Psychopharmacology
DOI 10.1007/s00213-008-1327-0

ORIGINAL INVESTIGATION

# Evaluation of a pharmacokinetic hypothesis for reduced locomotor stimulation from methamphetamine and cocaine in adolescent versus adult male C57BL/6J mice

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Received: 3 April 2008 / Accepted: 30 August 2008


**Abstract**
*Rationale* Adolescent mice display reduced locomotor stimulation to cocaine and amphetamine compared to adults, but the mechanisms are not known.
*Objectives* The primary aim of the current study is to test a possible pharmacokinetic explanation for the attenuated locomotor stimulation seen in adolescents. A secondary aim is to extend the current literature for acute methamphetamine in adolescents.
*Materials and methods* Male, adolescent (PN 30-35) and adult (PN 69-74) C57BL/6J mice were administered an intraperitoneal injection of cocaine (5, 15, 30 mg/kg) or methamphetamine (1, 2, 4 mg/kg) and euthanized 5, 10, 15, 30, 60, 120, or 240 min later. Home cage locomotor activity was recorded by video tracking, and drug concentration levels in brain and blood from the infraorbital sinus were measured using liquid chromatography combined with mass spectroscopy.
*Results* Both methamphetamine and cocaine increased locomotor activity in a dose–response fashion, but the magnitude of the increase was less in adolescents than adults. Concentration of methamphetamine in the brain was similar between ages across time points. Concentration of cocaine in the brain was significantly higher in adolescents than adults at 5 min, but similar at all other time points.
*Conclusions* Results suggest pharmacokinetics may make a small contribution to differential stimulation between adolescents and adult mice, but are unlikely the only factor. Developmental differences within the brain that effect pharmacodynamic properties of psychostimulants (e.g., number of receptor or transporters) represent alternatives.

**Keywords** Adolescent · Cocaine · Methamphetamine · Pharmacokinetics · Locomotor stimulation · Mice

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## Introduction

Human adolescents are prone to engage in risky behaviors such as taking drugs. This is disturbing since adolescents may be at a greater risk for addiction to drugs of abuse than adults (Estroff et al. 1989). Differential behavioral responses to psychostimulant drugs in adolescents versus adults have also been observed in rodents. For example, adolescent rodents display greatly reduced locomotor stimulation than adults after amphetamine or cocaine administration (Bolanos et al. 1998; Collins and Izenwasser 2002; Frantz et al. 2006; Lanier and Isaacson 1977; Laviola et al. 1999; Laviola et al. 1995; Spear and Brake 1983) but see (Parylak et al. 2008). Although neural adaptations associated with progressive increases in locomotor stimulation (i.e., sensitization) are widely regarded as relevant for addiction, the contribution of individual differences to the first acute locomotor response is not known and has been debated (Kalivas et al. 1998; Robinson and Berridge 2001). In any case, the differential behavioral response between adult and adolescent rodents is robust, and for that reason, it provides a useful model to explore potential mechanisms for differential behavioral responses to drugs between age groups.

One possible explanation for differential locomotor stimulation in adolescent versus adult rodents is that the two ages experience different levels of drug in their brain. Even though animals are given the same dose per kilogram body weight, cocaine and methamphetamine pharmacokinetics

might change with age. In fact, concentrations of amphetamine in adolescent rats have been found to be lower than adults up to 60 min after administration (Spear and Brake 1983). However, researchers concluded that attenuated stimulation in adolescents were not related to brain concentration levels based on the finding that younger rats had even lower concentrations in the brain, but no behavioral differences than adults.

Similar data are not available for methamphetamine. To the best of our knowledge, locomotor stimulation to methamphetamine between adolescents and adults has not been studied. Moreover, only one study has examined methamphetamine levels in the brains of adolescent versus adults and that was after four 10 mg/kg subcutaneous (s.c.) injections in Sprague–Dawley rats (Kokoshka et al. 2000). Using these data to generalize about possible pharmacokinetic contributions to differential locomotor stimulation is problematic, given that only one high dose was investigated and that the route of administration was different than the intraperitoneal (i.p.) route used in most other studies. Additionally, in Kokoshka et al. (2000), locomotor behavior of the rats was not examined making brain drug concentration and behavior correlations impossible.

Although more information is available about pharmacokinetics of cocaine in adolescents versus adult rodents, the data are still incomplete (see Table 1). First, no study has examined early time points (less than 10 min) when rapid changes are taking place. Second, the effect of different i.p. doses has not been examined within a single study. Third, only one study has looked at the time course of cocaine concentrations in the brain between age groups. In this study, dialysate levels of cocaine from the striatum after 20 mg/kg i.p. cocaine were compared between adolescents and adults and no differences found (Frantz et al. 2006). However, this conflicts with other studies showing lower cocaine levels in adolescents when whole brains are examined, but only for singular time points (Caster et al. 2005; McCarthy et al. 2004). Fourth, only one study has been preformed in mice (McCarthy et al. 2004). Given that strain and species differences in metabolism can occur (Azar et al. 1998; McCarthy et al. 2004), caution must be taken before generalizing the above findings for mice. Finally, to the best of our knowledge, no study has measured adolescent behavior and brain drug concentrations within the same animal. Therefore, within subject correlations have not been possible, only between subjects.

The aim of this study was to determine the extent to which pharmacokinetic differences between adolescents and adult C57BL/6J mice could account for differential acute locomotor stimulation to methamphetamine and cocaine between the age groups. Based on generalizations of past studies in mice and rats (see Table 1), we predicted that adolescents would have lower drug concentrations

**Table 1** Summary of the literature comparing psychostimulant pharmacokinetics in adolescent vs. adult rodents

| Author(s) | Drug and dose mg/kg | Strain | Age | Time point | Route admin. | Sampling technique | Results |
|---|---|---|---|---|---|---|---|
| Caster et al. (2005) | Cocaine 15 mg/kg given every hour for 3 h | Sprague–Dawley rats | PN 28, 42, 65 | 30 min after each injection | i.p. | Whole brain | No differences in brain cocaine levels, but blood plasma cocaine concentrations were higher in PN 65 than PN 28 and 42 |
| McCarthy et al. (2004) | Cocaine 20 mg/kg | C57BL/6 mice | PN 35, 63 | 15 min | i.p. | Whole brain | PN 35 had lower brain and blood cocaine concentrations |
| | Cocaine 20 mg/kg | CD-1 mice | PN 35, 63 | 15 min. | i.p. | Whole brain | PN 35 had lower blood, but not brain cocaine concentrations |
| Frantz et al. (2006) | Cocaine 20 mg/kg | Wistar rats | PN 37–52 and PN 75–90 | Every 10 min for 180 min | i.p. | Microdialysis from nucleus accumbens | No differences in brain dialysate levels of cocaine between age groups |
| | Cocaine 0.37, 0.74 and 2.92 mg/kg | Wistar rats | PN 37–52 and PN 75–90 | Every 10 min for 60 min | i.v. | Microdialysis, from nucleus accumbens | Adults showed higher dialysate levels of cocaine at 10 and 20 min |
| Kokoshka et al. (2000) | Methamphetamine 4 administrations of 10 mg/kg in 2 hr intervals | Sprague-Dawley rats | PN 40, 90 | 1 hr. after last injection | s.c. | Striatal tissue | PN 90 had higher methamphetamine concentrations in striatal tissue and blood plasma |
| Spear and Brake (1983) | Amphetamine 5 mg/kg | Sprague–Dawley rats | PN 25, 35, 45, adult | 15, 30, and 60 min. | i.p. | Whole brain | PN 25 and 35 had lower brain amphetamine levels than PN 45 at all time points |

relative to adults and that these lower concentrations would coincide with lower locomotor stimulation to cocaine and methamphetamine in adolescents versus adults.

## Materials and methods

### Subjects

Male, C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used. Adolescent mice were 21 days old at arrival and tested at 30–35 days of age. Adult mice were 60 days old at arrival and tested at 69–74 days of age. These are accepted time periods for adolescent and adult mice and rats (Spear and Brake 1983). Mice were housed in groups of 4 for 6 days after arrival and then housed singly for 5 days prior to the experiment. Single housing was necessary for video tracking (see below). Mice were not handled prior to the test day except for routine cage changes. Food and water were available at all times. All mice were housed on a 12-h reverse light–dark cycle (lights off at 10 A.M. and on at 10 P.M.). All testing was done at the onset of the dark cycle when animals are typically active. Room temperature was maintained at 21±1°C. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee and adhered to NIH guidelines. The University of Illinois is AALAC approved.

### Drugs

Both cocaine hydrochloride and methamphetamine hydrochloride were obtained from Sigma Aldrich (St. Louis, MO). Injection solutions were prepared according to the salt, not the base form. Drugs were dissolved in 0.9% saline and administered in a volume of 5 ml/kg. Doses and time points were chosen based on the literature (Azar et al. 1998; Benuck et al. 1987; Brien et al. 1978).

### Equipment

Mice were housed in custom-made home cages. Cages (18.5 cm×33.5 cm×16 cm) were constructed out of clear plastic with food and water access mounted on the side. Horizontal distance traveled in the home cage was recorded using TopScan (Clever Sys, Vienna, VA, USA) video tracking software. TopScan software was run on a Dell Precision 380 workstation (Dell Computer, Round Rock, TX, USA) which was connected to a Nuvico digital color quad interface (Nuvico, Englewood, NJ, USA) and an Osprey-2000 (Viewcast, Dallas, TX) or WinTV (Hauppauge Computer Works, Hauppauge, NY, USA) capture card. Four Panasonic WV-CP244 cameras (Panasonic, Secaucus, NJ, USA) mounted 152 cm above the cages were used to capture the video used for analysis.

### *Experiment 1—time course*

Previous studies have not established the time course for cocaine or methamphetamine concentrations in brain or blood in adolescents compared to adult mice. Therefore, one of the goals was to obtain these data for C57BL/6J. The purpose of these data was to identify time points when adolescent and adults display peak concentrations of drug in the brain, as well as time points when brain drug levels are similar between age groups.

### Methamphetamine

Mice were given a 2-mg/kg i.p. injection of methamphetamine and sampled at 5, 30, 60, 120, and 240 min postinjection ($n$=5, 3, 7, 4, 3 adults and 5, 4, 8, 4, 3 adolescents per time point, respectively). In each case, samples were taken from the infraorbital sinus and brain. Infraorbital sinus blood samples were collected using 44.7 μl heparinized capillary tubes and placed in microcentrifuge tubes containing 50 μl $ZnSO_4$ (5% in $H_2O$) and 50 μl $Ba(OH)_2$ (0.3 N). Immediately after taking the blood sample, mice were decapitated, and each hemisphere of the brain was collected and placed in microcentrifuge tubes containing 150 μl $ZnSO_4$ and 150 μl $Ba(OH)_2$. The $ZnSO_4$ and $Ba(OH)_2$ were added to precipitate out proteins and lyze cells. All tubes were kept on wet ice until processed.

### Cocaine

Mice were given a 30-mg/kg i.p. injection of cocaine and sampled at 5, 15, 30, or 60 min postinjection ($n$=6, 5, 6, 5, adults and 6, 6, 6, 6 adolescents per time point, respectively). Brain and blood samples were collected as before except 45 μl sodium fluoride (1% in $H_2O$) was added to each tube. Sodium fluoride was added to reduce molecular degradation of cocaine (Caster et al. 2005).

### *Experiment 2—locomotor activity*

Separate groups of animals ($n$=8 adolescents and eight adults) were used to measure the time course of locomotor stimulation to methamphetamine and cocaine. For this, TopScan was used to continuously record the home cage activity of animals before and after a series of two i.p. injections of saline or drug. The test began when TopScan was turned on at the beginning of the dark cycle. After 30 min of recording animals undisturbed, animals were given an i.p. injection of saline (0.9%) and immediately returned to home cages. After 60 min, animals were given

another injection, either 2 mg/kg methamphetamine or 30 mg/kg cocaine ($n$=4 adults and four adolescents per group).

*Experiment 3—dose response*

The 10-min time point for cocaine and 15-minute time point for methamphetamine were chosen for further analysis of drug concentrations in brain and blood for different doses because the pharmacokinetic data indicated concentration of drug in the brain would be close to the peak at these times in both age groups. A total of 96 additional animals were given 5, 15, or 30 mg/kg cocaine or 1, 2, or 4 mg/kg methamphetamine ($n$=8 / dose / age) and sampled after 10 or 15 min, respectively. In addition to collecting the drug concentration data, we also collected locomotor activity in the home cage up until the time of sampling.

*Liquid chromatography/mass spectrometry*

Preparation of stock solutions and standards

Stock cocaine and methamphetamine solutions were prepared in MilliQ water (Millipore Milli-Q Biocel water purification system, Billerica, MA, USA) to create 250 and 100 μg/ml solutions respectively. Stock solutions were used to prepare standard solutions of concentrations 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2, 3, 5, and 7 μg/mL for cocaine and 0.1, 0.5, 1, 5, 10, and 15 μg/ml for methamphetamine. All solutions were kept at 4°C and prepared fresh before each LC/MS run. Standard solutions were run in tandem with samples.

Sample preparation

Blood samples were centrifuged using an Eppendorf 5417R centrifuge (Hamburg, Germany) at 20,000×$g$ for 10 min and the supernatant transferred to a LC/MS vial (Agilent, Santa Clara, CA, USA). Brain samples were first homogenized for approximately 10 sec with a motorized pestle (Kontes, Vineland, NJ, USA). Water, 300 uL MilliQ, was added to brain cocaine samples before centrifuging in order to increase the volume of fluid for sampling. In order to aid in detecting low concentrations, water was not added to brains of mice that received methamphetamine. Brain samples were centrifuged at 20,000×$g$ for 15 min.

Instrumentation and chromatographic conditions

General LC/MS procedures followed Concheiro et al. (2006). An Agilent 1100 series LC system (Santa Clara, CA, USA) was used for sample separation and introduction to mass spectrometry. Samples were placed in a cooled sample tray (4°C) and injected (5 μL) into the Agilent column ZORBAX Eclipse XDB-C8 (4.6×150 mm, 5-micro) (Santa Clara, CA, USA) for cocaine and a Phenomenex Onyx Monolithic C18 column (100×4.6 mm; Torrance, CA, USA) for methamphetamine. The column was equilibrated with 95% solvent A (0.1% formic acid in $H_2O$) and 5% solvent B (ACN), and eluted at ambient temperature with a 300-μl/min flow rate. The linear gradient for cocaine is as follows: 1 min, 5% B; 8 min, 50% B; 13 min, 5% B. The linear gradient for methamphetamine was: 1 min, 5% B; 8 min, 100% B; 13 min, 5% B. A clean run of 100% ACN for 5 min, and then 5% ACN for 5 min was preformed after every ten samples followed by a blank run to ensure optimum column performance.

Positive ion mass spectra were acquired using an Agilent MSD Trap XCT Plus mass spectrometer equipped with an ESI source (Santa Clara, CA, USA). For best sensitivity, positive ESI signals from standard cocaine and methamphetamine solutions were tuned with the use of a Kd Scientific 789100A model syringe pump (Holliston, MA, USA) connected directly to ion source via PEEK tubing. Nitrogen was used as nebulizer gas (30 psi) and drying gas (9 l/min). The capillary voltage was set to 4.5 kV. The heated capillary of ESI source was kept at 350°C during the analysis. Software Chemstation for LC 3D system Rev B.01.03 (Agilent Technologies, Santa Clara, CA, USA) was used for LC/MS system control and data acquisition.

Data analysis

Statistical analysis was preformed with SAS 9.1 (SAS Institute, Cary, NC, USA). Adolescents and adults were compared for body mass and baseline locomotor activity using unpaired $t$ tests. The correlation between body mass and brain mass was analyzed using simple linear regression and polynomial regression. Distance traveled within 10 or 15 minute periods following injections and drug concentrations in the brain and the blood were analyzed using two-way analysis of variance with age and dose or age and time as factors. Pairwise differences were evaluated using Tukey or Scheffe post hoc tests. The relationship between locomotor stimulation and brain drug concentration was analyzed by analysis of covariance. In this model, locomotor activity was analyzed as the response, brain concentration as the continuous predictor (covariate), and age as the factor. For all tests, a $p$ value of $<0.05$ was considered significant.

## Results

Body mass

Adolescent body mass was 74% of adult body mass [16.4± 0.20 g SEM versus 22.3±0.16, $t(198)=23.1$, $P<0.0001$].

Adolescent brain mass was 95% of adult brain mass [404.4 mg±2.45 SEM versus 425.0±2.31 mg, $t(205)=6.1$, $P<0.0001$]. Brain mass was significantly correlated with body mass within each age group [adolescents: $R^2=0.20$, $t(103)=1.1$, $P<0.0001$) adults: $R^2=0.06$, $t(100)=2.4$, $P=0.017$] and among all individuals $R^2=0.26$, [$t(205)=8.44$, $P<0.0001$]. The second-order coefficient of the polynomial regression was significant [$t(204)=-1.99$, $P=0.048$] suggesting a curve or plateau in brain mass after approximately 20 g of body mass (Fig. 1).

## Methamphetamine

### *Experiment 1—time course*

Methamphetamine levels in the brain peaked slightly earlier and were slightly lower at 30 or 60 min postinjection in adolescent relative to adult animals though these effects were not significant (Fig. 2a). The effect of time was highly significant [$F(5,54)=4.23$, $P=0.003$], but there was no main effect of age [$F(1,54)=1.98$, $P=0.17$] or interaction.

### *Experiment 2—locomotor activity*

Under baseline conditions in home cages, in the absence of any injections, animals moved negligible distances compared to after injections of cocaine or methamphetamine (see Fig. 3). After a saline injection, there was a small increase in activity that returned to baseline within approximately 20 min. Baseline differences before or after saline between adolescents and adults were small but nonetheless statistically significant (adolescents slightly lower than adults, all $P<0.0001$). Note that because of the difference in the magnitude for locomotor activity after the drug doses, these small baseline differences have negligible quantitative effects on drug-induced locomotor activity (i.e., locomotor activity after drug minus after saline).

The distance traveled within a 90-min period after 2 mg/kg methamphetamine injection was significantly less in adolescents compared to adults [$F(2,18)=116.72$, $P<0.0001$] (Fig. 2d). The result was the same after subtracting distance traveled after saline within subjects.

### *Experiment 3—dose response*

Concentrations of methamphetamine in both brain and blood samples at the 15-min time point increased as a function of dose [brain, $F(2,42)=13.43$, $P<0.0001$; blood, $F(2,36)=12.63$, $P<0.0001$]; however, no age differences were observed at any dose [brain, $F(1,43=0.07$, $P>0.05$; blood $F(1,36)=0.96$, $P>0.05$] (Fig. 2b,c).

Locomotor activity at the 15-minute time point significantly increased with dose for both age groups [$F(3,38)=66.34$, $P<0.0001$] (Fig. 2e). The main effect of age was significant [$F(1,38)=9.34$, $P=0.004$] (Fig. 2e). Although the interaction between age and dose was not significant, Scheffe post hoc analysis indicated that adults differed from adolescents at 2 mg/kg ($P=0.002$), and there was a trend for 1 mg/kg ($P=0.09$) but not at 4 mg/kg ($P=0.29$), where the greatest amount of stimulation occurred.

Locomotor activity was significantly correlated with concentration of methamphetamine in the brain at the 15-min time point across all individuals and all doses [$R^2=0.25$, $P=0.005$]. Results of linear regression show a trend for lower activity for a given concentration of methamphetamine in the brain in adolescents compared to adults [$F(1,44)=2.64$, $P=0.11$] (Fig. 2f). Results were similar when locomotor activity was adjusted by subtracting distance traveled after saline within subjects.

## Cocaine

### *Experiment 1—time course*

Concentration of cocaine in the brain reached a higher peak, and the peak occurred earlier in adolescents compared to adults after 30 mg/kg i.p. injection (Fig. 4a). Because of the potential importance for the result at 5 min, that time point was repeated on three separate occasions. On each occasion, mean concentration was higher in adolescents than adults [overall $t$ statistic for 5-min time point was $t(22)=3.08$, $P=0.005$]. In the analysis with all other time points, where adolescents and adults did not differ (Fig. 4a), the interaction between age and time was nonsignificant [$F(4,66)=2.02$, $P=0.10$], but the Tukey or Scheffe post hoc difference between adolescent and adult at the 5-min time point was significant [$P<0.05$]. The main effect of time was

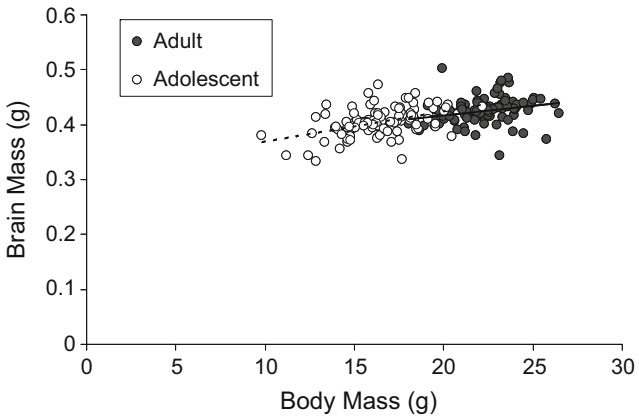


**Fig. 1** Brain–body mass allometry. Brain mass plotted against body mass for adolescent (*open circles*) and adult (*filled circles*) C57BL/6J male mice. Simple linear regression lines are shown separately for adults (*solid line*) and adolescents (*dashed line*)

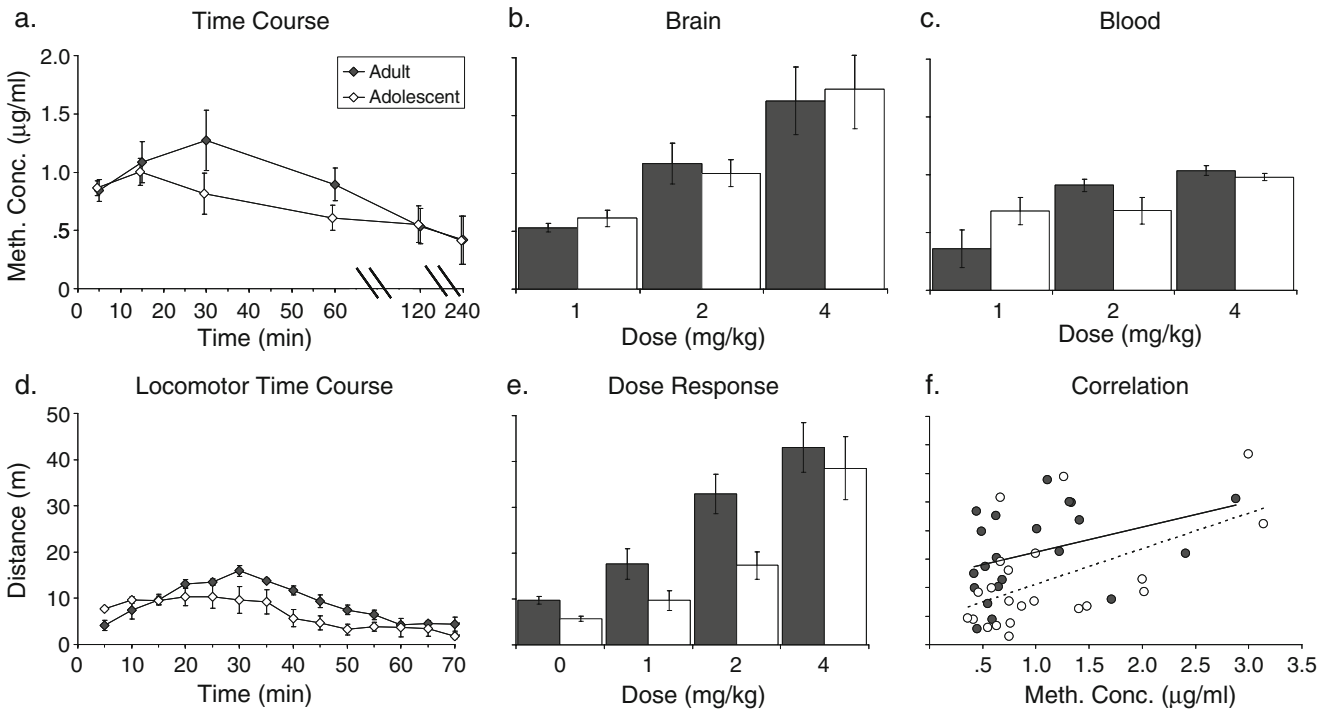


**Fig. 2** Methamphetamine data. **a** Time course for methamphetamine concentrations in the brain after i.p. injection of 2 mg/kg (adults *closed symbols*, adolescents *open symbols*; data from experiments 1 and 3 combined; $n$=5, 8, 3, 7, 4, 3 adults and 5, 8, 4, 8, 4, 3 adolescents per time point, respectively). Methamphetamine concentrations in the brain (**b**) and blood (**c**) at the 15 min time point after i.p. injection of 1, 2, or 4 mg/kg (adults *filled bars*, adolescents *open bars*; $n$=8 per bar). **d** Time course for locomotor activity after i.p. injection of 2 mg/kg ($n$=4 per age group). Only the first 70 min are shown to facilitate comparison with the pharmacokinetic data above. **e** Distance traveled in 15 min after 0, 1, 2, or 4 mg/kg ($n$=8 per bar). **f** Distance traveled plotted against brain–methamphetamine concentration, separately for adults (*open symbols*) and adolescents (*filled symbols*). The simple linear regression lines are shown separately for adults (*filled line*) and adolescents (*dashed line*). All *graphs in a row* share the same *y*-axis labels. *SE bars* shown

significant [$F$(4,66)=14.20, $P$<0.0001] and age nonsignificant [$F$(1,66)=1.48, $P$=0.07].

*Experiment 2—locomotor activity*

The distance traveled within a 90-minute period after 30 mg/kg cocaine injection was significantly less in adolescents compared to adults [$F$(1,18)=12.67, $P$=0.002] (Fig. 4b). The result was the same after subtracting distance traveled after saline within subjects.

*Experiment 3—dose response*

Concentrations of cocaine in both brain and blood samples at the 10-minute time point increased as a function of dose [brain, $F$(2,42)=39.32, $P$<0.0001; blood, $F$(2,42)=37.89, $P$<0.0001], however no age differences were observed at any dose [brain, $F$(1,42)=0.04, $P$>0.05; blood $F$(1,42)=0.83, $P$>0.05] (Fig. 4b,c).

Locomotor activity significantly increased with dose for both age groups [$F$(3,42)=34.39, $P$<0.0001] (Fig. 4e). Adults showed significantly greater locomotor stimulation from increasing doses than adolescents as evidenced by a

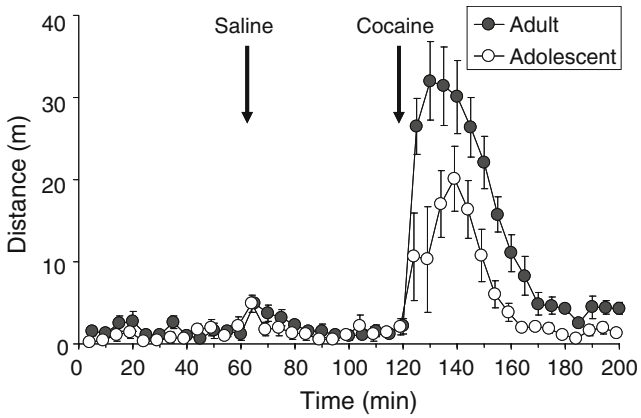


**Fig. 3** Locomotor activity before and after cocaine. Average distance traveled (meters, in 5 min bins; ±SE; $n$=4 per age group) in the home cage of adolescent (*open circles*) or adult (*filled circles*) mice starting at the onset of the dark cycle (when lights shut off) and ending 200 min later. The first 60 min shows baseline activity when animals were left undisturbed. An i.p. injection of saline was administered at 60 min (*first arrow*). At 120 min (*second arrow*), an i.p. injection of 30 mg/kg cocaine was administered. Other than removing animals for the injections, they were left undisturbed, and distance was measured continuously using video tracking software (see "Materials and methods" section)

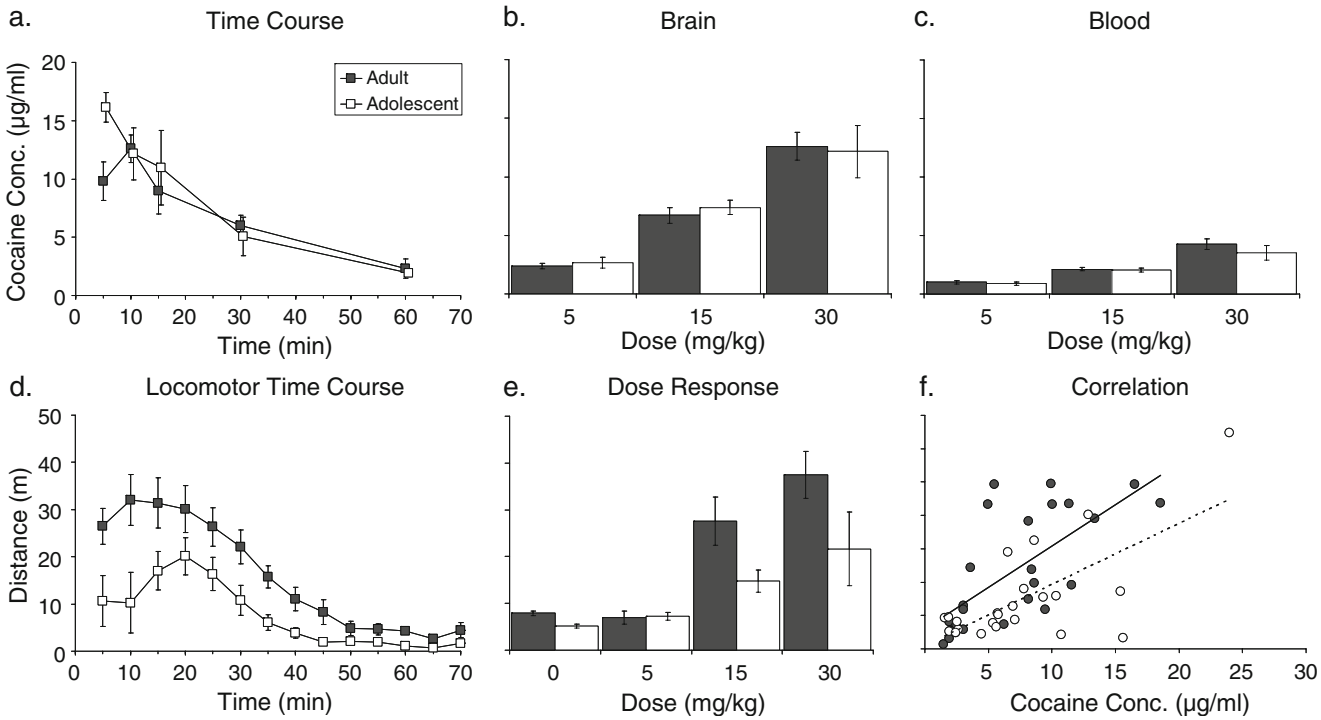


**Fig. 4** Cocaine data. **a** Time course for cocaine concentrations in the brain after i.p. injection of 30 mg/kg (adults *closed symbols*, adolescents *open symbols*; data from experiments 1 and 3 combined; $n$=6, 8, 5, 6, 5, adults and 6, 8, 6, 6, 6 adolescents per time point, respectively). Cocaine concentrations in the brain (**b**) and blood (**c**) at the 10-min time point after i.p. injection of 5, 15, or 30 mg/kg (adults *filled bars*, adolescents *open bars*; $n$=8 per bar). **d** Time course for locomotor activity after i.p. injection of 30 mg/kg ($n$=4 per age group). Note this is the same data as shown in Fig. 3, minutes 125–190. Only the first 70 min are shown here to facilitate comparison with the pharmacokinetic data above. **e** Distance traveled in 10 min after 0, 5, 15, or 30 mg/kg ($n$=8 per bar). **f** Distance traveled plotted against brain–cocaine concentration, separately for adults (*open symbols*) and adolescents (*filled symbols*). The simple linear regression lines are shown separately for adults (*filled line*) and adolescents (*dashed line*). All graphs in a row share the same *y*-axis labels. *SE bars* shown

significant main effect of age [$F(1,42)=10.55$, $P=0.002$] and interaction between age and dose [$F(3,42)=3.61$, $P=0.021$]. Scheffe post hoc analysis indicated that adults differed from adolescents at 15 mg/kg ($P=0.006$) and 30 mg/kg ($P=0.001$).

Locomotor activity in response to cocaine was significantly correlated with concentration of cocaine in the brain across individuals [$F(1,46)=36.98$, $P<0.0001$]. Results of analysis of covariance show that for a given concentration of cocaine in the brain, the level of activity was significantly lower in adolescents compared to adults [$F(1,45)=4.57$, $P=0.038$] (Fig. 4f). As before, baseline corrections did not change results.

## Blood–brain correlations

Concentration of cocaine in the brain was strongly correlated with infraorbital blood among individuals ($R^2=0.85$, $P<0.0001$; Fig. 5b). The correlation between blood and brain methamphetamine concentrations was significant

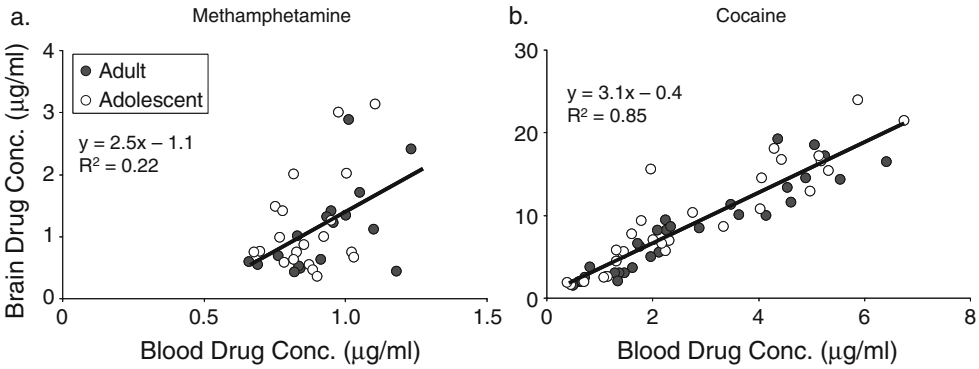


**Fig. 5** Correlation between brain and blood. Concentration of methamphetamine (**a**) and cocaine (**b**) in the brain plotted against infraorbital blood for adolescents (*open circles*) and adults (*filled circles*). Simple linear regression lines are shown for the combined adolescent and adult data

but not as strong in cocaine samples ($R^2$=0.22, $P$=0.004; Fig. 5a).

## Discussion

These data extend previous reports of reduced locomotor stimulation to cocaine (Collins and Izenwasser 2002; Frantz et al. 2006; Laviola et al. 1995) or amphetamines (Bolanos et al. 1998; Lanier and Isaacson 1977) in adolescents compared to adults. To the best of our knowledge, this is the first demonstration that the phenomenon is also true for methamphetamine in adolescent versus adult male C57BL/6J mice. Given that cocaine and methamphetamine share similar mechanisms of action, the result is not surprising. Regarding the primary question of whether pharmacokinetic differences could contribute to the behavioral differences, for methamphetamine, results suggest the pharmacokinetic contribution is likely small. The behavioral differences between the age groups were large and statistically significant (Fig. 2d,e), whereas the differences in brain concentrations were small and not significant (Fig. 2a,b). Moreover, levels of activity were lower in adolescents than adults for a fixed concentration of methamphetamine in the brain (Fig. 2f). Taken together, these results argue against the pharmacokinetic hypothesis for differential locomotor stimulation to methamphetamine.

Results for cocaine were more complex. Results establish for the first time that adolescents have a higher concentration of cocaine in their brain compared to adults, 5 min after an i.p. injection. The observation was replicated three times to confirm the new finding (Fig. 4a). The reason cocaine accumulated in the brain of adolescents more rapidly than adults may be because brain weight is a larger percentage of body weight in adolescents than adults (Fig. 1). Since cocaine is lipophilic, the initial peak in adolescents may represent a rapid redistribution of a lipophilic molecule in a highly perfused organ. Furthermore, this initial peak could indirectly contribute to differential locomotor stimulation. For example, it is possible that the higher peak and earlier rise in cocaine concentrations in the brains of adolescents compared to adults resulted in greater acute functional tolerance to cocaine. Acute tolerance refers to rapid neuroadaptations that occur leading to greater behavioral response when drug concentrations are rising than when falling. It is a well-documented phenomenon in alcohol exposure and has also been observed for the subjective effects of cocaine in humans (Foltin and Fischman 1991) as well as cardiovascular responses to cocaine in rats (Tella et al. 1999) and humans (Foltin and Fischman 1991). The higher peak in adolescents may act comparably to what would be a higher dose in adults. Since acute tolerance to alcohol has been shown to increase with dose in mice (Ponomarev and Crabbe 2004), the effect of the higher peak in adolescents may increase acute tolerance therefore decreasing locomotor stimulation.

An alternative explanation is that the higher peak concentration of cocaine produced stereotypic behavior in adolescents, resulting in reduced locomotor activity (Caster et al. 2005). Although stereotypy was not measured in the present study, indirect evidence suggests adolescents were not engaged in stereotyped behaviors. First, differences in locomotor activity between age groups were seen shortly after drug administration, and stereotyped behaviors typically occur after an initial locomotor activation phase (Rebec and Bashore 1984). Second, the dose response analysis showed that both adolescents and adults increased responding with dose (Figs. 2e and 4e). Given that the dose–response curve for the locomotor-stimulating effects of cocaine is known to follow an inverted U-shape with stereotypy contributing to the descending limb (Shuster et al. 1977; Tolliver and Carney 1994), if adolescents were engaged in stereotypy, an increase of dose would be expected to further decrease locomotion. Rather, the evidence suggests that adolescents are less sensitive than adults to the locomotor-stimulating effects of cocaine and methamphetamine. This is consistent with the conclusions of previous studies (Bolanos et al. 1998; Collins and Izenwasser 2002; Frantz et al. 2006; Lanier and Isaacson 1977; Laviola et al. 1999; Laviola et al. 1995; Spear and Brake 1983). It also explains why adolescents and adults showed similar levels of locomotor activity after the high methamphetamine dose (4 mg/kg; Fig. 2e). We have other unpublished data on the time course for locomotor stimulation to 4 mg/kg in adolescents versus adults and believe that this dose of methamphetamine is probably near the intersection of the descending limb of the U-shape curve in adults with the ascending limb in adolescents. Probably, had we used a higher dose of cocaine, we would have eventually reached the point of intersection where stimulation in adolescents is comparable to adults.

The finding that cocaine concentration in the brain is similar between adolescents and adults after 10 min is consistent with Frantz et al. (2006) and Caster et al. (2005) in rats. However, our results do not replicate the finding of McCarthy et al. (2004) which suggested adolescent male C57BL/6J mice have lower cocaine concentrations in the brain than adults 15 min after cocaine administration (see Table 1). The explanation for this discrepancy is not clear.

The current study used custom-made home cages conducive for video tracking to monitor locomotor activity in the animal's home environment. The finding that locomotor stimulation to cocaine was attenuated in adolescents compared to adults under these conditions is consistent with previous studies where animals were transferred to a new cage for activity measurements

(Bolanos et al. 1998; Collins and Izenwasser 2002; Frantz et al. 2006; Lanier and Isaacson 1977; Laviola et al. 1999; Laviola et al. 1995; Spear and Brake 1983). Although this suggests that the phenomenon of differential locomotor stimulation is robust across these two environments, it is important to note the methodological differences that could impact generalization of results. First, mice were singly housed for video tracking. Single housing has been shown to activate the hypothalamic–pituitary–adrenal axis and affect behavior in a number of tasks (Schrijver et al. 2002; Schrijver and Wurbel 2001). On the other hand, the single housing in these studies lasted over 50 days, whereas in this study, animals were singly housed for only 5 days. Another methodological consideration is that mice were not handled, other than to change cages, prior to the testing day. Handling has been shown to increase locomotor stimulation to cocaine in adolescent, but not adult, rats (Maldonado and Kirstein 2005a, b). Therefore, it is possible that had we handled the mice, the adolescents might have displayed higher levels of locomotor stimulation, more comparable to adults. Finally, testing in the home cage as opposed to a novel environment might have affected the magnitude of the behavioral difference between the age groups. While locomotor stimulation to psychostimulants has been observed in both types of environments (Ganea et al. 2007), adolescent rats have been shown to ambulate more than adults when placed in a novel environment in the absence of drug administration (Spear and Brake 1983).

### Blood and brain sample correlations

The finding that cocaine concentration in the brain is higher than in the blood is consistent with previous studies examining trunk blood and brain drug concentrations in adult mice (Benuck et al. 1987; Patrick et al. 1993; Reith et al. 1987) and rats (Nayak et al. 1976), but see McCarthy et al. (2004) who found higher concentrations of cocaine in blood than brain in C57BL/6J mice. Results suggest that sampling blood from the infraorbital sinus after cocaine administration accurately reflects concentrations of cocaine in the brain. The weaker correlation between blood and brain samples for methamphetamine compared to cocaine can be explained by a few key differences. First, the dose range was lower for methamphetamine than cocaine (1, 2, 4 mg/kg methamphetamine versus 5, 15, 30 mg/kg cocaine). Second, concentrations of methamphetamine in blood were lower than in brain, and some of the blood (but not brain) samples reached the limit of detection for LC/MS/MS.

### Limitations

It is important to note that the techniques used in this study do not differentiate pharmacologically active drug (e.g., drug bound to dopamine transporters) and the amount of drug that is inactive (e.g., drug that is not affecting cellular processes). It has been established that depot binding (e.g., drug binding to plasma protein, muscle, and fat), can affect the magnitude and duration of drug action (Fasano et al. 2005; Nayak et al. 1976). Given that white matter is increasing throughout adolescence (Giedd 2004), it is conceivable that there is a difference in depot binding within white matter between adolescents and adults. If so, then it is possible that adults and adolescents could have the same absolute concentration of drug in the brain while having differential amounts of pharmacologically active drug.

Another limitation for whole brain sampling is that it does not differentiate drug levels in areas of the brain that are more directly involved in locomotor stimulation from other regions. For example, dopaminergic projections from the ventral tegmental area to the striatum have been shown to be important in the motor activation effects of cocaine and methamphetamine (Rebec 2006). If there are any differences in distribution of cocaine or methamphetamine within the brain between adolescents and adults, then that could also explain behavioral differences.

Caution should be taken before generalizing results to other strains or other species given that previous studies have noted differences in pharamacokinetics between strains (Azar et al. 1998; McCarthy et al. 2004) and between rats and humans (Cho et al. 2001). While dose, strain, and type of administration all affect pharmacokinetics, our estimates of cocaine and methamphetamine concentrations in the brain and blood are within the range observed in other studies for methamphetamine (Brien et al. 1978; Fornai et al. 1999; Hendrickson et al. 2004; Won et al. 2001) and cocaine (Azar et al. 1998; Benuck et al. 1987; Miller et al. 1996; Pan and Hedaya 1998; Reith et al. 1987). Additionally, the primary routes of metabolism in mice (Boyer and Petersen 1992; Shuster et al. 1983) are similar to those in rats (Estevez et al. 1977) and humans (Bencharit et al. 2003; Brzezinski et al. 1997).

## Summary

These results add to the growing rodent literature on adolescents documenting differential behavioral responses to drugs of abuse compared to adults (Badanich et al. 2006; Bolanos et al. 1998; Collins and Izenwasser 2002; Laviola et al. 1995). We show, for the first time, that adolescents display reduced locomotor stimulation to methamphetamine, similar to cocaine and amphetamine that share similar mechanisms of action. Results do *not* support the pharmacokinetic hypothesis for differential locomotor stimulation to methamphetamine because locomotor stimulation was

significantly reduced in adolescents versus adults even though concentrations of drug in the brain were similar at all time points. However, for cocaine, the story was more complex. Adolescents experienced higher concentrations of cocaine in their brain compared to adults 5 min after an i.p injection. This is an important discovery because a higher concentration in adolescents compared to adults has never previously been reported (see Table 1), but no other study, to our knowledge sampled that early. The possible role this might have on affecting behavior at later time points is not clear, but acute functional tolerance represents a possible mechanism for future exploration. Nonetheless, given that the pharmacokinetic difference was only for the early time point and that locomotor activity differed at many later time points, the possible pharmacokinetic contributions are likely small.

**Acknowledgements** We wish to thank Nataliya Ryzhenko and Weronika Brzezinska for help with data collection. Special thanks to Lucas Li and Kelly Keating at the Metabolomics Center for their assistance with the LC/MS machine. Thanks also to Lisa Foster, Dack Shearer, Reid McClure, Marc Whiter, Donnell Parker, and Holly Fairfield for excellent animal care.

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