Published in final edited form as:
J Psychopharmacol. 2005 September ; 19(5): 443–447.

Heightened cocaine-induced locomotor activity in adolescent compared to adult female rats

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Abstract

Initiation and experimentation with illicit drugs often occurs in adolescence. Evidence suggests that adolescent rats are more sensitive to some of the effects of drugs of abuse than adult rats. The present study investigated whether adolescent and adult female Sprague Dawley rats differ in cocaine-induced locomotor activity. Animals were placed in the test environment for 30 minutes, and then administered an intraperitoneal (IP) injection of either cocaine (20 mg/kg) or saline (0.9%). Both adult and adolescent animals showed significant increases in locomotor activity as a result of cocaine administration compared to saline controls. Interestingly, cocaine induced significantly more locomotor activity in the adolescent females compared to the adults, demonstrating that cocaine acts differently in developing animals.

Keywords

adolescence; females; cocaine; locomotor activity

Introduction

In humans, adolescence is a time period when drugs are abused and addiction potential is high. Human drug use is frequently initiated during adolescence. A 2003 survey carried out by the National Institute on Drug Abuse indicated that, 3.6%, 5.1% and 7.7% of 8th, 10th and 12th graders, respectively, reported using cocaine in their lifetime. However, research in the field of drug abuse is carried out mainly using adult animal models, overlooking the importance of drug use on a developing system. During adolescence, brain maturation is still taking place, including the extensive over production and pruning of synapses (Huttenlocher, 1984), yet the effects of psychostimulants on the developing brain remain unclear in many aspects. The biological and behavioural effects as well as differences between developing males and females have yet to be elucidated.

Over the past decade cocaine abuse by women has increased so that approximately 30% of the 1.8 million people who abuse cocaine are women (Wetherington *et al.*, 1998). In addition, women initiate cocaine use and commence treatment programmes at earlier ages than men (Griffin *et al.*, 1989). Evidence from studies using adult animals suggests sex differences in

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how females and males react to cocaine. Female rats show exaggerated hypothalamic-pituitary-axis (HPA-axis) activation compared to males after acute administration of cocaine (Kuhn and Francis, 1997). Female rats develop self-administration of cocaine more rapidly than males (Lynch and Carroll, 1999), show less toxicity to cocaine (Craft and Stratmann, 1996), and exhibit greater cocaine-induced hyperactivity and stereotypic responses (Post *et al.*, 1981; Kalivas and Stewart, 1991; Chin *et al.*, 2001).

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

Adolescence is a critical time period in which brain maturation and development take place. One important 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, dopaminergic, serotonergic, GABAergic and adrenergic receptors (Lidow *et al.*, 1991; Lidow and Rakic, 1992). More specifically, the ontogenetic profile of the expression of dopamine (DA) receptors has been characterized in the rat striatum. There is an increase in DA receptor densities throughout development which peaks around PND 28–30 (Murrin and Zeng, 1986; Zeng *et al.*, 1988; Murrin and Zeng, 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 *et al.*, 1995; Andersen *et al.*, 2000). It has been reported that DA transporter (DAT) expression is highest during adolescence in human striatum (Meng *et al.*, 1999). However, animal studies revealed that DAT expression in nucleus accumbens septi (NAcc) and striatum increases with age peaking at PND 60 (Coulter *et al.*, 1997; Tarazi *et al.*, 1998). Interestingly, the most rapid increase in DAT occurs during adolescence (Coulter *et al.*, 1997; Tarazi *et al.*, 1998). It is well known that drugs of abuse have a direct effect on the dopaminergic system (Koob and Nestler, 1997). It has also been demonstrated that basal DA levels change during adolescence in both the striatum (Andersen and Gazzara, 1993), and NAcc (Philpot and Kirstein, 2004). Ontogenetic changes in basal activity likely impacts subsequent drug-induced changes in adolescent rats compared to those demonstrated in adults.

Few studies have focused on adolescence. Studies in the 1970s–1980s have shown that adolescent rats are unique in baseline behaviour and reactivity to pyschostimulants 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 behaviour than younger and older rats (Spear and Brake, 1983). Furthermore, adolescent female rats were more active than males; however, this effect was reduced when animals were not handled post-weaning (Bronstein *et al.*, 1975). Adolescent animals have been shown to respond less to the locomotor activating effects of acute cocaine than juvenile animals (Spear and Brick, 1979) and adult animals (Laviola *et al.*, 1995) and appeared unresponsive to amphetamine (AMPH) compared to both younger and older animals (Lanier and Isaacson, 1977). However, 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. A recent study showed that adolescent rats show significantly greater cocaine-induced locomotor activity compared to adult rats only when rats were handled prior to drug administration (Maldonado and Kirstein, 2005). The behavioural data presented above is mixed, in that both hyper and hyposensitivity are observed as a result of a dopamine (DA) agonist.

It is evident that drug use during adolescence results in unique behavioural and physiological effects. Since cocaine abuse is often initiated in adolescence, it is crucial to understand the effects of cocaine during this period of development. In this study, 20 mg/kg of cocaine, (i.e., a dose which has been shown to effectively increase locomotor activity (Laviola *et al.*, 1995)) was used to investigate the locomotor effects of acute cocaine administration on adolescent and adult female rats. It was hypothesized that adolescent female rats would show greater cocaine-induced behavioral hyperactivity compared to adults.

Material and methods

Subjects

Subjects consisted of adolescent (N = 13; postnatal day (PND) 35, average weight 128.8 g) and adult (N = 17; PND 60, average weight 215.8 g) female Sprague-Dawley rats derived from established breeding pairs in the laboratory at the University of South Florida (Tampa, FL). The date of birth was designated as 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 on PND 21. Animals were 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 AM. All animals were treated in accordance with the guidelines established by the National Institutes of Health (NIH).

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 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 analysed using Ethovision Video Tracking System created by Noldus (Netherlands). This software tracked and recorded the Total Distance Moved (TDM) (cm) of each animal during the testing session.

Procedure

All animals were handled in the colony room for 5 minutes once a day for 3 days prior to testing. On test day animals were weighed and placed in the locomotor activity apparatus for a total of 90 minutes. After a 20-minute habituation period, subjects received a single IP injection of saline, followed 10 minutes later by 20 mg/kg cocaine (a dose which has shown to induce a significant conditioned place preference in PND 35 animals; Badanich and Kirstein, 2003) or saline. After testing, a probe (3.8 mm diameter) connected to the Estrous Cycle Monitor Model EC40 (Fine Science Tools Inc.) was inserted into the vagina and the score obtained was recorded to determine estrous phase. This probe has previously been shown to be more reliable in adult rats than in adolescent rats (Woodson *et al.*, 2004). There were no significant correlations between estrous scores and cocaine-induced locomotor behaviour.

Statistical analyses

TDM was the dependant measure. A 2 (Age: PND 35, PND 60) × 2 (Drug: saline, cocaine) mixed factor ANOVA with time as a repeated measure was performed. Subsequent post hoc analyses were performed to isolate simple effects using Dunnett's test.

Results

Across time, locomotor activity differed as a function of age and drug. A 2 (Age) × 2 (Drug) mixed factor ANOVA with time as a repeated measure revealed significant main effect of

Drug, ($F(1,26) = 45.6$, $p < 0.05$), demonstrating that cocaine injected animals had significantly greater TDM than saline injected animals. There was also a significant effect of time, ($F(17,442) = 17.1$, $p < 0.05$), showing that initial placement in the apparatus and after the drug injection resulted in significantly more TDM than baseline activity. As shown in Fig. 1, there was a significant Time × Age × Drug interaction ($F(17,442) = 2.2$, $p < 0.05$). Adolescents ($F(17,68) = 14.2$, $p < 0.05$) and adults ($F(17,102) = 20.3$, $p < 0.05$) were initially active when placed in the novel environment. This variation was due to elevated locomotor levels in the novel environment (time point −15), which stabilized after 20 minutes in the apparatus ($p < 0.05$). Subsequent saline injections did not alter locomotor activity regardless of age (*ns*). Cocaine significantly increased TDM for both adolescent ($F(17,187) = 10$, $p < 0.05$) and adult ($F(17,255) = 5.6$, $p < 0.05$) rats, an effect that remained significant at the end of the trial. Relative to controls, adolescent and adult cocaine animals had increased TDM from 15–70 minutes post drug injection. Additionally, adolescent animals exhibited greater cocaine-induced locomotor activity at 25–45 minutes post drug injection when compared to cocaine treated adult rats ($p < 0.05$). Female adolescent animals showed significant increases in cocaine-induced locomotor activity compared to female adults indicating that female adolescents have a heightened sensitivity to cocaine.

Discussion

The current study demonstrates robust differences in cocaine-induced locomotor activity between adolescent and adult female rats. While both adolescent and adult females had dramatic increases in locomotor activity in response to cocaine, a significant accentuation of cocaine-induced locomotor activity was present in adolescent female rats. In both ages, cocaine-induced locomotor activity remained significantly elevated from saline controls at the end of the trial. Furthermore, control animals of both ages showed comparable locomotor responsivity (i.e., saline-treated) over the entire test. The adult locomotor scores reported in the present study as a result of saline or cocaine administration are consistent with results reported in the literature using comparable methodology (Carey *et al.*, 2004). The idea that lower cocaine-induced locomotor activity shown by adult animals could be due to the presence of stereotyped behaviours was considered. Although the ‘Noldus’ system does not detect the occurrence of compulsive stereotyped behaviour, it does measure the frequency of movement which is elevated during stereotyped behaviours. An analysis of the frequency moving in the present study (data not shown) did not reveal any difference between cocaine-treated animals, indicating that cocaine-treated adult rats were not engaging in more frequent movements than adolescent rats.

The present study shows that adolescent females are hyper-responsive to cocaine’s effects compared to adult female rats, a finding which previously has not been shown. Since the goal of the study was to investigate cocaine-induced locomotor activity, the methodology was designed to control for the novelty of the test environment and the stress of an injection, both of which could affect locomotor activity. By evaluating how locomotor activity changes as a result of these factors a clear effect of cocaine-induced locomotor activity was observed. Prior studies have found adolescent animals to be hypo-responsive to DA agonists compared to adults (Lanier and Isaacson, 1977; Spear and Brick, 1979). There are a number of methodological factors that may account for this finding. The methods employed in this study are different than those investigations in that the animals were handled prior to manipulation and were placed in the apparatus for 20 minutes before receiving an injection in order to parse out the effect of the novel environment on behaviour. This is of critical importance as many studies have shown that psychostimulant administration in a novel environment results in greater activity than when the drug is administered in the home cage (Badiani *et al.*, 1995a; Badiani *et al.*, 1995b; Browman *et al.*, 1998a; Browman *et al.*, 1998b). The present study found that both ages were more active when initially placed in the novel test environment and no

significant differences were obtained between ages throughout the habituation period. Additionally, no significant differences in TDM were obtained across age as a result of the stress of a saline injection (administered at time point zero). The present work recorded behaviour via computer software, whereas previous investigations used grid crossings, a method which has been shown to be less sensitive than the Noldus system (Vila *et al.*, 2004). A novel environment, the injection or similar stressors appear to critically impact cocaine-induced behaviour and the method of recording locomotor activity could be important in detecting these differences. It is important to consider that controlling for these factors may play a significant role in the clear accentuation of cocaine-induced locomotor activity in adolescent female rats as observed in the present study.

In conclusion, this study shows that adolescent females are more responsive to cocaine's locomotor activating effects. An explanation for cocaine-induced hyperactivity as observed in adolescence compared to adults could lie in the fact that adolescent brains are still undergoing development which might involve differences in the responsiveness of the dopaminergic system to drugs of abuse. More investigations should be directed towards uncovering neural substrates underlying the unique behavioural effects of cocaine during adolescence.

Acknowledgements

This work was supported by a grant from NIDA: RO1DA14024.

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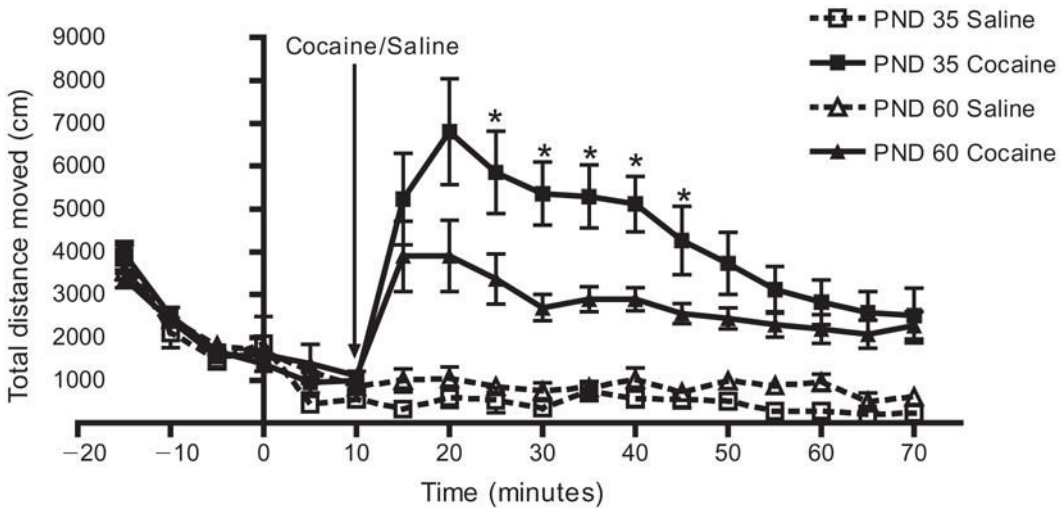

Figure 1. Locomotor differences between adolescent and adult female rats

Animals were placed in the test environment for 20 minutes before receiving saline IP (time point 0) followed by 20 mg/kg cocaine IP 10 minutes later (time point 10). Locomotor activity was measured by TDM (cm). PND 35 cocaine animals and PND 60 cocaine animals both show a significant increase in TDM compared to age-matched, saline-matched controls ($p < 0.05$). PND 35 animals showed an enhanced locomotor response compared to PND 60 ($p < 0.05$) (indicated by *)