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Handling alters cocaine-induced activity in adolescent but not adult male rats

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Abstract

The developmental period of adolescence is one that is characterized by increased levels of stress and vulnerability to drugs. Pre-test handling is an experimental manipulation that is used to acclimate animals prior to behavioral testing and exposure to a novel environment. Therefore, the present study was conducted in order to address the issue of pre-test handling of adolescent and adult male rats on subsequent cocaine-induced locomotor activity upon presentation to a novel environment. On days one through four, postnatal day (PND) 41–44 or PND 56–59, respectively, animals were handled b.i.d. for three minutes. On the fifth day, PND 45 or PND 60, animals were administered 30 mg/kg/ip cocaine or saline and immediately placed in a novel environment where locomotor activity was measured for 30 minutes. Cocaine increased locomotor activity similarly in all non-handled animals, regardless of age. Interestingly, adolescent animals expressed a differential effect when handled prior to an acute cocaine administration. Specifically, handling increased cocaine-induced locomotor activity in adolescent but not adult animals. These findings indicate that adolescent males that have been acclimated to the handling procedure experience significantly more behavioral reactivity than do adults to a high dose of cocaine upon exposure to a novel environment.

Keywords

Handling; Adolescent; Development; Cocaine; Locomotor activity; Novel environment

1. Introduction

It is well established that initiation of drug use in humans usually begins during adolescence. Illicit drug abuse is high among adolescents with a steady rise throughout the 1990s of frequent cocaine use among 12–17 year-olds, and remaining at stable levels in the early 2000s [1]. It has been estimated that among adolescents aged 12–17 there was a slight increase in the number of individuals who have ever used cocaine, from 2.3% in 2001 to 2.7% in 2002 [2]. The age of onset of drug-related behavior is an important predictor of later

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drug use [3,4]. From these trends in the number of adolescents involved in illicit drug use, namely the use of cocaine, it is important to further understand the influence that cocaine use can have on the developing adolescent.

Stress has been shown to alter responsivity to psychostimulant drugs, such as cocaine. It has been suggested that stress can increase the behavioral responsivity to psychostimulant drugs in animals and that these responses can modulate the rewarding effects of drugs of abuse [5–8]. Based on levels of locomotor activity animals separated into high- and low-responding groups differ in glucocorticoid levels, which can be altered pharmacologically among low-responder animals to elicit a high-responder behavioral profile [9]. Additionally, sensitivity to cocaine, as measured by rate of acquisition of self-administration, was increased among animals that were chronically exposed to stress [8]. This increase has been suggested to be dependent on the stress hormone, corticosterone, with levels required to rise above a “threshold critical for reward” [8]. That is, at lower doses, cocaine and corticosterone’s effects appear to be additive. Stress and psychostimulants appear to share and activate many of the same neurobiological substrates involved in the rewarding effects of drugs of abuse in adults [10]; however, this type of research using an adolescent animal model is limited.

Adolescence is a time of increased stress and anxiety. In an adolescent animal model using rodents, the periadolescent period can broadly be defined from postnatal day (PND) 28–42 [4]. Adolescence is also a period that is associated with increased risk to abuse drugs [4,11]. Periadolescent animals have been repeatedly described as possessing a “hyporesponsive” behavioral profile to an acute administration of psychostimulants [4,11–17]. It appears that adolescents are differentially affected by the locomotor activating effects of an acute administration of cocaine when compared to adult animals. Additionally, the effects of stress may modulate the behavioral response pattern elicited by adolescents to psychostimulant drugs.

Handling is an experimental manipulation that has been used either to acclimate animals prior to testing or to induce stress. Pre-test handling of male rats has been shown to exert an anxiolytic effect on locomotor activity and exploration [18–21], as well as alter subsequent responding in adult animals to footshock [22,23]. Importantly, handling of adolescent rats has been shown to result increased locomotor activity to amphetamine when compared to other types of stressors, such as chronic social stress [24]. Thus, handling appears to have profound effects on behavioral responsivity to multiple stimuli.

Since adolescence is a time of increased stress and anxiety as well as vulnerability to drug use, it would be interesting to determine whether a mild stressor, i.e., handling, differs in adolescent and adult behavioral responsiveness to an acute administration of cocaine. Therefore, the present study investigated the effects of prior handling on cocaine-induced locomotor activity in a novel environment in adolescent and adult male rats. The handling manipulation was expected to differentially affect locomotor activity when compared to non-handled animals.

2. Material and methods

2.1. Subjects

Sixty-four male Sprague–Dawley rats derived from established breeding pairs at the University of South Florida, Tampa (Harlan Laboratories, IN), were used in the present study. Litters were sexed and culled to 10 pups per litter on postnatal day (PND) 1, with the date of birth designated as PND 0. Pups were housed with their respective dams until PND 21 when they were weaned and housed in groups of three with same-sex littermates. The colony room was maintained in a humidity- and temperature-controlled vivarium on a 12:12 hour light/dark cycle, with lights on from 0700 to 1900 hr. Animals were allowed ad libitum access to food and water in the home cage. At the time of behavioral testing animals weighed between 180 and 345 g (PND 45, 180–247 g; PND 60, 285–345 g). Each animal was tested at only one age, PND 45 or PND 60. No more than one male pup per litter was used in any given condition. In all respects, maintenance and treatment of the animals were within the guidelines for animal care by the National Institutes of Health.

2.2. Apparatus

The apparatus consisted of an open circular field with a black Plexiglas floor ($D=96.5$ cm) and an opaque circular barrier made of Plexiglas ($H=45.7$ cm) in which animals were allowed free access to move about. A camera was suspended above the open field and movement (cm) of the animal was digitally recorded. This signal was tracked, quantified, and analyzed using an Ethovision video tracking system (Noldus Information Technology, Utrecht, Netherlands).

2.3. Procedure

Adolescent (PND 41–45) and young adult (PND 56–60) animals were randomly assigned to either the Handled (H) or Non-Handled (NH) treatment group. Handling was conducted in the colony room over a period of four consecutive days, (PND 41–44; PND 56–59) twice daily for three minutes (between 1000–1100 h, and 1600–1700 h, respectively). Handling consisted of removing an individual animal from the home cage and allowing it to sit and/or move about the experimenter's hands and arms for three minutes, after which the animal was returned to the home cage with littermates. Experimenters always wore a white lab coat to control for visual cues and gloves were not worn. Non-handled animals were left undisturbed until the target day, PND 45 or PND 60, except for regular cage maintenance, which occurred for all animals. On the target day, PND 45 or PND 60, animals were removed from the colony room, weighed, and administered an acute injection of either cocaine (30 mg/kg/ip; a dose which we have found to induce a significant conditioned place preference in PND 45 animals; unpublished data) or saline (0.9%), and immediately placed on the novel circular open field for 30 minutes and behavior recorded. All animals were challenged with an acute injection of cocaine between 10:00 a.m. and 2:00 p.m.

2.4. Design and analyses

Data for total distance moved (TDM; cm) for the entire 30 minute session were analyzed using a three factor between subjects ANOVA for Handling (2; Handled, Non-Handled),

Age (2; PND 45, PND 60) and Drug (2; saline, 30 mg/kg cocaine). Additionally, to conduct a detailed time course analysis, data for TDM were analyzed across the 30 minute trial in three-minute intervals using a four factor mixed-design ANOVA for Handling (2; Handled, Non-Handled), Age (2; PND 45, PND 60), Drug (2; 30 mg/kg cocaine, saline), with Time as a repeated measure. Subsequent planned comparisons (Fisher's protected least significant difference test, simple effects, and *t* tests) were used to isolate effects. Appropriate post hoc analyses were used to isolate handling effects (planned comparisons) and to control for familywise error.

3. Results

3.1. Collapsing across 30-minute trial

In general, cocaine increased TDM among all animals over the entire 30-minute period. A significant main effect of Drug was found among animals treated with cocaine as compared to saline-injected controls (Fig. 1; $F(1,56)=119.6$, $p<0.05$). Collapsing over the entire 30-minute period, cocaine-induced locomotor activity was similar in PND 45 and PND 60 animals. This effect was observed regardless of handling treatment. A significant main effect of Time was found ($F(9,504)=126.3$, $p<0.05$), with an increase in TDM in a novel environment.

3.2. Time course data (three-minute bins)

To further investigate the temporal effects of cocaine-induced locomotor activity on handling and age, data were analyzed across time in three-minute intervals. These data revealed a significant three-way interaction of Time by Age by Drug ($F(9,504)=2.4$, $p<0.05$). Subsequent post hoc analyses revealed that in a novel environment cocaine-treated adolescent animals increased TDM compared to cocaine-treated adult animals during minutes 9–18 of the trial. Cocaine-induced increases in TDM continued to be significantly elevated relative to saline controls even at the end of the 30-minute trial, as indicated by a significant Time by Drug interaction ($F(9,504)=2.8$, $p<0.05$). Furthermore, a trend for Time by Age ($F(9,504)=1.6$, $p=0.07$), indicating that adolescent animals had greater levels of locomotor activity relative to their adult counterparts, this effect is likely due to cocaine's effects given that saline animals did not appear to differ (see Fig. 2).

A priori planned comparisons for Handling were conducted because of the established anxiolytic properties handling has been shown to have on locomotor activity [18–21]. Repeated measures two-way ANOVA indicated no significant differences in cocaine-induced locomotor activity across time among NH animals, nor were there significant differences of age in TDM among NH-saline controls. In contrast, when adolescent and adult animals were handled prior to initial exposure to a novel environment, there was a significant increase in cocaine-induced locomotor activity among adolescent animals, relative to adult animals, from 6–18 minutes of the trial (Fig. 3, panel A—Non-Handled; panel B—Handled; $F(63,504)=2.47$, $p<0.05$). Additionally, subsequent planned comparisons indicated that in the first three minutes PND 60 H animals treated with saline, had a greater locomotor increase in a novel environment when compared to their NH counterparts. Taken

together, handling prior to an acute administration to cocaine appears to exert differential effects on cocaine-induced locomotor activity among adolescent and adult animals.

4. Discussion

Cocaine significantly increased TDM in comparison to controls regardless of age. All animals were significantly more active when initially placed in the novel environment in comparison to the end of the trial; however cocaine-induced increases in locomotor activity were still apparent at the end of the trial. Increased behavioral reactivity to stressful stimuli (e.g., novel environment) has been associated with an increased vulnerability to use drugs of abuse [5–8]. Stressors have also been suggested to increase locomotor responsivity to novelty and drugs of abuse, with increased levels of glucocorticoids being associated with increased rewarding effects of psychostimulants [5–8]. Additionally, glucocorticoids can increase the ability of cocaine to act as a positive reinforcer [5–8]. Locomotor activity is responsive to stress-related levels of glucocorticoids [9]. High doses of cocaine alone are able to activate the hypothalamic–pituitary–adrenal (HPA) axis, while lower doses only activate it in the presence of elevated plasma corticosterone, the two combined reaching a “threshold critical for reward” [8]. Any further increases in plasma corticosterone levels (e.g., mild stressor, such as a novel environment) would not affect the rewarding effects of cocaine [8]. Therefore, the present finding of increased levels of cocaine-induced locomotor activity may be indicative of increased reward being experienced by the animals in a novel environment.

Handling is a behavioral manipulation that is used prior to experimental regimens in order to acclimate animals to being manipulated by the experimenter. Handling has been shown to alter drug-induced locomotor responses [19]. In the present study, adolescent animals treated with cocaine were found to have greater increases in cocaine-induced locomotor activity relative to adult animals treated with cocaine. More specifically, handling increased cocaine-induced TDM among handled adolescent animals relative to their adult counterparts for several time points (e.g., 6–18 minutes after drug administration). Additionally, handling increased TDM at the first time point among saline treated handled and non-handled adults. Handling may have caused animals to have a greater locomotor response in a novel environment and they may be better able to habituate to the novel environment within the duration of the trial. Several researchers have suggested that animals that have been previously handled may be somewhat habituated to stress which they refer to as “fully adapted”, and that these same animals also express the greatest response to a novel stimulus [25]. Others found that previously handled animals exhibit a reduced neophobic response when tested in an open field [20], which may be due to an anxiolytic effect on open-field locomotor activity [18]. It may be that pre-test handling exerts a facilitatory reaction on locomotor activity when animals are presented with a novel environment.

Experiencing novelty in an inescapable novel environment may serve as a mild stressor to adult [26] and adolescent animals alike, and adolescent animals may be less neophobic. Stress-related hormones have been shown to modulate the effects of psychostimulants [5–8,24,26] and testing in an inescapable novel environment may potentiate this effect. Kabbaj et al. [24] have recently suggested that adolescent animals may be better able to cope with

mildly stressful stimuli, i.e., an inescapable novel environment. In a previous study, Adriani and Laviola [12] found that when adolescent animals were administered a high dose of amphetamine (10 mg/kg), they had greater increases in locomotor activity, relative to their adult counterparts. Similarly in the present study adolescent males that have been previously handled prior to an acute administration of a high dose (30 mg/kg) of cocaine were more responsive than adults. Therefore, when handled adolescent animals are administered a high dose of cocaine they express a hyperresponsive behavioral locomotor response when compared to their handled adult counterparts.

Adolescent animals are reported to have a unique behavioral profile in response to an acute administration of a psychostimulant. Periadolescent animals have been characterized as possessing a “hypo-responsive” locomotor reactivity to an acute psychostimulant administration [4,11–17]. The present study found a hyperresponsive behavioral response to an acute administration to cocaine only in handled adolescent (PND 45) animals. One of the main differences in the present study in comparison to many of these previous studies is that the hyperresponsive profile was found within the time course of a 30-minute session. The present study demonstrates the importance of considering the time course of cocaine’s effects when making comparisons across age and treatment. When data were examined over the entire trial it appeared that no significant differences were apparent regardless of age or treatment. Previous reports examined behavioral responsivity over the entire session and compared adolescents to adults and report a hypo-responsive behavioral response [12–14]. Collapsing the data across time in the present study reveals no differences in locomotor activity among adolescent and adult animals upon exposure to a novel environment. However, conducting a detailed time course analysis within the testing session more accurately reflects behavioral differences across age. This time course is important given that cocaine-induced activity in reward systems peak around this time interval in younger [27] and older animals [28,29].

It is possible that handling induced stress that was manifested in increased levels of locomotor activity in response to acute cocaine. This interpretation seems unlikely as previous data indicate that handling would increase cocaine-induced locomotor activity altogether in both aged-handled groups. Data from the present study do not support this notion of stress inducing increased levels of locomotor activity, as it was only among handled adolescent animals that a heightened locomotor response was evident when compared to handled adult animals. Finally, differences in locomotor activity among saline-control animals that were handled and not handled would be evident across the entire trial, and the present data show this is not the case.

Stress may be the modulating factor for the hypo-responsivity commonly observed in periadolescent animals. Indeed, it has very recently been proposed that the level of stress experienced by the periadolescent animal in a novel environment will contribute to the characteristic hypo-responsivity of adolescent animals [3]. When animals are tested in a familiar environment, this characteristic hypo-responsivity disappears. It has further been suggested that the behavioral pattern of periadolescent animals may even be enhanced in less-stressful conditions [3]. Prior handling may change the stress associated with an inescapable novel environment in periadolescent animals. This change in the associated

stress with an acute cocaine administration would thus manifest itself in a “hyperresponsive” behavioral profile, as shown in the present study.

Adolescent animals that have been handled may be somewhat habituated to stress and experience anxiolytic effects of the handling manipulation. Because animals at this age have been suggested to have better “coping strategies,” these animals may be better able to deal with the mild stress of a novel environment. Additionally, because stress has been shown to modulate the rewarding properties of psychostimulants, adolescent animals may have a greater reward-activated locomotor response to an acute administration of a high dose of cocaine. All of these factors may contribute to the increased vulnerability of adolescents to initiate drug use during this period of development. Future research should assess if this behavioral pattern is also evident throughout periadolescence.

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Fig. 1.

Total Distance Moved collapsed across the entire 30-minute session in PND 45 and PND 60 animals. Data indicate that overall cocaine-induced locomotor activity is similar in PND 45 and PND 60 animals when collapsed across the entire 30-minute period. *Indicates significantly differs from saline controls.

Fig. 2.

Total Distance Moved in three-minute intervals for PND 45 and PND 60 animals. A significant three way interaction (Time by Age by Drug) revealed significant differences of cocaine-induced TDM of PND 45 animals relative to PND 60 animals from 9–18 minutes of the trial. *Indicates significant difference between PND 45 cocaine animals from PND 60 cocaine animals.

Fig. 3.

Panel A: Total Distance Moved in three-minute intervals for PND 45 and PND 60 Non-Handled animals. Panel B: Total Distance Moved in three-minute intervals for PND 45 and PND 60 Handled animals. Data indicate that adolescent (PND 45) animals that are handled prior to exposure to an inescapable novel environment exhibit a significant increase in TDM from 6 to 18 minutes of the trial, when compared to their adult (PND 60) handled counterparts. At 12 minutes, NH PND 60 animals exhibited significantly more cocaine-induced locomotor activity than H PND 60 animals. #Indicates significant difference of cocaine-induced locomotor activity between Handled PND 45 animals and Handled PND 60 animals (6–18 minutes of trial).