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Original Article

ORIGINAL ARTICLE

Role of Dopamine 2 Receptor in Impaired Drug-Cue Extinction in Adolescent Rats

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Abstract

Adolescent drug users display resistance to treatment such as cue exposure therapy (CET), as well as increased liability to relapse. The basis of CET is extinction learning, which involves dopamine signaling in the medial prefrontal cortex (mPFC). This system undergoes dramatic alterations during adolescence. Therefore, we investigated extinction of a cocaine-associated cue in adolescent and adult rats. While cocaine self-administration and lever-alone extinction were not different between the two ages, we observed that cue extinction reduced cue-induced reinstatement in adult but not adolescent rats. Infusion of the selective dopamine 2 receptor (D2R)-like agonist quinpirole into the infralimbic cortex (IL) of the mPFC prior to cue extinction significantly reduced cue-induced reinstatement in adolescents. This effect was replicated by acute systemic treatment with the atypical antipsychotic aripiprazole (Abilify), a partial D2R-like agonist. These data suggest that adolescents may be more susceptible to relapse due to a deficit in cue extinction learning, and highlight the significance of D2R signaling in the IL for cue extinction during adolescence. These findings inspire new tactics for improving adolescent CET, with aripiprazole representing an exciting potential pharmacological adjunct for behavioral therapy.

Key words: adolescence, aripiprazole, dopamine, extinction, infralimbic cortex

Introduction

Drug addiction is a chronic, relapsing mental disorder characterized by loss of control over drug use, compulsive drug-seeking, and continued use despite serious adverse consequences (Camí and Farré 2003). It has been argued that mental disorders such as addiction should be defined as developmental disorders, due to the unique likelihood of onset during teenage and young adult years (Insel 2009). Indeed, adolescent drug users show higher resistance to therapeutic interventions and increased probability to relapse compared with adults, especially when faced with cues associated with the drug taking experience (Catalano et al. 1990; Perepletchikova et al. 2008; Ramo and Brown 2008; Winters et al. 2011).

Common behavioral treatments for addiction such as cue exposure therapy (CET) aim to reduce the craving elicited by drug-associated cues, based on the principle of extinction (Conklin and Tiffany 2002). Preclinical research using adult and adolescent rats suggests that the salience of drug-associated cues is strongly mediated across development by dopamine 1 receptor (D1R) activity on glutamatergic projections from the prelimbic cortex (PrL) of the medial prefrontal cortex (mPFC) to the core of the nucleus accumbens (NAc) (Kalivas and Duffy 1997; Brenhouse et al.

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2008). By comparison, studies using adult animals suggest that extinction learning is largely controlled by projections from the infralimbic cortex (IL) of the mPFC to the NAc shell, and may involve D1R and/or dopamine 2 receptor (D2R) signaling (Peters et al. 2008; Haaker et al. 2013; Mueller et al. 2013). Adolescence constitutes a period of dramatic maturation of the mPFC, including alterations in dopamine receptor density (Andersen et al. 2000), fiber infiltration (Kalsbeek et al. 1988), and dopamine availability (Wahlstrom et al. 2010). However, current understandings of adolescent extinction learning, particularly the significance of the mPFC, are relatively incomplete.

Based on clinical findings that adolescent drug users are more resistant to extinction-based therapies and more liable to cue-induced relapse, we hypothesized that adolescent vulnerability to addiction relates to a deficit in cue extinction. When re-exposed to environmental stimuli associated with the drug experience, this deficit would increase the likelihood of compulsive return to drug-seeking and drug taking for this population. To investigate this theory, we developed a preclinical paradigm that separates the critical components of adolescent drug abuse liability, namely: motivation to self-administer, amenability to therapeutic intervention (cue extinction), and propensity to relapse. Using this model, we also aimed to reduce relapse-like behavior by pharmacologically manipulating dopamine signaling in the IL at the time of cue extinction. Importantly, targeting cue extinction has stronger translational potential compared with targeting reinstatement, since relapse is difficult to pre-empt due to its unpredictability in the human scenario. We found that cue extinction was able to significantly reduce cue-induced reinstatement in adult rats but not in adolescents. We also observed that acutely enhancing D2R signaling in the adolescent IL by microinfusion of the D2R agonist quinpirole enhanced cue extinction learning to reduce subsequent cue-induced reinstatement the next day. A similar effect was observed following acute systemic treatment with the partial D2R-like agonist aripiprazole (Abilify). These results present aripiprazole as a promising adjunct to improve exposure-based therapy for adolescent drug users.

Materials and Methods

Animals and Surgery

Male Sprague-Dawley rats (N = 72; bred in-house) were individually housed under a 12:12 light/dark cycle (lights off 7 a.m.) with food and water available ad libitum. All testing was conducted during the dark phase. Rats were group-housed and handled 3 times prior to surgery. Rats were individually housed immediately following surgery for the duration of experimentation. Rats were aged postnatal day (P)34(±1) (adolescent) or P69(±1) (adult) at the commencement of self-administration. All procedures were performed in accordance with the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

For all experiments a catheter was implanted into the right jugular vein. Rats were anesthetized with isoflurane vaporized with oxygen and injected with meloxicam (3 mg/kg, ip). Catheters were constructed in-house as described previously (Kim et al. 2014) and consisted of guide cannulas (22 gauge, PlasticsOne, VA, USA) and three layers of Silastic tubing (adult length 14 cm; adolescent length 12 cm; Dow Corning, USA). Catheters were flushed daily for 2 days following surgery with 0.05 mL of heparinized saline (90 IU/mL; Pfizer, NY, USA) containing 10% Fisamox antibiotic (amoxicillin sodium; Aspen Australia, NSW, Australia). For the duration of experiments catheters were flushed daily with 0.05 mL of 10 and 50 IU/mL antibiotic-heparin solution before and after cocaine self-administration, respectively.

For the quinpirole experiment a double guide cannula (26 gauge, PlasticsOne) bilaterally targeting the IL (AP, +3.0 mm; ML ± 0.6 mm; DV −4.2 mm) was implanted stereotaxically (David Kopf Instruments, CA, USA) following jugular catheterization. The cannula was secured to the skull using dental cement (Vertex, MA, USA) combined with 4 anchoring screws (PlasticsOne). Obturators extending 1 mm below the guide cannula were checked and rats were weighed daily.

Adult Versus Adolescent Self-Administration, Extinction, and Reinstatement

Experimental design is depicted in Figure 1.

Cocaine Self-Administration

Rats were trained to self-administer cocaine (cocaine hydrochloride dissolved in saline; Johnson Matthey Macfarlan Smith, Edinburgh, UK) in standard operant conditioning chambers (29.5 × 32.5 × 23.5 cm; Med Associates, VT, USA) equipped with two retractable levers and a cue light above each lever. House lights remained off. Pressing on the active lever resulted in a 50 μL infusion of cocaine (0.3 mg/kg per infusion—concentration of cocaine dissolved in saline was customized for the weight of each rat updated every 3 days) over 2.7 s by activation of a pump (Med Associates). Infusions were paired with 2.7 s of illumination of the light located above the active lever, followed by a 17.3 s time-out period. A vanilla scent was present beneath the grid floor below the active lever to serve as a discriminatory cue for the active versus inactive lever. The vanilla scent was present whenever the active lever was presented, even during lever extinction and cue-induced reinstatement when active lever had no consequences, therefore, it was a mere discriminative cue for the location of the lever. Pressing on the inactive lever had no programmed consequences at any phase of experiment.

For all experiments, daily 2-h self-administration sessions were conducted. For the first 5 but no more than 7 days, rats received cocaine under a fixed ratio (FR) 1 requirement. For the final 5 days of self-administration, responding occurred under FR3. This was to ensure that lever pressing by rats was for cocaine, which would be indicated by an increase in lever pressing at FR3 reinforcement schedule. Patency was tested weekly using 0.03 mL of ketamine (100 mg/mL) for adult and 0.02 mL for adolescent rats immediately followed by 0.05 mL of 10 IU/mL antibiotic-heparin solution. Any rat that failed to show loss of muscle tone within 10 s was removed from the study. Any rat that failed to self-administer at least 7 infusions of cocaine/session averaged across the last 5 days of self-administration was removed from the study.

On the penultimate day of self-administration in the first experiment, approximately half the rats received a single progressive ratio (PR) session in which the number of active lever responses required to receive an infusion increased incrementally. Lever pressing during PR session indicates the animal's motivation to self-administer a drug by measuring how many lever presses an animal is willing to make for an infusion (Farid et al. 2012). The session ran for a maximum of 4 h, but terminated automatically if no response was made for 1 h. On the final day of self-administration rats went back onto FR3 for one 2-h session.

Lever Extinction

The day after the final self-administration session, rats received daily 1-h lever extinction session for 7 days, where pressing

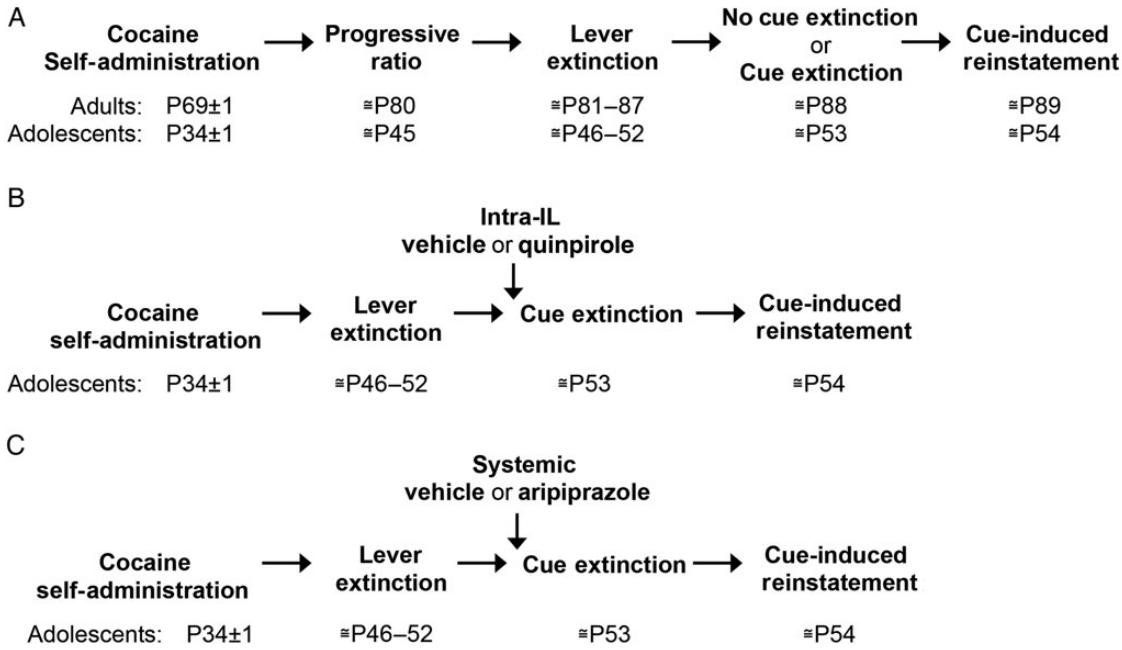

Figure 1. Experimental design. (*A*) Adult and adolescent rats underwent cue-paired cocaine self-administration. In the first experiment only, rats underwent a single PR session prior to the final day of self-administration. Rats then received lever extinction in absence of the cue. Rats were divided into groups for handling (No Cue Ext) or cue extinction (Cue Ext). Rats were tested the next day for cue-induced reinstatement. (*B*) Adolescent rats underwent cocaine self-administration and lever extinction as per the first experiment. Prior to cue extinction, rats received an infusion of vehicle or quinpirole (5 μg per hemisphere) into the infralimbic cortex (IL). Rats were tested the next day for cue-induced reinstatement. (*C*) Adolescent rats underwent cocaine self-administration and lever extinction as per the first two experiments. Prior to cue extinction, rats received a systemic injection of either vehicle or aripiprazole (5 mg/kg). Rats were tested the next day for cue-induced reinstatement.

either lever had no consequences. In other words, pressing on the previous active lever did not result in a cocaine infusion or a cue light illumination.

Cue Extinction

The day after the final lever extinction session, animals received a single cue extinction session without any levers present. This was to model CET in the clinic that typically does not involve re-enactment of drug taking actions but presentations of drug-associated cues. Following a 2-min baseline period, the 2.7 s cue light above the previously active lever was presented every 30 s 120 times. In the first experiment, rats were randomly assigned to one of two groups: a cue extinction group (Cue Ext) and a group that did not receive cue extinction but were handled for 2 min (No Cue Ext). In subsequent experiments all rats received cue extinction. Since there was no lever present for cue extinction, there was no vanilla scent present for this session.

Cue-induced Reinstatement

The day after cue extinction, rats were tested for cue-induced reinstatement. For 1 h, pressing the previously active lever resulted in illumination of the light above the lever under an FR3 schedule. If no lever response was made within the first 2 min, the cue light above the active lever illuminated automatically once. Reinstatement data greater than 5 standard deviations (SD) from the group mean were considered statistical outliers and were excluded from all analyses as previously reported (Kim et al. 2014). Overall, 2 rats from the quinpirole group in experiment 2, 1 rat from the vehicle group and 2 rats from the aripiprazole group in experiment 3 fell into this criterion and were excluded from the entire analyses. There were no rats that displayed reinstatement data >5 SD below their group mean.

Adolescent Intra-IL Quinpirole

A separate group of adolescent rats underwent cocaine self-administration followed by lever extinction as described for the first experiment. All rats then underwent cue extinction the following day. Prior to cue extinction, rats were treated using a bilateral intra-IL infusion. The infusion (0.5 μL per hemisphere) consisted of either vehicle (saline) or quinpirole (dissolved in saline; 2 μg/μL; Tocris, UK), and took place over 2 min. The infusion cannula extended 1 mm below the guide cannula, and remained in place for 2 min following the infusion. All rats then received cue extinction. All rats were tested for cue-induced reinstatement the next day. At the end of experimentation fresh frozen brains were sectioned and processed with cresyl violet to visualize cannula placement which are depicted in Fig. 4A.

Adolescent Systemic Aripiprazole

In the same design as the previous experiments, a separate group of adolescent rats underwent cue-paired cocaine self-administration, followed by lever extinction. All rats then underwent cue extinction the following day. Rats were treated with a systemic injection 30 min before the cue extinction session. The subcutaneous injection consisted of either vehicle (5% v/v Tween 80 in saline; Sigma-Aldrich Co., MO, USA) or aripiprazole (Alliance Biotech, India; 5 mg/kg; dose based on Feltenstein et al. 2007) suspended in vehicle. All rats were tested for cue-induced reinstatement the next day.

Data Analysis

Active lever presses with cocaine infusions and during the time-out period were summed into “active lever responses”. Self-administration, lever extinction, and reinstatement data

were analyzed using mixed-design repeated-measures analysis of variance (ANOVA). Significant interactions were followed up with further ANOVAs or *t*-tests as appropriate. Lever discrimination and PR data were analyzed using independent *t*-tests. Statistical tests were conducted using SPSS (IBM Corp., New York, USA), with acceptance for significance at $P \leq 0.05$.

Results

No Age Differences in Cocaine Consumption, Motivation to Self-Administer, or Lever Extinction

There was no difference between adult and adolescent rats in cocaine self-administration (Fig. 2). Analyses of active lever response data revealed a significant main effect of self-administration Day [$F_{9,\ 360} = 23.3$, $P < 0.05$], but no effect of Age, and no interaction between Day and Age (Fs < 1). Consistent with this, analyses of reward data revealed a significant main effect of Day [$F_{9,\ 360} = 8.8$, $P < 0.05$], with no effect of Age and no interaction (Ps > 0.05). Inactive lever response data showed no effect of Day or Age, and no interaction (Ps > 0.05). There also was no effect of Age on total active responses made over the PR session [$t_{(38)} = 1.1$, $P = 0.3$], or on PR breakpoint ($t < 1$).

Lever extinction was also similar across age groups (Fig. 2*D*). Analyses of active lever responses revealed a significant main effect of lever extinction Day [$F_{6,\ 240} = 7.0$, $P < 0.05$] but no effect of Age and no interaction (Fs < 1). This suggests that both adults and adolescent animals learned to inhibit drug-seeking over days, that is, lever extinction occurred. The same analyses of inactive lever response data revealed no effect of Day, Age, and no interaction (Ps > 0.05).

Age Differences in Cue-Induced Reinstatement

To analyze cue reinstatement we performed a 4-way ANOVA comparing active versus inactive lever pressing (Lever Type) on the last day of extinction versus cue reinstatement (Day), in different ages (Age) and cue extinction conditions (Cue Extinction) (Fig. 3A). This revealed significant main effects of Lever Type, Day, and Age (Ps < 0.05). There were also significant interactions between those factors and Cue Extinction (Ps < 0.05) hence analyses were split for each lever type for different age groups.

For adults, analyses of active lever revealed a main effect of Day [$F_{1,\ 22} = 12.8$, $P < 0.05$], and an interaction between Day and Cue Extinction [$F_{1,\ 22} = 4.8$, $P < 0.05$], but no main effect of Cue Extinction [$F_{1,\ 22} = 3.6$, $P = 0.07$]. Post hoc paired-sample *t*-tests of active lever responses comparing final lever extinction day versus cue-induced reinstatement revealed a significant difference between days for No Cue Ext adults [$t_{(12)} = 3.8$, $P < 0.05$], however, no difference for Cue Ext adults [$t_{(10)} = 1.1$, $P = 0.3$] (Fig. 3*B*). By comparison, analyses of adolescent active lever data revealed a

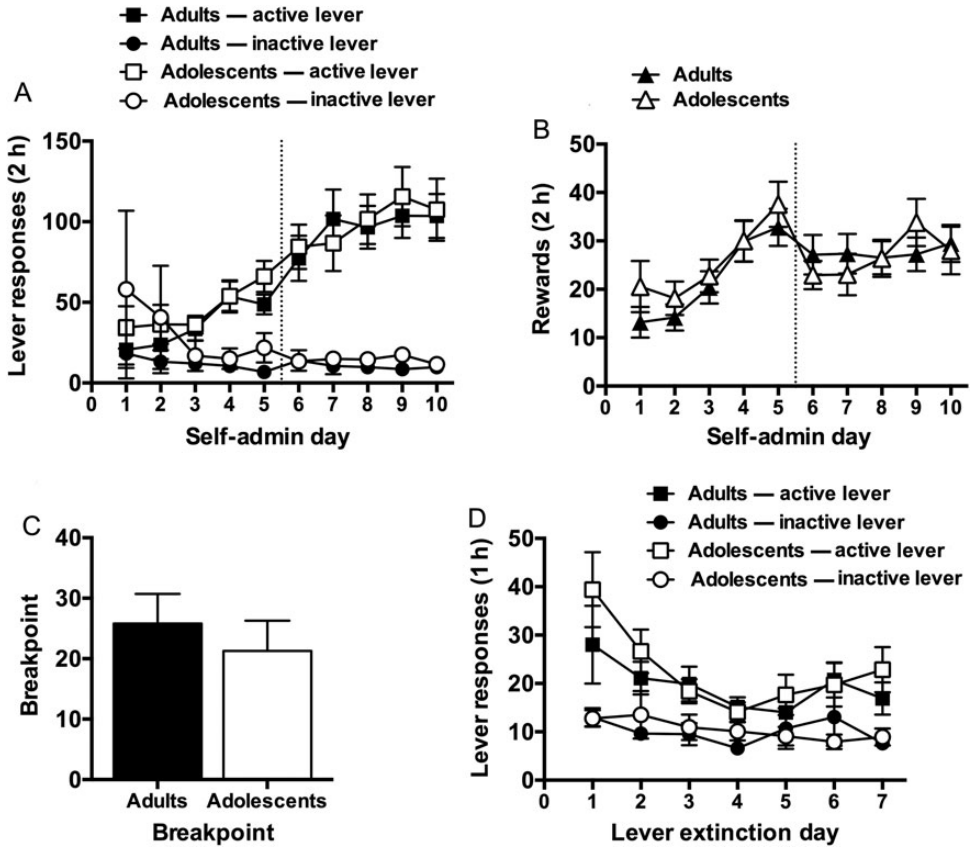

Figure 2. Cocaine self-administration was similar for adult and adolescent rats. Responding occurred on a FR 1 for the first 5 days, and increased to FR3 for the final 5 days of self-administration (broken line). (*A*) Mean (±SEM) daily lever responses. Responding on the active lever increased for both age groups over self-administration days ($P < 0.05$), while responding on the inactive lever remained low. (*B*) Mean (±SEM) daily rewards, that is, cocaine infusions (0.3 mg/kg per infusion) increased for both age groups over self-administration days ($P < 0.05$). (*C*) Once stable cocaine self-administration was established responding on a PR of reinforcement was similar across age groups, with adult and adolescent rats showing a similar number of maximum consecutive active lever presses to obtain a cocaine infusion (breakpoint). (*D*) Mean (±SEM) active lever responses decreased over lever extinction days ($P < 0.05$), with no difference between age groups by final lever extinction day. Inactive lever responding remained low relative to active lever responding across days. Adult $n = 24$; adolescent $n = 18$.

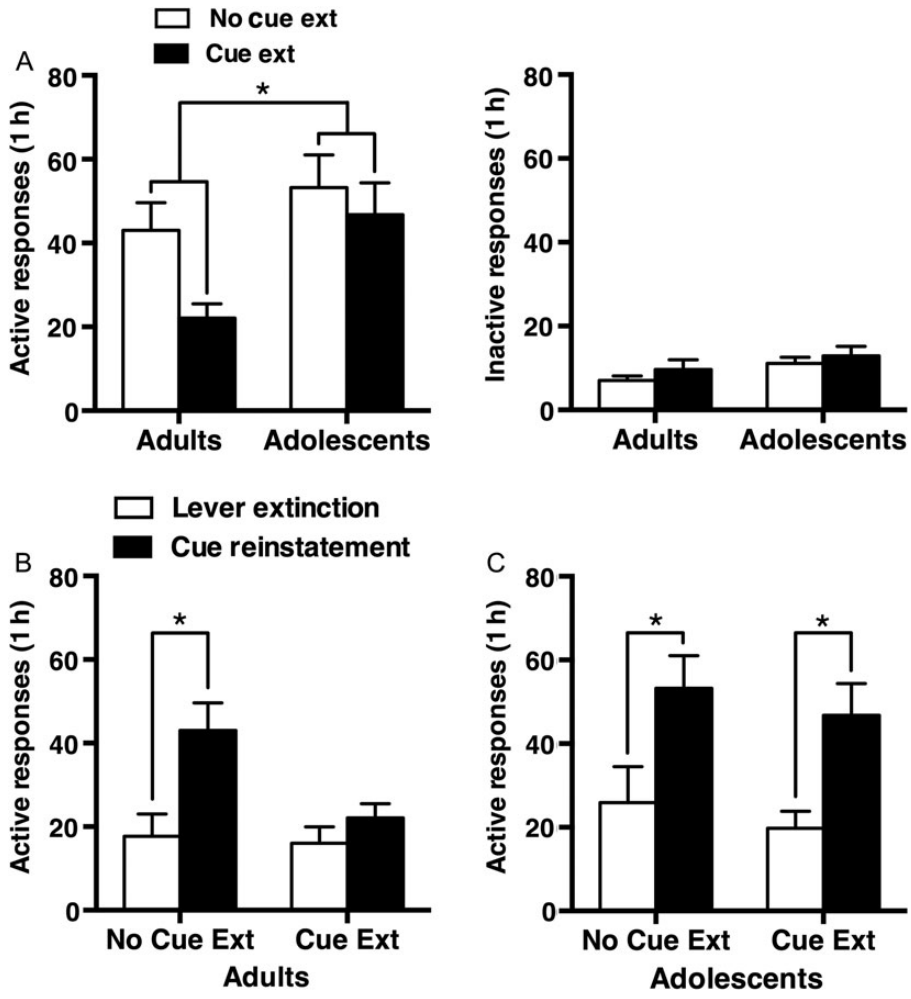

Figure 3. Age differences in cue-induced reinstatement following cue extinction. (*A*) Mean (+SEM) active lever responses made over one hour cue-induced reinstatement differed depending on age (*$P < 0.05$, main effect of Age, significant interaction of Age and Cue Extinction). There was no effect of day or cue extinction on inactive lever responding for both adults and adolescents. (*B*) Compared with the final day of lever extinction, adults that did not receive cue extinction (No Cue Ext) significantly reinstated cocaine seeking behavior following re-exposure to a drug-associated cue the next day (*$P < 0.05$, effect of Day in the No Cue Ext group) while adults that received cue extinction training did not. Adult No Cue Ext $n = 13$; adult Cue Ext $n = 11$. (*C*) Adolescents reinstated to the cue regardless of whether cue extinction training was received or not (*$P < 0.05$, main effect of Day). Adolescent No Cue Ext $n = 9$; adolescent Cue Ext $n = 9$.

significant main effect of Day [$F_{1,16} = 16.0$, $P < 0.05$], but no effect of Cue Extinction or an interaction ($Fs < 1$) (Fig. 3*C*). Analyses of inactive lever data found no effect of Day, Cue Extinction, and no interaction for either adults or adolescents ($Ps > 0.05$). These results indicate that cue extinction effectively reduced cue-induced reinstatement in adults but not in adolescents, and that this effect was not due to a generalized decrease in lever pressing activity.

Intra-IL Quinpirole or Systemic Aripiprazole at Cue Extinction Reduces Cue-Induced Reinstatement in Adolescent Rats

A separate group of adolescent rats underwent cocaine self-administration and lever extinction, and received an intra-IL infusion of the D2R-like agonist quinpirole (1 μg per hemisphere) or vehicle immediately prior to cue extinction. Rats were tested for cue-induced reinstatement the next day. Brains were processed for cannula placement verification following reinstatement and data from any rat with a cannula outside the IL were excluded from all analyses (Fig. 4*B*).

Treatment groups were comparable prior to intracranial infusion as indicated by analyses that showed no effect of Treatment (vehicle versus quinpirole) on any measure of cocaine self-administration or lever extinction ($Ps > 0.05$). Analyses of active lever on final lever extinction versus cue-induced reinstatement showed significant main effects of Day [$F_{1,\ 12} = 12.0$, $P < 0.05$], Treatment [$F_{1,\ 12} = 9.6$, $P < 0.05$], and a significant interaction [$F_{1,\ 12} = 6.1$, $P < 0.05$]. Post hoc *t*-tests revealed a significant increase in active lever responding at cue reinstatement compared with lever extinction for vehicle-treated rats [$t_{(7)} = 3.5$, $P < 0.05$], indicating that cue extinction was ineffective in this group. By comparison, there was no such difference across days for quinpirole-treated rats [$t_{(5)} = 2.4$, $P = 0.06$] (Fig. 4B). Analyses of inactive lever responses showed no effects ($Ps > 0.05$) (Figure). Together these results show that enhancing D2R activity in the IL improved cue extinction learning and thereby significantly reduced cue-induced reinstatement the next day.

We then aimed to replicate our quinpirole results using a pharmacological adjunct to cue extinction with strong translational potential. We chose aripiprazole, a widely used atypical antipsychotic with D2R partial agonist activity (Hirose and

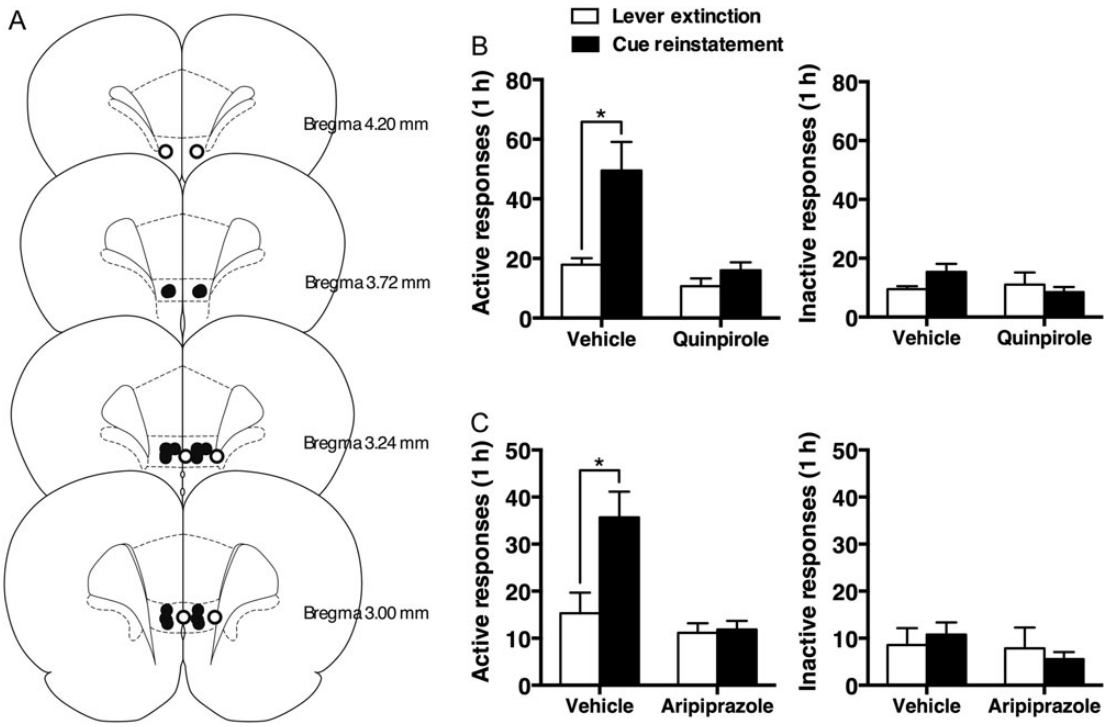

Figure 4. Enhancing D2R signaling at the time of cue extinction reduces cue-induced reinstatement in adolescent rats the next day. (*A*) Coronal sections illustrating intracranial cannula placements show that 14 rats had successful cannula tips within the infralimbic cortex (hits; filled circles) (misses; empty circles) (Paxinos and Watson 2013). (*B*) Analyses of mean active lever responses (+SEM, left panel) indicate that adolescent rats that received vehicle at the time of cue extinction showed reinstatement the next day (*$P < 0.05$), while rats that received quinpirole (5 μg per hemisphere) did not. Mean responses on the inactive lever (+SEM, right panel) remained low for both vehicle- and quinpirole-treated rats across final lever extinction and reinstatement test. Vehicle $n = 8$; quinpirole $n = 6$. (*C*) Adolescent rats that received vehicle at the time of cue extinction displayed cue reinstatement the next day (*$P < 0.05$), whereas rats that received aripiprazole (5 mg/kg) did not. Responding on the inactive lever remained low for both vehicle- and aripiprazole-treated rats over final lever extinction and reinstatement days. Vehicle $n = 9$; aripiprazole $n = 7$.

Kikuchi 2005). A separate group of adolescent rats underwent cocaine self-administration and lever extinction, and then received a systemic injection of aripiprazole (5 mg/kg) or vehicle 30 min prior to cue extinction. Cue-induced reinstatement was tested the next day.

Groups were comparable prior to treatment as indicated by analyses that showed no effect of Treatment (vehicle versus aripiprazole) on any measure of cocaine self-administration or lever extinction (*P*s > 0.05). Analyses of active lever responses made on the final lever extinction day versus cue reinstatement showed a significant main effect of Day [$F_{1,\ 14} = 10.5$, $P < 0.05$] a significant main effect of Treatment [$F_{1,\ 14} = 8.1$, $P < 0.05$], and a significant interaction [$F_{1,\ 14} = 9.1$, $P < 0.05$]. Post hoc paired-samples *t*-tests revealed a significant difference in active lever responses made on the final lever extinction day compared with cue reinstatement in vehicle-treated adolescents [$t_{(8)} = 3.7$, $P < 0.05$]. By comparison, there was no such difference across days in aripiprazole-treated rats ($t < 1$). Analyses of inactive lever responses made on final operant extinction day versus cue reinstatement revealed no effects (*P*s > 0.05) (Fig. 4C). These data indicate that acute aripiprazole at the time of cue extinction significantly reduced cue-induced reinstatement the next day, without affecting general lever responding.

Discussion

Understanding adolescent drug-cue extinction is critical to developing more effective treatment strategies for this vulnerable population. Our results show that adolescents are impaired in reducing cue-induced reinstatement following the extinction of cocaine-associated cue compared with adults. That is, we observed that adolescent rats that received cue extinction returned to drug-seeking when challenged with the cue the next day. By comparison, adult rats that received the same cue extinction session showed a significant decrease in cue-induced reinstatement. We found that the observed adolescent deficit in cocaine-associated cue extinction was ameliorated by acutely enhancing D2R signaling at the time of cue extinction training, with a potential mechanism for this effect identified in the IL of the mPFC. These results not only add to our understanding of the significance of the IL in adolescent drug-cue extinction learning, but also inspire novel approaches to improving adolescent exposure-based therapy in the clinical setting.

Adolescent Sensitivity to Drug-Associated Cues

In the present study, adolescent rats displayed impaired reduction of cue-induced reinstatement following cue extinction compared with adult rats. That is, while adult rats that received cue extinction training showed significantly reduced relapse-like behavior the next day, adolescent rats reinstated drug-seeking regardless of cue extinction training. Importantly, there were no observed age differences in extinction of lever pressing that were conducted in the absence of the cue. While one previous study shows that adolescent rats display increased responding during lever extinction following cocaine self-administration compared with adults, those results are confounded by age differences in overall cocaine consumption prior to lever extinction

(Anker and Carroll 2010). Adolescent rats have also been reported to show lower (Li and Frantz 2009) and equal (Schramm-Sapyta et al. 2011) responding during lever extinction. Those studies differed from our study in terms of methodology such as housing. Notably, there is evidence to suggest that individual housing from P21 effects anxiety and drug-seeking behavior during adulthood (Hall et al. 1998), although there were no adult isolated control groups. In the present study, all animals were bred and born in our facility, and rats assigned to adolescent groups were group housed until day of surgery (~P30), and were handled daily from the time of being individually housed, thus minimizing the potential stress of isolation from a young age. Therefore, our study suggests that acquisition, consolidation, and retrieval of operant extinction learning in the absence of the cocaine-associated cue occurs similarly in both adult and adolescent rats, as consecutive extinction sessions produced decreases in lever pressing for both ages. Thus the age difference in drug-associated extinction learning appears not to relate to operant responding, but to the extinction of drug-associated cues that is inferred from cue-induced reinstatement data. This specific age difference on drug-associated cue may be due to the dissociation in brain regions important for operant versus cue learning (Millan et al. 2011; Perry et al. 2014; Torregrossa et al. 2010).

In the present study, both adult and adolescent rats that did not receive cue extinction training displayed robust cue-induced reinstatement the next day. This is consistent with preclinical research in adult animals that shows re-exposure to the drug-associated cue triggers relapse-like behavior (Shaham et al. 2003). In previous preclinical investigations of adolescent drug self-administration, evidence for adolescent sensitivity to cue-induced reinstatement is mixed (Li and Frantz 2009; Anker and Carroll 2010). In those studies the drug-associated cue was never separately extinguished from lever responding, making interpretation of drug-cue sensitivity difficult. In one known study investigating adolescent extinction of drug-associated environmental cues, adolescent rats took longer to extinguish cocaine-associated contextual cues and displayed stronger reinstatement to those cues in a conditioned place preference paradigm (Brenhouse and Andersen 2008). Those findings are consistent with present results, which show for the first time in a self-administration paradigm that adolescents also display a deficit in extinction of a discrete cue associated with a self-administered drug. By comparison, our findings in adult rats are consistent with previous studies that show cue-alone extinction following lever-alone extinction reduces cue-induced reinstatement in adult rats (Torregrossa et al. 2010, 2013). It would be of great interest clinically for future studies to examine whether cue extinction without any lever extinction sessions reduces reinstatement of drug-seeking behaviors in adult rats, since it has already been shown that exposure to the cocaine-associated context can reduce drug-induced reinstatement in the absence of lever extinction (Kim et al. 2014).

Importantly, the present findings in adolescent rats directly model clinical evidence that CET is less effective in adolescent drug dependents (Catalano et al. 1990; Perepletchikova et al. 2008; Ramo and Brown 2008; Winters et al. 2011), which logically corresponds to higher relapse rates following therapy in this population (Ramo and Brown 2008). It should be noted that we did not observe enhanced cue-induced reinstatement per se in adolescents compared with adults, which may appear inconsistent with some human data that report adolescent humans show increased sensitivity to reward-associated cues in general (May et al. 2004; Ernst et al. 2005; Somerville et al. 2010). Critically, one study has specifically observed that adolescent drug users are more likely to relapse following craving induced by drug-associated cues (Ramo and Brown 2008), whereas, adult drug users are more likely to relapse when experiencing a negative physiological state such as withdrawal (Ramo and Brown 2008). In those studies, drug users did not necessarily undergo CET, whereas in our preclinical study, rats received lever and cue extinction. Therefore, we propose that adolescent vulnerability to addiction is at least partially due to a deficit in cue extinction that leads to increased likelihood of relapse, a hallmark of addiction. Combined with human research showing adolescent sensitivity to drug-associated cues, the present findings strongly suggest that drug use during adolescence leads to the formation of robust drug-cue associations that are difficult to extinguish.

It is important to note that cue extinction training was not given until late adolescence in the present study (Fig. 1). In the rat, P28–P56 is widely accepted as adolescence, with P70 as the onset of young adulthood (Spear 2000; Amorós-Aguilar et al. 2015; Saul et al. 2015). This relatively small developmental window is one of the reasons that preclinical adolescent addiction research is difficult to carry out. In the present study, self-administration occurred during early to mid-adolescence, and cue extinction occurred during late adolescence (~P53). We propose that self-administration during adolescence is most clinically relevant in terms of our model, and that cue extinction treatment during late adolescence still provides valuable insight into the effects of substance use during that vulnerable period.

The IL and Adolescent Cue Extinction

We observed that acutely enhancing D2R signaling in the IL of the mPFC during cue extinction reduces cue-induced reinstatement in adolescent rats. The IL was selected as a putative brain region important for adolescent cue extinction as a number of studies highlight a role for the IL in extinction of both aversive and reward-associated cues in adults (Peters et al. 2009). Indeed, in preclinical addiction studies, the IL has been strongly implicated in the extinction of operant responding (e.g., lever responding) and drug-associated contextual cues (Millan et al. 2011). However, the circuitry underlying extinction of discrete drug-associated cues is less clear. One study found that adult cue extinction was enhanced by systemic injection of the NMDA partial agonist D-cycloserine (DCS) (Torregrossa et al. 2010). Interestingly, this effect was observed via microinfusion into the NAc but not the mPFC (Torregrossa et al. 2010), though it may be that a lack of effect in the mPFC was due to targeting the whole region rather than the IL. In contrast, results of two studies examining contingent cue extinction in adult rats have suggested a role for the mPFC as a whole (Nic Dhonnchadha et al. 2013) and the IL specifically (Nic Dhonnchadha et al. 2012). However, those findings are confounded by lever pressing during cue extinction. Overall current understandings of the neural mechanisms underlying drug-cue extinction learning are relatively poor, as preclinical addiction research has largely ignored this aspect of addiction-related behaviors.

In fact, the neural basis of drug-associated cue extinction may be better understood from studies of fear extinction. While the vast majority of preclinical addiction literature focuses on extinction of operant and not cue memory, studies of conditioned fear focus largely on cue extinction in the absence of operant responding (Peters et al. 2009). Importantly, the IL has been implicated in fear extinction in both adults (Quirk and Mueller 2007) and adolescents (Kim et al. 2011). Furthermore, adolescents show a deficit in the consolidation of fear extinction learning comparable to the deficit in cocaine-cue extinction learning

observed in the present study (Kim et al. 2011; Pattwell et al. 2012; Ganella and Kim 2014). Our findings demonstrate for the first time that dopaminergic signaling via D2R in the IL is important for drug-cue extinction learning during adolescence. This is consistent with findings from fear conditioning in adult rats that show infusion of the D2R antagonist raclopride into the IL impaired retrieval of extinction the next day (Mueller et al. 2010). While further investigation is required to fully elucidate the neural basis of adolescent versus adult cue extinction learning in light of PFC maturation into late adolescence through adulthood, the present findings add invaluable novel data to this growing area of research, highlighting a role for dopaminergic signaling in the IL.

Translation to the Clinic: Aripiprazole

We sought to replicate the effect of intra-IL quinpirole using a pharmaceutical adjunct to cue extinction with strong translational potential. Therefore, we tested the effectiveness of aripiprazole, which is presently FDA-approved for the treatment of psychosis. We found that systemic administration of aripiprazole prior to cue extinction reduced relapse-like behavior in adolescents the next day.

Importantly, aripiprazole is already widely used in the treatment of psychosis not only for its efficacy but also because of its favorable safety profile and good tolerability (DeLeon et al. 2004). These factors make aripiprazole a compelling candidate for use in addiction treatment and in fact, aripiprazole is already in clinical trials for the treatment of cocaine dependence (Kim and Lawrence 2014). However, long-term use of aripiprazole in conjunction with abstinence has not generally shown beneficial results in non-psychotic patients (Brunetti et al. 2012). Evidence from preclinical relapse studies points to the benefits of short-term targeted use, with acute administration of aripiprazole reducing cocaine self-administration (Sørensen et al. 2008; Thomsen et al. 2008), as well as cue-induced and drug-primed reinstatement of cocaine-seeking following lever extinction (Feltenstein et al. 2007) or abstinence (Feltenstein et al. 2009). However, it should be noted that in those studies, treatment with aripiprazole occurred prior to reinstatement testing and drug-associated cues were never extinguished. Our results represent novel evidence for the efficacy of aripiprazole to block relapse specifically by improving cue extinction learning. This has important potential clinical implications, as therapy offers a controlled target for pharmacological intervention compared with relapse, which is often highly unpredictable.

Mechanism of Treatment Effects

We propose that the effects of quinpirole and aripiprazole in the present study are likely modulated through the D2R postsynaptically in the IL (Santana et al. 2009). Previous studies indicate that quinpirole decreases excitatory postsynaptic potentials (EPSPs) in PFC pyramidal cells both directly and by recruitment of local interneurons, consistent with post-synaptic D2R activation (Tseng and O'Donnell 2007). Similarly, the neuropsychological effects of aripiprazole involve D2R in the mesocorticolimbic dopamine pathway, which includes the IL (Stahl 2001; Burris 2002). However, aripiprazole differs from typical D2R agonists, as it does not produce motor behaviors associated with postsynaptic D2R activation. For instance, acute treatment does not induce contralateral rotation in striatal-lesioned rats or hyperlocomotion in reserpinized striatum-lesioned mice (Kikuchi et al. 1995). However, these experiments used adult rodents, and maturational differences in adolescent PFC may be associated with different drug effects. Importantly, aripiprazole is not a full D2R agonist but a partial D2R agonist. This means that when extracellular dopamine levels are low, it can act as a postsynaptic D2R agonist (Stahl 2001). Since the adolescent PFC is characterized by decreased dopamine availability (Wahlstrom et al. 2010), aripiprazole is likely acting as an agonist at postsynaptic D2Rs at this age. Computational models of PFC networks suggest that when D2R signaling is dominant, the PFC is in an "open gate" state where multiple inputs can have simultaneous representations in working memory (Seamans and Yang 2004). We suggest that activation of postsynaptic D2Rs shifts adolescent IL networks toward this more flexible state. In this way, acutely enhancing D2R signaling during cue extinction improves learning of the new inhibitory cue-no reward association.

It should be noted that while aripiprazole displays robust preferential binding to the D2R in both rats (Natesan et al. 2006) and humans (Mamo et al. 2007), it also exhibits partial agonist activity at the serotonin receptors 5HT1A and 5HT7 (DeLeon et al. 2004). Importantly, mPFC dopamine signaling is strongly mediated by the serotonin system (Benes et al. 2000). Indeed, serotonin fibers have been shown to interact with both dopamine afferents and gamma-aminobutyric acidergic interneurons in the mPFC (Taylor and Benes 1996), and to modulate the infiltration of fibers to this region (Taylor et al. 1998). This is of particular relevance given the infiltration of dopaminergic fibers occurring in the PFC during adolescence (Kalsbeek et al. 1988). However, "acute" treatment with aripiprazole during adolescence is unlikely to profoundly alter the course of dopamine afferent connectivity in the mPFC either directly or via serotonin modulation.

Importantly, the effects of treatment in the present study are not likely due to nonspecific effects during cue extinction such as sedation or stress. In fact, a single infusion of quinpirole into the mPFC has been shown to produce an anxiolytic response in mice tested drug-free the next day, with no effect on any anxiety measure at the time of treatment (Wall et al. 2003). In addition, a single intra-mPFC infusion of quinpirole has been found to produce no effect on locomotion compared with saline (Beyer and Steketee 2000). Studies using acute systemic aripiprazole in mice have similarly found no effect on locomotor activity (Viana et al. 2013). In fact acute aripiprazole has been shown to improve cognition in rats in terms of attentional functioning and response control (Carli et al. 2010), both of which are important in cue extinction learning. Acutely enhancing D2R signaling in the IL at the time of cue extinction therefore represents a promising tactic to enhance learning effectiveness per se and thereby reduce subsequent relapse to drug-associated cues.

Conclusion

Adolescence represents a unique period of risk for developing mental disorders, including drug addiction (Spear 2000). Our findings strongly suggest that adolescent vulnerability to addiction is explained at least in part by deficits in cue extinction that may lead to enhanced liability to relapse to drug-associated cues. Importantly, the present findings directly model clinical evidence that adolescent drug users are more resistant to exposure-based therapies and liable to relapse, especially to cues associated with the drug-taking experience, compared with their adult counterparts (Ramo and Brown 2008). The present study highlights a role for the D2R in the IL of the mPFC in mediating effective cue extinction learning in the adolescent rat. Since the neural correlates of adolescent behaviors are often conserved across species (Spear 2000), these findings inspire novel tactics

for pharmacologically enhancing extinction-based therapies for drug users who started during their adolescent years. Tailoring treatments to adolescent users will hopefully break the cycle of addiction for many living with substance abuse disorders.

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