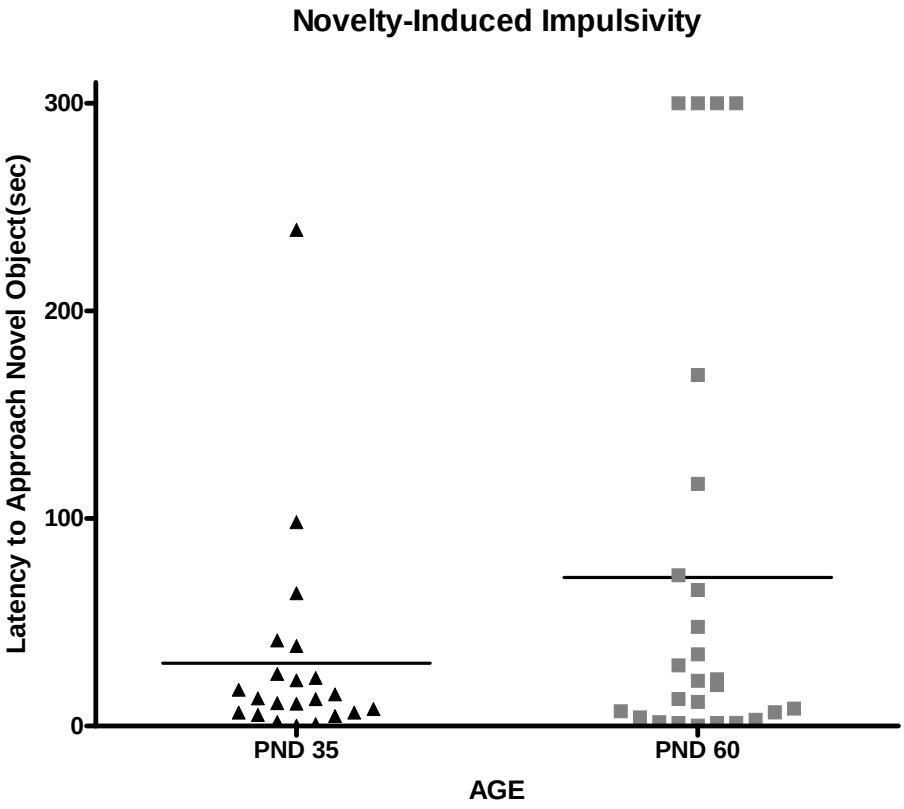
During exposure to a novel environment, adolescent (black triangles) and adult animals (grey squares) activity levels revealed a good distribution of scores allowing for reliable separation into high and low responders.

Appendix H: Novel Object Preference



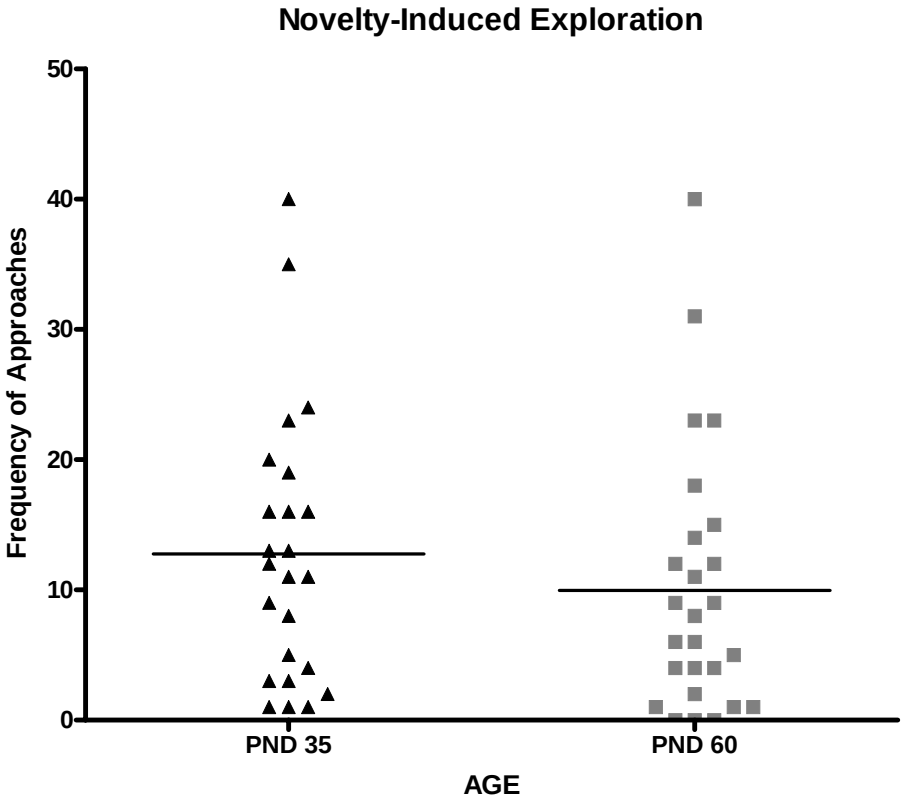
During test, adolescent (black triangles) and adult animals (grey squares) were reliably separated into high and low responders based on time spent interacting with the novel object.

Appendix I: Novelty-Induced Impulsivity



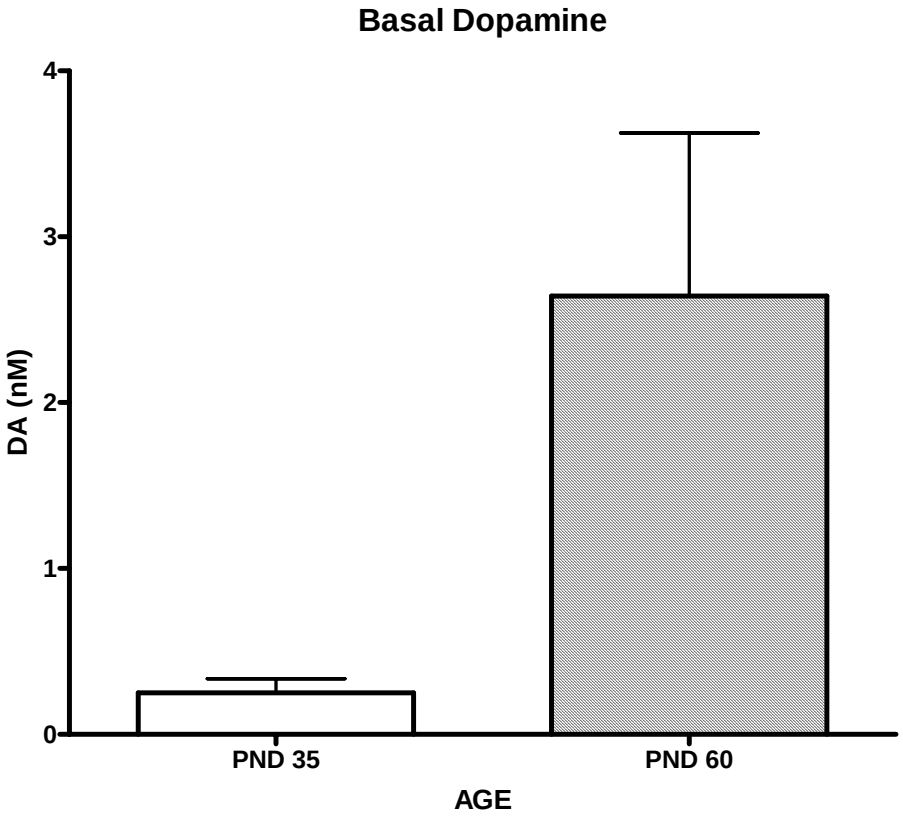
A measure of novelty-induced impulsivity (latency to approach the novel object) was used with the purpose of separating adolescent (black triangles) and adult (grey squares) animals into high and low responders.

Appendix J: Novelty-Induced Exploration



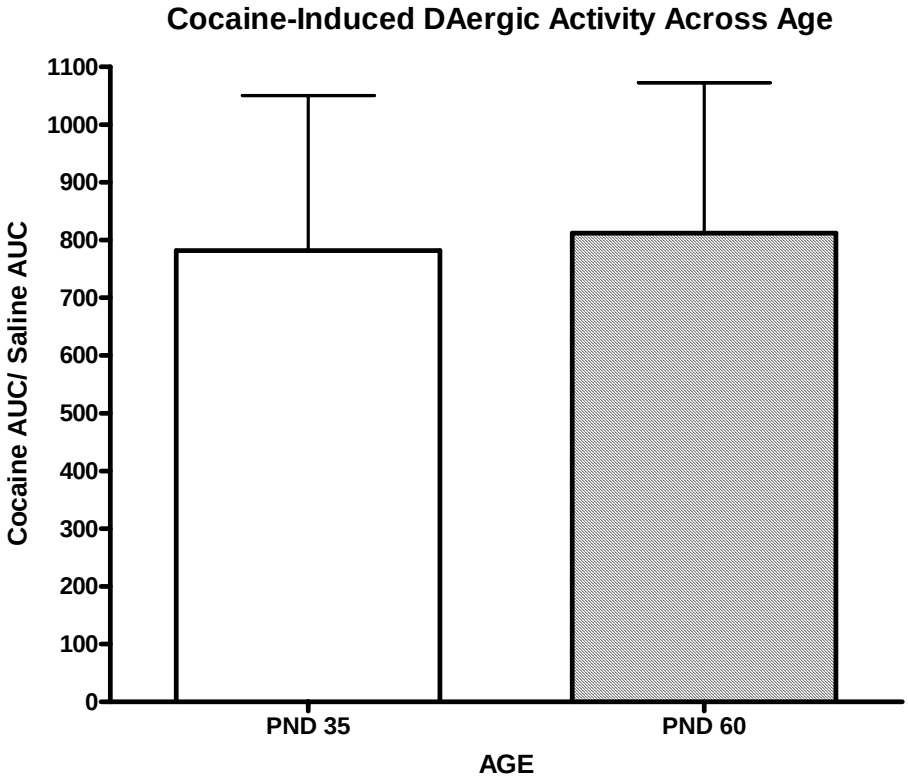
Animals were separated into high and low responding adolescent (black triangles) and adult (grey squares) animals based on the frequency to approach the novel object on test.

Appendix K: Basal Dopamine



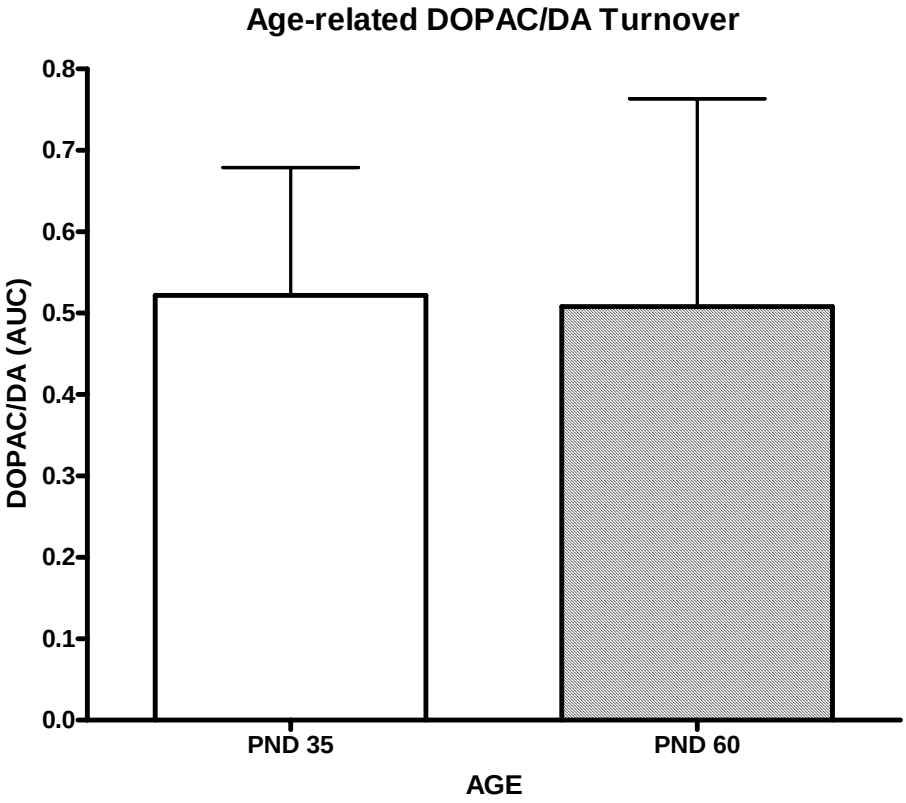
Adolescent animals (white bar) had significantly lower basal DA levels when compared to young adult animals (black lines) (note: values not corrected for probe recovery).

Appendix L: Cocaine-Induced DAergic Activity Across Age



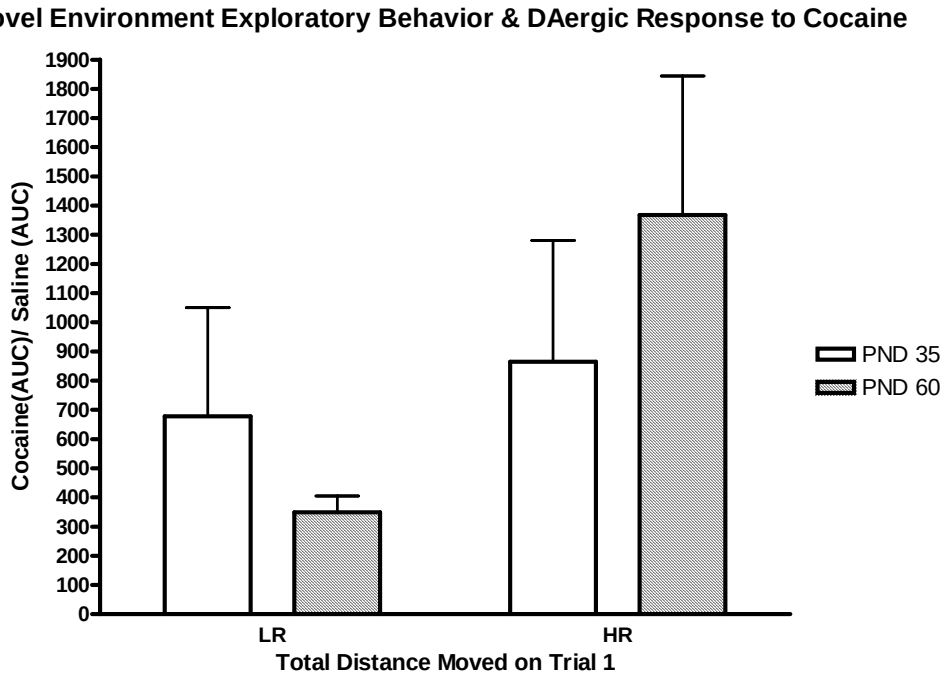
Cocaine-induced increases in DA were comparable across age.

Appendix M: Age-Related DOPAC/DA



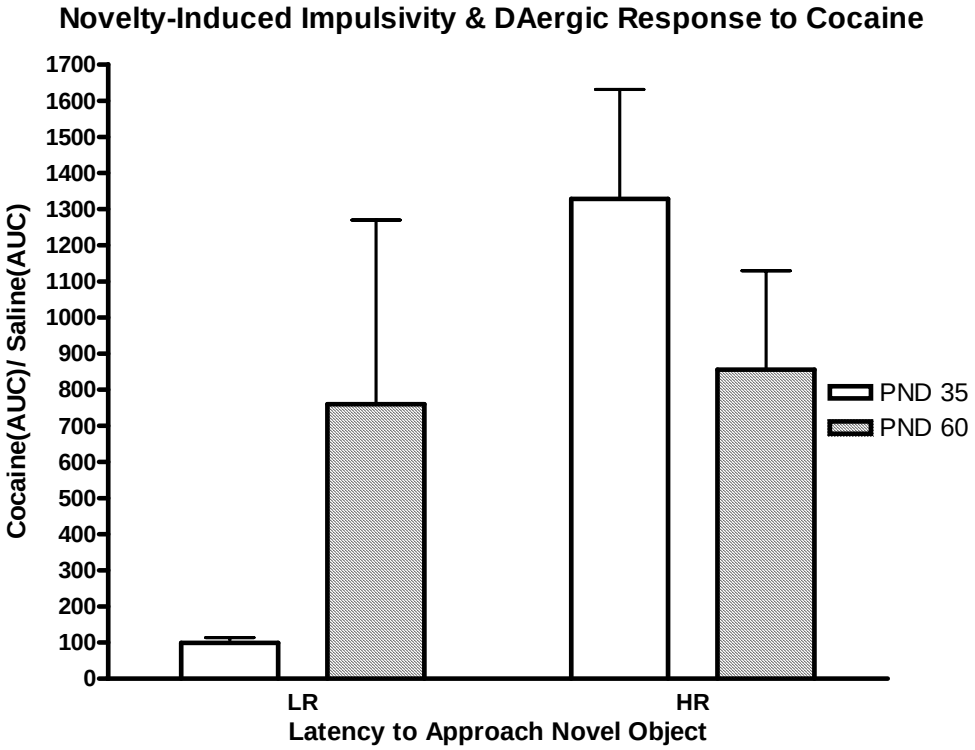
Turnover rates of DA (DOPAC to DA ratio) were comparable across age.

Appendix N: Novel Environment Behavior and the DAergic Response to Cocaine



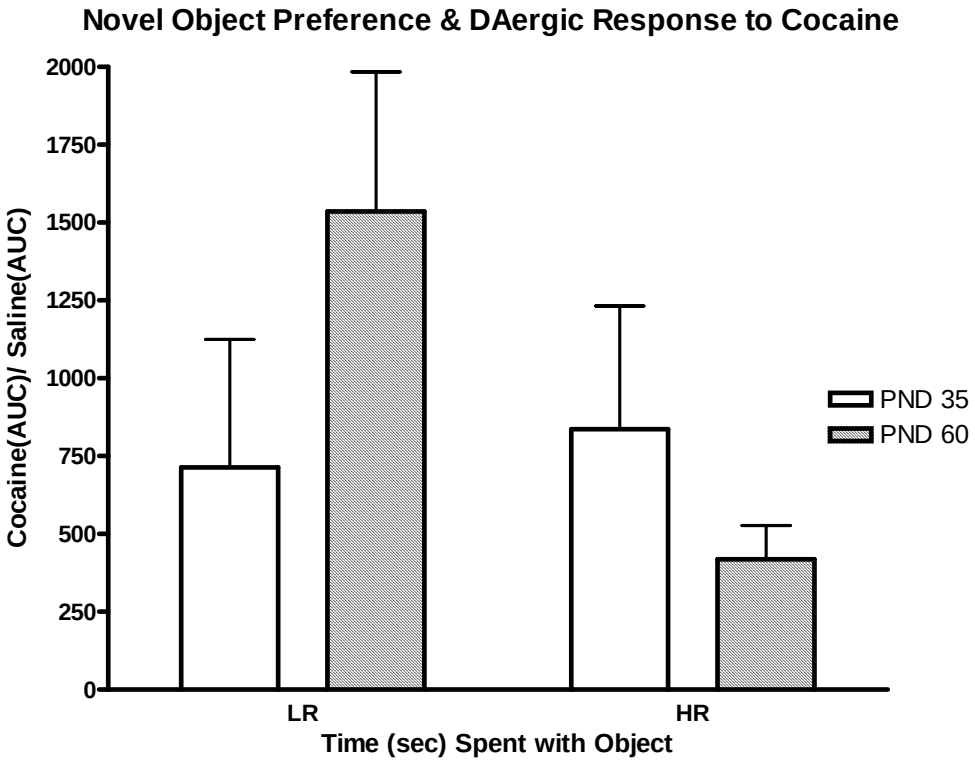
Adult animals (black lines) that exhibited greater novelty-induced locomotor activity (i.e., introduction to the novel environment on trial 1) had significantly higher cocaine-induced increases in DA compared to adults who scored lower on this behavioral measure. LR and HR adolescent animals (white bar) exhibited equal amounts of cocaine-induced DA efflux regardless of activity on trial one.

Appendix O: Novelty-Induced Impulsivity and the DAergic Response to Cocaine



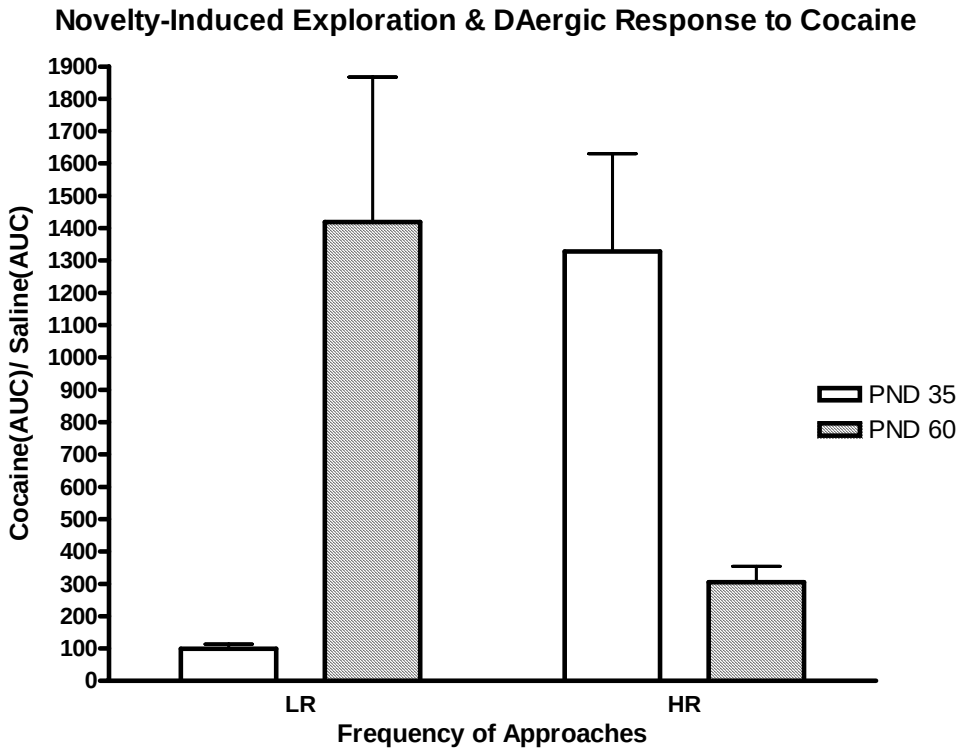
Less impulsive adolescent animals (white bar) had a significantly lower DAergic response to a challenge of cocaine compared to adolescents who were more impulsive. Both LR and HR young adults had comparable cocaine-induced increases in DA. (black lines).

Appendix P: Novel Object Preference and the DAergic Response to Cocaine



Adult animals (black lines) who had a greater novel object preference (i.e. spent more time with the object) had a significantly lower DAergic response to a challenge of cocaine. Both LR and HR adolescent animals exhibited comparable cocaine-induced increases in DA (white bar).

Appendix Q: Novelty-Induced Exploration and the DAergic Response to Cocaine



Adolescent animals (white bar) that had greater novelty-induced exploration scores (i.e. frequency of approaches) had a greater DAergic response to a challenge of cocaine compared to adolescent animals that approached the novel object less. Conversely, adult animals (black lines) that had greater novelty-induced exploration demonstrated lower DAergic responsivity to cocaine when compared to the LR adult animals.