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# Extinction of conditioned cues attenuates incubation of cocaine craving in adolescent and adult rats

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ABSTRACT

Relapse to drug use is often precipitated by exposure to drug associated cues that evoke craving. Cue-induced drug craving has been observed in both animals and humans to increase over the first few weeks of abstinence and remain high over extended periods, a phenomenon known as 'incubation of craving'. As adolescence represents a period of vulnerability to developing drug addiction, potentially due to persistent reactivity to drug associated cues, we first compared incubation of cocaine craving in adolescent and adult rats. Adolescent (P35) and adult (P70) rats were trained to lever press to obtain intravenous cocaine, with each drug delivery accompanied by a light cue that served as the conditioned stimulus (CS). Following acquisition of stable responding, rats were tested for cue-induced cocaine-seeking after either 1 or 30 days of abstinence. Additional groups of rats were also tested after 30 days of abstinence, however these rats were subjected to a cue extinction session 1 week into the abstinence period. Rats were injected with aripiprazole, a dopamine 2 receptor (D2R)-like partial agonist, or vehicle, 30 min prior to cue extinction. We found that adolescent and adult rats acquired and maintained a similar level of cocaine self-administration, and rats of both ages exhibited a higher level of cue-induced cocaine-seeking if they were tested after 30 days of abstinence compared to 1 day. Incubation of cocaine craving was significantly reduced to 1 day levels in both adults and adolescents that received cue extinction training. Administration of aripiprazole prior to cue extinction did not further reduce cue-induced drug-seeking. These results indicate that cue extinction training during abstinence may effectively reduce cue-induced relapse at a time when cue-induced drug craving is usually high.



## 1. Introduction

Drug addiction is a chronic, relapsing disorder typically characterised by periods of compulsive drug use interspersed with periods of abstinence (Koob & Volkow, 2010). It is estimated that around 40–60% of addicts will relapse within 1 year following treatment (McLellan, Lewis, O'Brien, & Kleber, 2000), making relapse prevention a significant challenge. One of the major precipitants of relapse is exposure to drug-associated cues such as people, places or paraphernalia associated with the drug-taking experience (Childress et al., 1999; Gawin & Kleber, 1986; O'Brien et al., 1992). Over time, these drug-associated cues acquire powerful conditioned reinforcing properties that can trigger craving (Ehrman, Robbins, Childress, & O'Brien, 1992). The motivational power of conditioned cues is long-lasting, as risk of relapse remains high even despite long periods of abstinence. In fact, in both animals and humans it has been observed that cue-induced craving for drugs actually intensifies over the first few weeks of abstinence and remains high over extended periods, a phenomenon termed 'incubation of craving' (Grimm, Hope, Wise, & Shaham, 2001; Li, Caprioli, & Marchant, 2015). This incubation effect is robust and has been observed for most drugs of abuse including cocaine (Pickens et al., 2011).

One way to reduce the incentive salience of drug-associated cues is by cue exposure therapy (CET) which works on the principle of extinction (Conklin & Tiffany, 2002). Extinction processes in CET typically involve repeated non-reinforced presentations of cues previously associated with the drug experience, which results in a reduction in the conditioned responses and craving evoked by

*Abbreviations:* ANOVA, analysis of variance; CET, cue exposure therapy; CS, conditioned stimulus; D2R, dopamine 2 receptor; EXT, extinction; FR, fixed ratio; REM, rapid eye movement; RM, repeated measures.

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the cue (O'Brien et al., 1990). In animal models of addiction, cue extinction is typically preceded by extinction of the operant response required for drug self-administration (e.g.: lever press or nose poke). We have begun to separate out these two different aspects of extinction (operant vs cue) by administering cue extinction in the absence of levers to more closely model CET that occurs in the clinic (Perry, Reed, Zbukvic, Kim, & Lawrence, 2016; Perry, Zbukvic, Kim, & Lawrence, 2014). In doing so, we have recently shown that cue extinction is significantly less effective in preventing reinstatement of cocaine-seeking in adolescent compared to adult rats (Zbukvic et al., 2016). This is consistent with clinical observations that adolescents are particularly vulnerable to developing drug addiction, more resistant to treatment and more liable to relapse (Catalano, Hawkins, Wells, Miller, & Brewer, 1990; Chen, Storr, & Anthony, 2009; Ramo & Brown, 2008; Spear, 2000). We also demonstrated that the efficacy of cue extinction could be dramatically improved in adolescents by manipulating dopamine signalling during cue extinction sessions (Zbukvic et al., 2016). Our findings suggest that adolescents are resistant to extinction of cocaine-associated cues compared to adults, and this can potentially be overcome by administering cue extinction in combination with pharmacotherapies that specifically target the dopaminergic system.

The first aim of the present study was to compare incubation of cocaine craving in adolescent and adult rats that self-administered cocaine and then received 1 or 30 days of forced abstinence. Given evidence for adolescents' deficit in extinction of drug-associated cues, we hypothesised that the magnitude of incubation would be greater in this age group. The second aim was to compare the effectiveness of cue extinction training administered during the abstinence period in reducing the incubation effect in adults and adolescents. Importantly, no animals received extinction of operant responding because we wanted to model CET in the clinic, which does not usually involve extinction of operant behaviours. In addition, we investigated whether systemic administration of aripiprazole, a partial agonist at D2-like receptors, would increase the efficacy of cue extinction training. We have previously shown that when aripiprazole is administered in conjunction with cue extinction training, cue-induced reinstatement is markedly reduced in adolescents (Zbukvic et al., 2016). It is not yet known whether aripiprazole in combination with cue extinction is similarly effective at preventing cue-induced cocaine-seeking following a period of abstinence.

## 2. Material and methods

### 2.1. Animals

62 Male Sprague-Dawley rats ($n$ = 33 adult, 29 adolescent) were bred in-house and individually housed in a 12 h reversed light-dark cycle (lights off at 7 a.m.) with food and water available *ad libitum*. All experiments were conducted during the dark phase of the light/dark cycle. Rats were postnatal day (P) 34 ± 1 (adolescent) or P69 ± 1 (adult) on the first day of self-administration. Rats were acclimated to handling for 3 days prior to surgery. All experiments were performed in accordance with the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

### 2.2. Surgery

Rats were anesthetized with isoflurane vaporised with oxygen (5% induction, 2% maintenance, Rhodia Organic Fine Ltd, Bristol, UK) and surgically implanted with an indwelling catheter into the right jugular vein as previously described (Kim et al., 2015). Catheters were constructed in-house and consisted of 22 gauge guide cannulas (Plastics One, VA, USA), 2 layers of Silastic tubing (adult length 14 cm, adolescent length 12 cm, Dow Corning, USA) and a catheter port made from dental cement (Vertex, MA, USA). Rats were administered Meloxicam for pain relief (0.05–0.1 mL of 5 mg/mL stock, *i.p.*, Boehringer Ingelheim, Germany) and were allowed 2 days recovery from surgery before cocaine self-administration commenced. Catheters were flushed twice daily with 0.9% saline containing 10 IU/mL heparin (Pfizer, NY, USA) prior to each session and 0.9% saline containing 90 IU/mL heparin containing antibiotic (10% Fisamox amoxicillin sodium, Aspen Australia, Australia) after each session. If catheter leakage was suspected, catheter patency was checked by intravenous infusion of 0.03 mL of ketamine (100 mg/mL) for adults and 0.02 mL for adolescents immediately followed by 0.05 mL heparinised saline (10 IU/mL).

### 2.3. Cocaine self-administration

Cocaine self-administration sessions took place in standard operant conditioning chambers (29.5 × 32.5 × 23.5 cm, Med Associates, VT, USA) equipped with 2 retractable levers and a cue light above each lever. Sessions began with the extension of both levers. Pressing on the active lever resulted in a 50 µl infusion of cocaine hydrochloride (Johnson Matthey Macfarlan Smith, Edinburgh, UK) dissolved in saline delivered over 2.7 s by activation of a pump (Med Associates, VT, USA). The concentration of cocaine was adjusted for each rat to account for differences in weight so that each infusion delivered a dose of 0.3 mg/kg, as described previously (Zbukvic et al., 2016). Cocaine infusions were paired with 2.7 s illumination of the light located above the active lever, followed by a 17.3 s time out period. Pressing on the inactive lever had no consequences. House lights remained off throughout all behavioural sessions. Rats were subjected to daily, 6 h self-administration sessions on a fixed ratio of 1 (FR1) schedule for the first 5 days, and then the response requirement was increased to FR3 for the last 5 days.

### 2.4. Experimental design

Following cocaine self-administration, adult and adolescent rats were randomly assigned to one of 4 experimental groups. Rats in the Day 1 group underwent a cue-induced drug-seeking test on the first day of abstinence from cocaine self-administration. Rats in the Day 30 NO EXT group underwent a cue-induced drug-seeking test on day 30 of abstinence from cocaine self-administration. Rats from these groups remained undisturbed in their home cages during the abstinence period, aside from their usual bedding changes. The two remaining groups underwent 1 session of cue-extinction training after 1 week of abstinence in their home cages. Rats were administered either vehicle (CS EXT Vehicle) or aripiprazole (CS EXT Aripiprazole) 30 min prior to their cue-extinction session. Rats from both these groups underwent a cue-induced drug-seeking test on day 30 of abstinence from cocaine self-administration (see Fig. 1 for timeline).

### 2.5. Cue extinction

Cue extinction sessions were designed to mimic cue-exposure therapy (CET) that is used in the treatment of addiction in the clinic, which typically involves presentation of drug-associated cues. 30 min prior to cue extinction sessions, rats were injected subcutaneously with either vehicle (5% v/v tween 80 in saline 1 mL/kg, Sigma-Aldrich, MO, USA) or aripiprazole (5 mg/kg, Alliance Biotech, India) suspended in vehicle. Rats were then placed in the same operant chamber where they self-administered