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Testosterone differentially alters cocaine-induced ambulatory and rearing behavioral responses in adult and adolescent rats

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ABSTRACT

Little is known about the physiological and behavioral effects of testosterone when co-administered with cocaine during adolescence. The present study aimed to determine whether exogenous testosterone administration differentially alters psychomotor responses to cocaine in adolescent and adult male rats. To this end, intact adolescent (30-days-old) and adult (60-day-old) male Fisher rats were pretreated with vehicle (sesame oil) or testosterone (5 or 10 mg/kg) 45 min prior to saline or cocaine (20 mg/kg) administration. Behavioral responses were monitored 1 h after drug treatment, and serum testosterone levels were determined. Serum testosterone levels were affected by age: saline- and cocaine-treated adults in the vehicle groups had higher serum testosterone levels than adolescent rats, but after co-administration of testosterone the adolescent rats had higher serum testosterone levels than the adults. Pretreatment with testosterone affected baseline activity in adolescent rats: 5 mg/kg of testosterone increased both rearing and ambulatory behaviors in saline-treated adolescent rats. After normalizing data to % saline, an interaction between hormone administration and cocaine-induced behavioral responses was observed; 5 mg/kg of testosterone decreased both ambulatory and rearing behaviors among adolescents whereas 10 mg/kg of testosterone decreased only rearing behaviors. Testosterone pretreatment did not alter cocaine-induced behavioral responses in adult rats. These findings suggest that adolescents are more sensitive than adults to an interaction between testosterone and cocaine, and, indirectly, suggest that androgen abuse may lessen cocaine-induced behavioral responses in younger cocaine users.

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1. Introduction

Adolescence is a critical period for the development of drug addiction in humans; most illicit drug use initiation begins between the ages of 12 and 20 (DeWit et al., 1997). From 2002 to 2008 abuse of both cocaine and anabolic–androgenic steroids (AAS) remained stable in this age group (www.NIDA.gov). According to the National Institute on Drug Abuse (2007) adolescents under age 18 account for 34% of new cocaine initiates. The fact that adolescents are more likely than adults to progress quickly from recreational cocaine use to dependence (Chen and Kandel, 1995, 2002; Reboussin and Anthony, 2006) suggests developmental differences in vulnerability to cocaine addiction. The abuse of AAS, which are synthetic derivatives of testosterone, has spread from elite Olympic, professional, college, or high school athletes to the general population (Buckley et al., 1988; Wilson, 1988). AAS abuse in non-athletes is particularly common among adolescent boys. For example, 2.5% of high school seniors reported the use of AAS (www.NIDA.gov). Among adolescents, cocaine was the drug most likely to be co-abused by AAS abusers — 33% of AAS users also reported using cocaine (DuRant et al., 1993). This co-abuse is also seen in adult men. A recent study of 227 men treated for cocaine dependence found that 9.3% were also steroid abusers (National Institute on Drug Abuse, 2004), with many of them indicating that their initial use of cocaine had been in an effort to minimize the negative effects of steroids, including insomnia, depression, and irritability (National Institute on Drug Abuse, 2004).

Although developmental differences in cocaine reward have been consistently reported, data on how development affects psychomotor responses to cocaine are less consistent. For example, while age differences in locomotor responses to acute cocaine administration have consistently been shown using low cocaine doses, several studies using high cocaine doses have shown a lack of age-related differences in cocaine behavioral responses (Maldonado and Kirstein, 2005; Caster et al., 2005; Collins and Izenwasser, 2002; Parylak et al., 2008). Chambers et al. (2003), Laviola et al. (1995), and Caster and Kuhn (2009) have postulated that this difference could be relevant to human addiction as the onset of drug taking early in adolescence is associated with higher abuse rates than onset later in adolescence or adulthood.

The confounding effects of cocaine and AAS co-addiction have yet to be determined. We know that testosterone inhibits electroencephalogram output and the behavioral effects of acute cocaine administration

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(van Luijtelaar et al., 1996). We also know that chronic exogenous testosterone administration prevents the enhancement of cocaine-induced locomotor activity produced acutely in adult male rats (Long et al., 1994; Martinez-Sanchis et al., 2002, and Salvador et al., 2000) and delays and reduces cocaine-induced stereotypic behavior in castrated rats (Chen et al., 2003; Chin et al., 2002). Cocaine administration has also been shown to decrease circulating serum testosterone levels (Festa et al., 2003). Although both substances individually lead to complex behavioral responses, little is known about the physiological and behavioral effects of testosterone when co-administered with cocaine during adolescence. The present study aimed to determine whether adolescent male rats differed from adult male rats in their behavioral responses to cocaine and to explore the extent to which co-administration of testosterone affected cocaine-induced responses. We postulate that in adolescent rats, because of developmental differences in testosterone metabolism, co-administration of testosterone and cocaine produces a more robust behavioral response than that in adult males.

2. Methods

2.1. Animals

Adult (60 days) and adolescent (30 days) intact male Fischer rats (Charles River Laboratories, Kingston, NY) were singly housed in animal cages with free access to food and water. Rats were maintained on a 12-h light/dark cycle (lights on at 9:00 a.m.) and handled daily after their arrival. Animals were randomly assigned to either saline or cocaine treatment groups and further subdivided into groups receiving either testosterone or vehicle. Each experimental group had a total of $n=8$ to 12 animals. All National Institutes of Health (NIH) and Institutional Animal Care and Use Committee (IACUC) guidelines for the care and use of laboratory animals were strictly followed.

2.2. Drug and hormone administration

Chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Forty-five minutes before cocaine or saline administration, rats received testosterone propionate (5 or 10 mg/kg) or vehicle (sesame oil) via subcutaneous injection. These two testosterone doses and the timing of their administration were chosen on the basis of previous studies showing that they produce the most robust locomotor effects (Festa et al., 2003). Cocaine was prepared daily in 0.9% saline solution and administered via intraperitoneal injection at a 20 mg/kg dose. This dose was chosen because it has reliably produced a significant increase in locomotor activity in male rats without producing a maximal locomotor responses (Festa and Quinones-Jenab, 2004; Festa et al., 2003, 2004).

2.3. Behavioral responses to cocaine

Measurements of behavioral responses were performed in the rats' home cages for 60 min after drug treatment. Locomotive activity was monitored with a Photobeam Activity System from San Diego Instruments (CA), as previously described (Niyomchai et al., 2005). Ambulation counts are produced by the interruption of two consecutive photobeams in the horizontal frame. Rearing counts represent vertical motion.

To assess stereotypic activity, rats were videotaped for 30 s, 30 min after cocaine or saline administration. The videotapes were later analyzed for behavioral stereotypy by three trained observers blinded to each animal's treatment group. The rating for cocaine-induced stereotypic behavior was based on a modification of the Creese and Iversen scale (1974). As summarized in Table 1, this scale consists of 10 scores ranging from 1, given to an animal that was asleep or inactive, to 10, given to an animal that exhibited splayed hind limbs. In the present study, a score of 10 was never warranted.

Table 1
Rating scale from Daunais and McGinty (1995).

Score	Behavior
1	Asleep, inactive
2	Alert, actively grooming
3	Increased sniffing in one location
4	Intermittent rearing and sniffing
5	Increased locomotion and sniffing
6	Intense sniffing in one location
7	Continuous pivoting and sniffing
8	Continuous rearing and sniffing
9	Maintained rearing and sniffing for >25 s
10	Splayed hind limbs

2.4. Testosterone radioimmunoassay

Animals were sacrificed by rapid decapitation after a brief (20-s) exposure to CO_2. Trunk blood was collected and centrifuged (at 3000 rpm for 30 min at 4 °C), and serum was extracted and stored at −80 °C until used. Serum testosterone levels were determined with a Coat-A-Count radioimmunoassay kit from Diagnostic Product Corporation (Los Angeles, CA). Intra-assay coefficient of variance averaged less than 10%. Results were determined using a log–logit analysis within GRAPHPAD PRISM (GraphPad Software, CA). Serum testosterone levels were expressed in ng/ml.

2.5. Data analysis

Behavioral response data and serum testosterone levels are presented as mean ± SEM. Stereotypic data are presented as median score ± semi-interquartile range. To analyze locomotor behaviors, because of possible baseline effects, ANOVAS were used to determine the effects of cocaine and hormone on behavioral responses within each age group as follows: Drug (saline or cocaine) × Testosterone dose (0, 5, and 10). Differences in serum testosterone levels and behavioral responses (once data were normalized to %-saline) were analyzed via a three-way ANOVA: drug (saline or cocaine) × testosterone dose (0, 5, and 10) × age (adult or adolescent). For time course analysis, behavioral responses were analyzed using three-way repeated measures analysis of variance (ANOVAs) for the variables age (adult or adolescent), drug (saline or cocaine), and time (18 5-min time blocks for acute cocaine/saline). Fisher LSD post hoc tests were used when appropriate. For stereotypic behavior, a Kruskal–Wallis test followed by a Dunn's post hoc analysis was used to assess the effects of testosterone dose on drug treatment. A p-value of <0.05 was considered significant in all statistical analyses.

3. Results

3.1. Effects of acute testosterone administration on serum testosterone levels

As shown in Fig. 1, in vehicle-treated controls, adults had higher serum testosterone levels than did adolescent rats ($F(2, 83)=187$, $p<0.01$). Furthermore, in both adult and adolescent rats serum testosterone levels were increased dose-dependently after testosterone administration ($F(2,83)=440.41$, $p<0.001$). A significant interaction of testosterone dose and age was observed ($F(2, 83)=235.00$, $p<0.001$). Serum testosterone levels in adolescent rats treated with 5 or 10 mg/kg testosterone plus saline were higher than in any other group ($p<0.05$ for all comparisons). A significant interaction between testosterone dose and drug treatment (saline or cocaine) was also observed ($F(2, 83)=50.48$, $p<0.05$). Serum testosterone levels were higher in cocaine-treated adult rats after 5 mg/kg testosterone than they were in adult rats treated

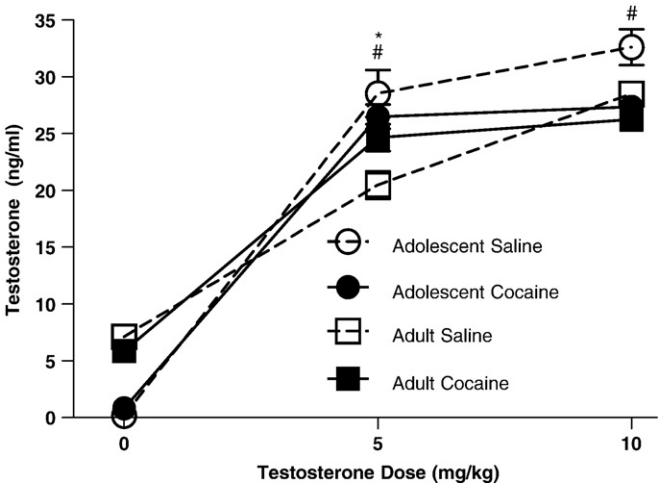

Fig. 1. Serum testosterone levels after acute testosterone administration. Data represent mean ± SEM serum levels of testosterone for both adult (square symbols) and adolescent (circular symbols) male rats in cocaine-treated (solid line, solid symbols) or saline-treated (dotted line, empty symbols) groups. *Represents statistically significant differences between saline and cocaine treatments. #Represents statistically significant differences between adolescent and adult rats. $N = 8–12$ per group.

with saline plus 5 mg/kg testosterone, but serum testosterone levels were lower in adolescent rats treated with cocaine plus 10 mg/kg testosterone than in either of their respective controls ($p < 0.05$ for all comparisons).

3.2. Effects of acute testosterone administration on ambulatory, rearing, and stereotypic behaviors

As shown in Fig. 2, regardless of the animal's age, cocaine increased overall ambulatory and rearing counts in rats ($F(1, 91) = 62.230$, $p < 0.01$; $F(1, 91) = 91.371$, $p < 0.01$, respectively). However, although in both age groups cocaine-induced behavioral responses across time (10 to 60 min; $p < 0.05$ for all comparisons), no statistically significant differences were observed between adolescent and adult rats. As shown in Fig. 3A and C, testosterone administration affected baseline ambulatory and rearing responses in adolescent rats ($F(2, 24) = 34.841$, $p < 0.01$; $F(2, 24) = 45.672$, $p < 0.01$, respectively); administration of 5 mg/kg of testosterone increased ambulatory and rearing responses in saline-treated adolescent rats as compared with the 0 and 10 mg/kg testosterone plus saline-treated groups ($p < 0.05$ for all comparisons).

To compensate for this baseline effect, data were normalized to percent control (Fig. 3B and D). In adolescent rats, testosterone dose-dependently altered the magnitude of cocaine-induced ambulatory and rearing responses ($F(2, 24) = 3.925$, $p < 0.05$; $F(2, 24) = 8.013$, $p < 0.05$, respectively; Fig. 3B and D). Five mg/kg testosterone reduced cocaine-induced ambulatory responses in adolescent rats, but 10 mg/kg testosterone significantly increased cocaine-induced ambulatory response as compared with the 5 mg/kg dose ($p < 0.05$ for all comparisons). On the other hand, adolescents who received either 5 or 10 mg/kg of testosterone had significantly lower rearing responses than did vehicle-treated rats.

Regardless of the dose, testosterone did not significantly alter cocaine-induced behavioral responses in adult rats. However, testosterone administration differentially affected the magnitude of cocaine-induced rearing responses between adult and adolescent rats. Adolescent rats treated with vehicle and cocaine had significantly higher %-change in rearing responses than did adults receiving the same treatment ($F(1, 16) = 10.938$, $p < 0.05$). The %-change in ambulatory responses was also higher in adolescents than adults, but this value fail to reach statistical significance. Although cocaine increased stereotypic scores ($F(1, 16) = 13.392$, $p < 0.01$; Fig. 4), no statistically significant

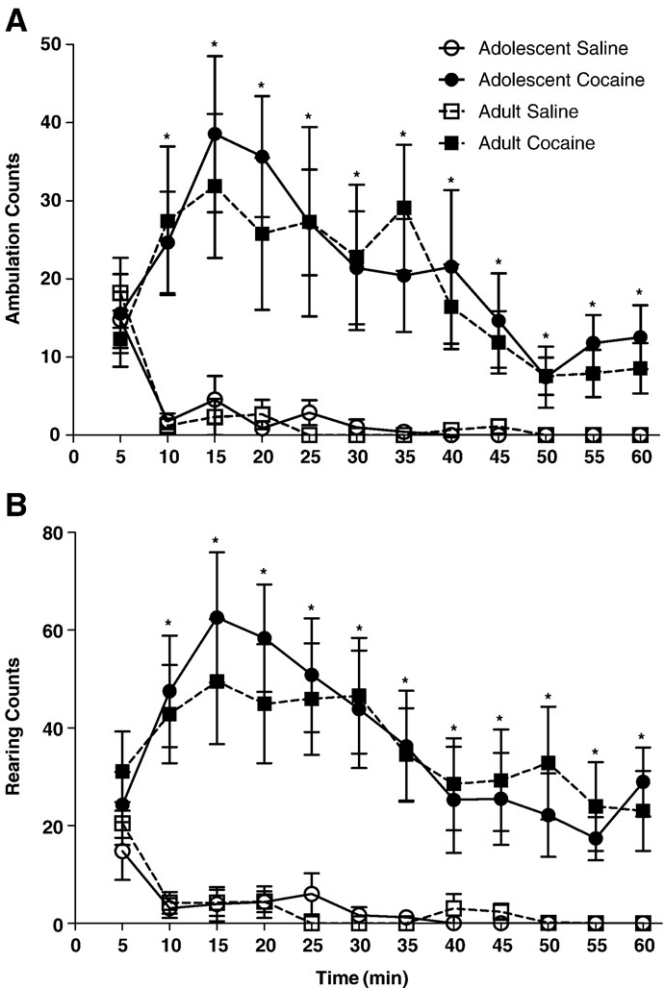

Fig. 2. Time course analysis of ambulatory (A) and rearing counts (B) in vehicle-treated adult and adolescent intact male rats after cocaine administration. Data represent mean ± SEM behavioral responses over a 1-h period (5-min intervals) in adult (square symbols) and adolescent (circular symbols) male rats in cocaine-treated (solid line, solid symbols) or saline-treated (dotted line, empty symbols) groups. *Represents statistically significant differences between saline and cocaine treatments. $N = 8–12$ per group.

differences in the effects of testosterone were observed across any of the experimental groups or between ages.

4. Discussion

The goal of our study was to determine whether adolescent male rats differed from adult male rats in their behavioral responses to cocaine and to explore the extent to which co-administration of testosterone affected cocaine-induced responses. Our results demonstrated that the magnitude of rearing responses in adolescent rats was higher than that of adult rats. Furthermore, testosterone affected cocaine-induced locomotor, but not stereotypic responses in a dose dependent manner only in adolescent rats. Taken together, these findings support the postulate that co-administration of testosterone and cocaine has more profound effects in adolescent than in adult male rats. However, a wider range of testosterone doses should be tested in the future to better establish the efficacy of testosterone in altering cocaine responses in adolescents.

Consistent with Maldonado and Kirstein (2005), cocaine's effects on locomotor responses did not differ between adolescent and adult rats, although it should be noted that their study used postnatal day (PN) 45 rats. Others have also shown no differences between adolescent and adult rats in cocaine-induced responses. For example, adolescent male rats starting on PN 37 acquired cocaine self-administration to the same

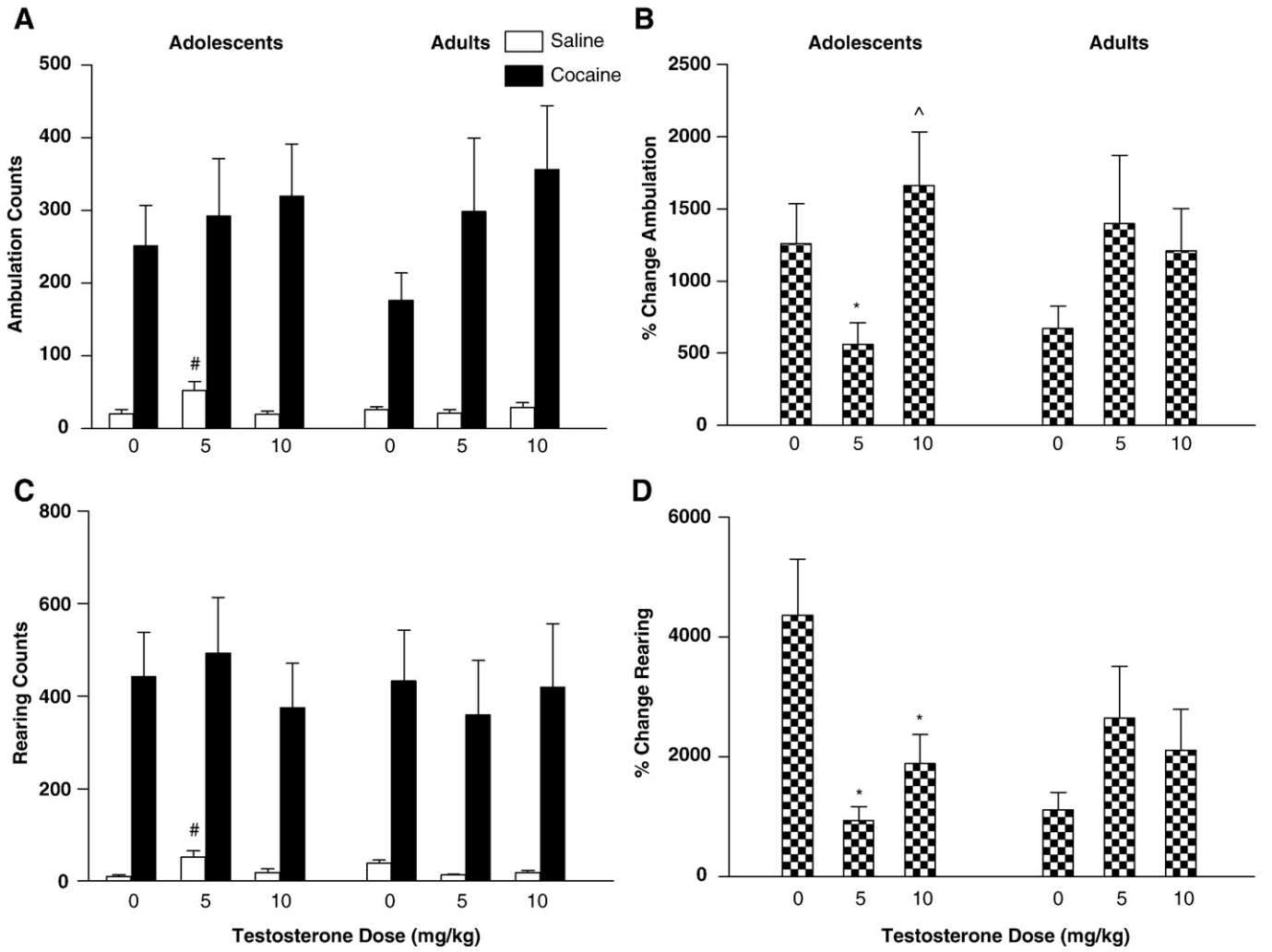

Fig. 3. Ambulatory (A, B) and rearing (C, D) behavioral responses in adult and adolescent intact male rats after acute testosterone and cocaine administration. Data in A and C represent the sum of mean ± SEM behavioral responses across 1 h after saline (white bars) or cocaine (solid bars) treatment. Data in C and D represent mean %-change ± SEM behavioral responses. Behavior was recorded for 1 h after drug treatment. ^Represents statistically significant differences between testosterone doses. *Represents statistically significant differences of %-change between testosterone doses. #Represents statistically significant differences between saline plus testosterone treated groups. $N = 8–12$ per group.

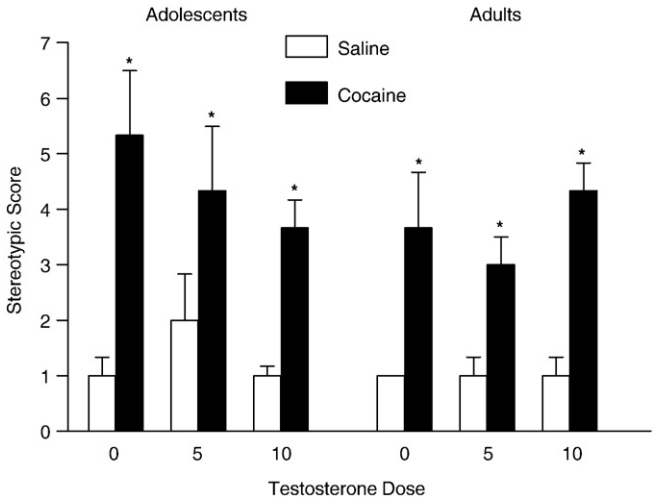

Fig. 4. Stereotypic activity, expressed as median stereotypic scores for adolescent and adult male rats after acute testosterone and cocaine administration. Activity was recorded for 30 s, 30 min after cocaine injection. Data represent stereotypic score as determined by three blinded observers for saline-treated (white bars) and cocaine-treated (black bars) animals. *Represents statistically significant differences between saline and cocaine treatments. $N = 8–12$ per group.

degree as an adult male rat under fixed ratio (FR) 1 response requirements (Belluzzi et al., 2005; Frantz et al., 2007). Kantak et al. (2007) showed that both PN 37 and adult rats have similar infusion across different cocaine doses and FR response requirements. However, younger adolescent rats (PN 28) have greater locomotor responses than either adults (Caster et al., 2005, 2007; Caster and Kuhn, 2009; Badanich et al., 2008) or mid-adolescent rats (PN 35; Badanich et al., 2008). Thus, at later adolescent stages, little difference in cocaine sensitivity between adolescent and adult males has been reported. However, even at young adolescent ages no differences in locomotor responses between these adolescents and adults are seen at higher doses of cocaine (ranging from 25 to 40 mg/kg; Caster et al., 2005; Collins and Izenwasser, 2002; Badanich et al., 2008; Caster and Kuhn, 2009). Thus, the lack of differences between adolescents and adults in our study may also rely on the higher cocaine doses used in this investigation.

Data analysis to determine the magnitude of change showed greater changes in rearing responses in adolescent than adult rats. Because rearing-response measurements are related to anxiety levels, it is feasible that age may impact cocaine's anxiolytic effects. However, Laviola et al. (1995) showed lower stereotypic activity, including rearing behaviors, between late adolescent (PN 35 to 39) and adult male rats, whereas we did not see changes in stereotypic activity between adolescent and adult rats. Thus, although their study and ours use similar cocaine doses,

the findings are contradictory. It is possible that strain differences, adolescent age (PN 30 vs. PN 35–39), and/or pretreatment with vehicle prior to cocaine may contribute to the studies' different results. Alternatively, contradictions between our study and that of Laviola et al. (1995) may reside in differences in the behavioral scales that were used; the Laviola et al. (1995) scale is more diverse than ours.

Long et al. (1994) reported that testosterone implantation in adult Wistar rats reduced cocaine-induced locomotor responses. Conversely, Martinez-Sanchis et al. (2002) found that the administration of exogenous testosterone resulted in an increase in cocaine-induced locomotor activity in Swiss–Webster mice. In our study, testosterone administration in adult male rats had little effect on ambulatory, rearing, or stereotypic responses. However, in adolescent male rats, testosterone administration altered behavioral baseline activity as well as cocaine-induced behavioral responses. The effects of testosterone were dose dependent, suggesting a receptor-mediated response. Furthermore, the dose response curve was non-monotonic, suggesting a dynamic interaction between testosterone doses and behavioral responses to cocaine.

As expected, in vehicle-treated controls, adults had higher serum testosterone levels than did adolescent rats. However, after exogenous administration of this gonadal hormone, adolescent rats had higher serum testosterone levels than adult rats. From PN 1 to adulthood there are gradual changes in the ontogenesis and enzyme activities of major cytochrome P450 (P450) monooxygenase isoforms and other key steroid-metabolizing enzyme changes (Borlak et al., 2004; Hulla and Juchau, 1989). Because these enzymes are at higher levels during adulthood, adult rats may be able to metabolize exogenous testosterone at a faster rate than adolescent rats. The level of 5-α-reductase isoenzymes, which catalyze the conversion of testosterone to the more potent androgen-DHT in the testes, peaks between PN 20 and 40. Therefore, given that 5-α-reductase isoenzymes are functional in PN 30 adolescent rats (Killian et al., 2003), metabolism of testosterone by the liver is likely to contribute to the observed differences in serum testosterone levels between adolescent and adult rats. It is feasible that testosterone's baseline effects on ambulation and rearing responses are in part mediated by the higher serum levels of testosterone in adolescent rats.

Cocaine has been shown to be hepatotoxic in rats and to affect liver enzymatic activity (Ndikum-Moffor and Roberts, 2003). Indeed, Vitcheva and Mitcheva (2007) found that cocaine treatment significantly decreased P450 in the liver by 17% as compared with controls, suggesting that cocaine may affect serum testosterone levels. Although it is not clear how cocaine affects liver function in adolescent rats, differences in serum testosterone levels between saline- and cocaine-treated adolescent rats may in part be due to cocaine's effects on liver enzymes.

On the basis of our results, we postulate that in humans co-administration of testosterone and cocaine may have detrimental effects. For example, in adolescents attenuation of cocaine's desired behavioral effects by testosterone and/or decreases in serum testosterone levels by cocaine may lead to overdoses of cocaine and/or testosterone as well as other clinical complications. Thus, adolescents may use higher doses of cocaine or testosterone to achieve greater subjective effects of cocaine or the desired physiological responses to testosterone (i.e., increased muscle mass). Finally, normal spermatogenesis requires physiological levels of testosterone — testosterone interacts with androgen receptors in Sertoli cells to activate specific genes necessary for the differentiation process in spermatogenesis (Griffin, 2004). Thus, down-regulation of serum testosterone levels by cocaine may lead to reproductive and developmental consequences for the cocaine-using adolescent. These important clinical issues in young males need further investigation.

Epidemiological studies have indicated that adolescents are more likely than adults to progress quickly from recreational cocaine use to dependence (Chen and Kandel, 1995; Kandel et al., 1997; Reboussin and Anthony, 2006), and adolescents have a high rate of co-dependence of testosterone and cocaine abuse. Our preclinical data suggest that the consequences of co-administration of cocaine and testosterone may differ according to the subject's developmental stage. In fact, age-related differences in the interaction between cocaine and testosterone may affect vulnerability to cocaine and its neurophysiological adaptations. Further studies are needed to determine the long-term effects of chronic testosterone and/or AAS and cocaine in adolescents as compared with adults. However, based on our results we postulate that adolescent males, due to their differential metabolism of exogenous testosterone and response to cocaine, may have susceptibility for using high doses of cocaine given that testosterone inhibits some desired cocaine-induced responses.

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