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Ontogeny of cocaine-induced behaviors and cocaine pharmacokinetics in male and female neonatal, preweanling, and adult rats

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Abstract

Rationale: Ontogenetic differences in the behavioral responsiveness to cocaine have often been attributed to the maturation of dopaminergic elements (e.g., dopamine transporters, D2^{High} receptors, receptor coupling, etc.).

Objective: The purpose of this study was to determine whether ontogenetic changes in cocaine pharmacokinetics might contribute to age-dependent differences in behavioral responsiveness.

Methods: Male and female neonatal (PD 5), preweanling (PD 10 and PD 20), and adult (PD 70) rats were injected (IP) with cocaine or saline and various behaviors (e.g., locomotor activity, forelimb paddle, vertical activity, head-down sniffing, etc.) were measured for 90 min. In a separate experiment, the dorsal striata of young and adult rats were removed at 10 time points after IP cocaine administration. Peak cocaine values, cocaine half-life, and dopamine levels were determined using HPLC.

Results: When converted to percent of saline controls, PD 5 and PD 10 rats were generally more sensitive to cocaine than older rats, but this effect varied according to the behavior being assessed. Peak cocaine values did not differ according to age or sex, but cocaine half-life in brain was approximately 2× longer in PD 5 and PD 10 rats than adults. Cocaine pharmacokinetics did not differ between PD 20 and PD 70 rats.

Conclusions: Differences in the cocaine-induced behavioral responsiveness of very young rats (PD 5 and PD 10) and adults may be attributable, at least in part, to pharmacokinetic factors; whereas, age-dependent behavioral differences between the late preweanling period and adulthood cannot readily be ascribed to cocaine pharmacokinetics.

Keywords

Cocaine; Behavior; Ontogeny; Pharmacokinetics; Half-Life

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Conflict of Interest

All authors declare no conflict of interest.

Introduction

The ontogeny of behavioral sensitivity to cocaine and other psychostimulants has been studied extensively in adolescent and adult rats (for reviews, see Spear and Brake 1983; Spear 2000); however, much less attention has been given to neonatal and preweanling rats. Initial studies indicated that the ontogeny of behavioral responsiveness to psychostimulants can be represented by a U-shaped curve, with preweanling and adult rats showing robust cocaine-induced locomotor activity while adolescent rats exhibit a more muted response (Spear 1979; Spear and Brake 1983; see also Bolanos et al. 1998; Frantz et al. 2006). In contrast, more recent studies suggest that acute cocaine treatment causes hyperresponsiveness, rather than hyporesponsiveness, in adolescent rats (Caster et al. 2005; Catlow and Kirstein 2005; Badanich et al. 2008). Our analysis of this phenomenon suggests a somewhat different pattern of drug action, as we reported that preweanling rats tested on postnatal day (PD) 20 were more sensitive to the effects of cocaine than adult rats, while adolescent rats showed less sensitivity to cocaine than the other age groups (McDougall et al. 2015). Importantly, we converted our data to percent of same-age saline controls (see also Badanich et al. 2008; White et al. 2008; Koek et al. 2012), which produces a different pattern of drug effects than when raw scores are analyzed (McDougall et al. 2015).

Across early ontogeny, some dopamine (DA) mediated behaviors (e.g., roll curling) are present during the neonatal period and disappear with increasing age, while other behaviors (e.g., rearing) are initially absent and only emerge as the animal matures (Spear and Brick 1979; Moody and Spear 1992). Still other behaviors (e.g., head-up sniffing) are apparent at an early age and show quantitative changes across early ontogeny and into adulthood. The manifestation of these behaviors is often differentially affected by psychostimulant administration. The reason for these age-dependent behavioral differences has often been attributed to the maturation of dopaminergic elements (e.g., DA transporters, D₂^{High} receptors, receptor coupling, neurotransmitter levels, etc.) presumed to contribute to the neural mechanisms underlying the expression of the behavior (e.g., see Lin and Walters 1994; Grigoriadis et al. 1996; Andersen 2003; Walker et al. 2010; McDougall et al. 2015). Less frequently discussed is the potential impact of drug pharmacokinetics on the ontogeny of psychostimulant action (for exceptions, see Walker et al. 2010; McDougall et al. 2011). Unfortunately, few studies have systematically examined the pharmacokinetics of psychostimulant drugs across early ontogeny; however, in a critical exception, Lal and Feldmüller (1975) reported that the brain half-life of d-amphetamine was over twice as long in preweanling rats (PD 12) than adults. The results of this study suggest that pharmacokinetic factors like drug half-life may contribute to ontogenetic differences in cocaine responsiveness.

The purpose of the present study was to do a detailed behavioral assessment of male and female neonatal (PD 5), preweanling (PD 10 and PD 20), and young adult (PD 70) rats after a single injection of saline or 15 mg/kg cocaine. Various behaviors were assessed [e.g., locomotor activity, forelimb paddle, head-up sniffing, vertical activity, head-down sniffing (a measure of stereotypy), grooming, etc.] and data were presented untransformed and as percent of same-age/same-sex saline controls. In a separate experiment, the peak values and half-life of cocaine and benzoylecgonine (an active metabolite of cocaine) were measured in

the dorsal striatum of male and female PD 5, PD 10, PD 20, and PD 70 rats at 10 different time points. The relationship between cocaine-induced locomotor activity and peak striatal cocaine values at the different ages was compared.

Materials and methods

Subjects

Subjects were 658 male and female Sprague-Dawley rats. Young rats (males, $N = 228$; females, $N = 228$) were born and bred at California State University, San Bernardino (CSUSB). Litters were culled to 10 pups on postnatal day (PD) 3. Male ($N = 96$) and female ($N = 106$) adult rats were purchased from Charles River (Hollister, CA). Adult rats were allowed to acclimate to the CSUSB vivarium for a minimum of 14 days before behavioral testing. Adult rats were group housed with conspecifics, whereas preweanling rats were kept with the dam and littermates. Food and water were freely available. The colony room was maintained at 22–23°C and kept under a 12 L:12 D cycle. Subjects were cared for according to the “Guide for the Care and Use of Laboratory Animals” (National Research Council 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

For preweanling (PD 9–10 and PD 19–20) and adult (PD 69–70) rats, behavioral testing was done in activity monitoring chambers that consisted of acrylic walls, a plastic floor, and an open top (Coulbourn Instruments, Whitehall, PA). Each chamber included an X–Y photobeam array, with 16 photocells and detectors, that was used to determine distance traveled (a measure of locomotor activity) in the margin and the center of the activity monitoring chambers. In order to equate for differences in body size (see also Campbell et al. 1969; Shalaby and Spear 1980), preweanling rats were tested in smaller chambers (26 × 26 × 41 cm) than adult rats (41 × 41 × 41 cm). For neonatal rats (PD 4–5), behavioral testing occurred in clear 12 cm diameter cylinders (Hall 1979; Moody and Spear 1992) located inside clear Wessels Warming Chambers (Braintree Scientific, Braintree, MA).

Drugs

For the behavioral and pharmacokinetic experiments, (–)-cocaine hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in saline and injected intraperitoneally (IP) at a volume of 6 ml/kg (PD 5), 4 ml/kg (PD 10), 2 ml/kg (PD 20), or 1 ml/kg (PD 70). In the pharmacokinetic experiment, separate groups of PD 20 rats were injected subcutaneously (SC) with saline or cocaine. Cocaine propyl ester hydrochloride was used as the standard (Sigma-Aldrich).

Behavioral procedures

On the habituation day, male and female rats (PD 4, PD 9, PD 19, or PD 69) were weighed and placed in holding cages for 30 min. Rats were then taken to the experimental room, injected with saline, and placed in the testing chambers for an additional 30 min. When in either the holding cages or test chambers, PD 4–5 rats were maintained at 34 °C (Hall 1979),

whereas PD 9–10 rats were maintained at 30 °C (Campbell et al. 1969). Rats tested at PD 19–20 or PD 69–70 were kept at room temperature.

After 24 h (i.e., on PD 5, PD 10, PD 20, and PD 70), the same basic procedure was repeated, except that rats ($n = 8$ per group) were injected with saline or 15 mg/kg cocaine immediately before being placed in the testing chambers for 90 min. In addition to continuously measuring distance traveled in the margin and center of the testing chambers, various discrete behaviors were quantified using the fixed interval momentary time sampling method described by Cameron et al. (1988). For a given rat, the presence or absence of each behavior was determined during a 15 s interval that occurred every 2 min for the entire 90-min testing session. The behaviors monitored were based on studies conducted by Spear and colleagues (Spear and Brick 1979; Moody and Spear 1992), and included vertical activity (wall climbing and rearing), grooming, head-up sniffing, head-down sniffing (a measure of stereotypy), mouthing, licking, pivoting, head lift, roll/curl, and forelimb paddle. In all cases, behaviors were quantified by observers blind to treatment conditions.

Cocaine pharmacokinetics and dorsal striatal DA levels

On PD 5, PD 10, PD 20, and PD 70, rats were injected (IP) with 15 mg/kg cocaine and returned to their home cages if time permitted. After various time intervals (0, 2.5, 5, 10, 20, 30, 60, 90, 150, or 210 min), rats were killed by rapid decapitation and dorsal striatal sections were dissected bilaterally on an ice-cold dissection plate and stored at –80 °C. In order to detect possible sex differences in cocaine pharmacokinetics, twice as many rats were tested on PD 70 than at the younger ages.

On the day of assay, frozen dorsal striatal samples were sonicated in 200 µl of acetonitrile with 20 µl of cocaine propyl ester hydrochloride (80 µM) added as an internal standard. Tissue was centrifuged at $20,000 \times g$ for 15 min at 4 °C. Twenty microliters of the resulting extracts were assayed for DA using high-performance liquid chromatography (HPLC) with electrochemical detection (MD-150 column and Coulochem III detector; Thermo Fisher Scientific, Waltham, MA). The mobile phase consisted of 75 mM NaH_2PO_4 , 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 7 % acetonitrile (pH 3.1) and was pumped at a rate of 0.5 ml/min. To measure cocaine and benzoylecgonine levels, the remaining supernatant was mixed with 350 ml of chloroform:ethanol (4:1) and 50 ml 0.1 M NaHCO_3 and centrifuged again for 15 min (Gulley et al. 2003). The top aqueous layer was removed and the lower organic layer was allowed to dry. When completely dry, 200 ml of the mobile phase (0.1 M KH_2PO_4 and 40% acetonitrile, pH 2.7) was added and 100 ml of the resulting solution was injected into the HPLC system (Alliance HPLC system with a 2489 UV detector, Waters, Milford, MA). A Hypersil ODS C18 column (100 mm $\times$ 4.6 mm) was used for the separation; the flow rate was set at 1.5 ml/min and absorbance was monitored at 235 nm.

Data analysis

Litter effects were minimized by assigning no more than one subject from each litter to a particular group (Holson and Pearce 1992). For the behavioral experiment, distance traveled (untransformed data and % of control), percent center distance [%CD = (center distance /

total distance) * 100], and forelimb paddle were analyzed using repeated measures analyses of variance (ANOVAs). Significant higher order interactions (e.g., Age × Sex × Drug × Time Block) were further analyzed using lower order ANOVAs. When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the Greenhouse-Geisser epsilon statistic was used to adjust degrees of freedom (Geisser and Greenhouse 1958). Corrected degrees of freedom were rounded to the nearest whole number and, in each case, are indicated by a superscripted "a" in the parenthetical statistical reports. Other behavioral data (e.g., head-down sniffing and vertical activity) were collapsed across the testing session and analyzed using multifactor between-subject ANOVAs.

For the neurochemistry experiment, single- and multifactor ANOVAs were used to analyze dorsal striatal DA concentrations, as well as cocaine and benzoylecgonine half-life and peak concentrations. Pharmacokinetic properties of cocaine and benzoylecgonine were estimated using a nonlinear curve fitting program (Prism, GraphPad Software, San Diego, CA). Tissue elimination half-life and peak concentrations were calculated using the following formula:

$$Y = Y_0 * \exp(-K * X)$$

where Y_0 is the cocaine value when time (X) is zero, and K is the rate constant. Linear regression was used to assess the relationship between dorsal striatal cocaine levels measured at various time points after injection (5, 10, 20, 30, 60 and 90 min) and distance traveled scores of an independent sample of rats tested for 5 min at the same time points. The individual values used in the linear regression were group means of male and female rats at each age. When appropriate, post hoc analysis of both the behavioral and neurochemistry data was done using Tukey tests ($P < 0.05$).

Results

Locomotion

Untransformed data—Relative to the saline groups, cocaine increased the distance traveled of male and female rats (Fig. 1) [Drug main effect, $F_{1,84} = 138.24$, $P < 0.001$]. At PD 10 and PD 70, differences between the cocaine- and saline-treated rats were evident on time blocks 1–9; whereas, cocaine significantly enhanced the distance traveled scores of PD 20 rats on time blocks 1–6 [^aAge × Drug × Time Block interaction, $F_{5,221} = 3.80$, $P < 0.01$]. Locomotion varied according to age [Age main effect $F_{2,84} = 47.35$, $P < 0.001$], since saline- and cocaine-treated PD 70 rats exhibited greater distance traveled scores than similarly-treated rats tested on PD 10 or PD 20 (Fig 1) [Age × Drug interaction, $F_{2,84} = 17.89$, $P < 0.001$]. Among cocaine-treated rats, significant differences between PD 70 rats and the younger age groups were apparent on time blocks 1–9; whereas, saline-treated PD 70 rats differed from PD 10 and PD 20 rats on time blocks 1–6 [^aAge × Drug × Time Block interaction]. Distance traveled did not vary according to sex.

An omnibus ANOVA analyzing percent center distance (%CD) showed that PD 70 rats locomoted relatively greater distances in the center portion of the testing chamber than PD 10 or PD 20 rats (Fig. 2) [Age main effect, $F_{2,84} = 11.51$, $P < 0.001$]. Additionally, male rats had significantly greater %CD scores than female rats [Sex main effect, $F_{1,84} = 5.00$,

$P<0.05$], while cocaine-treated rats had larger %CD scores than rats given saline [Drug main effect, $F_{1,84}=17.59$, $P<0.001$]. In the latter case, cocaine only caused a significant enhancement of %CD scores in PD 70 rats and not in the two younger age groups [Age $\times$ Drug interaction, $F_{2,84}=8.92$, $P<0.001$].

In our youngest age group, cocaine increased the number of forelimb paddles exhibited by male and female rats on PD 5 (Fig. 3) [Drug main effect, $F_{1,28}=38.82$, $P<0.001$]. Relative to the saline group, cocaine-induced forelimb paddles were significantly elevated on time blocks 1–9 [Drug $\times$ Time Block interaction, $F_{8,224}=2.53$, $P<0.05$]. No sex differences were apparent.

Percent of saline controls—When distance traveled data were transformed to percent of saline controls (%DT), a different pattern of effects emerged (Fig. 4). More specifically, rats tested at PD 10 and PD 20 exhibited greater cocaine-induced distance traveled scores, relative to their saline controls, than PD 70 rats [Age main effect, $F_{2,42}=4.70$, $P<0.05$]. This effect varied according to sex, as male rats tested at PD 10 had greater %DT scores on time blocks 1–3 than male rats tested at PD 20 or PD 70 [^aAge $\times$ Sex $\times$ Time Block interaction, $F_{7,154}=5.06$, $P<0.001$]. On time blocks 4 and 5, male PD 20 rats had greater %DT scores than PD 70 rats. This effect reversed itself at the end of the testing session, as male PD 20 rats had smaller %DT scores on time blocks 7–9 than male PD 70 rats. Female rats responded differently than males, as females tested at PD 10 had greater %DT scores on time blocks 1 and 8 than female PD 20 and PD 70 rats [^aAge $\times$ Sex $\times$ Time Block interaction, $F_{7,154}=5.06$, $P<0.001$]. Among females, PD 20 rats exhibited greater %DT scores than both PD 70 rats on time blocks 2–4 and PD 10 rats on time blocks 3 and 4. On no time blocks were the %DT scores of female PD 70 rats greater than the scores of the younger age groups.

Additional behaviors (untransformed data)

Head-down sniffing—Head-down sniffing, which is a measure of stereotypy, was significantly elevated after cocaine administration (Table 1) [Drug main effect, $F_{1,112}=629.99$, $P<0.001$]. Cocaine-treated rats tested on PD 5, PD 10, and PD 20 exhibited more head-down sniffing than PD 70 rats [Age $\times$ Drug interaction, $F_{3,112}=13.32$, $P<0.001$]. Nearly the opposite was the case with saline-treated rats, since the basal number of head-down sniffing counts was significantly greater on PD 70 than PD 5 or PD 10.

Vertical activity—Overall, cocaine stimulated more vertical activity than saline [Drug main effect, $F_{1,112}=71.52$, $P<0.001$], and PD 70 rats exhibited greater vertical activity than the three younger age groups (Table 1) [Age main effect, $F_{3,112}=38.28$, $P<0.001$]. This age difference was only evident in cocaine-treated rats (i.e., the saline groups did not differ amongst themselves) [Age $\times$ Drug interaction, $F_{3,112}=15.00$, $P<0.001$].

Grooming—PD 70 rats groomed more than all other groups, while PD 20 rats groomed more than PD 10 and PD 5 rats (Table 1) [Age main effect, $F_{3,112}=38.05$, $P<0.001$]. Cocaine significantly depressed the grooming of PD 20 rats, relative to both cocaine-treated

PD 70 rats and the PD 20 saline control group, while not altering grooming at any other age [Age $\times$ Drug interaction, $F_{3,112}=4.64$, $P<0.01$].

Head-up sniffing—When compared to PD 70 rats, the basal head-up sniffing of saline-treated PD 5 and PD 10 rats was almost non-existent (Table 1) [Age $\times$ Drug interaction, $F_{3,112}=5.40$, $P<0.01$]. Relative to saline controls, cocaine increased the head-up sniffing of all age groups [Drug main effect, $F_{1,112}=95.18$, $P<0.001$]. Cocaine-treated PD 70 rats exhibited more head-up sniffing than all of the younger age groups, while PD 20 rats showed more head-up sniffing than PD 5 rats.

Licking—Among saline-treated rats of all ages, basal levels of licking approached zero (Table 1). With the exception of PD 70 rats, cocaine increased the licking of all age groups [Age $\times$ Drug interaction, $F_{3,112}=4.65$, $P<0.01$].

Mouthing—Saline-treated rats, regardless of age, exhibited similar levels of mouthing behavior (Table 1). Cocaine significantly increased the occurrences of mouthing in PD 10 rats, but not in any of the other age groups [Age $\times$ Drug interaction, $F_{3,112}=6.13$, $P<0.001$].

Head lift—Cocaine significantly increased the number of head lifts exhibited by PD 5 and PD 10 rats (Table 2) [Drug main effect, $F_{1,56}=70.12$, $P<0.001$].

Pivoting—Although basal levels of pivoting did not differ between PD 5 and PD 10 rats (Table 2), cocaine caused a significant increase in pivoting that was greater in PD 5 rats than PD 10 rats [Age $\times$ Drug interaction, $F_{1,56}=12.55$, $P<0.001$].

Roll/Curl—Overall, PD 5 rats exhibited more roll/curls than PD 10 rats (Table 2) [Age main effect, $F_{1,56}=14.46$, $P<0.001$], and cocaine-treated rats had more roll/curls than saline controls [Drug main effect, $F_{1,56}=16.51$, $P<0.001$].

Sex differences—In terms of the “additional behaviors” reported in this section (e.g., head-down sniffing, vertical activity, head lift, etc.), neither the main effects nor interactions involving the sex variable were statistically significant.

Additional behaviors (percent of saline controls)

In order to directly compare the locomotion of all age groups, forelimb paddle (PD 5) and distance traveled data (PD 10, PD 20, and PD 70) were transformed to percent of saline controls (% *behavior*). Using this transformation, the %locomotion scores of PD 10 rats were greater than PD 20 or PD 70 rats [Age main effect, $F_{3,60}=8.73$, $P<0.001$], with the PD 5 rats being intermediate between and not significantly different from the PD 10 and older age groups (Table 3). When calculated as percent of saline controls, PD 10 rats also exhibited significantly more %head-down sniffing, %head-up sniffing, %licking, and %mouthing than PD 70 rats (Table 3). More specifically, %head-down sniffing significantly declined in a progressive manner from PD 10 $>$ PD 5 $>$ PD 20 $>$ PD 70 [Age main effect, $F_{3,60}=169.45$, $P<0.001$]. Additionally, PD 10 rats showed greater %head-up sniffing than PD 20 or PD 70 rats [Age main effect, $F_{2,45}=15.30$, $P<0.001$]; PD 10 rats had larger %mouthing scores than all other age groups [Age main effect, $F_{3,60}=12.94$, $P<0.001$]; and PD 5 and PD 10 rats

exhibited greater %licking scores than PD 70 rats [Age main effect, $F_{3,60}=4.58$, $P<0.01$ (for PD 5 vs. PD 70, the Tukey probability value was $P=0.061$)]. Vertical activity and grooming evidenced different patterns of effects (Table 3), as PD 70 rats had larger %vertical activity scores than PD 20 rats [Age main effect, $t_{30}=2.42$, $P<0.05$], while cocaine produced a significantly greater reduction in the %grooming scores of PD 20 rats than adults [Age main effect, $F_{2,45}=4.63$, $P<0.05$].

Dorsal striatal DA concentrations

Basal DA concentrations in the dorsal striatum increased with age (Fig. 5), as PD 20 rats had more dorsal striatal DA than PD 5 and PD 10 rats, while PD 70 rats had greater DA levels than all other age groups [Age main effect, $F_{3,43}=16.42$, $P<0.001$]. Cocaine (15 mg/kg) administration did not alter DA values at any time point after injection (2.5–210 min), nor did DA concentrations vary according to sex.

Cocaine pharmacokinetics

Effects of age—Peak cocaine concentrations in the dorsal striatum did not differ according to age or sex (upper graph, Fig. 6), although a post hoc test comparing peak cocaine values of PD 5 and PD 70 rats was marginally significant (Tukey tests, $P=0.07$). In contrast, cocaine half-life did vary according to age (upper graph, Fig. 7), as the dorsal striatal cocaine half-life of PD 5 rats ($M = 80.7$ min) was significantly longer than PD 20 ($M = 46.0$ min) or PD 70 ($M = 38.1$ min) rats (lower graph, Fig. 6) [Age main effect, $F_{3,40}=8.58$, $P<0.001$]. The cocaine half-life of PD 10 rats ($M = 71.9$ min) was also significantly greater than PD 70 rats.

When collapsed across all age groups, peak cocaine concentrations ($3.22 \mu\text{g/g}$ tissue) in the dorsal striatum were $12\times$ greater than peak benzoylecgonine levels ($0.26 \mu\text{g/g}$ tissue), which was a statistically significant effect [$F_{1,29}=126.61$, $P<0.001$]. Peak benzoylecgonine values varied neither with age nor sex, while the half-life of this metabolite was significantly longer at PD 5 than at PD 20 or PD 70 (Table 4) [Age main effect, $F_{3,25}=4.35$, $P<0.05$]. None of these effects varied according to sex.

Effects of route of administration and drug volume—In PD 20 rats, an SC injection of cocaine, when compared to an IP injection, resulted in lower peak cocaine concentrations and a longer half-life (Table 5) [Route main effects, $F_{1,9}=21.96$, $P<0.01$; $F_{1,9}=35.60$, $P<0.001$, respectively]. Route of administration did not differentially affect peak benzoylecgonine levels, but half-life of the metabolite was significantly longer when cocaine was injected SC [Route main effect, $F_{1,7}=22.83$, $P<0.01$]. Intraperitoneally administering cocaine (15 mg/kg) at two different injection volumes (2 vs. 5 ml/kg) did not differentially affect the peak values or half-life of cocaine or benzoylecgonine (Table 5). The pharmacokinetic effects caused by route of administration did not differ according to sex.

Recovery of standards—Recovery of the standard (cocaine propyl ester hydrochloride) was consistent across age groups, ranging from 84.4 to 85.5% ($\pm 1\%$).

Relationship between cocaine levels and distance traveled scores

Among the groups of male and female rats tested at PD 20 [$r = 0.90$, $P < 0.001$] and PD 70 [$r = 0.64$, $P < 0.05$], there were positive correlations between dorsal striatal cocaine levels and distance traveled (Fig. 8). At both ages, the slope of the regression line was significantly non-zero [$F_{1,10} = 45.39$, $P < 0.001$; $F_{1,10} = 6.82$, $P < 0.05$, respectively]. For PD 10 rats, the correlation between cocaine levels and distance traveled ($r = 0.43$) was nonsignificant.

Discussion

The early ontogeny of cocaine sensitivity is difficult to study because of the substantial age-dependent differences in body size and motoric capability of the species being tested. The impact of these physiological factors is evidenced in the untransformed behavioral data of our Sprague-Dawley rats. For example, adult rats (PD 70) exhibited significantly more basal and cocaine-stimulated locomotor activity than rats tested on PD 10 or PD 20. Similar age-dependent differences were evident when vertical activity, grooming, and head-up sniffing were assessed. In order to reduce the impact of physiological factors, data can be transformed to percent of same-age/same-sex saline controls from the habituation or test day (Badanich et al. 2008; White et al. 2008; Koek et al. 2012). After this transformation, it was evident that both male and female PD 20 rats were more sensitive to the locomotor-activating effects of 15 mg/kg cocaine than were PD 70 rats. This effect was especially apparent during the first 60 min of testing (see also McDougall et al. 2015). At various time points, PD 10 rats also exhibited more cocaine-induced locomotor activity (relative to their saline controls) than adult rats. Therefore, these results support our hypothesis that preweanling rats (PD 10 and PD 20) are more sensitive than adult rats to the locomotor stimulating effects of 15 mg/kg cocaine – a conclusion that is consistent with previous studies using both cocaine and amphetamine (Campbell et al. 1969; Lanier and Isaacson 1977; McDougall et al. 2015).

The behavioral repertoire of rats changes across early ontogeny and into adulthood. Some form of forward locomotion (forelimb paddle and locomotor activity) is evident from the neonatal period to adulthood, while other behaviors (head lift, pivot, and roll/curl) exclusively occur in their juvenile form during the neonatal and early preweanling periods. Additional behaviors (head-down sniffing, vertical activity, grooming, and head-up sniffing) emerge during early ontogeny and are only fully expressed as the rat matures (for additional discussion, see Spear and Brick 1979; Moody and Spear 1992). Forelimb paddle, a behavior observed in our youngest age group (PD 5), increased 592% after cocaine treatment, which was not significantly different from the relative increases in locomotion exhibited by PD 10 (757%) and PD 70 (445%) rats. Amphetamine may produce even more pronounced age-dependent behavioral effects, as Sobrian et al. (1974) reported that amphetamine-treated PD 4 rats were more active than older age groups. Among our two youngest age groups (PD 5 and PD 10), 15 mg/kg cocaine increased the occurrence of head lifts, pivots, roll/curls, head-up sniffing, and vertical activity. The latter two effects are notable, since head-up sniffing and vertical activity were not part of the normal behavioral repertoire of saline-treated PD 5 or PD 10 rats. In older rats, cocaine robustly increased the vertical activity of PD 70 rats, and the head-up sniffing of both PD 20 and PD 70 rats.

In terms of stereotypy, 15 mg/kg cocaine increased the head-down sniffing of all age groups, but the effect was strongest in neonatal and preweanling rats. Cocaine-induced licking also occurred in greater frequency in younger rats than adults. When converted to percent of saline controls, the cocaine-induced head-down sniffing and licking of PD 5 and PD 10 rats was dramatically greater than in PD 70 rats. Other indirect (amphetamine) and direct (apomorphine) DA agonists produce stereotyped sniffing and licking in neonatal rats (Abrams and Bruno 1992), and this stereotypy persists substantially longer in young rats than adults (Lal and Sourkes 1973). Although conclusions based on a single dose of a drug must be considered tentative, the present results suggest that stereotypy produced by 15 mg/kg cocaine is more readily expressed in very young rats (PD 5 and PD 10) than adults.

Cocaine pharmacokinetics also differed according to age. In adult rats, we found that peak cocaine values (3.55 µg/g tissue) were reached approximately 5–10 min after injection, while the half-life of cocaine was 38.1 min. These values are in accordance with past research, as peak cocaine values are typically observed 5–15 min after drug administration (Benuck et al. 1987; Lau et al. 1991; Bowman et al. 1999), and cocaine half-life ranges between 16 min and 72 min depending on the species being tested, drug dose, and laboratory conditions (Benuck et al. 1987; Lau et al. 1991). In terms of young rats, cocaine half-life varied substantially across early ontogeny, while peak cocaine values did not differ according to age. Specifically, cocaine half-life in the dorsal striatum of PD 5 and PD 10 rats was approximately 2× longer than in adult rats. Consistent with this finding, Bowman et al. (1999) reported that cocaine levels in whole brain samples persisted longer in PD 7 rats than adults. In PD 20 rats, administering cocaine SC resulted in longer half-life values than when the drug was delivered IP (for similar results using adult rats, see Lau et al. 1991). Conversely, injection volume (2 vs. 5 ml/kg) did not alter peak cocaine values or half-life in the dorsal striatum of PD 20 rats. The latter finding is meaningful since injection volumes used in ontogenetic studies are not standardized and often vary widely (e.g., Lin and Walters 1994; Ujike et al. 1995; Tirelli 2001; McDougall et al. 2015). As with cocaine, the half-life of benzoylecgonine was longer in PD 5 and PD 10 rats than adults. That being said, benzoylecgonine concentrations in brain were 12-fold lower than cocaine, which is an often reported finding (Benuck et al. 1987; Browne et al. 1991; Lau et al. 1991; Gulley et al. 2003). In serum, benzoylecgonine concentrations are typically greater than cocaine values (Benuck et al. 1987; Lau et al. 1991), leading Browne et al. (1991) to conclude that cocaine is more stable in brain than in blood. Our results extend these findings by showing that cocaine is stable in brain during both early ontogeny and adulthood.

In addition to assessing peak cocaine levels, we also quantified dorsal striatal DA concentrations at various time points after cocaine treatment. Not surprisingly, basal DA values differed according to age, as dorsal striatal DA levels were approximately 3× greater on PD 70 than PD 5 or PD 10 (see also Giorgi et al., 1987; Broaddus and Bennett, 1990). DA values on PD 20 were roughly equidistant between the younger and older age groups. Interestingly, cocaine did not alter DA concentrations at any time point. It is well established that cocaine increases DA overflow in microdialysis preparations (Hurd and Ungerstedt 1989; Martin-Fardon et al. 1996; Dewey et al. 1997), but cocaine's effects on DA levels in striatal or accumbal tissue homogenates is ambiguous. For example, Festa et al. (2004) reported that DA levels in the striatum were elevated 15 min after cocaine treatment, but

cocaine-induced DA changes in the nucleus accumbens were sex-dependent (i.e., DA levels were enhanced in males and depressed in females). Consistent with our results, various other studies found that acute cocaine administration does not increase or decrease DA levels in the dorsal striatum (Yu et al. 1990; Hadfield and Milio 1992; Alburges and Wamsley 1993).

Although eight male and eight female rats were tested at each age, there were few statistically significant sex effects. An absence of sex differences is common in prepubertal age groups (Frantz et al. 1996; Bowman et al. 1997; Snyder et al. 1998; McDougall et al. 2013), but psychostimulants (e.g., cocaine, amphetamine, and methamphetamine) typically produce greater locomotor activity in adult female rats than adult males (Sell et al. 2000; Schindler and Carmona 2002; Festa et al. 2004; Milesi-Hallé et al. 2005, 2007; McDougall et al. 2015). For unknown reasons, no such sex differences were observed in the present study. In the dorsal striatum, peak cocaine values and cocaine half-life also did not differ according to sex, which is seemingly consistent with the absence of sex-dependent behavioral differences. Importantly, Festa et al. (2004) reported that 20 mg/kg cocaine (IP) caused more locomotion in adult female rats than males, yet serum and brain levels of cocaine did not differ between the sexes (van Haaren et al. 1997; Bowman et al. 1999; Festa et al. 2004). Thus, it appears that cocaine pharmacokinetics cannot account for sex-dependent differences in behavioral responsiveness (for alternative explanations of cocaine-induced sex differences, see Becker et al. 2001; Festa et al. 2004). Despite a general absence of sex effects, we did find that the topography of locomotor activity varied according to both age and sex. Specifically, cocaine-treated PD 70 rats had larger %CD (Percent Center Distance) scores than both cocaine-treated young rats and saline-treated PD 70 controls. Among PD 70 rats, cocaine-treated male rats had greater %CD scores than female rats. Enhanced movement or time spent in the center of the testing arena, relative to the margin, is frequently interpreted as reflecting reduced anxiety (Treit and Fundytus 1989; Simon et al. 1994), therefore it is possible that cocaine decreased the anxiety of male PD 70 rats more than females, while not altering the anxiety of the younger age groups.

In conclusion, cocaine quickly reached peak levels in brain and then was more rapidly metabolized in older rats (PD 70 and PD 20) than younger rats (PD 10 and PD 5). Time-dependent alterations in cocaine levels appeared to have behavioral impact, since dorsal striatal cocaine values were positively correlated with distance traveled scores in both PD 20 and PD 70 rats. When data were transformed to percent of saline controls, PD 5 and PD 10 rats were more sensitive to the effects of cocaine than were adult rats. These results are consistent with the longer cocaine half-life evident in our two youngest age groups. In general, the present results suggest that behavioral differences between very young rats (PD 5 and PD 10) and adults may be attributable, at least in part, to pharmacokinetic factors. Conversely, cocaine pharmacokinetics (i.e., peak cocaine values and half-life) did not differ between PD 20 rats and adults. The latter result suggests that age-dependent behavioral differences between the late preweanling period and adulthood cannot readily be ascribed to cocaine pharmacokinetics.

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Fig. 1.

Mean ($\pm$ SEM) distance traveled scores of male and female rats ($n = 8$ per group) on the test day. Prewanling (PD 10 and PD 20) and adult rats (PD 70) were injected with saline or 15 mg/kg cocaine immediately before testing. The inset shows data collapsed across the 9 time blocks. 'a' Significantly different from saline-treated rats of the same age. 'b' Significantly different from PD 20 rats in the same treatment condition. 'c' Significantly different from PD 10 rats in the same treatment condition.

Fig. 2.

Mean ($\pm$ SEM) % center distance scores of male and female rats ($n = 8$ per group) on the test day. These are the same rats as described in Fig. 1. 'a' Significantly different from saline-treated rats of the same age. 'b' Significantly different from PD 20 rats in the same treatment condition. 'c' Significantly different from PD 10 rats in the same treatment condition.

Fig. 3.

Mean ($\pm$ SEM) forelimb paddles of male and female PD 5 rats ($n = 8$ per group) on the test day. The neonatal rats (PD 5) were injected with saline or 15 mg/kg cocaine immediately before testing. The inset shows data collapsed across the 9 time blocks. Filled circle = 15 mg/kg cocaine-male; filled triangle = 15 mg/kg cocaine-female; open circle = saline-male; open triangle = saline-female. 'a' Significantly different from saline-treated rats.

Fig. 4.

Percent distance traveled scores (%DT; $\pm$ SEM) of male and female rats ($n = 8$ per group) on the test day. Dashed lines represent control values (100%). These are the same rats as described in Fig. 1. 'a' Significantly different from PD 70 rats of the same sex. 'b' Significantly different from PD 20 rats of the same sex. 'c' Significantly different from PD 10 rats of the same sex.

Fig. 5.

Mean (±SEM) DA levels in the dorsal striatum of neonatal rats (PD 5), preweanling (PD 10 and PD 20) and adult rats (PD 70). Brains were removed 0–210 min after cocaine injection.

‘a’ Significantly different from PD 70 rats. ‘b’ Significantly different from PD 20 rats.

Fig. 6. Mean ($\pm$ SEM) peak cocaine levels (upper graph) and cocaine half-life (lower graph) of male and female neonatal rats (PD 5), preweanling (PD 10 and PD 20) and adult rats (PD 70). 'a' Significantly different from PD 70 rats. 'b' Significantly different from PD 20 rats.

Fig. 7. Nonlinear regression showing the concentration-time curves for rats at different postnatal ages (upper graph), PD 20 rats injected via different routes of administration (middle graph), and PD 20 rats injected with cocaine at different volumes (lower graph).

Fig. 8.

Scatterplots representing the relationship between dorsal striatal cocaine levels and distance traveled scores on PD 10 (upper graph), PD 20 (middle graph) and PD 70 (lower graph). Each point represents the mean scores of separate groups of male and female rats tested 5, 10, 20, 30, 60 and 90 min after cocaine injections. The straight line is the linear regression estimate.

Table 1

Effects of cocaine (15 mg/kg, IP) on various discrete behaviors [mean (SEM)] of male and female rats ($n = 8$ rats per group) tested on PD 5, PD 10, PD 20, or PD 70

Treatment	PD 5	PD 10		PD 20		PD 70	
	Male	Female	Male	Female	Male	Female	Female
Head-Down Sniffing							
Saline	1.9 (0.6)	1.2 (0.5) ^b	1.6 (0.4)	1.4 (0.4) ^b	4.2 (0.5)	3.6 (1.0)	9.2 (2.7)
Cocaine	32.4 (2.3)	28.9 (1.4) ^{ab}	36.7 (1.2)	32.6 (1.6) ^{ab}	31.5 (2.9)	31.6 (3.3) ^{ab}	20.4 (3.2)
Vertical Activity							
Saline	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.1 (0.1)	2.0 (1.0)	2.2 (0.9)	5.2 (1.4)
Cocaine	3.6 (1.4)	2.0 (0.7) ^{bd}	8.2 (1.4)	10.4 (2.4) ^{ab}	3.6 (1.9)	5.1 (2.8) ^b	23.0 (3.6)
Grooming							
Saline	0.1 (0.1)	0.0 (0.1) ^{bc}	0.9 (0.4)	1.0 (0.3) ^{bc}	5.9 (0.9)	5.1 (1.0)	5.1 (1.1)
Cocaine	0.1 (0.1)	0.4 (0.3) ^b	0.4 (0.3)	0.7 (0.2) ^b	1.5 (0.3)	1.7 (1.0) ^{ab}	7.1 (1.8)
Head-Up Sniffing							
Saline	0.0 (0.0)	0.1 (0.1) ^b	0.9 (0.4)	0.6 (0.4) ^b	4.6 (1.3)	4.4 (1.3)	9.4 (2.8)
Cocaine	9.2 (2.2)	7.6 (1.8) ^{abc}	9.5 (2.5)	13.2 (3.2) ^{ab}	15.9 (4.9)	18.2 (5.2) ^{ab}	35.0 (2.5)
Licking							
Saline	0.1 (0.1)	0.3 (0.2)	0.1 (0.1)	0.2 (0.1)	0.4 (0.3)	0.2 (0.2)	0.9 (0.4)
Cocaine	5.5 (2.1)	3.9 (0.8) ^{ab}	4.9 (1.6)	3.6 (1.3)	7.8 (3.0)	7.4 (4.0) ^{ab}	0.4 (0.2)
Mouthing							
Saline	3.2 (1.0)	2.6 (0.7)	2.0 (0.7)	2.2 (0.9)	2.1 (0.5)	1.3 (0.6)	2.0 (0.7)
Cocaine	2.1 (0.5)	2.2 (0.8)	4.3 (1.1)	5.1 (1.4) ^{abce}	0.8 (0.5)	0.7 (0.5)	0.6 (0.4)
							0.8 (0.2)

^aSignificantly different from male and female saline-treated rats of the same age.

^bSignificantly different from male and female PD 70 rats in the same drug treatment condition.

^cSignificantly different from male and female PD 20 rats in the same drug treatment condition.

^dSignificantly different from male and female PD 10 rats in the same drug treatment condition.

Significantly different from male and female PD 5 rats in the same drug treatment condition.

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Table 2

Effects of cocaine (15 mg/kg, IP) on various discrete behaviors of male and female rats ($n = 8$ rats per group) tested on PD 5 or PD 10

Treatment	PD 5		PD 10	
	Male	Female	Male	Female
Head Lift				
Saline	2.1 (0.5)	2.6 (0.8)	2.0 (0.7)	1.8 (0.5)
Cocaine	9.9 (1.7)	10.7 (1.4) ^a	6.5 (1.1)	8.7 (1.8) ^a
Pivot				
Saline	3.0 (1.5)	2.3 (0.5)	1.6 (0.5)	2.2 (0.6)
Cocaine	22.7 (1.5)	25.1 (2.3)	16.3 (2.1)	14.9 (1.8) ^a
Roll/Curl				
Saline	1.8 (0.7)	3.4 (1.0) ^b	0.4 (0.3)	0.8 (0.2)
Cocaine	8.2 (2.2)	6.1 (1.3) ^{ab}	3.6 (1.5)	2.0 (0.8) ^a

^aSignificantly different from male and female saline-treated rats of the same age.

^bSignificantly different from male and female PD 10 rats in the same drug treatment condition.

Table 3

Effects of cocaine, expressed as percent of saline controls [mean (SEM)], on various discrete behaviors of male and female rats ($n = 8$ rats per group) tested on PD 5, PD 10, PD 20, or PD 70

%Behavior	PD 5	PD 10	PD 20	PD 70
%Locomotion	592% (74)	757% (235) ^{ab}	400% (125)	445% (175)
%Head-Down Sniffing	1,995% (114) ^{ab}	2,313% (69) ^{abc}	811% (58) ^a	272% (29)
%Vertical Activity	*	*	206% (78)	428% (47) ^b
%Grooming	*	56% (17)	29% (10) ^a	95% (17)
%Head-Up Sniffing	*	1,598% (310) ^{ab}	379% (78)	312% (24)
%Licking	2,830% (911) ^a	3,950% (883) ^a	2,520% (874)	41% (16)
%Mouthing	77% (18)	225% (40) ^{abc}	44% (22)	31% (10)
%Head Lift	448% (48)	402% (57)		
%Pivot	930% (68)	832% (86)		
%Roll/Curl	324% (74)	617% (223)		

Note: Locomotion was defined as either distance traveled (PD 10, PD 20, and PD 70) or forelimb paddles (PD 5);

* Value is out of scale.

^aSignificantly different from male and female PD 70 rats in the same drug treatment condition.

^bSignificantly different from male and female PD 20 rats in the same drug treatment condition.

^cSignificantly different from male and female PD 5 rats in the same drug treatment condition.

Table 4

Effects of age on the peak levels and half-life of benzoylecgonine in male and female rats ($n = 8$ sets of rats per group)

Benzoylecgonine	PD 5	PD 10	PD 20	PD 70
Peak Levels	0.206 (0.05)	0.274 (0.05)	0.287 (0.02)	0.258 (0.02)
Half-Life	182.11 (55.3) ^{ab}	100.68 (15.3)	68.90 (5.0)	57.96 (8.5)

^aSignificantly different from male and female PD 70 rats

^bSignificantly different from male and female PD 20 rats

Table 5

Effect of route of administration (IP vs. SC) and drug volume (2 vs. 5 ml/kg, IP) on the peak levels and half-life of cocaine and benzoylecgonine in male and female rats ($n = 5$ –8 sets of rats per group) tested on PD 20

Compound	Route of Administration		Drug Volume	
	IP	SC	2 ml/kg, IP	5 ml/kg, IP
Peak Levels				
Cocaine	3.46 (0.25)	1.44 (0.27) ^a	3.46 (0.25)	3.68 (0.48)
Benzoylecgonine	0.27 (0.03)	0.19 (0.02)	0.27 (0.03)	0.31 (0.04)
Half-Life				
Cocaine	50.30 (6.26)	282.78 (48.7) ^a	50.30 (6.26)	41.13 (2.59)
Benzoylecgonine	75.37 (5.98)	590.4 (197.4) ^a	75.37 (5.98)	59.85 (7.23)

^aSignificantly different from male and female PD 20 rats given an IP injection of 15 mg/kg cocaine.