

Attenuated incubation of cocaine seeking in male rats trained to self-administer cocaine during periadolescence

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Abstract

Rationale and objectives Although onset of drug use during adolescence appears to increase long-term vulnerability to drug dependence in humans, relatively little is known about extinction and reinstatement of drug seeking after periadolescent onset of drug self-administration in laboratory animals. Furthermore, although cue-induced reinstatement of cocaine seeking increases progressively during abstinence from cocaine self-administration in adult subjects, this “incubation of cocaine craving” remains unexplored after adolescent drug intake in animal models.

Materials and methods We allowed periadolescent (post-natal day (PND) 35 at start) and adult (PND 83–95 at start) male Wistar rats to self-administer cocaine (0.36 mg/kg/infusion) in 2-h daily sessions on a fixed ratio 1 schedule of reinforcement over 14 days. Then, we compared extinction and cue-induced or cocaine priming-induced reinstatement (10 mg/kg cocaine, intraperitoneal) of cocaine seeking in both age groups after 30 days of abstinence in home cages. In separate cohorts, we tested for time-dependent increases in cue-induced reinstatement over approximately 1, 14, 30, or 60 days of abstinence in both age groups.

Results Adolescent and adult rats self-administered similar amounts of cocaine. Subsequent cue-induced reinstatement was lower in the adolescent-onset group after a 30-day abstinence period, but cocaine priming-induced reinstatement did not differ across ages. Also, extinction responding and time-dependent increases in cue-induced reinstatement (incubation) were less pronounced in rats that took cocaine as adolescents compared with adults.

Conclusions Surprisingly, these results may reflect resistance among adolescent subjects to some enduring effects of drug self-administration, such as reward learning.

Keywords Adolescence · Periadolescence · Cocaine · Cue-induced reinstatement · Drug priming · Extinction · Incubation

Introduction

Adolescence among humans is a developmental stage associated with vulnerability to drug use and abuse (Laviola et al. 1999; O'Malley and Johnston 2007). People aged 18 to 20 years report the highest rates of illicit drug use compared to other age groups, and individuals who initiate drug taking at age 14 or younger are almost five times more likely to classify themselves as drug-dependent in adulthood than those who first tried drugs after age 18 (SAMHSA 2006). However, whether or not these phenomena can be attributed to biological vulnerability of adolescent development is unclear, especially given that a manifestation of vulnerability to drugs among humans might be adolescent onset of drug use; if so, few vulnerable individuals would be included in any survey group of adult-onset drug users (Shram et al. 2007). Animal models of adolescent drug intake may help to identify the potential role of biological vulnerability in drug abuse.

In rodents, adolescence can be defined as a transitional period sometime between postnatal days (PND) 28 and 60 (Smith 2003; Spear 2000a; Spear and Brake 1983) and shares several key characteristics with primate adolescence (Crews et al. 2007; Laviola et al. 1999; Smith 2003; Spear 2000a). As a gold standard measure of drug abuse liability (Meisch 1982; Schuster and Thompson 1969), drug self-

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administration has been used to study adolescent vulnerability to drug reward and reinforcement. Intravenous (i.v.) drug self-administration by adolescent rodents varies across drugs and subject populations (Belluzzi et al. 2005; Doherty et al. 2009; Frantz et al. 2007; Kantak et al. 2007; Levin et al. 2003, 2007; Shahbazi et al. 2008; Shram et al. 2007) and thus requires further investigation. The first aim of the present study was to replicate and extend our previous results that no age differences exist in cocaine self-administration. We allowed periadolescent (PND 35 at start) and adult (PND 83–95 at start) male Wistar rats to acquire lever pressing maintained by i.v. infusions of cocaine. We also compared outcomes using two different drug-dosing methods, changing either volume or concentration of the i.v. drug infusions to account for daily changes in body weight.

Long-lasting vulnerability to relapse to drug seeking or drug taking during periods of abstinence is one of the major challenges for treatment of drug addiction (Chung and Maisto 2006; Hunt et al. 1971; Mezinis et al. 2001; Sayette et al. 2000), yet relapse or reinstatement of drug seeking after adolescent drug use remains almost entirely unexplored in animal models. To the best of our knowledge, only one study explicitly analyzed extinction and reinstatement in animals that acquired drug self-administration as adolescents (Shram et al. 2007); nicotine-induced reinstatement of nicotine seeking did not differ across age groups, but Wistar rats that took nicotine as adolescents extinguished their drug seeking faster than those that took the drug as adults. The second aim of the present study was to compare patterns of extinction and cue-induced or drug priming-induced reinstatement of cocaine seeking after a 30-day abstinence period (Grimm et al. 2001; Shaham et al. 2003) in rats that self-administered cocaine during adolescence (adolescent-onset groups) vs. adulthood (adult-onset groups).

Finally, incubation of drug craving refers to time-dependent increases in cue-induced reinstatement of drug seeking after abstinence from drug self-administration in rodents (Grimm et al. 2001; Lu et al. 2004b). Although incubation may contribute to the persistent vulnerability to relapse observed in human drug addicts (Chung and Maisto 2006), it has not been examined after adolescent onset of drug self-administration in rats. Therefore, the third aim of the present study was to compare time-dependent changes in cue-induced reinstatement after 1, 14, 30, or 60 days of abstinence from cocaine self-administration during adolescence vs. adulthood.

Based on previous data, we predicted that neither age nor titration method would affect cocaine self-administration (Crombag et al. 2008; Frantz et al. 2007). However, based on reports of heightened vulnerability to drug dependence after adolescent onset of drug use among humans, we predicted that rats that acquired cocaine self-administration

as periadolescents would show higher levels of reinstatement and more robust time-dependent increases in cue-induced reinstatement of cocaine seeking (incubation of cocaine craving) compared with rats that acquired self-administration as adults.

Materials and methods

Subjects

Male Wistar rats (Charles River Laboratories, Inc., Wilmington, MA, USA) arrived in the laboratory at either PND 22 or 70–82 and were housed in groups of two to three in a humidity-(50%) and temperature-controlled (22°C) vivarium on a 12/12-h light/dark cycle (reverse cycle, with lights on at 1900 hours). Rats acclimated to these conditions for 6–8 days prior to the start of experiments. Food and water were available ad libitum except during self-administration sessions. All subjects were observed and/or weighed daily to assess general health and responsiveness to drug exposure. All procedures were conducted in strict adherence to the *Principles of Laboratory Animal Care* and the *National Institute of Health Guide for the Care and Use of Laboratory Animals* (NRC 2003).

Drugs

Cocaine hydrogen chloride was obtained from Mallinckrodt Inc. (Hazelwood, MO, USA). For rats receiving variable concentrations to account for differences in body weight, the concentration of cocaine stock solution in sterile saline was 2.5 mg/ml and was diluted for individual subjects. For the variable volume group, the concentration of stock solution was 1.25 mg/ml (see below for details on dosing methods). Methohexital sodium was obtained from Eli Lilly (Indianapolis, IN, USA).

Surgery

The i.v. catheters for drug self-administration were made as previously described (Caine et al. 1993), with minor modifications including a shorter length of tubing inserted into the jugular vein for adolescents (2 cm) compared with adults (4 cm) (Shahbazi et al. 2008). Rats were anesthetized with an isoflurane/oxygen vapor mixture (4–5% for initial anesthetization and 1.5–3% during surgery), and catheter tubing was passed subcutaneously from the animal's back to the right jugular vein, inserted into the vein previously punctured with a 25-gauge needle, and tied gently with suture thread. During recovery, rats received approximately 0.2 ml timentin (ticarcillin disodium and clavulanate potassium; 100 mg/ml, i.v.) twice daily on the first 2 days post-surgery, then once daily throughout the experiment.

Catheters were also flushed daily with approximately 0.4 ml heparinized saline (100 USP units/1 ml). Catheter patency was confirmed in all subjects by full loss of muscle tone within 5 s of i.v. infusion of the short-acting anesthetic agent, 1% methohexital sodium, 1 day before the first and after the last self-administration session. Subjects that failed either patency test were eliminated from the study.

Equipment

Behavioral tests were conducted in operant conditioning chambers enclosed in sound-attenuating, ventilated environmental cubicles (Med Associates, Inc., St. Albans, VT, USA). To start each session, a house light and white noise were turned on, and two levers were extended into the chamber. Lever presses on the inactive lever were recorded but had no scheduled consequences. Presses on the active lever triggered a syringe pump (Med Associates, Inc., St. Albans, VT, USA) to deliver drug solution via a stainless steel swivel (Instech Laboratories, Inc., Plymouth Meeting, PA, USA) and polyethylene tubing attached to a catheter portal on each animal's back. Each reinforced response lit a cue light above the lever which stayed on for the duration of the infusion. The cue light, house light, and white noise were not present during a 20-s time-out (TO20) after each infusion. Drug delivery and data collection were controlled by Med Associates software (Med PC IV).

Self-administration

Following a 6–7-day post-surgical recovery, spontaneous acquisition of cocaine self-administration began (PND 35 or 83–95), with daily 2-h sessions over 14 days conducted during the dark phase of the light–dark cycle. Non-contingent drug injections were not administered in this phase of experimentation. Lever pressing on the active lever was reinforced by i.v. infusion of cocaine (0.36 mg/kg/infusion) under a fixed ratio 1 TO20 schedule of reinforcement.

Infusion conditions

Two different methods to account for differences in body weight across age groups were compared in order to verify that a switch from a previous method of adjusting concentration (Frantz et al. 2007) to a preferred current method of adjusting infusion volume would not alter behavioral outcomes. Thus, we tested the influence of varying concentration vs. varying volume of drug infusions on self-administration. Adolescent and adult subjects were assigned to either variable concentration or variable volume conditions. For variable concentration groups, the concentration of cocaine solution for each rat was titrated according to body weight, but the volume was fixed such

that all subjects received 0.1 ml cocaine solution over 4 s per infusion (approximately 0.12 ml/kg/s for adolescent and 0.06 ml/kg/s for adults). For the variable volume groups, the volume of cocaine solution for each rat was titrated according to body weight (0.07 ml/kg/s), but the concentration was fixed such that all subjects received the same concentration of cocaine solution. The volume and duration were based on a standard 0.1 ml/4 s per infusion for a 350-g rat.

Abstinence period

After 14 days of self-administration, separate groups of rats remained in their home cages for 20–24 h, 14–15, 30–31, or 60–61 days, under normal housing conditions. Rats were handled twice per week during this abstinence period. Cue-induced reinstatement was tested at each abstinence period, but drug-induced reinstatement was tested only after 30–31 days of abstinence in different experimental groups.

Extinction and reinstatement

After the abstinence from cocaine self-administration, a within-session extinction and reinstatement test was conducted (Grimm et al. 2001, 2003). Six 1-h extinction sessions were followed by a 1-h cue- or drug-induced reinstatement test. During extinction, rats were connected to the metal coil tether but not the infusion tubing; white noise remained off, and the house light remained on. Neither cue lights nor TO signals were presented after presses on either lever. Five-minute breaks occurred between each successive session during which the two levers retracted and the house light turned off.

For cue-induced reinstatement tested after various abstinence periods, rats in the adolescent-onset groups were PND 49–50, 63–64, 79–80, or 109–110, whereas adult-onset groups were PND 97–110, 111–124, 127–140, or 157–171. Cue-induced reinstatement tests began with the onset of the house light, white noise, and a 5-s cue light, followed by a 20-s TO during which the house light, white noise, and cue light were turned off. During the remainder of cue-induced reinstatement sessions, presses on the active lever produced cue sequences identical to those presented during cocaine self-administration, and the pump went on although no syringe was loaded. The only difference between self-administration and cue-induced reinstatement sessions was that drug solution was not infused during reinstatement.

For drug priming-induced reinstatement tested 30–31 days after drug self-administration, rats in the adolescent-onset group were PND 79–80, whereas the adult-onset group was PND 127–140. Rats were taken out of the chambers after the last extinction session, injected with 10 mg/kg cocaine (intraperitoneal (i.p.)), and placed back into the chamber immediately. This dose was chosen for two reasons. First,

10 mg/kg cocaine (i.p.) is a commonly used dose for cocaine priming-induced reinstatement (Schenk and Partridge 1999; Soria et al. 2008). Second, we tested 10 mg/kg followed by either 3 or 30 mg/kg cocaine priming-induced reinstatement on successive days in the same animals, and only 10 mg/kg cocaine (i.p.) induced reliable reinstatement. While 3 mg/kg did not induce reinstatement in either age group, 30 mg/kg caused stereotyped behaviors in subjects from each age group (These preliminary data are confounded by the previous day's test and are not shown.). Parameters for drug priming-induced reinstatement sessions were identical to extinction sessions. No control injections of saline were administered before the drug priming-induced reinstatement test.

Data analysis

For drug self-administration sessions, the number of drug infusions per session was analyzed using a three-way mixed-measures analysis of variance (ANOVA) with age and infusion condition (variable concentration vs. variable volume) as between-subjects factors and day as a within-subjects factor. Total drug intake (mg/kg) summed over the entire 14 days of cocaine self-administration was also compared using a two-way ANOVA with age and infusion condition as between-subjects factors. The number of lever presses per session was also analyzed using a three-way ANOVA with age as a between-subjects factor and day and lever (active vs. inactive) as within-subjects factors (Data were collapsed across infusion condition for the active vs. inactive levers analysis because no differences were observed on other measures; see "Results").

For extinction sessions, the number of active or inactive lever presses per session was analyzed using a two-way mixed-measures ANOVA with age as a between-subjects factor and session as a within-subjects factor. To test for cue- or drug-induced reinstatement of lever pressing, the number of active lever presses in the last extinction session was compared directly with active lever presses during the reinstatement session using a two-way age \times session ANOVA with age as a between-subjects factor and session (extinction vs. reinstatement) as a within-subjects factor. To test our hypothesis regarding age differences in reinstatement directly, the number of active lever presses was also compared across age groups using unpaired *t* tests.

For analysis of time-dependent changes in cocaine seeking, total extinction responses on the active lever summed over all six 1-h extinction sessions were analyzed using a two-way between-subjects age \times abstinence period ANOVA. Time-dependent changes in the number of active lever presses per reinstatement session were also analyzed using a two-way age \times abstinence period ANOVA. Per the analytical methods of Grimm et al. (2001, 2003), one-way

ANOVAs were also conducted on each age group separately to test specifically for time-dependent increases in reinstatement. Unpaired two-sided *t* tests with Bonferroni correction were used for post hoc comparisons as appropriate. Results were considered significant if $p < 0.05$.

Results

Influence of age and infusion condition on cocaine self-administration

All age and titration groups showed similar patterns of reliable cocaine self-administration, which increased gradually in the first week and stabilized during the second week of testing ($n = 7\text{--}9/\text{group}$). A three-way age \times infusion condition \times days ANOVA revealed that only the main effect of days was significant ($F_{(13, 364)} = 11.12$, $p < 0.001$) but no other main effects nor interactions. Total drug intake (mg/kg) over 14 days of self-administration did not differ across age or infusion condition either (adolescent variable concentration group, 612 ± 50 mg/kg; adult variable concentration group, 704 ± 67 mg/kg; adolescent variable volume group, 666 ± 72 mg/kg; and adult variable volume group, 613 ± 33 mg/kg). Additional groups were added for subsequent extinction and reinstatement tests ($n = 9\text{--}16/\text{group}$), and all showed similar initial patterns of cocaine self-administration. Thus, we collapsed data across infusion conditions and subsequent abstinence period groups to show similar lever presses and total intake in all adolescent vs. adult rats (Fig. 1a). Moreover, despite relatively high rates of non-reinforced responding on the active lever, rats clearly discriminated between active and inactive levers (Fig. 1b) suggesting reliable self-administration. However, no age difference was observed in either total active lever presses or inactive lever presses. A three-way age \times days \times lever ANOVA revealed the main effects of day ($F_{(13, 1638)} = 3.24$, $p < 0.001$), lever ($F_{(1, 126)} = 271.07$, $p < 0.001$), and day \times lever interaction ($F_{(13, 1638)} = 4.22$, $p < 0.001$) but no other main effects nor interactions.

Extinction and reinstatement after a 30-day abstinence period

No age difference was observed in cocaine self-administration (Fig. 1a) or extinction sessions (see below). However, after a 30-day abstinence period, rats in the adolescent-onset group showed lower levels of cue-induced reinstatement than the adult-onset group (Fig. 2, $n = 11$ or $12/\text{group}$), in contrast to similar levels of drug-induced reinstatement (Fig. 3, $n = 6$ or $10/\text{group}$).

During extinction tests before cue-induced reinstatement, both age groups exhibited more active lever pressing than

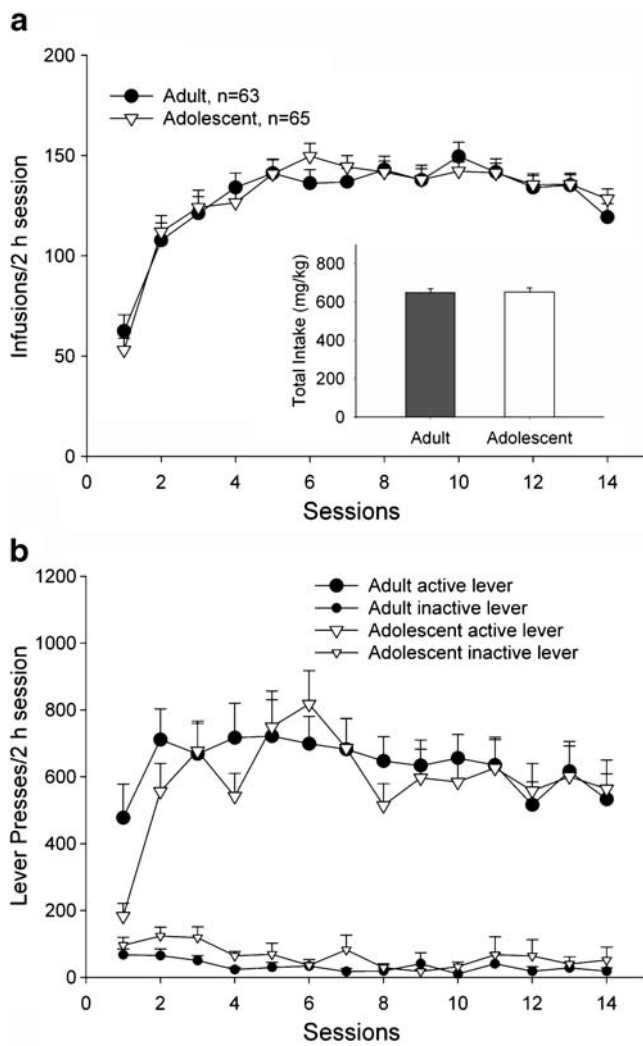


Fig. 1 Fourteen consecutive daily 2-h cocaine self-administration sessions in adolescent vs. adult male rats. **a** Number of infusions during 14 daily self-administration sessions. Points represent mean \pm SEM ($n=63$ or 65 /group). Inset shows total cocaine intake summed across the entire 14 days of self-administration. Bars represent mean \pm SEM. **b** Number of active vs. inactive lever presses during self-administration. Points represent mean \pm SEM ($n=63$ or 65 /group)

inactive lever pressing in the first 1-h session (Fig. 2). Lever pressing gradually declined in both age groups by the sixth 1-h extinction session. A two-way age \times session ANOVA on extinction responding revealed a significant main effect of session ($F_{(5, 105)}=21.704, p<0.001$), but neither the main effect of age nor the age \times session interaction was significant. During the cue-induced reinstatement test, re-exposure to drug-associated cues triggered lever-pressing behavior in both age groups. A two-way age \times session ANOVA on active lever presses in the last extinction session vs. the reinstatement session revealed the main effects of session ($F_{(1, 21)}=23.257, p<0.001$) and age ($F_{(1, 21)}=50.840, p=0.03$) but no interaction. Moreover, the adolescent-onset group exhibited less lever pressing than adults during the

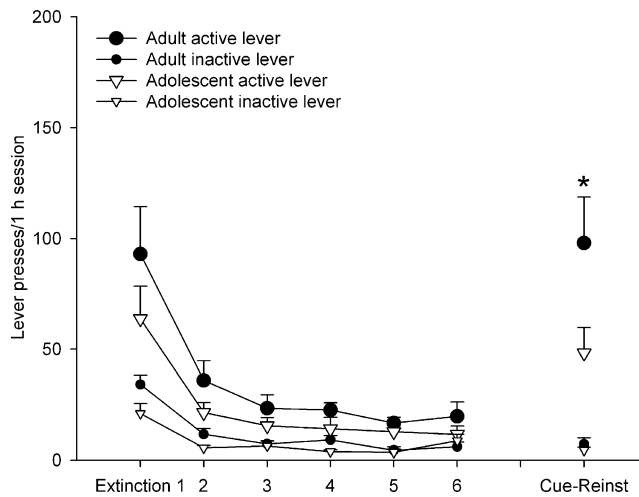


Fig. 2 Lever pressing in extinction and cue-induced reinstatement after 30 days of abstinence in adolescent- vs. adult-onset age groups. Points represent mean \pm SEM lever presses on the drug-associated (active) lever or the inactive lever ($n=11$ or 12 /group). Adolescents showed a lower level of cue-induced reinstatement than adults (asterisk indicates $p<0.05$ on targeted t test)

single cue-induced reinstatement session, confirmed by a targeted t test ($t_{(21)}=2.139, p=0.044$). In contrast, inactive lever presses did not differ between the last extinction session and reinstatement.

During extinction tests before drug-induced reinstatement, subjects exhibited similar rates of lever pressing and extinction (Fig. 3). Subsequent i.p. injections of cocaine triggered similar levels of reinstatement in both age groups. A two-way age \times session ANOVA comparing active lever presses in the last extinction session with the reinstatement session revealed only a main effect of session ($F_{(1, 14)}=6.164, p=0.026$) but no main effect of age nor a significant

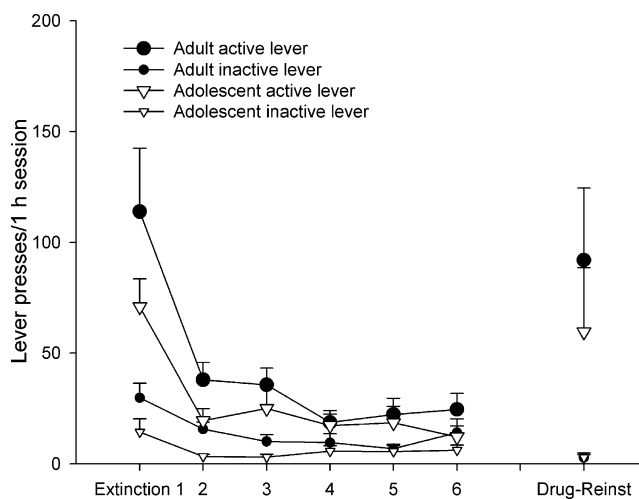


Fig. 3 Lever pressing in extinction and drug-induced reinstatement after 30 days of abstinence in adolescent- vs. adult-onset age groups. Points represent mean \pm SEM ($n=6$ or 10 /group). No age differences were observed

age \times session interaction. Inactive lever presses did not differ across age or session.

Time-dependent increases in cue-induced reinstatement

During extinction tests at approximately 1-, 14-, 30-, or 60-day abstinence, adolescent-onset groups showed an overall lower level of responding compared with adult-onset groups (Fig. 4; $n=9-16/\text{group}$). A two-way ANOVA on the total number of active lever presses summed over all six 1-h extinction sessions revealed a main effect of age ($F_{(1,86)}=10.30$, $p=0.002$) and abstinence period ($F_{(3,86)}=5.77$, $p=0.001$) but no age \times abstinence period interaction.

With regard to cue-induced reinstatement after 1-day abstinence, both age groups showed similarly low levels of cue-induced lever pressing (Fig. 5). Subsequently, the adult-onset groups demonstrated more robust increases in drug seeking from 1 to 60 days of abstinence compared with the adolescent-onset groups. A two-way ANOVA on active lever presses showed main effects of age ($F_{(1,86)}=17.29$, $p<0.001$), abstinence period ($F_{(3,86)}=13.82$, $p<0.001$), and age \times abstinence period interaction ($F_{(3,86)}=2.87$, $p=0.041$). Post hoc t tests showed that adolescent-onset groups responded at lower levels than adults after both 30 ($F_{(1,21)}=4.57$, $p=0.044$) and 60 days ($F_{(1,26)}=15.61$, $p=0.001$) of abstinence. Separate one-way ANOVAs on each age group revealed a significant effect of abstinence period among adults ($F_{(3,40)}=10.802$, $p<0.001$) and adolescent-onset groups ($F_{(3,46)}=3.195$, $p=0.032$). Post hoc tests on the adult-onset groups confirmed that reinstatement was higher at 60 days than at 1 or 14 days of abstinence (1 vs. 60, $p<0.001$; 14 vs. 60, $p=0.008$). However, post hoc tests on

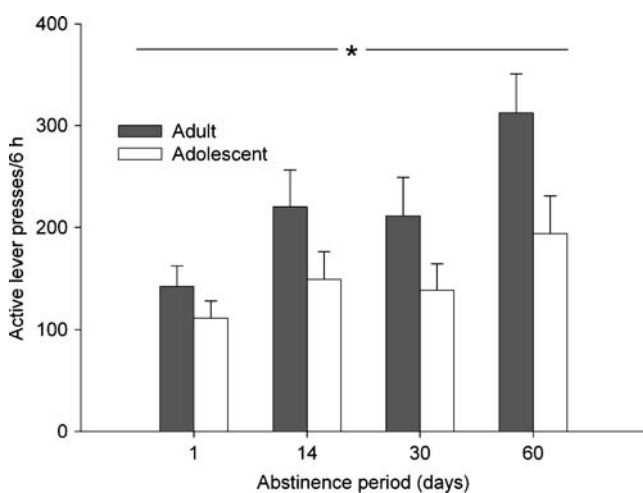


Fig. 4 Total extinction responses summed over six 1-h sessions conducted after 1, 14, 30, or 60 days of abstinence from cocaine. Bars represent mean \pm SEM ($n=9-16/\text{group}$). Adolescent-onset groups showed lower levels of extinction responding compared with adults (asterisk indicates overall main effect of age, $p<0.05$)

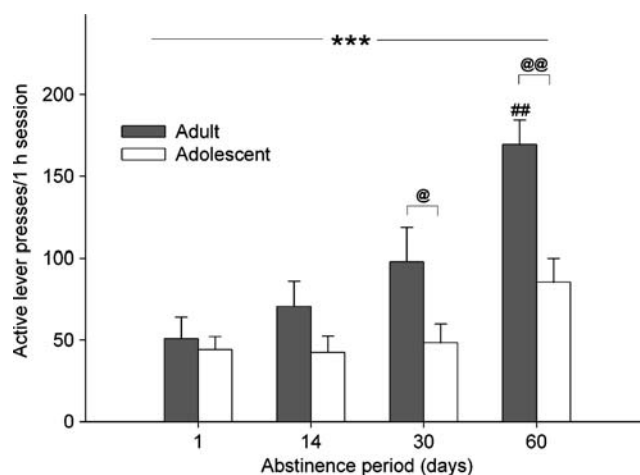


Fig. 5 Time-dependent increases in cue-induced reinstatement after adult but not periadolescent onset of cocaine self-administration. Bars represent mean \pm SEM ($n=9-16/\text{group}$). Age groups showed similar levels of cue-induced reinstatement at 1-day abstinence, but adolescent-onset groups showed overall lower levels of reinstatement (three asterisks indicates an overall main effect of age, $p<0.001$). Only adult groups showed time-dependent increases in reinstatement (two pound signs higher cue-induced reinstatement after 60- compared with 1- or 14-day abstinence). Adolescents showed less cue-induced reinstatement than adults after 30- and 60-day abstinence periods (one commercial at symbol $p<0.05$, two commercial at symbols $p<0.01$ on targeted t tests)

the adolescent-onset groups did not reveal specific differences suggesting a weak overall effect.

Discussion

Results from the present experiment are consistent with previous studies from our group and others demonstrating that periadolescent and adult male rats acquire cocaine self-administration similarly (Frantz et al. 2007; Kantak et al. 2007). On the other hand, striking age differences emerged in the test of time-dependent increases in cocaine seeking during drug abstinence, such that no age differences were observed in reinstatement after 1-day abstinence, but cue-induced reinstatement remained low in adolescent-onset rats while increasing significantly in adult-onset rats through a 60-day abstinence period. Drug priming-induced reinstatement after a 30-day abstinence period did not differ across ages, but only one cocaine dose was tested.

During 14 daily 2-h self-administration sessions, adolescent and adult rats took similar amounts of cocaine. These results confirm several reports and our own preliminary data that no age differences exist in cocaine self-administration behavior (Belluzzi et al. 2005; Frantz et al. 2007; Frantz and Parsons 2000; Kantak et al. 2007; Kerstetter and Kantak 2007). Moreover, small variations in the infusion parameters (variable volume vs. variable

concentration methods) had no effect on cocaine self-administration, as shown previously in adult rats (Crombag et al. 2008), despite the fact that rapid infusion enhances the reinforcing effects of drugs in a self-administration model with non-human primates (Kato et al. 1987; Panlilio et al. 1998). Overall, we conclude that cocaine has similar acute reinforcing effects in adolescent and adult male rats.

Despite similar rates of cocaine self-administration across age groups, extinction responding was lower in adolescent-onset rats than adult-onset rats during extinction tests after 1-, 14-, 30-, and 60-day abstinence periods. These age differences fail to support our hypothesis of adolescent vulnerability but do corroborate results from Wistar rats in another test of extinction and reinstatement after adolescent drug self-administration (Shram et al. 2007). Given that lower rates of extinction responding have been interpreted as lower motivation to obtain drugs or lower conditioned incentive motivational effects of a drug-associated environment (Bossert et al. 2004; Fuchs et al. 2005, 2008), these results lead to the unexpected conclusion that adolescent onset of drug self-administration does not heighten vulnerability to long-term motivation toward drug intake.

A major emphasis of the present study was comparing incubation of drug craving after cocaine self-administration during adolescence vs. adulthood, measured as time-dependent increases in cue-induced reinstatement of drug seeking after abstinence from drug self-administration. Similar to extinction responding, the present results are surprising in light of evidence supporting the hypothesis that adolescent onset of drug intake heightens vulnerability to drug addiction (Laviola et al. 1999; SAMHSA 2006). Whereas both age groups showed similarly low levels of cue-induced reinstatement of cocaine seeking 1 day after cocaine self-administration, adolescent rats showed only weak increases in cue-induced reinstatement of drug seeking from 1 to 60 days in abstinence, while adults demonstrated significant increases over the same time period. In other words, adolescent subjects failed to demonstrate the robust incubation of cocaine craving that has been observed repeatedly in adult subjects (Grimm et al. 2001; Lu et al. 2004a, b).

The present results could reflect resistance among adolescent subjects to some enduring effects of drug self-administration. As such, they are consistent with other recent reports. For example, stimulus-reward learning remains intact after cocaine self-administration during adolescence but is impaired after cocaine self-administration in adulthood (Kerstetter and Kantak 2007). Also, both somatic and affective signs of abstinence from nicotine appear less intense in adolescent compared with adult rats (O'Dell et al. 2004, 2007; Shram et al. 2008). Finally, after morphine self-administration by adolescent or adult rats, cue-induced

reinstatement is attenuated in the younger cohort (Doherty et al. 2009). On the other hand, adolescent resistance to long-term drug effects clearly contradicts prior reports and hypotheses on adolescent vulnerability to addiction (Laviola et al. 2003; Smith 2003; Spear 2000b). Particularly with regard to cocaine, adolescent vulnerability has included specific neurocognitive deficits such as impaired spatial memory, altered inhibitory avoidance, and attentional challenges, as well as premature death (Black et al. 2006; Santucci 2008; Santucci et al. 2004).

An alternative explanation for the present age differences in reinstatement is that subjects taking cocaine during adolescence experience a generalized reward devaluation later in adulthood, as observed after juvenile/pre-adolescent methylphenidate exposure (Andersen et al. 2002; Bolanos et al. 2003; Mague et al. 2005). However, not only is stimulus-reward learning intact after adolescent cocaine self-administration (Kerstetter and Kantak 2007), but also cocaine-induced devaluation of natural rewards is less affected by cocaine treatment in adolescence than adulthood (Schramm-Sapyta et al. 2006). Additionally, sucrose-stimulated dopamine efflux in the nucleus accumbens remains intact after cocaine treatment in adolescence but not in adulthood (Catlow and Kirstein 2007). Thus, evidence available at this time does not support a general diminution in reward-related processing after adolescent cocaine self-administration.

Drug-induced reinstatement after a 30-day abstinence period did not differ across age groups, consistent with the other test of drug-induced reinstatement after adolescent drug intake (Shram et al. 2007). Possibly, drug injection (but not cue presentation) is such a strong trigger of drug seeking that it fails to reveal differential sensitivity across development, as postulated previously with regard to the reinforcing effects of cocaine (Frantz et al. 2007). A potential limitation of the present study, however, is that only one highly effective cocaine dose was tested. A full dose-effect analysis will be required to explore drug priming-induced reinstatement thoroughly and reach justifiable conclusions on the topic.

If confirmed in future studies, age differences in cue-induced but not drug-induced reinstatement might suggest a developmental dissociation between neural pathways mediating cue- vs. drug-induced reinstatement. For example, the amygdala has been identified as a brain region critical for both cue-induced reinstatement and its time-dependent increase during drug abstinence but less involved in drug-induced reinstatement of drug seeking (Lu et al. 2005, 2007; Quirk and Gehlert 2003; See 2005, for review). Moreover, glutamatergic projections from the prefrontal cortex to the nucleus accumbens are involved in reinstatement to drug seeking (Conrad et al. 2008; Kalivas and O'Brien 2008; Kalivas et al. 2005; Koya et al. 2008), as are

local changes in the nucleus accumbens (Grimm et al. 2003; Hollander and Carelli 2005, 2007; Kalivas and O'Brien 2008). Thus, periadolescent anatomical and functional remodeling in such areas draws attention for future research (Alexander and Goldman 1978; Brenhouse et al. 2008; Teicher et al. 1991, 1998; Tseng and O'Donnell 2007).

In summary, our data suggest that adolescence might be a developmental stage associated with resistance to the long-term motivation to seek cocaine or to the salience of drug-associated cues. These results counter predictions based on human survey data and rodent models other than self-administration showing that early onset of drug intake increases chances of later addiction. Thus, our results may support the idea that social factors and other variables not modeled in most rodent drug self-administration studies influence drug use and abuse by human adolescents. As a note of caution, the validity of extinction, reinstatement, and incubation of drug-seeking behavior in rodents as models of human drug-related behavior has been questioned (Katz and Higgins 2003). Overall, if future studies from multiple lines of research converge on the conclusion that adolescence is a period of relative biological resistance to some long-term drug effects, then intervention programs targeting adolescent drug abusers may have high success rates.

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