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Behavioral and Neurochemical Responses to Cocaine in Periadolescent and Adult Rats

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Although recreational drug use by human adolescents is a well-known and long-standing problem, relatively little is known regarding differences in behavioral and physiological responses to abused substances in adolescent *vs* adult animals. The present study compared effects of the psychomotor stimulant, cocaine, in periadolescent (postnatal days 37–52) and adult (postnatal days 75–90) male Wistar rats. Locomotion and motor stereotypy were recorded after acute and repeated cocaine injections (0, 10, or 20 mg/kg cocaine, intraperitoneal (i.p.), four injections spaced 5 days apart). Spontaneous acquisition of intravenous (i.v.) cocaine self-administration was investigated in two dose groups (~0.37 or 0.74 mg/kg/infusion) over 14 days. Dopamine levels in the nucleus accumbens were recorded under basal conditions (no net flux method) and after cocaine administration (~0.37, 0.74, and 2.92 mg/kg/i.v. infusion or 20 mg/kg i.p.) using *in vivo* microdialysis. The locomotor data are in partial agreement with previous reports of hyposensitivity to acute cocaine in periadolescent *vs* adult rats; periadolescents were less active overall than adults. Moreover, adult rats exhibited significant locomotor sensitization after repeated injection of 10 mg/kg cocaine, whereas periadolescents required the high dose of 20 mg/kg cocaine to demonstrate sensitization. Neither age group showed sensitization of motor stereotypies. No age-related difference was observed in acquisition of cocaine self-administration, or in basal or cocaine-stimulated nucleus accumbens dopamine. These experiments imply a developmental dissociation between the motor activating and reinforcing effects of cocaine. Similarities in dopamine levels across age groups suggest that age-specific motor responses to cocaine are not mediated by dopamine in the nucleus accumbens.
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INTRODUCTION

Almost 1.5 million Americans abuse cocaine (SAMHSA, 2003). Most cocaine users are adolescents or had their first contact with cocaine during adolescence (Johanson and Fischman, 1989; SAMHSA, 2003). In fact, early exposure to drugs of abuse might be a strong predictor of later drug use and dependence (Johanson and Fischman, 1989; Kandel and Davies, 1992). Cocaine use among adolescents typically involves rapid escalation to high levels of intake (Estroff *et al*, 1989), perhaps related to reportedly less intense euphoric and stimulatory effects of the drug in young cocaine users compared with adults (Koob *et al*, 1994). Nevertheless, laboratory investigations have yet to determine whether or not these trends reflect developmental changes in the physiological effects of cocaine. The present study aimed to compare behavioral and neurochemical effects of cocaine in adolescent *vs* adult rodents.

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Whereas adolescence in humans and non-human primates extends over several years, rodent models of this developmental stage, termed 'periadolescence,' are limited to the 2 weeks between approximately 35 and 50 days of age (postnatal days (PND) 35–50; Spear and Brake, 1983; Spear, 2000). Other estimates of rodent adolescence shift earlier by a week or so, such as PND 28–42 (Collins and Izenwasser, 2004; Maldonado and Kirstein, 2005a, b). In many species including rodents, this transition from youth to adulthood entails physiological maturations such as gonadarche and adrenarche, as well as ethological factors such as departing from the early postnatal home environment and changing social companions from immediate family members to other age-mates (Campbell *et al*, 2000, for review).

Several behavioral characteristics are common in both rodent and primate periadolescence. Examples include a high amount of time spent in social interaction and play behavior (Brown, 1990; Panskepp, 1981), high levels of risk-taking, sensation-seeking, or novelty-seeking (Adriani *et al*, 1998; Zuckerman, 1992), and general hyperactivity or hyperexploration of a novel environment (Bronstein, 1972, 1979; Caza and Spear, 1980; but see Bauer and Duncan, 1975; Frantz and Van Hartesveldt, 1999). Mesocorticolimbic dopamine circuitry is integral to these behaviors (Bardo,

1998; Burns *et al*, 1996; Critchley *et al*, 2000; Hooks *et al*, 1994; Russell, 2000) and is also implicated in the motor-activating and reinforcing effects of stimulants (Koob, 1992; Wise and Bozarth, 1987). This system and related inputs continue to mature throughout periadolescence (Campbell *et al*, 2000, for review). For instance, during the periadolescent period or just prior, dopamine receptors are transiently overexpressed in striatal regions (Gelbard *et al*, 1989; Seeman *et al*, 1987; Tarazi *et al*, 1998, 1999, 1995), levels of basal or receptor-stimulated second messenger activities fluctuate (Andersen, 2002), voltammetric measures of dopamine release and uptake remain lower than in adults (Stamford, 1989), and dopaminergic innervation of the prefrontal cortex increases (Kalsbeek *et al*, 1988). Such reorganization of behavior and neural circuitry may underlie developmental alterations in responsiveness to a variety of agents including cocaine. Coupled with data on human drug use during adolescence, these findings have led to the hypothesis that periadolescence is a period of heightened vulnerability to the addictive properties of cocaine and other drugs (Campbell *et al*, 2000; Laviola *et al*, 1999).

For several decades, most reports indicated that periadolescent rats are *less* responsive than younger or older rats to psychomotor stimulants. Periadolescent Sprague–Dawley and Long Evans rats ranging from PND 28 to 45 were less sensitive to motor activation induced by several indirect or mixed dopamine agonists including cocaine, amphetamine, and apomorphine (Bolanos *et al*, 1998; Lanier and Isaacson, 1977; Laviola *et al*, 1995; Shalaby and Spear, 1980; Snyder *et al*, 1998; Spear and Brake, 1983). After repeated, intermittent injection of cocaine, periadolescent rats (PND 35–45) also exhibited similar or a lower degree of sensitization of cocaine-induced locomotion compared with weanlings (PND 21–27) or adults (Collins and Izenwasser, 2002; Laviola *et al*, 1995; Snyder *et al*, 1998) as well as a similar low level or lower degree of sensitization of some cocaine-induced motor stereotypies (Laviola *et al*, 1995; Snyder *et al*, 1998). In terms of neuroendocrine effects, plasma corticosterone responses were lower in PND 35–38 rats after acute or repeated cocaine injections compared with PND 60–70 adults (Laviola *et al*, 1995), and also in PND 33–43 *vs* >PND 70 mice after acute amphetamine (Adriani and Laviola, 2000). During classical conditioning, PND 35 rats were resistant to taste aversions conditioned by amphetamine injection, whereas juvenile (PND 18) and young adult (PND 52) rats displayed strong aversions (Infurna and Spear, 1979).

However, not all studies point to a periadolescent hyposensitivity to psychostimulants. A recent study revealed similar locomotor activation by cocaine in PND 45 *vs* 60 rats (Maldonado and Kirstein, 2005a, b), although handling procedures or the later adolescent/earlier adult ages of testing may have contributed to this result. During operant conditioning, PND 27, 37, and 90 rats did not differ significantly in their rates of nose-poking behavior reinforced by intravenous (i.v.) cocaine infusions (Belluzzi *et al*, 2005). In terms of stimulant-related reward, PND 34–37 and 65–78 adult rats displayed the same preference for a cocaine-paired environment in a conditioned place preference paradigm, and PND 30–49 *mice* displayed no preference for an amphetamine-paired environment (Adriani and Laviola, 2003; Campbell *et al*, 2000; but see Laviola *et al*, 1994).

Some recent experiments even demonstrate adolescents to be *more sensitive* than adults to stimulants. A sensitization study in CD-1 outbred mice revealed significant locomotor sensitization after repeated amphetamine injection in PND 33–43 mice but not their adult (>PND 60) counterparts (Adriani *et al*, 1998). Adolescents may also be more sensitive than their adult counterparts to nicotine (Adriani *et al*, 2004, 2002; Elliott *et al*, 2005; Faraday *et al*, 2003; Vastola *et al*, 2002; but see Schochet *et al*, 2004). In an operant behavior paradigm, PND 27 male rats acquired stable nose-poking behavior maintained by a combination of nicotine and components of tobacco smoke faster than PND 90 adults, and self-administered more of the drug combination (Belluzzi *et al*, 2005). A trend toward higher nicotine self-administration among late adolescent/young adult (PND 54–62) female rats compared with older adult females (>PND 90) was also reported (Levin *et al*, 2003). In terms of neurochemistry, PND 33–43 male rats demonstrated neurochemical sensitization after repeated amphetamine injection when their adult (>PND 70) counterparts did not (Laviola *et al*, 2001). Similarly, Bolanos *et al* (1998) reported neurochemical *super*sensitivity in acetylcholinergic response to dopamine agonists at PND 35, compared with adults (age not specified). Thus, generalizations *cannot* be made regarding differential sensitivity among periadolescent and adult subjects with regard to the behavioral and neurochemical effects of psychomotor stimulant drugs.

The present study directly compared the motor stimulant and reinforcing effects of *cocaine* in periadolescent *vs* adult male Wistar rats. The first experiment aimed to re-evaluate the motor response to cocaine in periadolescents (PND 37–39 at start) *vs* adults (PND 73–75 at start) using a different rat strain and longer time interval between injections than previous studies (Bolanos *et al*, 1998; Collins and Izenwasser, 2002, 2004; Laviola *et al*, 1995; Snyder *et al*, 1998). This experiment provided a context in which to analyze cocaine-related behavioral reinforcement via i.v. cocaine self-administration using a lever-pressing paradigm and a longer acquisition period than previously utilized (Belluzzi *et al*, 2005). Because the locomotor stimulant and reinforcing effects of cocaine are mediated in part by dopamine in the nucleus accumbens (Koob, 1992; Wise, 1996; Wise and Bozarth, 1987), the final experiment investigated nucleus accumbens dopamine levels in both age groups using *in vivo* microdialysis. Basal extracellular levels of dopamine were estimated by calculating the concentration at which no net exchange of analyte occurred across the dialysis membrane (ie no net flux; Lonnroth *et al*, 1987). Dopaminergic responses to i.v. or intraperitoneal (i.p.) cocaine injection were also compared, and dialysate levels of cocaine were assessed for potential age-related differences in cocaine bioavailability or clearance.

MATERIALS AND METHODS

Subjects

Male Wistar rats (Charles River Laboratories, Wilmington, MA) arrived at postnatal day (PND) 21–23 or 60–62 and

were housed in groups of 2–3 in a humidity- and temperature-controlled (22°C) vivarium on a 12 h light/dark cycle. Lights came on at 0600 hours for i.p. injection experiments and data were collected in the light. Lights went off at 1000 hours for i.v. injection experiments and data were collected in the dark. (Phases were based on relevant studies.) Rats acclimated for 5–7 days before experiments and had *ad libitum* access to food and water throughout experimentation. Subjects were observed and/or weighed daily to assess health and drug responsiveness. All procedures adhered to the 'Principles of Laboratory Animal Care' and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs

Cocaine HCl was obtained from the National Institute on Drug Abuse (Washington, DC, USA). Methohexital sodium (1%, Brevital Sodium) was from Eli Lilly (Indianapolis, IN). All chemicals for artificial cerebrospinal fluid (aCSF), HPLC mobile phase, and chromatographic standards were from Sigma Chemicals (St Louis, MO), except acetonitrile from VWR Pharmaceuticals (San Francisco, CA) and EDTA from JT Baker Chemical Co. (Phillipsburg, NJ).

Experiment I: Effects of Acute and Repeated Cocaine Injection on Motor Activity

Motor activity was measured in 23 × 46 cm plastic cages either 20 or 28 cm high for periadolescent or adult rats, respectively, located in a room separate from the vivarium. Each cage was surrounded by two sets of photocell beams (San Diego Instruments, La Jolla, CA): a low set positioned 4 cm above the floor and a high set positioned 12 or 17 cm above the floor for periadolescents or adults, respectively. Each low set contained eight photocells spaced evenly along the long axis and four photocells along the short axis. Each high set had eight photocells on the short axis. Photocell-beam interruptions were recorded in 5-min bins by a DOS-based computer system. 'Matrix crossings' were calculated from beam interruptions in each octant.

Animals were handled and weighed daily for 3–5 days before the first test at PND 37–39 or 73–75, and were acclimated to the testing room for 15 min ($n = 6$–10 per group). Sessions began when rats were placed individually in a test cage for a 30-min habituation period. Subsequently, each rat was removed, injected with cocaine (10 or 20 mg/kg, i.p.) or saline vehicle (1 ml/kg), and returned for 120 min. Testing was conducted during the light phase to facilitate observation of motor activity and to replicate previous experiments (Bolanos *et al*, 1998; Infurna and Spear, 1979; Laviola *et al*, 1995; Snyder *et al*, 1998).

Stereotyped behaviors (sniffing, rearing, grooming, head-bobbing, and oral stereotypy) were scored during habituation and for 60 min after injection. Experimenters rated each rat every 10 s for a total of six possible scores per min, or 180 possible scores per 30 min (O'Dell *et al*, 1996). 'Lying Still' was recorded in the absence of stereotypy or locomotion.

To assess age differences in repeated intermittent effects of cocaine, all subjects underwent the same procedure 5, 10, and 15 days later, receiving the same dose on all 4 days but no injections between test days. Injections were always given in the testing environment. Automated measures were taken on all days, but experimenter ratings were conducted only on the first and last days.

Experiment II: Acquisition of Cocaine Self-Administration

Equipment. The i.v. catheters used for self-administration were constructed as described (Caine *et al*, 1993), with minor modifications (Emmett-Oglesby and Lane, 1992). Alterations in catheters for periadolescents included a shorter length of silastic tubing inserted into the vein (2.0 *vs* 3.5 cm) because the distance from vein incision to the atrium of the heart was shorter in smaller immature rats. Also a cotton mesh 'back plate' was used instead of plastic mesh because the dermal tissue of developing rats may have been subject to tearing by hard plastic. Self-administration chambers consisted of operant boxes enclosed in sound-attenuating, ventilated environmental cubicles (MED Associates Inc., St Albans, VT or BRS/LVE, Laurel, MD). Two levers extended into the chamber to start each session. Pressing on the 'active lever' initiated a syringe pump with a 5 r.p.m. motor (Model A; Razel Scientific Instruments, Stanford, CT) for 4 s to deliver 0.1 ml drug solution via a stainless steel liquid swivel and polyethylene tube attached to the catheter on the animal's back. Each reinforced response lit a cue light above the lever, which stayed on throughout a 20-s time out (TO) period. Lever-presses during TO were recorded but not reinforced. Pressing on the 'inactive lever' was also recorded but not reinforced. Drug delivery and data collection were controlled by a DOS-based computer system.

Surgical procedures. At PND 29–31 for periadolescents or PND 67–69 for adults (mean body weight: 126.6, $n = 21$ or 302.8 g, $n = 21$, respectively), rats were implanted with i.v. catheters (Caine *et al*, 1993). Briefly, rats were anesthetized with a 1–2% halothane/oxygen vapor and catheter tubing was passed subcutaneously from the back to the right jugular vein, inserted into the vein previously punctured with a 25-gauge needle, and tied gently with suture thread. During recovery, periadolescent and adult rats received 0.1 or 0.2 ml, respectively, of the antibiotic ticarcillin (100 mg/ml, i.v.) twice daily for 2 days post-surgery, then once daily during testing. Catheters were also flushed daily with heparinized saline (30 USP units/ml).

Self-administration testing. Following a 7- to 9-day post-surgical recovery, spontaneous acquisition of cocaine self-administration began (PND 38 or 76). Two-hour sessions were conducted daily for 14 days during the dark phase. (Dark phase testing maximized the likelihood rats would encounter the cocaine-associated lever.) Non-contingent injections were never administered. Lever pressing on the active lever was reinforced by i.v. injection of cocaine HCl under a fixed-ratio 1 TO20 sec schedule of reinforcement in dose groups receiving either ~0.37 or ~0.74 mg/kg/infusion. (These doses approximated 0.125 or 0.25 mg/infusion in previous experiments from this laboratory in which drug solutions were not titrated for body weight.) To account for differences in body weight between age groups,

the concentration of cocaine solution for periadolescent rats was titrated on days 1 and 8 to match the mean adult dose in mg/kg/infusion.

Patency of the i.v. catheters was tested 1 day before the first and immediately after the last test session by administering an ultra-short-acting barbiturate anesthetic (Brevital Sodium, 1% methohexital sodium; Eli Lily, Indianapolis, IN) through the catheter. If muscle tone was not lost within 3 s, the catheter was presumed defective and the subject was not included in analyses.

Experiment III: Basal and Cocaine-Stimulated Dopamine Levels in the Nucleus Accumbens

Apparatus. Microdialysis probes were constructed according to a procedure modified from Parsons *et al* (1991). Briefly, a 300 μm o.d. regenerated cellulose dialysis membrane (13 000 molecular weight cutoff; Spectrum Laboratories Inc., Laguna Beach, CA) was fitted over two lines of silica tubing (40 μm i.d., 105 μm o.d., Polymicro Technology, Phoenix, AZ) held in an aluminum shaft. The membrane was sealed to the silica using fast-drying epoxy for an active membrane length of 2 mm.

Dopamine was separated from 5 μl dialysate volumes injected onto a microbore HPLC system with a 1 × 100 mm column (3 μm packing material, C18 stationary phase; Keystone, Bellefonte, PA). Mobile phase was composed of a 50 mM NaH_2PO_4 (monohydrate) buffer (pH 3.92) with 17% acetonitrile, 0.27 mM Na_2-EDTA, 0.4% triethylamine, and 3.27 mM decane sulfonic acid, and was pumped at 35 μl/min. An amperometric detector (EG&G PARC Model 400, Princeton, NJ) using dual glassy carbon working electrodes was set at 700 and −10 mV against a Ag/AgCl reference electrode (BAS, West Lafayette, IN). Dopamine concentrations were determined with an external calibration curve. Standard solutions contained 0.25 mM ascorbic acid to minimize analyte auto-oxidation.

Cocaine was separated from 3 μl volumes of dialysate injected onto an HPLC system equipped with a 1 × 100 mm column (3 μm packing material, C18 stationary phase; Sepstix, BAS, West Lafayette, IN). Mobile phase was composed of a 48 mM NaH_2PO_4 (monohydrate) buffer (pH 6.0) with 14% acetonitrile, 12% methanol, and 1.0% triethylamine and pumped at 30 μl/min. A Spectra Physics Spectra Focus scanning multiple-wavelength ultraviolet detector was set with a flow cell absorption pathlength of 3 mm, total cell volume of 1.2 μl, and absorbance wavelength of 225 nm. Cocaine concentrations were determined using an external calibration curve.

Surgical procedures. Two different routes of cocaine administration (i.v. and i.p.) were tested in separate groups ($n = 5$–10 per age group). For i.v. cocaine injections, rats were surgically implanted with i.v. catheters at PND 30–33 or 70–74 as described above and were allowed 5–7 days recovery. For both i.v. and i.p. cocaine injection groups, microdialysis probes were implanted 1 day before testing. Thus, rats were anesthetized (1–2% halothane or isofluorane vapor) and placed in a stereotaxic instrument at PND 38–40 or 70–72 (i.p. group) and PND 37–40 or 77–80 (i.v. group). Microdialysis probes were implanted into the nucleus accumbens at coordinates of AP +1.7, ML ±1.2, DV −7.9 or −8.1, for periadolescent or adult rats, respectively, with measurements from dura and flat skull. Probes were secured to the skull with methylmethacrylate over skull screws (Small Parts Inc., Miami Lakes, FL). Animals were allowed 6–12 h recovery in the dialysis sampling environment (23 × 46 × 28 cm plastic cages) while the probes were perfused with aCSF at 0.2 μl/min. For the i.v. administration group, microdialysis sampling consisted of two segments (described below) under low illumination during the dark phase. For the i.p. administration group, sampling consisted of just one segment (described below) under fluorescent light in the light phase. Different phases of the light/dark cycle matched the behavioral analyses of cocaine self-administration (dark phase i.v. administration) and motor activation (light phase i.p. administration).

Estimation of basal dopamine levels in the nucleus accumbens. The no net flux method was used to estimate extracellular levels of dopamine. Thus, concentrations of dopamine above and below the anticipated extracellular concentration in the nucleus accumbens were perfused through the probe. Resultant dialysate concentrations were compared to the perfusate concentrations to generate a series of points interpolated to determine the concentration at which there was no net analyte flux (Lonnroth *et al*, 1987; Parsons and Justice, 1992). Specifically, the perfusate flow rate was increased to 0.6 μl/min 1 h before dialysate sampling. Then, baseline samples were collected at 15-min intervals for 1 h. The perfusate was then changed to an aCSF containing dopamine (2.5, 5.0, or 10 nM), allowed to equilibrate for 15 min, then samples were collected for another 1 h before the perfusate was changed to a different concentration of dopamine. Dopamine concentrations were presented in varied order with all subjects receiving all concentrations.

Dopamine levels in the nucleus accumbens following i.v. cocaine injection. Cocaine injections were given i.v. in order to measure cocaine-related increases in extracellular dopamine in the nucleus accumbens. After the no net flux analysis, the flow rate was increased to 1.0 μl/min to facilitate sample separation for dopamine and cocaine quantitation in the dialysate. Following 1 h equilibration, baseline samples were collected at 10-min intervals for 60 min, then three i.v. infusions of cocaine were given in succession (~0.37, 0.74, or 2.92 mg/kg) each followed by 60 min of dialysate sampling, except the last infusion followed by 90 min sampling. Concentrations of cocaine were titrated across age groups to account for body weight. Injection volume determined dose within age groups. Thus, solutions of approximately 1.00 or 2.50 mg/ml for periadolescent or adult rats, respectively, were administered over 2, 4, or 16 s (Harvard Apparatus pump, South Natuck, MA).

Dopamine levels in the nucleus accumbens following i.p. cocaine injection. The perfusate flow rate was increased to 1.0 μl/min after recovery from probe implantation. Sixty minutes later, six baseline samples were collected at 10-min intervals. Rats were then briefly removed, injected with 20 mg/kg cocaine i.p., and returned immediately to the test cage to continue sampling for 2 h.

Data Analysis and Histology

Motor activity. Automated measures of *acute* cocaine-induced locomotion (matrix crossings) were analyzed using three-way analyses of variance (ANOVAs) with factors of age × cocaine dose × time. (Time was a repeated measure tested in 5 min blocks over the first 1 h of testing *and* in two 1/2 h intervals of 0–30 min and 30–60 min post-injection.) Matrix crossings after *repeated* cocaine were summed over the 2 h test and analyzed using a three-way ANOVA with main factors of age × cocaine dose × day. (Day was a repeated measure for days 0, 5, 10, and 15). Two-way and one-way ANOVAs followed by *post hoc* analyses (simple effects or *t*-tests with Bonferroni's correction) were conducted as appropriate. In all cases, *p*-values <0.05 were considered significant. Observational measures of sniffing, grooming, and rearing were summed over the 1 h observation period and analyzed as above.

Self-administration data. Rats were defined as having acquired self-administration when their lever-pressing behavior met the following criteria for at least three successive sessions and throughout the remainder of the acquisition period: (a) the number of responses on the active lever exceeded two times the number of presses on the inactive lever and (b) the number of responses on the active lever was greater than 12. (Twelve is the average number of non-reinforced lever-presses made by cocaine-naïve periadolescent and adult rats in the absence of cocaine; data not shown.) See Donny *et al* (1998) for a similar definition of acquisition. The proportion of rats in each age group that acquired self-administration was compared for each test day using Fisher's exact test. Lever pressing on active and inactive levers was also compared using a three-way ANOVA on lever × age × days (repeated measure) for all rats. Finally, total cocaine intake (mg/kg) was compared among all rats using unpaired *t*-tests between age groups.

Microdialysis sampling. For the no net flux analysis, dialysate dopamine concentrations were estimated using the mean of four samples at each perfusate dopamine concentration (0, 2.5, 5.0, and 10.0 nM). First-order regressions provided the slope and intercept values to calculate the point of no net flux (zero intercept on the *y*-axis), which estimates extracellular dopamine concentration (Parsons and Justice, 1992). The *in vivo* probe recovery was calculated as the slope of the no net flux regression line. Values were compared using unpaired *t*-tests between age groups. For cocaine-stimulated dopamine, concentrations before cocaine injections did not differ between age groups, so percentage of baseline data were analyzed using two-way ANOVAs on age × time (repeated measure) and simple effects *post hoc* analyses as appropriate. Separate analyses were carried out for i.p. and i.v. routes of administration. Brain levels of cocaine (nM) were also analyzed with two-way repeated measures ANOVA and simple effects testing. Concentrations were not adjusted for probe efficiency.

Histology. Probe placement was verified by mounting 40 μm brain slices, staining with cresyl violet, and examining under a microscope. Only those subjects with placement in the core/shell region medial to the anterior commissure between 1.00 and 2.70 mm anterior to bregma were included. Distinctions between core and shell were not investigated, but probe placements were more in the core than the shell in 60% of periadolescent subjects and 81% of adults, with remaining placements more in the shell.

RESULTS

Experiment I: Effects of Acute and Repeated Cocaine Injection on Motor Activity

Locomotor behavior. Periadolescent and adult rats demonstrated similar patterns of locomotor response to *acute cocaine injection* (Figure 1a–c). Matrix crossings increased dose-dependently after 10 or 20 mg/kg cocaine injections for 30 or 60 min, respectively, regardless of age group. Adult rats exhibited more matrix crossings during the second 30 min post-cocaine interval, regardless of drug dose, but variability within age groups obscured overall interactions between age, dose, and time effects. Thus, three-way ANOVA on the first 30 min post-cocaine revealed significant

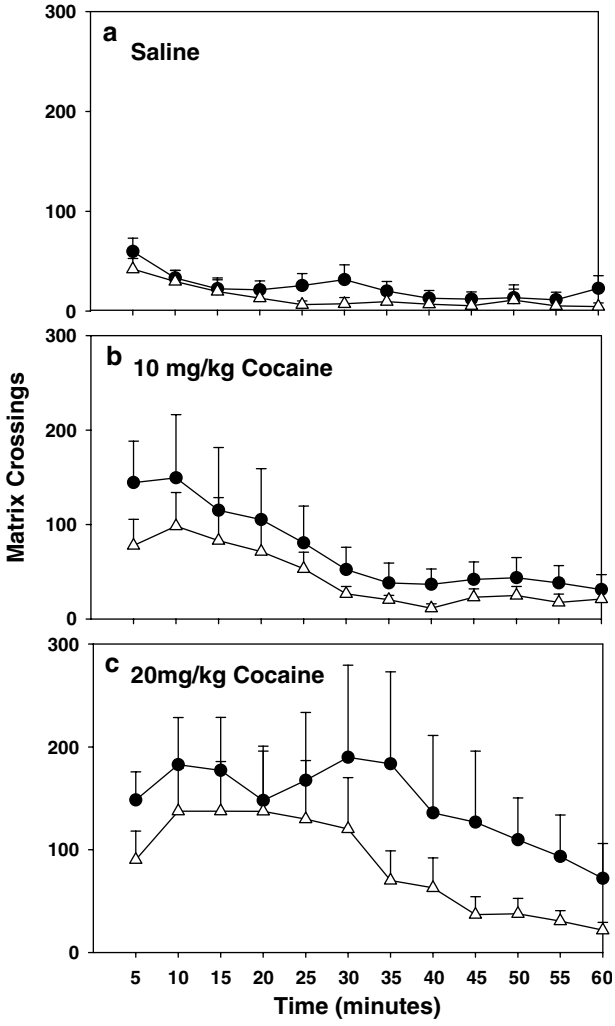

Figure 1 Number of matrix crossings in 5 min intervals over 1 h test by periadolescent (open triangles) and adult (closed circles) rats administered 1 ml/kg saline vehicle (a), 10 mg/kg cocaine (b), or 20 mg/kg cocaine (c). Rats were habituated to the test cage for 30 min before injection at time 0. Points represent means ± SEM ($n = 7$–10). Periadolescent rats exhibited fewer crossings than adults in the 30–60 min period, regardless of drug dose.

main effects of dose ($F(2,45) = 8.69$, $p < 0.001$) and time ($F(5,225) = 2.76$, $p < 0.05$), a significant dose × time interaction ($F(10,225) = 2.59$, $p < 0.01$), but no main effect of age, nor a significant three-way interaction. Three-way ANOVA on the second 30 min post-cocaine interval (30–60 min post-cocaine) revealed significant main effects of age ($F(1,45) = 4.72$, $p < 0.05$), dose ($F(2,45) = 6.92$, $p < 0.001$), time ($F(5,225) = 4.07$, $p < 0.01$), a significant dose × time interaction ($F(10,225) = 3.56$, $p < 0.001$), but no significant three-way interaction. All groups habituated similarly to the test environment for 30 min before injection (data not shown).

After *repeated cocaine injections*, matrix crossings increased in an age- and dose-specific manner (Figure 2a–c). A three-way ANOVA revealed a significant age × dose × day interaction ($F(6,108) = 5.49$, $p < 0.001$) as well as significant main effects of dose ($F(2,36) = 25.92$, $p < 0.001$) and days ($F(3,108) = 9.69$, $p < 0.001$). Subsequent two- and one-way ANOVAs and *post hoc* comparisons revealed specific effects. First, at the 10 mg/kg cocaine dose, adult rats showed significantly more matrix crossings on day 15 than day 0 ($p < 0.05$, one-way ANOVA on days ($F(3,18) = 4.144$, $p < 0.02$)). In contrast, no significant sensitization occurred among periadolescents at this dose. However, at the 20 mg/kg dose, adults failed to show sensitized responding, whereas periadolescents showed more matrix crossings on days 10 and 15 compared with day 0 ($p < 0.02$, one-way ANOVA on days ($F(3,21) = 8.47$, $p < 0.001$)). Second, age comparisons on specific test days indicated that adult rats exhibited significantly more matrix crossings than periadolescents under three conditions: day 10 (third injection) of 10 mg/kg cocaine, day 15 (fourth injection) of 10 mg/kg cocaine, and day 5 (second injection) of 20 mg/kg cocaine. Finally, adult rat responses to 20 mg/kg cocaine on day 10 were surprisingly low; given that we did not record motor stereotypies on day 10, we cannot rule out the possibility that stereotyped behavior interfered with matrix crossings.

Stereotyped behaviors. Observations of motor stereotypies demonstrated that *acute cocaine injection* dose-dependently increased some of the observed behaviors (data not shown). *Sniffing* was increased by cocaine; a two-way age × dose ANOVA revealed a significant main effect of dose ($F(2,45) = 5.96$, $p < 0.01$), with follow-up one-way ANOVA and *t*-tests with Bonferroni's correction indicating significant differences between 0 and 20 mg/kg doses. Neither the main effect of age nor an age × dose interaction was significant.

With regard to acute effects of cocaine on *grooming*, a significant age × dose interaction ($F(2,45) = 3.18$, $p = 0.05$) followed by one-way ANOVA and *t*-tests with Bonferroni's correction indicated differences among periadolescents such that grooming was increased in a dose-dependent manner between 0 and 10 or 20 mg/kg doses. Main effects of age and dose were not significant, although a trend toward significant main effect of dose ($F(2,45) = 2.89$, $p = 0.06$) suggests increased grooming induced by cocaine, regardless of age group.

With regard to observed *rearing* behavior, no significant effects of acute cocaine were observed, although a trend toward main effect of dose ($F(2,45) = 2.74$, $p = 0.08$) suggests increased rearing by cocaine, regardless of age group. These results together reveal behavior-specific effects of cocaine on motor stereotypies in periadolescent and adult male rats alike.

After *repeated cocaine injections*, observed motor stereotypies did not reveal robust sensitization (data not shown). Three-way ANOVAs with age × dose × days (repeated measure) as factors on sniffing, grooming, or rearing did not reveal significant interactions. With regard to *sniffing*, a significant main effect of dose ($F(2,40) = 7.85$, $p < 0.001$) with follow-up testing showed that 20 mg/kg cocaine elevated sniffing regardless of age group or test day. With regard to *grooming*, a trend toward main effect of days ($F(1,37) = 3.89$, $p = 0.06$) suggested a *decline* on day 15 compared with day 0, regardless of age or dose group. Also a main effect of age on grooming ($F(1,37) = 4.38$, $p < 0.05$) revealed significantly *more* grooming behavior by periadolescents over adults, regardless of cocaine dose or test day. With regard to *rearing*, the main effect of dose ($F(2,37) = 10.68$, $p < 0.001$) with follow-up testing indicated increased rearing induced by 20 mg/kg cocaine compared with saline or 10 mg/kg cocaine, regardless of age group or test day.

Experiment II: Acquisition of Cocaine Self-Administration

Individual Fisher's exact tests conducted on the difference in proportions of rats acquiring self-administration on each day of testing revealed no difference between age groups in rate of acquisition of cocaine self-administration (Figure 3 and Table 1). Moreover, active lever pressing

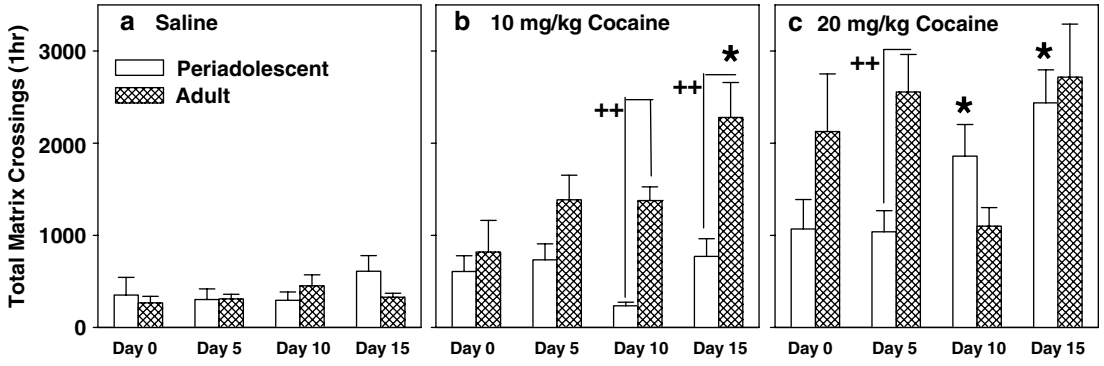

Figure 2 Total matrix crossings in repeated 2 h test sessions in periadolescent (open bars) or adult rats (hatched bars) administered saline vehicle (1 ml/kg; a), 10 mg/kg cocaine (b), or 20 mg/kg cocaine (c) on days 0, 5, 10, and 15. Rats were habituated for 30 min before each injection. Bars represent means ± SEM ($n = 6$–8). Significant differences from age- and dose-matched day 0 groups are indicated (*$p < 0.05$), as well as significant differences between age groups ($^{++}p < 0.01$).

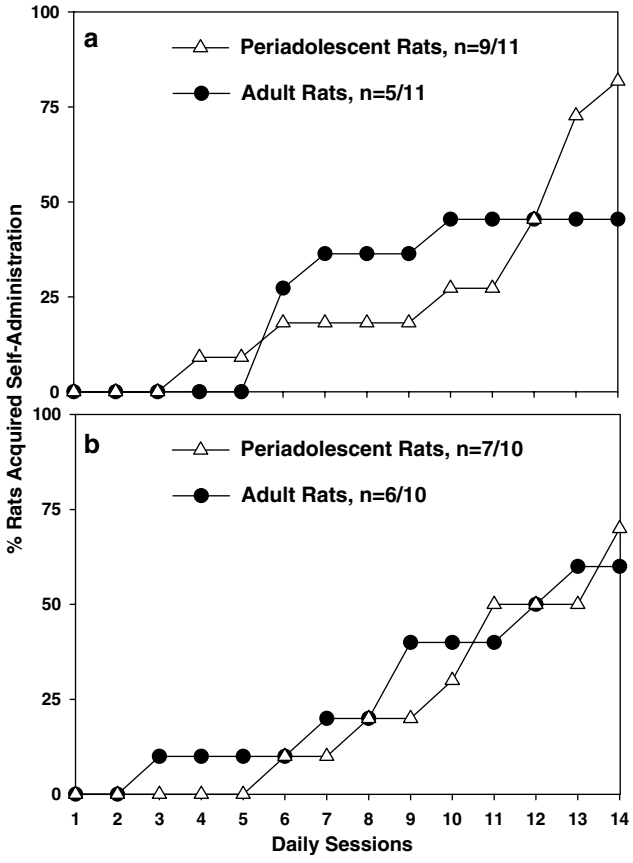

Figure 3 Percent of rats acquiring self-administration of a low dose of cocaine (0.37 mg/kg/infusion; $n=11$ per age group (a)) or high dose (0.74 mg/kg/infusion; $n=10$ per age groups) across daily test sessions in periadolescent (open triangles) or adult (closed circles) rats. Final proportions on day 14 are indicated in legends. (See Materials and methods for definition of acquisition.)

did not differ between age groups (Figure 4a and b). To analyze active and inactive lever pressing separately at each dose, we conducted two-way ANOVAs with days (repeated measure) × age as factors (results in Table 2). For both cocaine doses, main effects of days on active lever-presses showed increased responding across acquisition. At the low dose, a main effect of days on inactive lever-presses revealed decreased responding over days. Interestingly, a main effect of age on inactive lever-presses in the low-dose cocaine group revealed higher inactive lever pressing by periadolescent over adult rats, regardless of test day. Total cocaine intake (mg/kg) across the 14-day acquisition test did not differ across dose or age groups (Table 3).

Catheter surgery and cocaine self-administration did not impede weight gain in adolescent or adult rats, compared with age-matched, drug-naïve control rats from another experiment. Among the present rats that acquired self-administration (with highest cocaine intake), initial average body weights were 143.8 ± 3.1 and 338.0 ± 3.4 g for periadolescent ($n=16$) and adult rats ($n=11$), respectively, and final body weights on the last day of self-administration were 236.3 ± 4.4 and 397.0 ± 6.1 g. For matched controls, initial body weights were 147.5 ± 3.6 and 372.4 ± 5.9 g for periadolescent ($n=11$) and adult ($n=15$) groups, respectively, and final body weights were 237.0 ± 4.5 and 413.5 ± 6.9 g. (Values are mean ± SEM.)

Experiment III: Basal and Cocaine-Stimulated Dopamine Levels in the Nucleus Accumbens

As revealed by no net flux methodology, neither basal extracellular levels of dopamine nor *in vivo* recovery of dopamine differed across age groups (Figure 5). Estimated extracellular dopamine concentrations (nM) were

Table 1 Results of Fisher's Exact Test for a 2 × 2 Contingency Table on the Difference in Proportions of Rats Acquiring Self-Administration over 14 Days Acquisition Testing

	0.37 mg/kg/infusion cocaine			0.74 mg/kg/infusion cocaine		
Day	Periadolescent, acquired (out of 11)	Adult, acquired (out of 11)	*p*-value	Periadolescent, acquired (out of 10)	Adult, acquired (out of 10)	*p*-value
1	0	0	>0.9999	0	0	>0.9999
2	0	0	>0.9999	0	0	>0.9999
3	0	0	>0.9999	0	1	>0.9999
4	1	0	>0.9999	0	1	>0.9999
5	1	0	>0.9999	0	1	>0.9999
6	2	3	>0.9999	1	1	>0.9999
7	2	4	0.6531	1	2	>0.9999
8	2	4	0.6531	2	2	>0.9999
9	2	4	0.6531	2	4	0.6285
10	3	5	0.6594	3	4	>0.9999
11	3	5	0.6594	5	4	>0.9999
12	5	5	>0.9999	5	5	>0.9999
13	8	5	0.3870	5	6	>0.9999
14	9	5	0.1827	7	6	>0.9999

5.39 ± 1.03 ($n = 9$) and 4.77 ± 0.66 ($n = 8$) for periadolescent and adult rats, respectively ($T(15) = -0.52$). The slopes of the no net flux regression lines, used to estimate *in vivo* probe recovery, were 0.55 ± 0.08 and 0.71 ± 0.05 for periadolescent and adult rats, respectively ($T(15) = 1.59$).

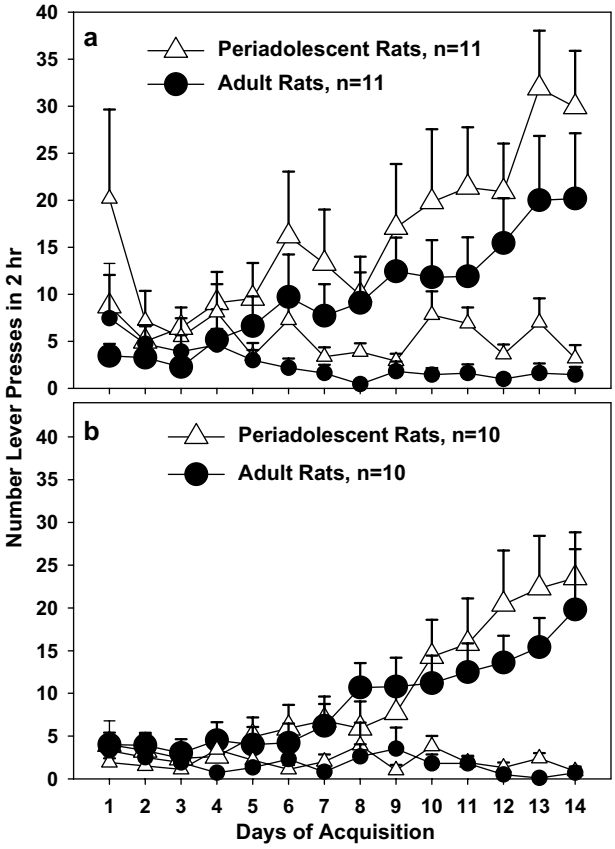

Figure 4 Number of presses on the active lever (large symbols) or inactive lever (small symbols) during cocaine self-administration in periadolescent (open triangles) and adult rats (closed circles) with access to a low-dose cocaine (0.37 mg/kg/infusion (a)) or high-dose (0.74 mg/kg/infusion (b)). Points represent means ± SEM ($n = 10$–11).

In the same rats, i.v. cocaine dose-dependently increased dopamine in the nucleus accumbens to a similar degree in periadolescent and adult rats (Figure 6a). Baseline dialysate dopamine was similar between ages (6.20 ± 0.90 and 7.13 ± 1.33 nM for periadolescent and adult rats, respectively; values not adjusted for probe efficiency), so data were analyzed as a percentage of baseline. A significant main effect of time ($F(26,416) = 30.39$, $p < 0.0001$) and simple effects analyses specified increases in dopamine for 10, 20, or 30 min after the low, mid-, or high dose, respectively. Neither the main effect of age ($F(1,16) = 0.025$) nor the age × time interaction ($F(26,416) = 1.30$) was significant.

I.v. cocaine produced higher brain cocaine in adults than periadolescents, after the mid-range and high-dose injections (Figure 6b). A trend toward a main effect of age did not reach significance ($F(1,7) = 4.84$, $p = 0.06$), but the main effect of time ($F(20,140) = 67.39$, $p < 0.0001$) and the age × time interaction were significant ($F(20,140) = 3.50$, $p < 0.0001$). Follow-up analyses revealed greater brain cocaine in adults for 20 min after the 0.74 and 2.92 mg/kg i.v. injections.

I.p. cocaine increased dopamine similarly across ages (Figure 7a). Baseline dopamine did not differ (1.99 and 2.24 nM for periadolescents and adults, respectively), so data were analyzed as a percentage of baseline. Neither the main effect of age ($F(1,11) = 0.002$, $p = 0.96$) nor the age × time interaction ($F(17,187) = 0.48$, $p = 0.96$) was significant, although the main effect of time was significant ($F(17,187) = 20.96$, $p < 0.0001$); cocaine increased dopamine for 40 min post-injection.

Table 3 Cocaine Intake (mg/kg)

	Periadolescent	Adult
0.37 mg/kg/infusion cocaine $T(16) = -1.32$, $p = 0.21$	82.72 ± 24.39 ($n = 11$)	46.35 ± 13.00 ($n = 11$)
0.74 mg/kg/infusion cocaine $T(18) = -0.29$, $p = 0.98$	111.56 ± 23.79 ($n = 10$)	81.73 ± 23.26 ($n = 10$)

Table 2 Results of Two-Way ANOVAs (Days (Repeated Measure) × Age) on Lever Pressing on the Active Lever (Left Columns) or Inactive Lever (Right Columns) over 14 Days Acquisition Testing in Periadolescent *vs* Adult Rats

Active lever				Inactive lever			
Effect	**df**	**F**	***p***	**Effect**	**df**	**F**	***p***
Low dose							
Days	13, 260	12.57	<0.0001*	Days	13, 260	3.76	<0.0001*
Age	1, 20	1.25	0.28	Age	1, 20	5.43	0.030*
Days × age	13, 260	0.63	0.83	Days × age	13, 260	1.022	0.43
High dose							
Days	13, 234	16.11	<0.0001*	Days	13, 234	1.24	0.25
Age	1, 18	0.093	0.76	Age	1, 18	0.12	0.74
Days × age	13, 234	1.16	0.31	Days × age	13, 234	1.51	0.12

* indicates $p < 0.05$.

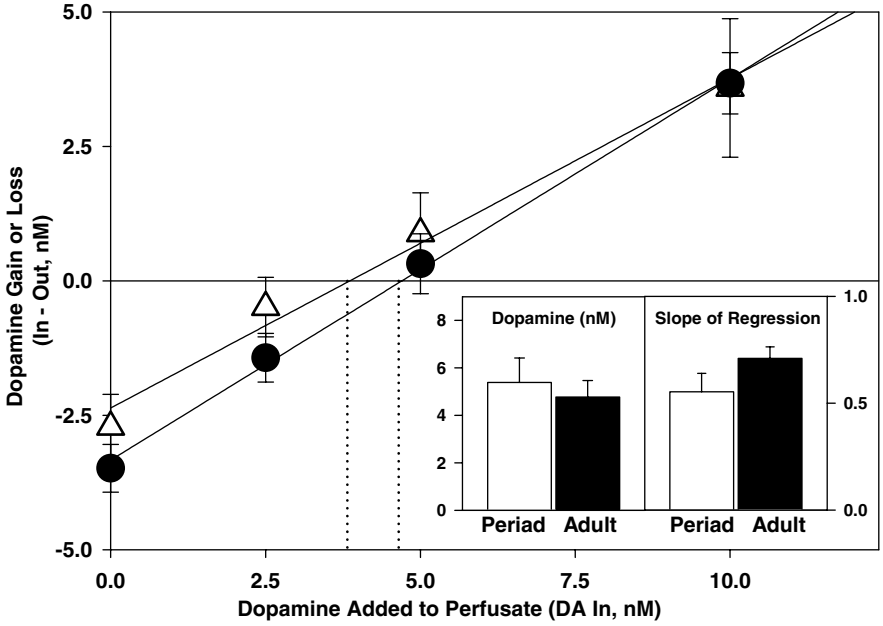

Figure 5 Dopamine gain or loss to the brain as a function of perfusate concentration for periadolescent (open triangles, $n=9$) or adult (closed circles, $n=8$) rats. The point of no net flux (zero on the *y*-axis) is the estimated extracellular concentration of dopamine. The left panel of inset graphs indicates average estimated dopamine concentrations based on *individual regression functions* for periadolescent (open bar) and adult (filled bar) rats. The right panel shows average slope of individual regression lines, an indicator of *in vivo* dopamine clearance.

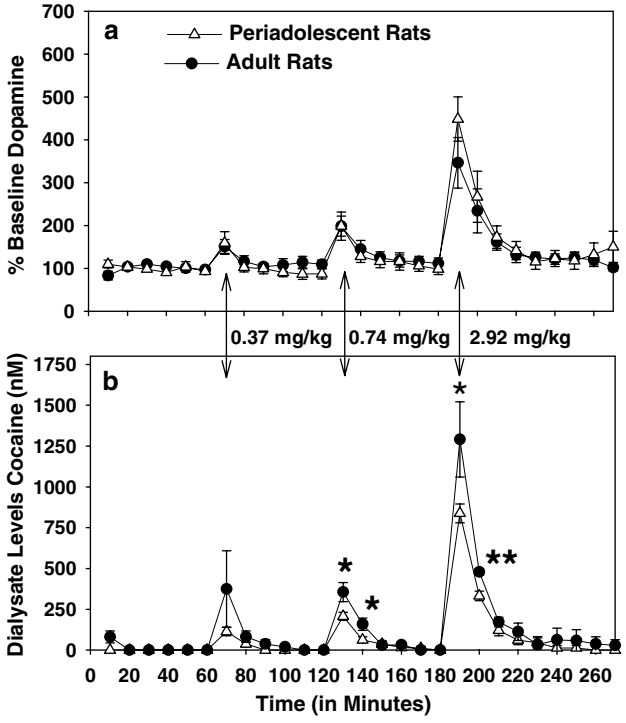

Figure 6 Percent basal dopamine concentrations ((a), $n=8$–10) and dialysate levels of cocaine ((b), $n=4$–5) in 10 min intervals for periadolescent (open triangles) or adult (closed circles) rats at baseline or following i.v. injection of approximately 0.37, 0.74, or 2.92 mg/kg cocaine. Significant differences between age groups are indicated (*$p<0.05$, **$p<0.01$).

I.p. cocaine injections also produced similar brain cocaine across ages (Figure 7b). The main effect of age was not significant ($F(1,9)=1.068$) nor was age × time interaction ($F(11,99)=0.32$, $p=0.98$). A significant main effect of time ($F(11,99)=52.24$, $p<0.0001$) and a follow-up test revealed significant increases in brain cocaine for 70 min post-injection.

DISCUSSION

The present results are in partial agreement with previous reports of periadolescent hyposensitivity to i.p. cocaine-induced motor activity compared with younger or older rats. Nevertheless, extracellular levels of dopamine in the nucleus accumbens were similar across age groups under basal conditions and after acute i.p. cocaine injection, suggesting that age differences in locomotor stimulant effects are not mediated by dopamine efflux in the nucleus accumbens. Age-related differences in cocaine-induced locomotor behavior were not paralleled by rate of acquisition of i.v. cocaine self-administration, nor by differences in the amount of cocaine self-administered. Furthermore, extracellular levels of dopamine in the nucleus accumbens stimulated by i.v. cocaine were similar across age groups. Together, these findings present a developmental dissociation between the locomotor and reinforcing effects of cocaine; periadolescent rats displayed lower sensitivity to the locomotor effects of the drug, but similar sensitivity to the reinforcing effects of the drug.

A lower sensitivity of periadolescent rats to the *acute* motor stimulatory effects of cocaine was previously manifest in behaviors such as matrix crossings (Snyder *et al*, 1998; Spear and Brick, 1979), rearing (Laviola *et al*, 1995), stereotyped sniffing (Laviola *et al*, 1995; Spear and Brick, 1979), and head-scanning (Laviola *et al*, 1995). In the present experiment, matrix crossings were lower in periadolescent compared with adult rats during the second

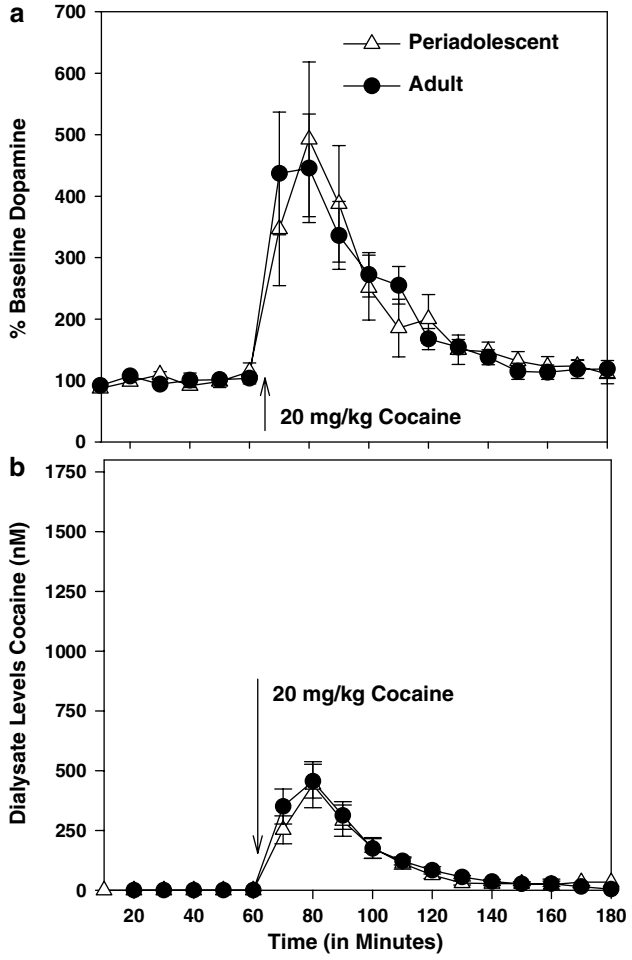

Figure 7 Percent basal dopamine concentrations ((a), $n = 5–8$) and dialysate levels of cocaine ((b), $n = 5–6$) in 10 min intervals for periadolescent (open triangles) or adult (closed circles) rats at baseline or following i.p. injection of 20 mg/kg cocaine.

30 min interval after acute injection, regardless of drug dose, but motor stereotypies were mainly similar across age groups, with only grooming showing evidence of *higher* rates among periadolescents *vs* adults. These data support recent assertions that periadolescent hyposensitivity to dopaminergic compounds is detectable but less robust than previously postulated (Maldonado and Kirstein, 2005b). Strain differences could also explain the less robust hyposensitivity presently observed in Wistar rats *vs* that previously seen with Sprague–Dawley rats. Also our subjects were handled and weighed for several days before experimentation, which may have elevated responding in periadolescents to near adult levels; experimenter handling before cocaine injections increased cocaine-induced locomotion in periadolescent but not adult rats (Maldonado and Kirstein, 2005a, b).

In previous studies, a lower sensitivity of periadolescent rats to the motor effects of cocaine extended to a lesser degree of motor sensitization after repeated cocaine injections (Collins and Izenwasser, 2002; Laviola *et al*, 1995; Snyder *et al*, 1998). In the present extended dosing regimen, locomotion by periadolescents failed to sensitize after repeated administration of 10 mg/kg cocaine, when adult locomotion sensitized. Also periadolescents exhibited less motor activity than adults after the third and fourth 10 mg/kg injections and after the second injection of 20 mg/kg cocaine. However, periadolescents did eventually show locomotor sensitization after the third and fourth 20 mg/kg cocaine injections. Neither periadolescent nor adult rats showed sensitization of motor stereotypies, perhaps again owing to differences in strain and dosing schedule.

Behavioral and neurochemical sensitization have been linked to vulnerability to drug reinforcement (Robinson and Berridge, 2000). Thus, periadolescent hyposensitivity to locomotor sensitization predicts hyposensitivity to cocaine-related behavioral reinforcement, but this prediction was not supported in the present experiments. Although more periadolescent rats in the low-dose cocaine condition tended to acquire self-administration compared to adults, the difference in proportions was not significant and therefore periadolescent and adult rats demonstrated the same rate of acquisition. Furthermore, active lever pressing and cocaine intake were similar across age groups for the doses of cocaine tested. A separate analysis was conducted to evaluate the influence of cocaine concentration, infusion volume, and infusion duration on operant behavior in both age groups and demonstrated that these factors did not change the observation of no age-related difference in the acquisition or maintenance of cocaine self-administration (data not shown). In another study, cocaine intake over a short 5-day nose-poke acquisition test did not differ significantly between PND 27, 37, and 90 rats (Belluzzi *et al*, 2005). Also cocaine- or morphine-conditioned place preference was indistinguishable between periadolescent and adult Sprague–Dawley rats (Campbell *et al*, 2000). Although we cannot rule out the possibility that lower cocaine doses might demonstrate significant age-related differences in reward and reinforcement paradigms, these data sets imply that although cocaine-related motor activity and behavioral reinforcement (or reward) share neural circuitry, they are not inextricably linked.

Notably, periadolescent rats in the present low cocaine dose group pressed the inactive lever significantly more than their adult counterparts. High inactive lever pressing may represent impulsivity or low stimulus control among periadolescents, an idea consistent with hypothesized immature systems for behavioral suppression in adolescent subjects (Adriani and Laviola, 2003; Laviola *et al*, 2003).

The principal action of cocaine is to block monoamine reuptake (Kuhar *et al*, 1991). Although other transporters and brain regions are involved (Bardo, 1998, for review), blockade of dopamine transporters in the nucleus accumbens appears necessary for cocaine's acute and chronic effects (Church and Justice, 1987; Hurd and Ungerstedt, 1989; Kelly *et al*, 1975; Koob, 1992; White, 1998; Wise and Bozarth, 1987). Consistent with the present results on behavioral reinforcement but not locomotion, periadolescent and adult rats exhibited similar nucleus accumbens basal and cocaine-stimulated dopamine levels. These results suggest that dopamine transporter expression and function are likewise similar across age groups. Accordingly, insofar as the slope of the no-net-flux line of regression reflects *in vivo* uptake (Parsons and Justice, 1994), no differences in dopamine clearance by uptake mechanisms such as transporters were demonstrated in our experiments. In

corroboration, similar basal expression of dopamine transporters was reported for dorsal and ventral striatum of periadolescent and adult Sprague–Dawley rats (Collins and Izenwasser, 2002). On the other hand, *repeated* i.p. cocaine injection increased dopamine transporter expression in adult Sprague–Dawley rats but not periadolescents (Collins and Izenwasser, 2002), providing a potential explanation for sensitization in adult but not periadolescent rats at our mid-range cocaine dose. Age differences in acute motor effects of cocaine are left unexplained.

One caveat in the present neurochemical analysis must be considered: a distinction between core and shell subregions of the nucleus accumbens is not reported. Dopaminergic activity in the core of the nucleus accumbens is closely aligned with motor activity and instrumental learning (Kelley *et al*, 1997; Kelley and Swanson, 1997; Maldonado-Irizarry and Kelley, 1994; Pulvirenti *et al*, 1994), whereas dopamine in the shell appears responsive to primary rewards (Carlezon and Wise, 1996; Di Chiara *et al*, 1993; Tanda *et al*, 1997). Based on our behavioral results, the core but not shell is predicted to show age-related differences in extracellular dopamine after i.p. cocaine.

Postsynaptic to dopamine terminals, GABAergic neurons in the striatum integrate dopamine signals in part via complex interactions between D1- and D2-like dopamine receptors (Fontana *et al*, 1993; Hu and White, 1997; Ikemoto *et al*, 1997). Thus, transient overexpression of D1 and D2 dopamine receptors during periadolescence (eg Teicher *et al*, 1995) and altered receptor coupling with cAMP (Andersen, 2002) may regulate responsiveness to cocaine. Moreover, projections between the frontal cortex, midbrain, and nucleus accumbens modulate the effects of acute and repeated cocaine (Vanderschuren and Kalivas, 2000, for review), and these regions mature just before or during periadolescence (Alexander and Goldman, 1978; Insel *et al*, 1990; Kalsbeek *et al*, 1988; Teicher *et al*, 1998, 1991). On the molecular level, age-specific sensitivity of transcription factors to drug exposure may have extensive and enduring effects on neural signaling. For example, periadolescent male CD-1 mice showed higher deltaFosB upregulation in the nucleus accumbens than adults after repeated cocaine or amphetamine injections (Ehrlich *et al*, 2002). Future experiments will define roles for these cellular and molecular pathways in mediating developmental changes in cocaine responsivity.

Pharmacokinetic mechanisms are not likely to mediate periadolescent-specific behavioral effects of psychostimulants. Despite evidence for periadolescent motor hyposensitivity to stimulants (Adriani and Laviola, 2000; Bolanos *et al*, 1998; Infurna and Spear, 1979; Spear and Brake, 1983, 1979), systemic amphetamine injections produced a monotonic rise in post-mortem amphetamine brain concentrations across ontogeny that did not correlate with the developmental trajectory of behavioral responding (Spear and Brake, 1983). In other experiments, age-specific effects of dopaminergic ligands were maintained across systemic and intracerebral routes of administration, implying pharmacodynamic rather than pharmacokinetic mechanisms (Campbell *et al*, 1988; Frantz and Van Hartesveldt, 1995). Our data showed no age difference in dialysate cocaine after i.p. cocaine injections. Although we did observe lower dialysate cocaine levels in periadolescent *vs* adult rats after a high-dose i.v. cocaine injection, behavioral reinforcement mediated by i.v. cocaine did not differ across age groups.

Overall, a developmental dissociation between cocaine-stimulated locomotor activity and reinforced behavior may be practical from an ethological perspective. In periadolescence, mature reinforcement circuitry used to assign hedonic value to stimuli may be necessary for survival. On the other hand, continued flexibility in motor reactivity throughout adolescence may facilitate relocation from the early home environment to a new territory. Furthermore, even if reinforcement circuitry is not fully mature by periadolescence, its direct activation with a stimulus as strong as i.v. cocaine may not provide an assay sensitive enough to detect age-related qualitative differences in reinforcement processing. In contrast, motor activation by doses of cocaine over the present extended dosing regimen appears to be sensitive to developmental changes.

Although extrapolation from rodent to human psychopharmacology may be tenuous, the present data suggest that human adolescents experience the same vulnerability to cocaine reinforcement, and thereby cocaine dependence, as adults. Compounded by altered responsivity to stress, immature rational decision-making skills, high novelty-seeking, and social factors leading to drug experimentation such as increased free time, cash flow, and independence, human adolescents may be particularly vulnerable to initiating an addiction cycle with cocaine or other psychomotor stimulant drugs (Gruber, 2001; Laviola *et al*, 2003; Spear, 2000; Steinberg, 1999).

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