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Novelty-induced locomotion is positively associated with cocaine ingestion in adolescent rats; anxiety is correlated in adults

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ABSTRACT

The present studies assessed the roles of sex, age, novelty-seeking and plus-maze behavior on cocaine drinking in rats. Cocaine/saccharin solution was available in three daily, 5-hour sessions then a saccharin-only solution was also available in following sessions. In the one-bottle drinking phase, early and late adolescent males, post-natal day 28 (PN28) and PN42, consumed more cocaine/saccharin solution than young adults (PN65), but females did not exhibit significant age differences. Adolescents of both sexes consumed more cocaine/saccharin than adults during choice drinking. Saccharin availability in the two-bottle trials decreased cocaine/saccharin consumption in PN28 and PN65 rats. After a drug-free period, cocaine-stimulated locomotion was lower in cocaine/saccharin drinking than saccharin-only males, indicating tolerance. We tested the hypothesis that individual differences in pre-screened behavioral traits would correlate with cocaine/saccharin consumption in PN28 and PN65 male rats. High locomotor responses to novelty were associated with greater cocaine/saccharin drinking in adults in one-bottle sessions. In the subsequent choice drinking phase, correlations were age-specific. Adolescents with high novelty-induced locomotion and adults that spent less time on open arms of the elevated plus-maze drank more cocaine/saccharin. Thus, behavioral phenotypes correlated with individual differences in cocaine/saccharin consumption in an age-related manner.

1. Introduction

Humans and animals find sweet-tasting substances rewarding (Berridge, 1996). The reinforcing properties of drugs of abuse depend upon the same neural systems that normally mediate natural rewards like sweet taste (Hyman et al., 2006). Thus, drugs and sweet tastants compete for the same reward circuit. One's likelihood to choose drugs over natural rewards is influenced by a complex interaction of biologic variables including developmental stage, gender, personality traits and environment. Although using drugs compulsively instead of seeking natural rewards is a hallmark of addiction, animal models of drug taking infrequently use choice paradigms. Comparing choice between natural and drug rewards is important because consumption of a sweet-tasting solution can surpass the reward of self-administered i.v. cocaine, a gold-standard animal model of drug addiction (Lenoir et al., 2007). The present studies have examined how sex, age and personality traits are related to the choice between sweet taste (saccharin solution) and sweet taste combined with cocaine (cocaine/saccharin solution). We sought to characterize which of these three factors predispose animals to choose in favor of cocaine.

The present voluntary cocaine drinking paradigm provided a way to examine the choice between sweet taste and sweet taste plus drug. Oral cocaine has been shown to be rewarding and reinforcing. It established conditioned place preference when administered by gavage or when self-administered in a schedule-induced polydipsia paradigm (Seidman et al., 1992). Rats (Jentsch et al., 1998) and primates (Macenski and Meisch, 1998) lever pressed to drink cocaine solutions and cocaine drinking was not devalued by pairing with LiCl (Miles et al., 2003). Cocaine ingestion in humans produced similar peak plasma concentrations and a subjective "high" rating greater than that resulting from intranasal administration of the same dose (Van Dyke et al., 1978). Humans found the subjective and reinforcing effects of oral cocaine to be qualitatively similar to i.v. cocaine but 10-fold less potent (Smith et al., 2001). Oral cocaine was reinforcing in humans performing a vigilance task but not during a relaxation activity (Jones et al., 2001). In rodents, oral cocaine (7.5 and 15 mg/kg) produced maximal serum concentrations of cocaine and area under the curve values equivalent to those following the more commonly used i.p. route of administration of the same doses (Falk et al., 1991; Lau et al., 1991). The i.p. and p.o. routes were equipotent for inducing deficits in fine motor control during an operant task (Lau et al., 1991). Consumption of 29.8 mg/kg cocaine over a 3-hour session (the lowest

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dose tested) increased locomotor behavior (Lau et al., 1992). Stromberg et al. (2001) found detectable levels of benzoylecgonine (364 mg/dl), the primary cocaine metabolite, in blood of a rat that drank 12.6 mg/kg cocaine in a 1-hour session. Thus, the pharmacokinetics of cocaine ingestion is similar to other routes of administration and produces subjective effects similar to more addictive routes of administration.

Sex, age and personality traits are known to influence drug abuse. For psychostimulants, male addicts outnumber women, but women progress to addiction more rapidly (Lynch et al., 2002). Age of onset is another critical determinant of the development of addiction. The onset of substance abuse during adolescence is correlated with a greater severity of addiction involving increased rates of morbidity and mortality (Spear, 2000; Chambers et al., 2003). Escalation of drug use is more rapid and the risk of dependence is greater in adolescents than adults (Estroff et al., 1989; Robins and Przybeck, 1995; Chen et al., 1997; Clark et al., 1998). Risk taking, impulsivity, sensation seeking and anxiety disorders are behavioral traits associated with drug use, especially during adolescence (Zuckerman, 1986; Wills et al., 1994; Costello et al., 2003; Kelley et al., 2004; Kreek et al., 2005; Lopez et al., 2005). The present studies have investigated how these specific biologic differences influence the choice between natural and drug reward in rats. We predicted that adolescents would ingest more cocaine than adults, that females would ingest more cocaine than males and that novelty-seeking would predict higher propensity to ingest cocaine.

2. Materials and methods

2.1. Subjects

Male and female CD rats, purchased from Charles River Laboratories (Raleigh, NC, U.S.A.), were shipped and received at post-natal ages 21, 35 and 58 days, 1 week prior to experimentation. They were segregated by sex and age and were housed two or three per cage in suspended, ventilated plastic cages in the same room where cocaine drinking occurred. Food and water were provided *ad libitum* and the room observed a 12:12 light:dark cycle with lights on at 0530 h. Females were used without regard to estrous state to avoid potential behavioral disruptions, because we have shown that the estrous cycle testing by vaginal lavage affects cocaine-stimulated motor behavior (Walker et al., 2002). Animal care was performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 865-23, Bethesda, MD, U.S.A.) and experimental procedures were approved by the Institutional Animal Care and Use Committee.

2.2. Sex and age differences in voluntary cocaine/saccharin consumption: one solution drinking

Rats were tested at ages that span the entire time frame of adolescence and into early adulthood in rats (Spear, 2000). Rats of post-natal age (PN) 28, 42, and 65 days were weighed and separated into individual drinking cages with wire tops for three consecutive days at 1700 h. Cocaine/saccharin and saccharin solutions were placed in 100 mL Techniplast® bottles with tight fitting stainless steel spouts. The tip of the sipper tube had an internal diameter of 2 mm. Rat chow was also available during this test session. Each drinking bottle was weighed before and after each day's session to determine the volume consumed from each bottle. Rats were returned to communal cages at 2200 h. Animals were weighed daily to determine the mg/kg cocaine consumed. Fig. 1 shows a flow chart of the experiments. One bottle containing either 500 mg/L saccharin in water or 500 mg/L cocaine in the same saccharin solution was placed on the wire top of each cage. Rats in the "cocaine" group had the cocaine/saccharin solution available and rats in the "saccharin" group drank saccharin solution

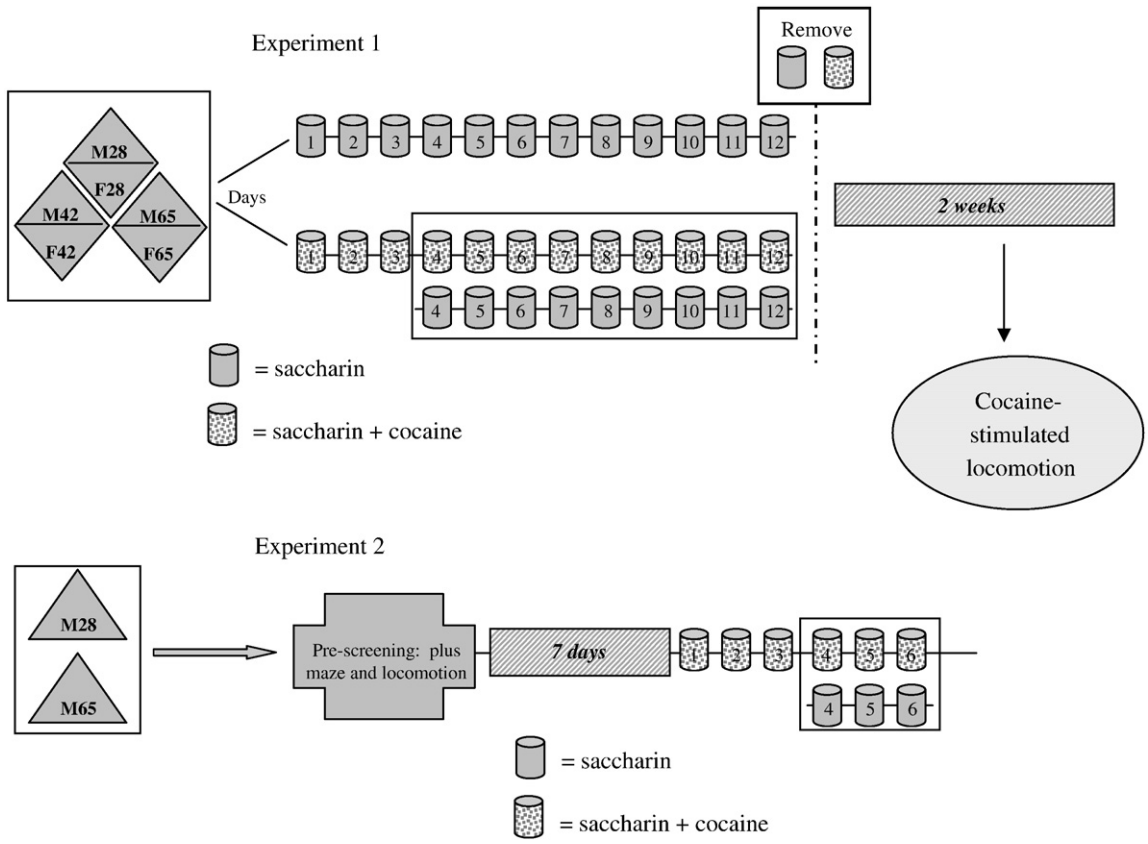

Fig. 1. Timelines of the two voluntary cocaine drinking experiments.

only. This initial 3 days of one-bottle exposure was designed to acquaint the cocaine group with its taste and pharmacological effects and thereby overcome any neophobia.

2.3. Choice drinking

After the initial three sessions of one-bottle cocaine/saccharin drinking, the cocaine group was presented with an additional bottle containing the saccharin vehicle for nine consecutive daily sessions. The saccharin bottle was always placed on the same side of the overhead wire rack opposite the cocaine/saccharin bottle. The saccharin control group continued with sessions of one bottle of saccharin available. Rat chow was always available to both groups.

2.4. Locomotor challenge

To determine if voluntary cocaine/saccharin drinking induced age- or sex-specific enduring effects on behavior, cocaine/saccharin and saccharin drinking groups were evaluated for locomotor behavior in a novel open-field (described below). Two weeks after drinking sessions ended for rats in the age and sex study, activity data were recorded for 1 h during habituation to the novel environment and then for another hour following an injection of cocaine (10 mg/kg, i.p.).

2.5. Pre-screening for behavioral traits

These experiments examined whether pre-existing behavioral traits are correlated with cocaine/saccharin intake. Behaviors in the open-field (locomotor response to novelty) and on the elevated plus-maze (anxiety) were determined in rats prior to cocaine/saccharin drinking. Because large numbers of rats were required to establish these correlations, we focused these studies on the sex (male) and ages (PN28 and 65) that exhibited the largest difference in cocaine/saccharin drinking in the prior experiment. Naïve male rats at PN28 and 65 were tested in an open-field activity monitor and on an elevated plus-maze 5–7 days before cocaine/saccharin drinking began. Two pre-screened traits were assessed for each rat: horizontal distance traveled during 15 min exposure to a novel open-field and percent time spent on the open arms of an elevated plus-maze. These rats began one-bottle cocaine/saccharin drinking at PN35 and 72. The procedure described above for one- and then two-bottle choice drinking was also followed for this experiment, except there was no saccharin-only control group. The duration of the choice period was reduced from that used in the first experiment to match the 3 days of one-bottle drinking and to resolve cocaine/saccharin and saccharin drinking during a more discrete period of early choice drinking. The data for the first three choice days were averaged to indicate the cocaine/saccharin and saccharin consumption for each rat.

2.6. Locomotor activity

Motor activity was determined in open-field photocell devices (Kinder Scientific, Inc., Poway, CA). The devices were comprised of an open Plexiglas arena (40 cm for each dimension) with corn cob bedding on the floor. Computer software supplied by the manufacturer recorded interruptions of photobeams spaced 1 in. apart. Horizontal ambulations were defined by the software as relocations of the entire body of the rat. Assignment to test chambers was counterbalanced across days with respect to sex and age. Habituation test sessions began when rats were placed in the open arena without injection. After this 1-hour session all rats were injected with 10 mg/kg cocaine i.p. and another 1-hour activity recording session began. For the behavioral trait pre-screening experiment, individual rats were sequentially placed in a single open-field device for 15 min without injection.

2.7. Elevated plus-maze

The relative amount of time spent on open arms of the elevated plus-maze (EPM) is a commonly used measure of anxiety in rodents (Pellow et al., 1985). The maze is made of sealed wood arranged in a plus shape which is elevated 90 cm above the floor. Two of the arms (north and south) are open and are 50×10 cm. The other two arms (east and west) are enclosed with walls that are 36 cm high on the two long sides. The areas surrounding the EPM were made visually neutral by hanging white curtains on all four sides. The room was dimly lit by incandescent light so that the brightness on the open arms was approximately 7 lx, and in the closed arms approximately 3.5 lx. A video camera using “night shot” mode recorded the rat's behavior from above the maze. The videotaped sessions were analyzed later to score entries into and time spent in each arm. A rat was considered to be in one of the arms if all four limbs were in that region. Otherwise, it was considered to be in the middle. Open arm time was calculated as sec in open arms/(sec in open+sec in closed arms).

2.8. Determination of cocaine solution stability

The chemical stability of cocaine in refrigerated cocaine/saccharin solutions was determined. Two cocaine/saccharin solutions were prepared in water daily, as described, for 5 days and stored in the same lab refrigerator where the experimental solutions were stored. Cocaine concentrations were determined by HPLC with fluorescence detection using excitation and emission wavelengths of 230 and 316 nm, respectively according to previously published methods (Sun et al., 2000). The HPLC system included a Dynamax SD-200 pump and FL-2 fluorometric detector (Rainin Instrument, Woburn, MA) controlled by ChromGraph DA-5 software (BAS, West Lafayette, IN) that digitized and stored data. The mobile phase was comprised of 76% 25.8 mM sodium acetate and 100 mM tetrabutylammonium phosphate (pH 2.6), 14% methanol and 10% acetonitrile. The mobile phase flowed through a Unijet PEEK ODS, 3 μm, 100 mm×2 mm I.D. column (BAS, MF-8957) at 0.6 mL/min. A cocaine stock solution (1 mg/mL) was prepared in mobile phase and stored at −80 °C. Six working standards were diluted from the stock solution and 10 μl of each was injected to calculate a standard curve. The same volumes of the test solutions were injected and cocaine concentrations were determined from the standard curve.

2.9. Drugs and chemicals

Saccharin sodium (Sigma-Aldrich, St. Louis, MO) solutions were prepared at 500 mg/L (0.05%) in tap water. Cocaine HCl (Sigma-Aldrich, St. Louis, MO) was dissolved in the saccharin solution at a concentration of 500 mg/L (0.05%). At the end of the cocaine/saccharin drinking sessions, cocaine/saccharin bottles were refrigerated overnight until 1 h before the next session. Cocaine/saccharin solutions were used for 5 days. Cocaine/saccharin consumption is reported as the total mg of cocaine in the fluid volume (saccharin was also present at the same dilution) that the rat drank divided by the rat's body weight in kg (mg/kg). Saccharin consumption refers to drinking of saccharin-only solution, and was determined similarly.

HPLC grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ) and acetonitrile was purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). Tetrabutylammonium phosphate was purchased from Sigma (St. Louis, MO) and sodium acetate from Mallinckrodt.

2.10. Data analysis

Data are expressed as the mean±SEM. The average cocaine or saccharin consumption (mg/kg) for the 3 days of one-bottle drinking was determined for each rat. Consumption of each solution during the

Table 1
Consumption of either cocaine/saccharin or saccharin solution (mg/kg)

	Days	Females			Males		
		PN28	PN42	PN65	PN28	PN42	PN65
One-bottle drinking							
Saccharin	1–3	61.7±6.9	47.3±8.4	45.2±5.9	63.1±6.7	50.4±6.6	42.2±3.3
Saccharin	4–9	67.5±5.7	53.2±10.2	54.9±6.1	70.4±8.3	58.4±8.8	48.2±5.8
Cocaine/saccharin	1–3	31.5±3.6	28.7±3.8	22.8±1.4	38.2±2.5*	25.3±2.6*	16.9±2.9
Two-bottle drinking							
Cocaine/saccharin	4–9	20.2±1.8	25.8±4.5*	13.9±1.7	28.9±3.1*	23.7±3.6*	10.4±0.9
Saccharin	4–9	48.2±11.2	34.1±12.8	19.3±4.2	29.0±6.4	19.3±4.2	20.5±3.4

Values for saccharin and cocaine/saccharin consumption are means±SEM (N=6/group). Cocaine/saccharin consumption is reported as mg cocaine/kg body weight. Drinking the same dose of cocaine/saccharin and saccharin represents consumption of the same volume of each solution. Only the groups that drank cocaine/saccharin in the one-bottle drinking phase were subsequently presented with a choice in the two-bottle phase. An asterisk indicates a significant difference compared to PN65 of the same sex.

choice drinking phase was determined as the average of the following 9 days. Three-way ANOVA tested the effects of solution being consumed (cocaine/saccharin or saccharin), age and sex on consumption during one-bottle drinking. Age differences in consumption for each sex were investigated because of the large literature showing sex differences in psychostimulant effects in rodents. Cocaine/saccharin consumption was evaluated by ANOVA with repeated measures for drinking phase (one and two bottle). In the locomotor activity challenge experiment, the effects of sex, age, chronic exposure (cocaine/saccharin or saccharin drinking) on horizontal ambulations were determined using three-way ANOVA with repeated measures on trial. Differences between groups were determined within each sex and drinking group by post-hoc analysis using Newman–Keuls multiple comparison test.

In the behavioral pre-screen experiment, age differences in the pre-screened and consumption measures were assessed by t-test. Although multiple measures of behavior in both the open-field and elevated plus-maze were available, this number was reduced to two in order to decrease the number of comparisons to restrict type I errors. The influences of the two pre-screen phenotypes (distance traveled in the novel open-field and open arm time on the elevated plus-maze) on one- and two-bottle cocaine/saccharin drinking and on saccharin consumption during two-bottle trials were determined by multiple regression with age as a covariate using JMP 7 (SAS Institute, Cary, NC). Age was crossed with each behavioral measure to determine whether the interaction term indicated significantly different slopes in each age group. Correlations between pre-screened traits and consumption at each age are shown with r^2 values, the fraction of the variance in the consumption explained by the pre-screened trait (Prism 5, Graphpad Software, Inc., CA).

3. Results

3.1. Stability of cocaine in cocaine/saccharin solutions

To confirm that cocaine did not degrade in our solutions while refrigerated for 5 days, cocaine concentrations in cocaine/saccharin solutions were determined by HPLC with fluorescence detection. The calculated cocaine concentrations for cocaine/saccharin solutions refrigerated for 1–5 days were 0.52, 0.58, 0.55, 0.52, and 0.55 mg/mL, respectively (average of duplicates) (0.5 mg/mL, expected). Thus, cocaine concentration in this aqueous solution was stable across the time frame employed in these studies.

3.2. Cocaine/saccharin and saccharin consumption during one-bottle trials

Fig. 1 provides a flow chart for experiment 1. Male and female rats aged PN28, 42, and 65 began one-bottle drinking sessions, lasting 5 h on three consecutive days. Separate groups were given saccharin or cocaine/saccharin solutions. The saccharin drinking groups continued one-bottle drinking sessions on days 4–9, while the cocaine/saccharin groups received a choice of cocaine/saccharin and saccharin on days 4–9. The average consumption of each solution during both drinking phases is shown in Table 1. Three-way ANOVA of one-bottle drinking data from each age and sex indicated two main effects but no significant interactions. Rats offered saccharin consumed more solution (52 mg/kg) than did those offered cocaine/saccharin (27 mg/kg) indicating a main effect of solution consumed ($F(1,60)$= 72, $p<0.001$). ANOVA also indicated a main effect of age for the consumption of both solutions ($F(2,60)=11.4$, $p<0.001$). Post-hoc

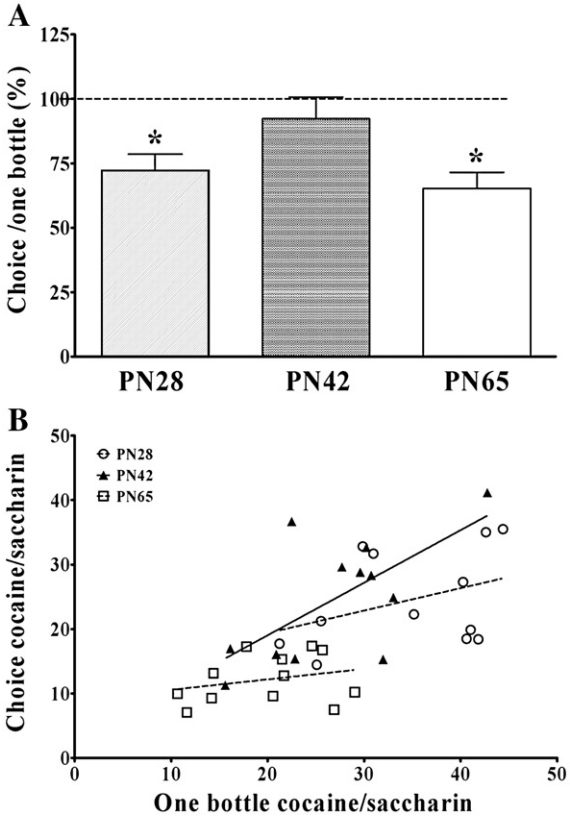

Fig. 2. (A) Percent cocaine/saccharin consumption in the two compared to the one-bottle drinking phase. The relative consumption within individual rats was determined and averaged for male and female rats at each age (N=12/group). Consumption decreased in PN28 and PN65 rats ($p<0.05$). (B) Linear regression analysis comparing cocaine/saccharin consumption (mg/kg) in one- and two-bottle drinking phases in individual rats by age groups of male and female rats. The correlation was significant only for the PN42 rats ($r^2=0.42$, $p=0.02$).

analysis indicated that PN28 rats consumed more solution than PN42 and PN65 rats during these one-bottle sessions ($p<0.05$).

We analyzed age differences for consumption of each solution in each sex because of the large literature describing sex differences in psychostimulant effects. One-way ANOVA of cocaine/saccharin drinking in males indicated a highly significant effect of age ($F(2,15)=16.0$, $p<0.001$). Post-hoc analysis indicated that PN28 male rats consumed a larger dose of cocaine/saccharin than PN42 and PN65 males and that PN42 consumed more than PN65 during these one-bottle sessions ($p<0.05$). In contrast, no effect of age on cocaine/saccharin drinking was found in the females ($F(2,15)=2.04$, $p=0.16$).

Table 1 also shows average saccharin solution consumption over the initial three and final nine sessions. The youngest rats drank more saccharin than the other age groups in the initial three sessions. Two-way ANOVA indicated an overall effect of age ($F(2,30)=4.38$, $p=0.02$), but no effect of sex ($p=0.89$) and no interaction with age ($p=0.91$). The main effect of age did not reach statistical significance in the males ($F(2,15)=3.2$, $p=0.07$) or females ($F(2,15)=1.6$, $p=0.23$) when analyzed separately by one-way ANOVAs. Over the final nine sessions, the age effect was no longer significant ($p=0.08$) and there was no age or sex difference or interaction. Thus, age-related differences were stronger for cocaine/saccharin than saccharin consumption.

3.3. Change in cocaine/saccharin consumption in one- and two-bottle trials

Rats that drank cocaine/saccharin exclusively were then presented with an additional bottle containing the saccharin solution on the fourth through ninth days. The addition of the saccharin-only bottle reduced consumption of cocaine/saccharin in all rats from 27.3 mg/kg (when cocaine/saccharin was the only solution available) to 20.5 mg/kg, a 25% decrease. The addition of the saccharin-only bottle increased total fluid intake (cocaine/saccharin plus saccharin consumption) to 50 mg/kg in all rats.

The effects of sex and age on cocaine/saccharin consumption were determined by ANOVA with repeated measures on drinking phase (one vs two bottle). There was no three-way interaction. Age interacted with drinking phase (age×phase, $F(2,30)=3.35$, $p=0.049$). Post-hoc analysis of this interaction indicated that cocaine/saccharin consumption decreased in the PN28 and PN65, but not the PN42 rats ($p<0.05$), indicating better age-specific retention of cocaine/saccharin drinking despite the addition of the saccharin-only bottle in late adolescent rats. Fig. 2A shows this result as percent consumption in the two-bottle phase relative to the one-bottle consumption within each rat as a transformation of data from Table 1. Age and sex did interact ($F(2,30)=3.8$, $p=0.033$) because the youngest and oldest males exhibited the highest and lowest consumption levels, respectively. Post-hoc analysis showed that PN28 males consumed more than PN65 males and females and that PN65 males consumed less cocaine/saccharin than PN28 and PN42 males and females ($p<0.05$). This effect demonstrated a greater developmental change in cocaine drinking in males. Analysis of main effects showed that cocaine/saccharin consumption was less in the two-bottle than the one-bottle phase ($F(1,30)=27$, $p<0.001$). Age significantly affected cocaine/saccharin consumption ($F(2,30)=17$, $p<0.001$) and post-hoc tests showed that PN65 rats drank significantly less cocaine/saccharin than PN28 and PN42 rats ($p<0.05$). The main effect of sex was not significant ($p=0.97$). In summary, this analysis of one- and two-bottle cocaine/saccharin drinking showed that adolescents consumed more than adults, male rats showed a greater developmental change in consumption, and addition of the saccharin-only solution reduced cocaine/saccharin consumption overall and in an age-specific manner.

To consider the age by drinking phase interaction further, cocaine/saccharin consumption during one- and two-bottle tests was compared in individual rats of each age. Fig. 2B shows that a regression analysis indicated a significant relationship between one- and two-bottle drinking only in the PN42 rats ($r^2=0.42$, $p=0.02$). Thus, initial and choice intake levels were related only in the late adolescent rats, the only age group that did not exhibit an attenuation of cocaine/saccharin consumption when the saccharin-only bottle was added.

3.4. Age and sex differences in cocaine/saccharin and saccharin consumption in two-bottle trials

Similar to results in the one-bottle cocaine/saccharin drinking, adolescent male and female rats drank more cocaine/saccharin solution than the adults (effect of age: $F(2,30)=12.31$, $p<0.001$). Sex did not affect cocaine/saccharin drinking ($F(1,30)=0.18$, $p=0.68$) and

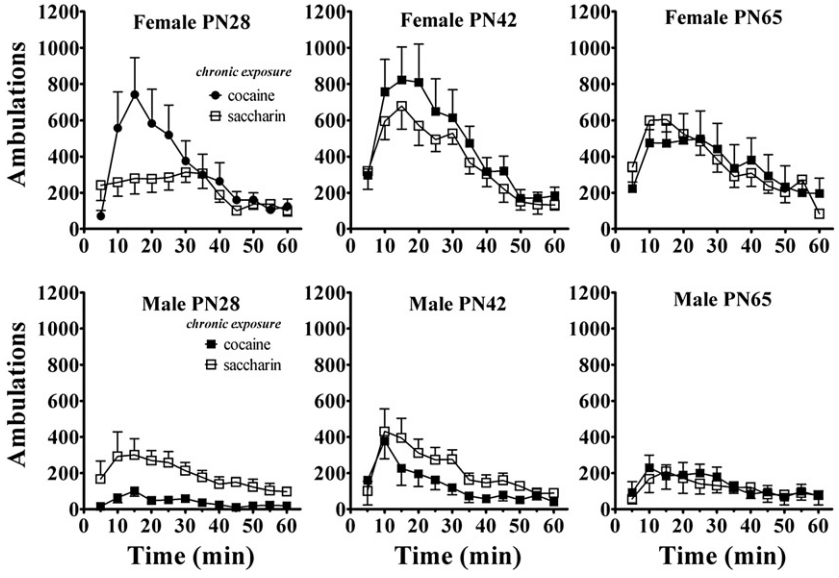

Fig. 3. Age and sex-related differences in cocaine-stimulated locomotion in rats that drank only saccharin or chose between cocaine/saccharin and saccharin ($N=6$/group). Male and female rats began drinking one of the two solutions at the three ages indicated. After the one- and two-bottle drinking experiments (Figs. 2 and 3) and an extended period without testing (rat ages were then 55, 69 and 92 days), rats were placed in open-field activity monitors for 1 h and then injected with 10 mg/kg cocaine i.p. Horizontal activity was recorded for 1 h and reported as the number of ambulations. ANOVA indicated a significant main effect of sex and a significant interaction effect of sex and chronic exposure (see Results). Prior cocaine/saccharin drinking significantly attenuated cocaine-stimulated locomotion in males ($p=0.017$) but not females.

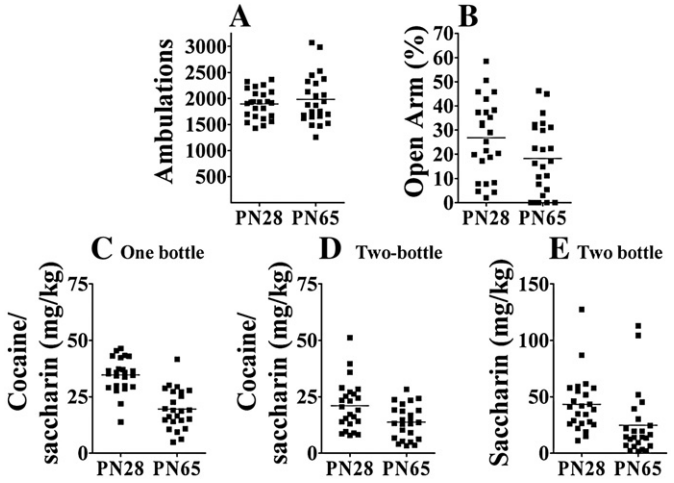

Fig. 4. Scattergrams of behavioral traits and cocaine/saccharin and saccharin drinking in young and adult rats (N=24/age). Pre-screening prior to beginning cocaine/saccharin drinking produced individual data for horizontal ambulations in a novel environment (A) and percent of time spent in open arms of an elevated plus-maze (B). No age difference was found for either of these behavioral traits. The average cocaine consumption was greater for adolescent than adult rats during 3 days of one-bottle cocaine/saccharin drinking (C). A similar age difference was also found for cocaine/saccharin drinking during three choice drinking sessions (D). Adolescents also drank significantly more saccharin solution during these choice drinking sessions (E).

sex and age did not interact (F(2,30)=2.65, p=0.087). Unlike one-bottle drinking, age did significantly affect cocaine/saccharin drinking in females (F(2,15)=3.93, p=0.042). PN42 females consumed more cocaine than PN65 females (p<0.05). Age significantly affected cocaine/saccharin drinking in males (F(2,15)=11.51, p<0.001) as both adolescent male groups consumed significantly more cocaine than adults (p<0.05), but were not different from each other.

Two-way ANOVA indicated that female rats consumed significantly more saccharin solution than males (F(1,30)=4.31, p=0.046). A significant main effect of age was also found when males and females were analyzed together (F(2,30)=3.98, p=0.029) but the interaction of sex and age was not significant (p=0.24). Age did not significantly affect saccharin drinking in either males (F(2,15)=1.18, p=0.33) or females (F(2,15)=3.15, p=0.07) when analyzed separately.

3.5. Voluntary cocaine/saccharin drinking induced persistent sex differences in cocaine-stimulated behavior

Motor activity during habituation to a novel environment and cocaine-stimulated locomotion were compared in the cocaine/saccharin and saccharin control rats, 2 weeks after the drinking sessions ended. The effect of voluntarily consumed cocaine on motor behavior was compared to that in age- and sex-matched controls that consumed only saccharin. Prior cocaine/saccharin drinking did not affect horizontal activity relative to any age- or sex-matched saccharin control group during habituation to the novel environment (data not shown).

Following habituation all rats were injected with cocaine (10 mg/kg, i.p.). Fig. 3 shows that cocaine-stimulated locomotion demonstrated a typical time course of onset and offset of motor stimulation (F(11,638)=52, p<0.001 for main effect of time). Cocaine induced more ambulations in females than males as ANOVA confirmed a significant main effect of sex (F(1,58)=33.4, p<0.001). The main effects of age and chronic drinking solution did not reach significance. Females that drank cocaine exhibited the highest, while males that drank cocaine, the lowest,

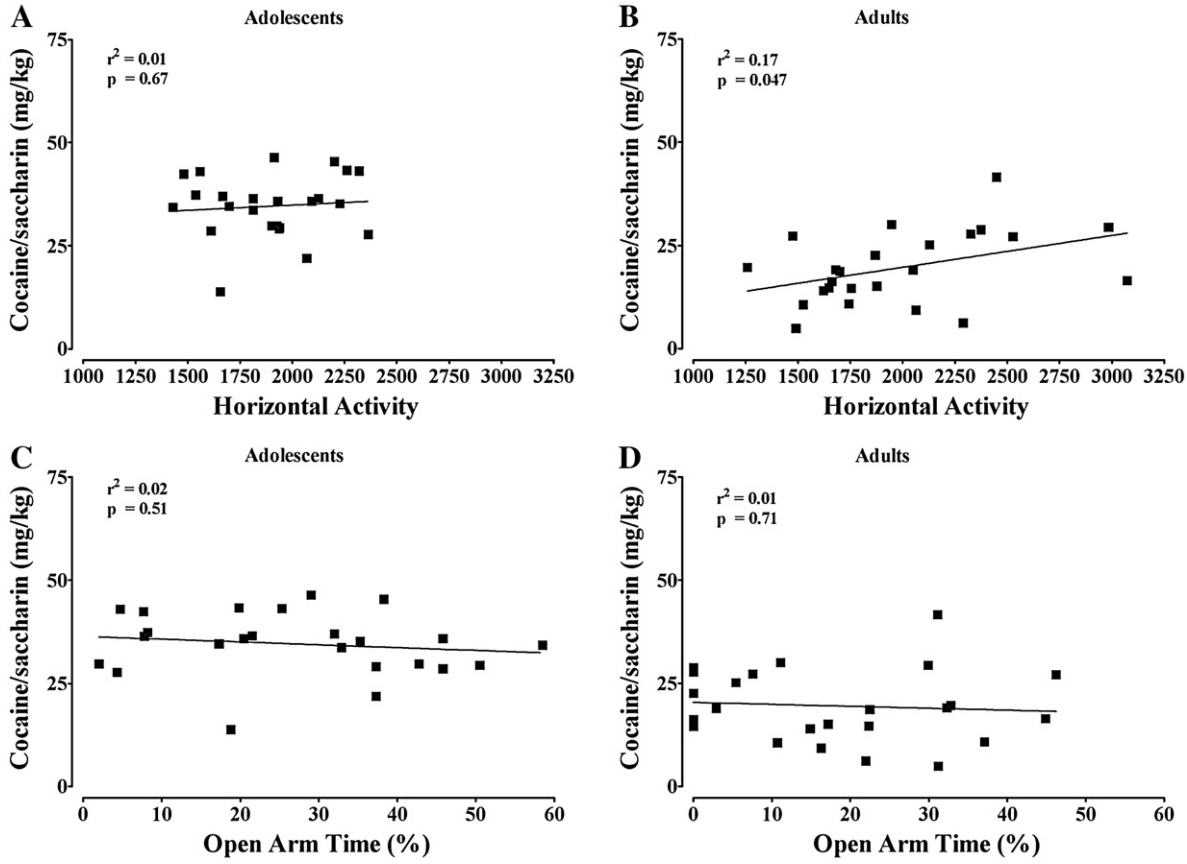

Fig. 5. The relationships between pre-screened traits and cocaine/saccharin drinking during one-bottle sessions (N=24/age). Each panel displays the r^2 (fraction of variance of y accounted for by x) and p values (significant when p<0.05) for the regression lines. Horizontal activity in the novel open-field was significantly associated with cocaine/saccharin drinking in adults. Neither trait correlated with one-bottle cocaine/saccharin drinking in adolescents.

cocaine-stimulated locomotion. This resulted in a significant interaction of chronic drinking solution and sex ($p=0.049$), indicating that prior cocaine drinking induced an enduring, sex-specific effect on cocaine-stimulated behavior. The time course for ambulations varied with sex, age and chronic drinking solution ($F(22,638)=1.65$, $p=0.031$).

Analyzing the data for each sex individually indicated that prior cocaine/saccharin drinking significantly attenuated cocaine-stimulated ambulations in males ($F(1,28)=6.32$, $p=0.017$) but did not significantly affect activity in females ($p=0.31$). There was no main effect of age on ambulations in males or females and the interaction of chronic drinking solution and age was not significant in either sex.

3.6. Behavioral phenotypes are similar in adolescents and adults

To determine if novelty-seeking and/or anxiety are correlated with drug intake in adolescence and adulthood, two behavioral phenotypes were determined before drinking began: locomotor activity in a novel open-field and percent open arm time in the elevated plus-maze. The consumption measures (cocaine/saccharin and saccharin-only intake) were subsequently determined during one- and/or two-bottle drinking experiments as previously described. Column graphs of each trait and consumption measure are displayed in Fig. 4 to indicate the variability by age. Fig. 4A and B shows the horizontal activity in the novel environment and percent open arm time, respectively, for PN28 and 65 rats. The lines in each column indicate the mean of the values. There were no age differences for horizontal activity ($p=0.44$) or open arm time ($p=0.063$).

Fig. 4C shows the average amount of cocaine consumed by each rat in the three initial cocaine/saccharin drinking sessions that began 5–7 days after the behavioral testing. The results of this experiment replicate the first experiment: adolescent rats drank more cocaine/saccharin than adults in one-bottle sessions ($p<0.001$). Fig. 4D shows that PN28 rats also drank more cocaine/saccharin solution during subsequent choice drinking sessions ($p=0.009$). Finally, Fig. 4E indicates that adolescent rats drank more saccharin solution than adults during choice sessions ($p<0.02$).

3.7. Behavioral phenotype and age associations with cocaine/saccharin drinking in adults during one-bottle sessions

Multiple regression analysis examined the relationships between consumption measures and each behavioral phenotype using age as a covariate. The regression model incorporating age, total locomotion and their interaction term was significant ($F(3,44)=13.1$, $p<0.001$) and accounted for 51% of the variance in one-bottle drinking data. Age significantly correlated with one-bottle cocaine drinking ($p<0.001$) and there was not a main effect of total locomotion ($p=0.15$) or interaction with age ($p=0.47$). Each panel in Figs. 5–7 displays the r^2 and p values indicating the probability of a significant association between the two variables. Fig. 5A indicates no relationship between novelty locomotion and one-bottle cocaine/saccharin drinking in adolescent rats. In contrast, novelty locomotion did account for 17% of the variance in cocaine/saccharin drinking in adult rats and this was a significant, positive correlation, $p=0.047$ (Fig. 5B). The regression model incorporating age, percent open arm time on the elevated plus-maze and their interaction term was significant ($F(3,44)=13.1$, $p<0.001$) and accounted for 47% of the variance in the one-bottle cocaine/saccharin drinking data. Age significantly correlated with one-bottle cocaine drinking ($p<0.001$) and there was not a main effect of percent open arm time ($p=0.49$) or interaction with age ($p=0.90$).

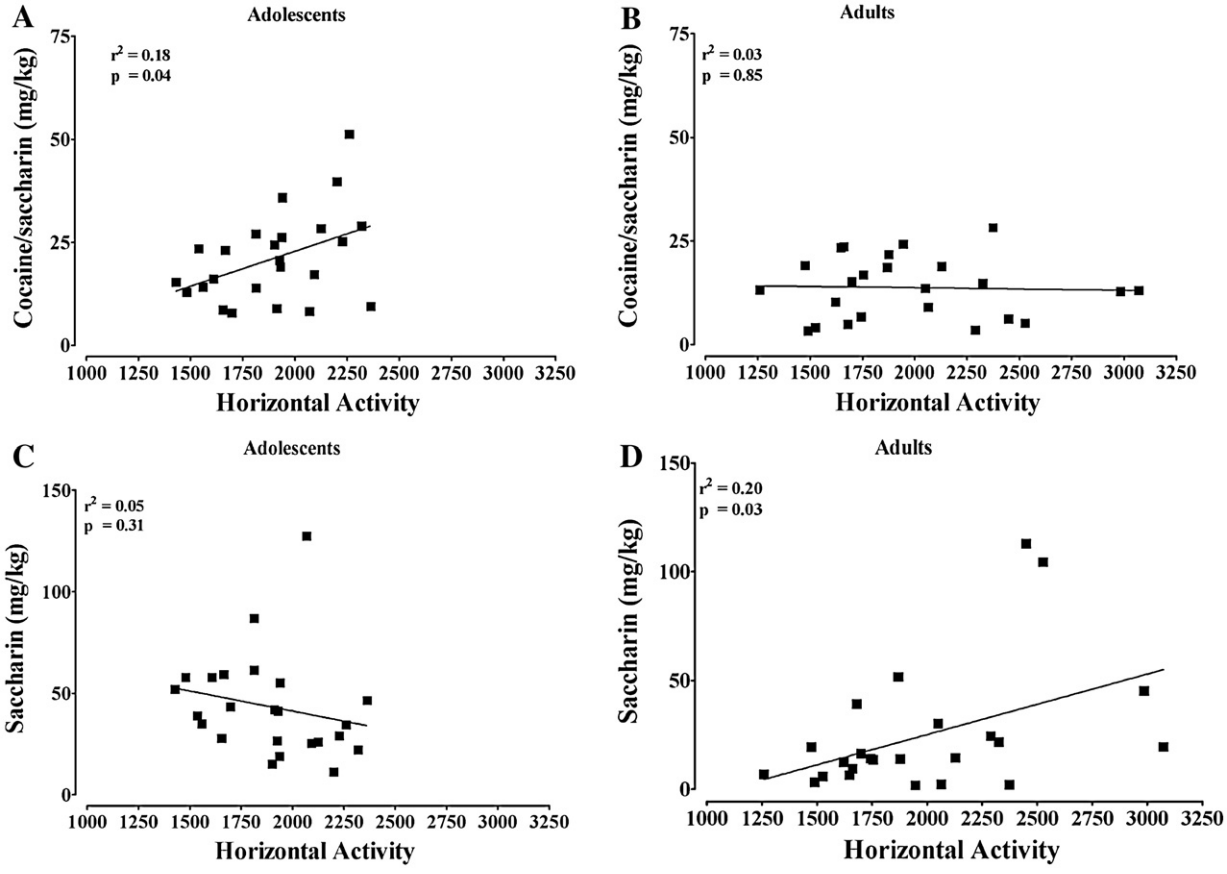

Fig. 6. Novelty exploration is correlated with cocaine/saccharin and saccharin drinking during choice sessions ($N=24$/age). The average cocaine/saccharin and saccharin-only consumptions for each rat during the initial three choice sessions are shown. Linear regression analysis was performed as in Fig. 5. Horizontal activity was significantly correlated with cocaine/saccharin (A) but not saccharin (C) drinking in adolescents. Horizontal activity was positively correlated with saccharin drinking in adults (D), but was not associated with cocaine/saccharin (B) drinking in adults.

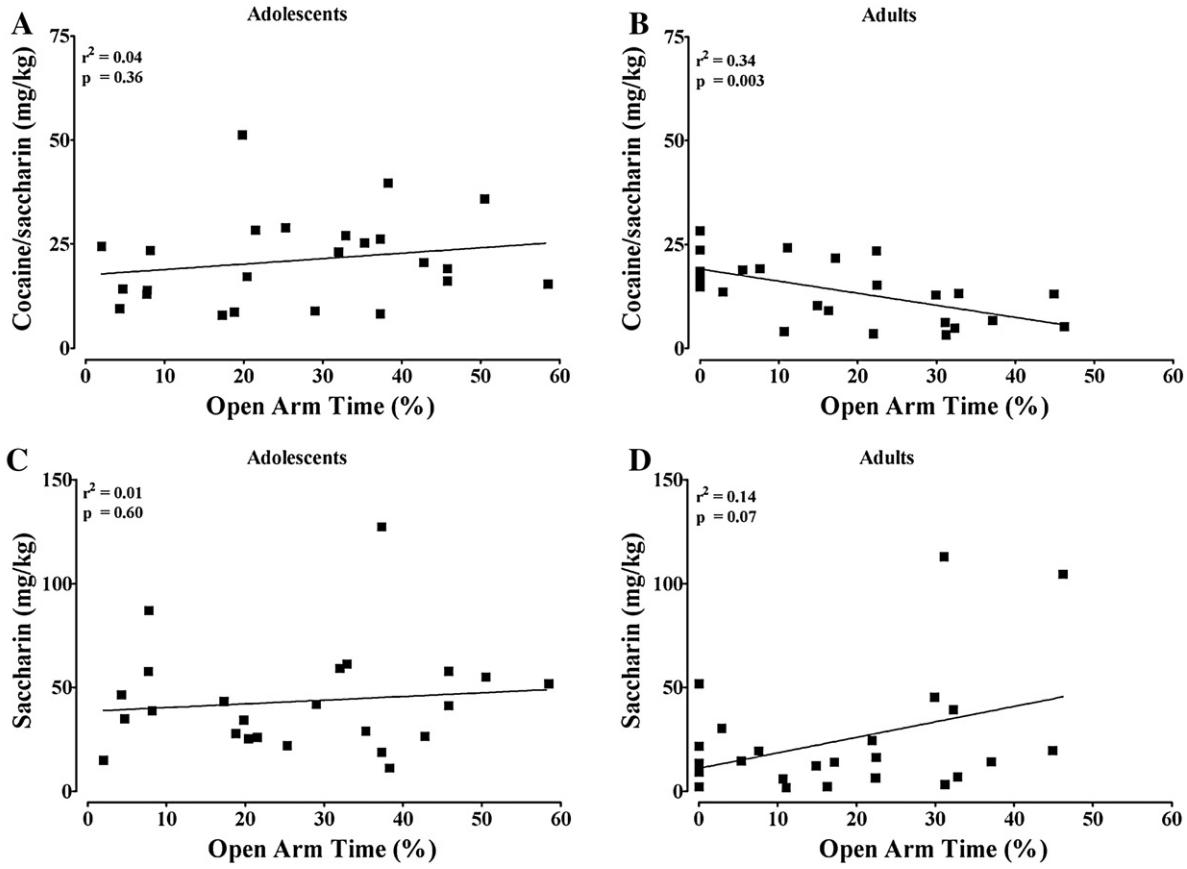

Fig. 7. Correlations between open arm time on the elevated plus-maze and cocaine/saccharin (A and B) and saccharin drinking (C and D) during choice sessions (N=24/age). Open arm time was correlated with cocaine/saccharin drinking in adults (B) only and only during choice drinking.

Fig. 5C and D shows that open arm time was not correlated with cocaine/saccharin drinking in adolescents or adults, respectively. For one-bottle drinking data, age was the primary determinant of cocaine/saccharin drinking.

3.8. Novelty exploration correlates with cocaine/saccharin drinking in adolescents during choice trials

The choice drinking period was restricted to 3 days in this experiment to resolve effects in a discrete period of development and to examine factors operating during early drug choice. Data from the three choice trials were averaged to determine the dose of cocaine each rat consumed. For cocaine/saccharin drinking in the choice phase, the regression model incorporating age, total locomotion and their interaction term was significant ($F(3,44)=4.8$, $p=0.006$) and accounted for 24% of the variance in cocaine/saccharin drinking. Age significantly correlated with cocaine/saccharin drinking ($p<0.001$) and there was a main effect of total locomotion ($p=0.043$) and an interaction with age ($p=0.03$). Fig. 6A and B shows the significant age effect in the relationship between novelty exploration and cocaine/saccharin drinking. The significant interaction term indicates that the slopes of the regression lines are different in Fig. 6A and B. The positive slope of the line for adolescent rats indicates that higher activity levels in the novel environment were significantly correlated with drinking greater doses of cocaine ($r^2=0.18$, $p=0.04$), although no relationship existed for the adults (Fig. 6B).

For saccharin drinking in the choice phase, the regression model incorporating age, total locomotion and their interaction term was significant ($F(3,44)=4.32$, $p=0.009$) and accounted for 22% of the variance in saccharin drinking. Age significantly correlated with saccharin drinking ($p<0.015$) and there was not a main effect of total locomotion ($p=0.72$). The interaction of novelty-seeking and age was significant ($p=0.043$), indicating opposite relationships at each age. In adults (Fig. 7D) but not adolescents (Fig. 7C), locomotion in the novel environment was positively correlated with saccharin drinking ($p=0.03$).

3.9. Elevated plus-maze behavior in adults correlates with cocaine/saccharin consumption during choice trials

The relationships between open arm time, age and cocaine/saccharin and saccharin drinking are shown in Fig. 7. For cocaine/saccharin drinking in the choice phase, the regression model incorporating age, percent open arm time and their interaction term was significant ($F(3,44)=4.97$, $p=0.005$) and accounted for 25% of the variance in cocaine/saccharin drinking. Age significantly correlated with cocaine/saccharin drinking ($p=0.004$), there was no main effect of percent open arm time ($p=0.34$) but an interaction with age ($p=0.016$) was found. A significant association between open arm time and cocaine/saccharin drinking was found in adult rats, $p=0.003$ (Fig. 7B). This negative correlation indicated that the adults that spent a lower percentage of time on the open arms of the maze consumed higher cocaine doses. Percent open arm time accounted for 34% of the variance in adult cocaine/saccharin drinking, but was not correlated in the adolescents.

For saccharin drinking in the choice phase, the regression model incorporating age, percent open arm time and their interaction term was significant ($F(3,44)=3.43$, $p=0.025$) and accounted for 19% of the variance in cocaine/saccharin drinking. Age ($p=0.07$) and percent open arm time ($p=0.07$) were weakly associated with saccharin drinking but did not interact ($p=0.25$). Percent open arm time tended to be positively correlated with saccharin drinking in adults (Fig. 7D)

($p=0.07$). Open arm time was not associated with drinking saccharin solution in adolescents (Fig. 7C).

4. Discussion

The present study determined the effects of age, sex and behavioral traits on voluntary cocaine/saccharin drinking in rats. This paradigm was an effective method for exposing adolescents to pharmacologically effective doses of cocaine as they consumed more cocaine than adults and the cocaine exposure resulted in enduring, sex-specific behavioral effects as cocaine-stimulated locomotion was attenuated only in males. Female rats consumed more saccharin-only but not more cocaine/saccharin than males. Finally, we showed that the behavioral traits of novelty-seeking and anxiety-like behavior on the plus-maze were correlated with cocaine/saccharin drinking in age and context-specific ways. In the choice drinking context, novelty-seeking was associated with cocaine/saccharin consumption in adolescents while open arm time on the plus-maze was related to adult cocaine/saccharin drinking.

The present results support the feasibility of using ingestion in the brief rodent adolescent period to study exposure and drug taking during early development. Young rats voluntarily consumed a cocaine/saccharin solution without invasive manipulations like surgery, water deprivation or prolonged forced exposure. The 5-hour cocaine/saccharin availability allowed the animals to be drug-free for most of the day and to gain weight normally. Voluntary cocaine/saccharin consumption produced an enduring effect on subsequent, drug-stimulated behavior, thereby demonstrating consumption of pharmacologically effective doses. We assayed solutions refrigerated for 5 days and verified that cocaine did not degrade, which would have yielded lower consumed doses than expected.

This paradigm provides a choice between two reinforcers, saccharin and cocaine/saccharin solutions. This is unlike the majority of i.v. drug self-administration studies in which the only reinforcer is the drug. Cocaine/saccharin consumption reflects a balance of its reinforcing and aversive components and, during one-bottle exposure, thirst. Although rats drank more of the saccharin-only vehicle than the cocaine/saccharin solution, they did voluntarily consume cocaine in amounts that changed subsequent responses to a cocaine challenge. Although the present studies did not confirm previous reports of the reinforcing properties of oral cocaine, it is reasonable to speculate that the aversive effects of cocaine did not outweigh the combination of the reinforcing effects of cocaine plus saccharin.

Lenoir et al. (2007) have demonstrated that under a variety of conditions rats choose to consume sweet-tasting saccharin solution over i.v. cocaine self-administration, indicating that sweet taste is powerfully rewarding. Other examples demonstrate that the presence of an alternate reinforcer reduces consumption of a drug of abuse. Lactating female rats will choose the reward of pups over cocaine at certain postpartum periods, indicating that pup suckling is more rewarding than cocaine at least in certain individuals (Hecht et al., 1999; Mattson et al., 2001; Mattson et al., 2003; Ferris et al., 2005). Saccharin decreased oral phencyclidine consumption in male and female monkeys (Cosgrove and Carroll, 2003). Access to a glucose/saccharin solution decreased cocaine-seeking and drug-induced relapse (Liu and Grigson, 2005). Exercise is an effective reinforcer in operant paradigms (Iversen, 1993) and its availability decreased oral amphetamine intake (Kanarek et al., 1995), ethanol drinking in ethanol-preferring rats (McMillan et al., 1995) and i.v. cocaine self-administration (Cosgrove et al., 2002). In the present paradigm the bottle of saccharin was useful because it obviated the need to drink cocaine/saccharin for thirst, making cocaine/saccharin consumption during choice tests voluntary and likely reflecting intake of desired cocaine levels. The late adolescent rats did not reduce their intake of cocaine/saccharin when the saccharin-only bottle was added, suggesting an age-specific lack of distraction by the alternate reinforcer.

The relative insensitivity of adolescents to the aversive aspects of cocaine could have contributed to their drinking more cocaine/saccharin solution in the present study. Both the local anesthetic effect of cocaine and its bitter taste could be responsible for decreasing the palatability of cocaine/saccharin. Cocaine also has central aversive properties (Ettenberg and Geist, 1991). This laboratory has shown that cocaine is less aversive in adolescent male rats using the conditioned taste aversion paradigm (Schramm-Sapyta et al., 2006). High levels of consumption favor progression to compulsive drug use in animal models (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004). If adolescent humans are also less sensitive to the aversive properties of cocaine (and other drugs) this could lead to consumption of higher drug doses and potentially enhance progression to addiction. In fact, it has been suggested that human adolescents exhibit a triad of enhanced response to rewards, decreased sensitivity to punishment and an inefficient system that regulates approach/avoidance (Ernst et al., 2005).

The finding that adolescent rats consumed more cocaine/saccharin than adults is consistent with literature indicating that adolescents are particularly vulnerable to drugs of abuse. We have reported that acute cocaine administration stimulates more ambulatory behavior in adolescent than adult male rats (Caster et al., 2005) and that a single high dose of cocaine elicits more behavioral sensitization in adolescent males (Caster et al., 2007). The onset of puberty in rats attenuates cocaine-stimulated locomotion in males while stimulating behavior in females (Parylak, 2008). This dichotomous effect of puberty on cocaine-stimulated locomotion reflects the greater decrease in cocaine/saccharin consumption in male rats observed in this study. Female rats that start nicotine self-administration during very early adulthood take more nicotine than females that start later (Levin et al., 2003). Adolescent mice are particularly vulnerable to nicotine self-administration (Adriani et al., 2002; Adriani et al., 2004). Nicotine ± acetaldehyde was more reinforcing in adolescent than adult rats (Belluzzi et al., 2005a). In contrast to the present and other studies showing greater intake/vulnerability of adolescents, adolescent rats do not self-administer more i.v. cocaine than adults (Leslie et al., 2004; Belluzzi et al., 2005b; Kerstetter and Kantak, 2007; Frantz et al., 2007; Kantak et al., 2007). The reason for this disparity is unclear. It could reflect a developmental difference in choice between a natural and drug reinforcer or a different balance of reinforcing and aversive effects of cocaine when delivered orally and intravenously. Regardless of whether adolescents take more cocaine, there is evidence that it may have more profound neurological effects in adolescents. We have shown greater cocaine-stimulated dopamine overflow in adolescent rats (Walker and Kuhn, 2008), an effect that is directly relevant to drug reinforcement (Di Chiara and Imperato, 1988; Koob, 1992; Wise, 1998).

Cocaine/saccharin drinking during early adolescence attenuated cocaine-stimulated locomotion after a period of drug abstinence in males. Since drug sensitization depends largely on whether exposure is continuous or intermittent (Nelson and Ellison, 1978; Post, 1980; King et al., 1992; Davidson et al., 2005), the slowed absorption kinetics of oral cocaine relative to intranasal application (Van Dyke et al., 1978) may explain why cocaine-stimulated locomotion was attenuated and not sensitized in males in the present study. Females did not show this adaptation after cocaine/saccharin drinking. While the tolerance induced in male rats was consistent with previous studies of human adolescent stimulant exposures (Carlezon et al., 2003), the observed sex differences in chronic effects in rats warrant close examination of sequelae from stimulant exposures to adolescent human females.

Abundant preclinical and clinical evidence indicate that certain aspects of drug responses and addiction may be more severe in females (Lynch et al., 2002; Roth et al., 2004; Carroll et al., 2004). Contradictory reports in animal literature did not find evidence for sex differences in cocaine discrimination or self-administration (Haney et al., 1995; Roberts et al., 1989; Craft and Stratman, 1996; Caine et al., 2004). The present results did not find evidence of greater cocaine/

saccharin consumption in females. The most striking sex difference in the present work was that cocaine drinking decreased subsequent cocaine-stimulated locomotion in males but not females. We have reported that female rats have significantly greater dopamine uptake and release rates in striatum and that cocaine induces greater electrically-stimulated dopamine efflux in female striatum (Walker et al., 2000; Walker et al., 2006). However, these studies were conducted after intraperitoneal administration of cocaine, and it is possible that the dopamine dynamics of males and females do not differ after routes of administration that slow absorption (such as the oral route).

The present results indicate that voluntary cocaine/saccharin drinking was associated with novelty-seeking. Novelty-induced locomotion was positively correlated with cocaine/saccharin consumption during choice trials in adolescents, while cocaine/saccharin consumption in adults was correlated with novelty-seeking during one-bottle drinking. Exploration in novel environments in rodents is thought to model novelty or sensation seeking in humans (Bardo et al., 1996; Bardo and Dwoskin, 2004; Cain et al., 2005). Novelty-seeking and risk taking are highly associated with the development of substance use disorder (SUD) in young males (Wills et al., 1994; Masse and Tremblay, 1997; Wills et al., 1998). A related trait, neurobehavioral disinhibition, has also been shown to be a risk factor for the development of SUD in boys (Tarter et al., 2004). Animal studies support a positive relationship between novelty responding and amphetamine and cocaine self-administration (Piazza et al., 1989; Pierre and Vezina, 1997; Piazza et al., 2000; Marinelli and White, 2000; Klebaur et al., 2001; Pelloux et al., 2004). The dopaminergic neurons of the substantia nigra and ventral tegmental area play prominent roles in novelty-seeking as well as reinforcement (Le Moal and Simon, 1991; Spanagel and Weiss, 1999). The present findings extend the previous results by showing that novelty-seeking is also related to oral cocaine/saccharin consumption and that this trait can be identified early in adolescence.

While anxiety is often comorbid with substance use disorders (Le Moal and Simon, 1991; Karlsgodt et al., 2003; Watkins et al., 2004; Patkar et al., 2004; Fox et al., 2005), separating trait anxiety from the state anxiety produced by drug use/withdrawal is difficult in human populations. Our present correlation between plus-maze performance as a measure of anxiety and cocaine/saccharin drinking in adult rats shows that individual differences in anxiety prior to drug exposure are related to subsequent drug intake. Similarly, Homberg et al. (2002) found that the rats that displayed the most anxiety behaviors (self-grooming on the elevated plus-maze), also had the highest break points on a progressive ratio schedule for cocaine self-administration. Unlike adults, plus-maze performance was not associated with cocaine/saccharin drinking in adolescents. This may be related to the lesser processing of both aversive drug effects and stress in adolescence. Adolescent rats have less cocaine-induced aversion (Schramm-Sapyta et al., 2006), less hangover-induced anxiety after alcohol intoxication (Doremus et al., 2003), and fewer physiologic symptoms of nicotine withdrawal than adults (O'Dell et al., 2004). Stressful stimuli seem to induce different, and typically less neuronal change/plasticity in adolescents (Kellogg et al., 1998; Viau et al., 2005; Romeo et al., 2006).

In conclusion, susceptibility to drug taking is dependent on complex interactions among behavioral traits, sex and developmental stage. The behavioral traits of novelty-seeking and elevated plus-maze behavior correlated with cocaine/saccharin drinking, but in age- and context-specific ways. These findings suggest that individual differences in novelty-seeking might play a greater role in drug taking in adolescents, as is widely hypothesized, and introduces the novel finding that a role for anxiety-like behavior can be demonstrated in adults. The ability to determine the role of individual differences in model systems offers the opportunity to elucidate the neurobiologic mechanisms responsible.

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References

Adriani W, Macri S, Pacifici R, Laviola G. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacology 2002;27:212–24.
Adriani W, Granstrem O, Macri S, Izykenova G, Dambinova S, Laviola G. Behavioral and neurochemical vulnerability during adolescence in mice: studies with nicotine. Neuropsychopharmacology 2004;29:869–78.
Bardo MT, Dwoskin LP. Biological connection between novelty- and drug-seeking motivational systems. Motivational factors in the etiology of drug abuse, vol. 50. ; 2004. p. 127–58.
Bardo MT, Donohew RL, Harrington NG. Psychobiology of novelty seeking and drug seeking behavior. Behav Brain Res 1996;77:23–43.
Belluzzi JD, Wang RH, Leslie FM. Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacology 2005a;30:705–12.
Belluzzi JD, Wang RH, Leslie FM. Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacology 2005b;30:705–12.
Berridge KC. Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev 1996;20:1-25.
Cain ME, Saucier DA, Bardo MT. Novelty seeking and drug use: contribution of an animal model. Exp Clin Psychopharmacol 2005;13:367–75.
Caine SB, Bowen CA, Yu G, Zuzga D, Negus SS, Mello NK. Effect of gonadectomy and gonadal hormone replacement on cocaine self-administration in female and male rats. Neuropsychopharmacology 2004;29:929–42.
Carlezon WA, Mague SD, Andersen SL. Enduring behavioral effects of early exposure to methylphenidate in rats. Biol Psychol 2003;54:1330–7.
Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. Trends Pharmacol Sci 2004;25:273–9.
Caster JM, Walker QD, Kuhn CM. Enhanced behavioral response to repeated-dose cocaine in adolescent rats. Psychopharmacology (Berl) 2005;183:218–25.
Caster JM, Walker QD, Kuhn CM. A single high dose of cocaine induces differential sensitization to specific behaviors across adolescence. Psychopharmacology 2007;193: 247–60.
Chambers RA, Taylor JR, Potenza MN. Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. American Journal of Psychiatry 2003;160:1041–52.
Chen K, Kandel DB, Davies M. Relationships between frequency and quantity of marijuana use and last year proxy dependence among adolescents and adults in the United States. Drug Alcohol Depend 1997;46:53–67.
Clark DB, Kirisci L, Tarter RE. Adolescent versus adult onset and the development of substance use disorders in males. Drug Alcohol Depend 1998;49:115–21.
Cosgrove KP, Carroll ME. Effects of a non-drug reinforcer, saccharin, on oral self-administration of phencyclidine in male and female rhesus monkeys. Psychopharmacology 2003;170:9-16.
Cosgrove KP, Hunter RG, Carroll ME. Wheel-running attenuates intravenous cocaine self-administration in rats — sex differences. Pharmacol Biochem Behav 2002;73: 663–71.
Costello EJ, Mustillo S, Erkanli A, Keeler G, Angold A. Prevalence and development of psychiatric disorders in childhood and adolescence. Arch Gen Psychiatry 2003;60: 837–44.
Craft RM, Stratman JA. Discriminative stimulus effects of cocaine in female versus male rats. Drug Alcohol Depend 1996;42:27–37.
Davidson C, Lee TH, Ellinwood EH. Acute and chronic continuous methamphetamine have different long-term behavioral and neurochemical consequences. Neurochem Int 2005;46:189–203.
Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. Science 2004;305:1014–7.
Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 1988;85:5274–8.
Doremus TL, Brunell SC, Varlinskaya EI, Spear LP. Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. Pharmacol Biochem Behav 2003;75:411–8.
Ernst M, Pine DS, Hardin M. Triadic model of the neurobiology of motivated behavior. Psychol Med 2005;35:1-14.
Estroff TW, Schwartz RH, Hoffmann NG. Adolescent cocaine abuse — addictive potential, behavioral and psychiatric effects. Clin Pediatr 1989;28:550–5.
Ettenberg A, Geist TD. Animal-model for investigating the anxiogenic effects of self-administered cocaine. Psychopharmacology 1991;103:455–61.
Falk JL, Ma F, Lau CE. chronic oral cocaine self-administration — pharmacokinetics and effects on spontaneous and discriminative motor functions. J Pharmacol Exp Ther 1991;257:457–65.
Ferris CF, Kulkarni P, Sullivan JM, Harder JA, Messenger TL, Febo M. Pup suckling is more rewarding than cocaine: evidence from functional magnetic resonance imaging and three-dimensional computational analysis. J Neurosci 2005;25: 149–56.
Fox HC, Talih M, Malison R, Anderson GM, Kreek MJ, Sinha R. Frequency of recent cocaine and alcohol use affects drug craving and associated responses to stress and drug-related cues. Psychoneuroendocrinology 2005;30:880–91.

Frantz KJ, O'Dell LE, Parsons LH. Behavioral and neurochemical responses to cocaine in periadolescent and adult rats. Neuropsychopharmacology 2007;32:625–37.
Haney M, Maccari S, Le Moal M, Simon H, Piazza PV. Social stress increases the acquisition of cocaine self-administration in male and female rats. Brain Res 1995;698: 46–52.
Hecht GS, Spear NE, Spear LP. Changes in progressive ratio responding for intravenous cocaine throughout the reproductive process in female rats. Dev Psychobiol 1999;35: 136–45.
Homberg JR, Van Den AM, Raaso HS, Wardeh G, Binnekade R, Schoffelmeer AN, et al. Enhanced motivation to self-administer cocaine is predicted by self-grooming behaviour and relates to dopamine release in the rat medial prefrontal cortex and amygdala. Eur J Neurosci 2002;15:1542–50.
Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 2006;29:565–98.
Iversen IH. Techniques for establishing schedules with wheel running as reinforcement in rats. J Exp Anal Behav 1993;60:219–38.
Jentsch JD, Henry PJ, Mason PA, Merritt JH, Ziriax JM. Establishing orally self-administered cocaine as a reinforcer in rats using home-cage pre-exposure. Prog Neuro-Psychopharmacol Biol Psychiatry 1998;22:229–39.
Jones HE, Garrett BE, Griffiths RR. Reinforcing effects of oral cocaine: contextual determinants. Psychopharmacology (Berl) 2001;154:143–52.
Kanarek RB, Marks-Kaufman R, D'Anci KE, Przypek J. Exercise attenuates oral intake of amphetamine in rats. Pharmacol Biochem Behav 1995;51:725–9.
Kantak KM, Goodrich CM, Uribe V. Influence of sex, estrous cycle, and drug-onset age on cocaine self-administration in rats. (*Rattus norvegicus*). Exp Clin Psychopharmacol 2007;15:37–47.
Karlsgodt KH, Lukas SE, Elman I. Psychosocial stress and the duration of cocaine use in non-treatment seeking individuals with cocaine dependence. Am J Drug Alcohol Abuse 2003;29:539–51.
Kelley AE, Schochet T, Landry CF. Risk taking and novelty seeking in adolescence — introduction to part I. Adolescent brain development: vulnerabilities and opportunities, vol. 1021. ; 2004. p. 27–32.
Kellogg CK, Awatramani GB, Piekut DT. Adolescent development alters stressor-induced Fos immunoreactivity in rat brain. Neuroscience 1998;83:681–9.
Kerstetter KA, Kantak KM. Differential effects of self-administered cocaine in adolescent and adult rats on stimulus–reward learning. Psychopharmacology 2007;194:403–11.
King GR, Joyner C, Lee T, Kuhn C, Ellinwood EH. Intermittent and continuous cocaine administration — residual behavioral states during withdrawal. Pharmacol Biochem Behav 1992;43:243–8.
Klebaur JE, Bevins RA, Segar TM, Bardo MT. Individual differences in behavioral responses to novelty and amphetamine self-administration in male and female rats. Behavioural Pharmacology 2001;12:267–75.
Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 1992;13:177–84.
Kreek MJ, Nielsen DA, Butelman ER, LaForge KS. Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat Neurosci 2005;8:1450–7.
Lau CE, Imam A, Ma F, Falk JL. Acute effects of cocaine on spontaneous and discriminative motor functions — relation to route of administration and pharmacokinetics. J Pharmacol Exp Ther 1991;257:444–56.
Lau CE, Falk JL, King GR. Oral cocaine self-administration — relation of locomotor-activity to pharmacokinetics. Pharmacol Biochem Behav 1992;43:45–51.
Le Moal M, Simon H. Mesocorticolimbic dopaminergic network — functional and regulatory roles. Physiol Rev 1991;71:155–234.
Lenoir M, Serre F, Cantin L, Ahmed SH. Intense sweetness surpasses cocaine reward. PLoS ONE 2007;2:e698.
Leslie FM, Loughlin SE, Wang RH, Perez L, Lotfipour S, Belluzzi JD. Adolescent development of forebrain stimulant responsiveness — insights from animal studies. Adolescent brain development: vulnerabilities and opportunities, vol. 1021. ; 2004. p. 148–59.
Levin ED, Rezvani AH, Montoya D, Rose JE, Swartzwelder HS. Adolescent-onset nicotine self-administration modeled in female rats. Psychopharmacology (Berl) 2003;169: 141–9.
Liu C, Grigson PS. Brief access to sweets protect against relapse to cocaine-seeking. Brain Res 2005;1049:128–31.
Lopez B, Turner RJ, Saavedra LM. Anxiety and risk for substance dependence among late adolescents/young adults. J Anxiety Disord 2005;19:275–94.
Lynch WJ, Roth ME, Carroll ME. Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology 2002;164:121–37.
Macenski MJ, Meisch RA. Ratio size and cocaine concentration effects on oral cocaine-reinforced behavior. J Exp Anal Behav 1998;70:185–201.
Marinelli M, White FJ. Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. J Neurosci 2000;20:8876–85.
Masse LC, Tremblay RE. Behavior of boys in kindergarten and the onset of substance use during adolescence. Arch Gen Psychiatry 1997;54:62–8.
Mattson BJ, Williams S, Rosenblatt JS, Morrell JJ. Comparison of two positive reinforcing stimuli: pups and cocaine throughout the postpartum period. Behav Neurosci 2001;115:683–94.
Mattson BJ, Williams SE, Rosenblatt JS, Morrell JI. Preferences for cocaine- or pup-associated chambers differentiates otherwise behaviorally identical postpartum maternal rats. Psychopharmacology 2003;167:1–8.
McMillan DE, McClure GYH, Hardwick WC. Effects of access to a running wheel on food, water and ethanol intake in rats bred to accept ethanol. Drug Alcohol Depend 1995;40:1–7.
Miles FJ, Everitt BJ, Dickinson A. Oral cocaine seeking by rats: action or habit? Behav Neurosci 2003;117:927–38.
Nelson LR, Ellison G. Enhanced stereotypes after repeated injections but not continuous amphetamines. Neuropharmacology 1978;17:1081–4.
O'Dell LE, Bruijnzeel AW, Ghozland S, Markou A, Koob GF. Nicotine withdrawal in adolescent and adult rats. Ann NY Acad Sci 2004;1021:167–74.
Parylak SL, Caster JM, Walker QD, Kuhn CM. Gonadal steroids mediate the opposite changes in cocaine-induced locomotion across adolescence in male and female rats. Pharmacol Biochem & Behav 2008;89:314–23.
Patkar AA, Thornton CC, Mannelli P, Hill KP, Gottheil E, Vergare MJ, et al. Comparison of pretreatment characteristics and treatment outcomes for alcohol-, cocaine-, and multisubstance-dependent patients. J Addict Dis 2004;23:93-109.
Pelloux Y, Costentin J, Duterte-Boucher D. Differential effects of novelty exposure on place preference conditioning to amphetamine and its oral consumption. Psychopharmacology (Berl) 2004;171:277–85.
Pellow S, Chopin P, File SE, Briley M. Validation of open–closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
Piazza PV, Deminiere JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. Science 1989;245:1511–3.
Piazza PV, Deroche-Gamonent V, Rouge-Pont F, Le Moal M. Vertical shifts in self-administration dose–response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci 2000;20:4226–32.
Pierre PJ, Vezina P. Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology (Berl) 1997;129:277–84.
Post RM. Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance. Life Sci 1980;26:1275–82.
Roberts DC, Bennett SA, Vickers GJ. The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. Psychopharmacology 1989;98:408–11.
Robins LN, Przybeck TR. Age of onset of drug use as a factor in drug and other disorders. NIDA Res Monogr 1995;56:178–92.
Romeo RD, Karatsoreos IN, McEwen BS. Pubertal maturation and time of day differentially affect behavioral and neuroendocrine responses following an acute stressor. Horm Behav 2006;50:463–8.
Roth ME, Cosgrove KP, Carroll ME. Sex differences in the vulnerability to drug abuse: a review of preclinical studies. Neurosci Biobehav Rev 2004;28:533–46.
Schramm-Sapyta NL, Morris RW, Kuhn CM. Adolescent rats are protected from the conditioned aversive properties of cocaine and lithium chloride. Pharmacol Biochem Behav 2006;84:344–52.
Seidman MH, Lau CE, Chen RY, Falk JL. Orally self-administered cocaine — reinforcing efficacy by the place preference method. Pharmacol Biochem Behav 1992;43:235–41.
Smith BJ, Jones HE, Griffiths RR. Physiological, subjective and reinforcing effects of oral and intravenous cocaine in humans. Psychopharmacology (Berl) 2001;156:435–44.
Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. Trends Neurosci 1999;22:521–7.
Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.
Stromberg MF, Mackler SA, Volpicelli JR, O'Brien CP, Dewey SL. The effect of gamma-vinyl-GABA on the consumption of concurrently available oral cocaine and ethanol in the rat. Pharmacol Biochem Behav 2001;68:291–9.
Sun L, Hall G, Lau CE. High-performance liquid chromatographic determination of cocaine and its metabolites in serum microsamples with fluorimetric detection and its application to pharmacokinetics in rats. J Chromatogr B, Biomed Sci Appl 2000;745:315–23.
Tarter RE, Kirisci L, Reynolds M, Mezzich A. Neurobehavior disinhibition in childhood predicts suicide potential and substance use disorder by young adulthood. Drug Alcohol Depend 2004;76:S45–52.
Van Dyke C, Jatlow P, Ungerer J, Barash PG, Byck R. Oral cocaine: plasma concentrations and central effects. Science 1978;200:211–3.
Vanderschuren LJ, Everitt BJ. Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 2004;305:1017–9.
Viau V, Bingham B, Davis J, Lee P, Wong M. Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. Endocrinology 2005;146:137–46.
Walker QD, Kuhn CM. Cocaine increases stimulated dopamine release more in periadolescent than adult rats. Neurotoxicol Teratol 2008;30:412–8.
Walker QD, Nelson CJ, Smith D, Kuhn CM. Vaginal lavage attenuates cocaine-stimulated activity and establishes place preference in rats. Pharmacol Biochem Behav 2002;73: 743–52.
Walker QD, Rooney MB, Wightman RM, Kuhn CM. Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. Neuroscience 2000;95:1061–70.
Walker QD, Ray R, Kuhn CM. Sex differences in neurochemical effects of dopaminergic drugs in rat striatum. Neuropsychopharmacology 2006;31:1193–202.
Watkins KE, Hunter SB, Wenzel SL, Tu WL, Paddock SM, Griffin A, et al. Prevalence and characteristics of clients with co-occurring disorders in outpatient substance abuse treatment. Am J Drug Alcohol Abuse 2004;30:749–64.
Wills TA, Vaccaro D, McNamara G. Novelty seeking, risk taking, and related constructs as predictors of adolescent substance use: an application of Cloninger's theory. J Subst Abuse 1994;6:1-20.
Wills TA, Windle M, Cleary SD. Temperament and novelty seeking in adolescent substance use: convergence of dimensions of temperament with constructs from Cloninger's theory. J Pers Soc Psychol 1998;74:387–406.
Wise RA. Drug-activation of brain reward pathways. Drug Alcohol Depend 1998;51: 13–22.
Zuckerman M. Sensation-seeking and the endogenous deficit theory of drug abuse. NIDA Res Monogr 1986;74:59–70.