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Enhancing the salience of dullness: Behavioral and pharmacological strategies to facilitate extinction of drug-cue associations in adolescent rats

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Abstract

Extinction of drug-seeking is an integral part of addiction treatment, and can profoundly reverse or ameliorate the harmful consequences of drug use. These consequences may be the most deleterious during adolescence. The studies presented here build from recent evidence that adolescent rats are more resistant to extinction training than adults, and therefore may require unique treatment strategies. We used unbiased place-conditioning in male rats to show that passive, un-explicit extinction pairings resulted in delayed extinction in 40-day-old adolescents relative to 80-day-old adults. However, explicit-pairing of a previously cocaine-associated context with the *absence* of drug produces extinction in adolescents as rapidly as in adults. These data suggest that successful extinction of drug-paired associations in adolescents may be facilitated by stronger acquisition of a new (extinction) memory. Drug-paired associations are largely controlled by the prelimbic prefrontal cortex (plPFC) and its influence on the nucleus accumbens. This pathway mediates the motivational salience attributed to incoming stimuli through the D1 dopamine receptor. D1 receptors on plPFC outputs to the accumbens are transiently overproduced during adolescence. Since D1 receptors are selectively responsive to potent stimuli, we hypothesized that the adolescent plPFC hinders competition between potent drug-paired associations and the subtler, drug-free information necessary for extinction. To harness this unique profile of the adolescent plPFC, we aimed to increase the salience of unrewarded extinction memories by activating plPFC D1 receptors during extinction training. In a second study, extinction of drug-cue associations was facilitated in adolescents by elevating dopamine and norepinephrine in the PFC during extinction training with atomoxetine. In a third study, direct microinjection of the D1 receptor agonist SKF38393 mimicked this effect, also facilitating extinction in adolescent subjects. Furthermore, pharmacological intervention attenuated subsequent drug-primed reinstatement of cocaine-conditioned preferences. We establish a potential direction for distinct strategies to treat this vulnerable population.

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Keywords

place conditioning; cocaine; prefrontal cortex; reinstatement; adolescence

Complete and sustained cessation of drug taking is the immediate goal of addiction treatment. Adolescents (age 12-17 years) are the most at risk for addictive processes, with the relative risk for cocaine dependence being highest if the initial exposure occurred during early adolescence (O'Brien and Anthony, 2005). While many avenues of addiction treatment are being pursued (Taylor et al., 2009, Jupp and Lawrence), extinction training is a common approach in humans (Jupp and Lawrence). Extinction training aims to attenuate conditioned responses to cocaine-related stimuli that are associated with craving (O'Brien et al., 1990, Foltin and Haney, 2000). However, treatment of drug abuse in adolescents has proven difficult (Schell et al., 2005). Consistent with these clinical observations, our preclinical studies in rats suggest that extinction training may need to be optimized for the treatment of adolescent drug abusing populations (Brenhouse and Andersen, 2008). Taken together, treatment of addiction in adolescents may require distinct approaches from those used for adults.

Extinction of a conditioned association requires the formation of a new association between the conditioned stimulus (CS) and the *absence* of the unconditioned stimulus (US). New associations can be formed through either explicit or un-explicit pairings of a context with the lack of US (Mueller and Stewart, 2000). Adolescent rats require 75% longer than adults to extinguish conditioned associations using un-explicit extinction pairings [repeated exposure to both sides of a place-preference apparatus (Brenhouse and Andersen, 2008)], but whether explicit pairings may provide a more salient "new" conditioned memory to compete effectively with strongly held drug-cue associations is not known. The first study investigated whether explicit pairings may provide the necessary heightened salience to facilitate extinction. In addition, our previous study demonstrated that adolescents are more likely to 'relapse' following extinction (e.g. a greater preference for a previously cocaine-paired environment upon drug-primed reinstatement). This study also endeavored to reduce reinstatement to cocaine-associated cues in both adolescents and adults.

The prelimbic prefrontal cortex (plPFC) mediates salience attribution to environmental stimuli and regulates drug-cue associations and motivation (Seamans and Yang, 2004, Ventura et al., 2007). Inactivation of a cortical region including the plPFC immediately prior to extinction training trials attenuates extinction of place preferences for cues associated with amphetamine (Hsu and Packard, 2008), however the mechanism underlying this effect is not known. Within the plPFC, dopamine D1 receptors (D1R) on glutamatergic projections to the nucleus accumbens (NAc) mediate the salience attributed to drug-cue associations (Kalivas and Duffy, 1997). In rats that show increased drug-seeking, D1R activity on these projections is elevated, causing stimuli that are predictive of drugs to become exceedingly salient at the expense of other information (Everitt and Wolf, 2002, Kalivas et al., 2005). Elevated dopaminergic activity in the plPFC is also necessary to reinstate drug seeking after a period of extinction (Sanchez et al., 2003, Graham et al., 2006).

The PFC undergoes extensive development between childhood, adolescence, and adulthood, which mirrors the development of affective and motivational processing (Alexander and Goldman, 1978, Fuster, 1980, Kalsbeek et al., 1988). More importantly, D1R expression on plPFC-NAc projections peaks during adolescence in the rat, followed by a marked reduction that is complete by adulthood (Andersen et al., 2000, Brenhouse et al., 2008). Therefore, this cresting of D1R density during adolescence reveals a unique period of development when the PFC DA receptor profile dangerously resembles one of an addicted brain. Drug exposure

during this adolescent peak in D1R may program the pLPFC to a “pro- addictive state” in part by forming strong drug associations that are highly resistant to extinction and reinstate more robustly (Brenhouse et al., 2008).

Here, we aimed to harness the unique dopaminergic receptor profile of the pLPFC in adolescents to facilitate extinction of drug-cue associations in this vulnerable population. We hypothesized that adolescent resistance to extinction is due to strong-held memories of potent rewards, which compete with the process of forming new memories. If adolescent elevations in D1R do confer greater vulnerability to addictive processes, then using either a strategic training paradigm (e.g., explicit pairing) or pharmacological intervention to enhance the salience of the *absence* of drug (i.e., a relative ‘dullness’ compared to cocaine exposure) is predicted to facilitate the acquisition of a new extinction memory. In the first study, adolescents were exposed to a previously drug-associated environment that was explicitly paired with the absence of drug, compared to being passively exposed (e.g., non-explicitly paired) to both drug-paired and non-drug paired environments as in our earlier study (Brenhouse and Andersen, 2008). To shed light on a novel mechanism for adolescent extinction treatment, the second study aimed to enhance D1R stimulation during the formation of a new, non-drug association. The non-stimulant atomoxetine was administered during extinction training to pharmacologically manipulate pLPFC function by increasing extracellular norepinephrine and dopamine (Bymaster et al., 2002). Finally, we investigated the pLPFC D1R specifically as a unique treatment target through microinjections of a D1 agonist during extinction training to an un-explicitly paired environment.

Methods

Subjects

Male Sprague-Dawley rats were obtained from Charles River (Boston, MA). Subjects were P38 at the start of conditioning (adolescence) or P75 (adulthood for Experiment 1 only). Food and water were made available *ad libitum* in constant temperature and humidity conditions on a 12-hour light/dark cycle (light period 0700-1900). All experiments were conducted in accordance with the 1996 Guide for the Care and use of Laboratory Animals (NIH) and McLean-approved IACUC protocols. Prior to testing, all rats were given a minimum of one week to acclimate to our facilities, and were handled for a minimum of three days.

Cocaine place conditioning

Adolescent rats were conditioned to 20mg/kg cocaine using an unbiased place conditioning protocol, as previously described (Brenhouse and Andersen, 2008). This dose of cocaine produces reliable place preferences in our hands and others at this stage (Badanich et al., 2006, Brenhouse et al., 2008). The conditioning apparatus consisted of two large (24 × 18 × 33cm) side compartments and a small (12 × 18 × 33cm) middle gray compartment, with the large compartments differing in color, lighting, and floor texture (Med Associates, Georgia, VT). On Day 1 rats were screened for initial side biases, where subjects were free to explore the entire apparatus for 30 minutes after a 5-minute adaptation period of confinement to the middle compartment. Seven rats (of 72) displayed an initial strong preference for one chamber (>18 of 30 min) during screening day and were eliminated from testing. Following screening, rats received two days of conditioning (Days 2 and 3), during which subjects were injected with saline (1 ml/kg, i.p.) and placed in a side chamber for one hour, and four hours later injected with cocaine (20 mg/kg, i.p.) and placed in the opposite chamber for one hour. Vehicle injections always preceded cocaine injections to obviate any after-effects of cocaine during conditioning to saline (Carlezon, 2003). On Day 4, rats were allowed access to the entire apparatus in a drug-free state for 30 minutes to test for conditioned preferences.

Preference scores were determined by calculating the ratio of time spent in the drug-paired side to the total time spent in both the drug and saline-paired sides (Brenhouse and Andersen, 2008). Eight rats (of 65) failed to form a preference for the drug-paired chamber after the 2 days of conditioning and were also eliminated from further extinction testing (Brenhouse and Andersen, 2008). Groups were organized such that average baseline preferences for one particular chamber were minimized, and average conditioned preferences were similar between groups.

Experiment 1: Differential effects of two training strategies on adolescent extinction

We recently reported that adolescents display delayed extinction compared to adults using a repeated testing paradigm to drug-associated environments using an unpaired approach (Brenhouse and Andersen, 2008). Here, we determined whether explicitly pairing (EP) a previously drug-paired environment with the absence of the drug would facilitate extinction compared with passive exposure (un-paired; UP) to the drug-paired environment. Nineteen adolescent rats and 15 adult rats were conditioned to 20 mg/kg cocaine as described above. Rats were treated in an UP (n=9 adolescents; n=8 adults) extinction paradigm as described previously (Brenhouse and Andersen, 2008), or in an EP (n=10 adolescents, n=7 adults) paradigm (see Figure 1). Briefly, twenty-four hours after the initial preference test, rats were again introduced to the entire apparatus in a drug-free state for 30 min, and time spent in each chamber was recorded. This test was repeated daily until each animal achieved extinction, defined as a reduction in preference score to 0.55 or lower for two consecutive days. A 0.55 preference score corresponds to within 10% of average baseline values, and represents a 50% reduction in time spent in the initially preferred compartment, based on group averages (Sanchez et al., 2003, Brenhouse and Andersen, 2008). UP testing ceased after 7 days if a subject had not yet reached extinction criterion, since we previously showed that adolescents require an average of 7 days to reach extinction in an UP paradigm (Brenhouse and Andersen, 2008). More importantly, truncating UP testing to 7 days allowed for UP and EP groups to undergo a similar number of extinction sessions before reinstatement, while allowing as many UP subjects as possible to achieve extinction. A separate group of rats received EP, wherein extinction trials were similar to conditioning trials: beginning 24 hours after the initial place preference test, rats were exposed in the morning for 30 min to one chamber, then four hours later exposed for 30 min to the other chamber. Both chambers were paired with saline injection, and rats did not receive cocaine during this period. EP trials were conducted for four days, which corresponds to the number of days required by adults to reach extinction in an UP paradigm (Brenhouse and Andersen, 2008). On the fifth day, rats were given an additional 30-min drug-free test session and allowed access to all three compartments of the apparatus. Preference scores from the fifth day of UP were compared to preference scores upon retest after EP. Two-way analysis of variance (ANOVA) compared the scores after extinction training between UP and EP groups across the repeated measures of baseline, initial preference, and extinction scores.

After achieving extinction, rats were further tested for drug-primed reinstatement. Twenty-four hours following the last extinction trial (for UP subjects) or following retest (for EP subjects), subjects were administered 5 mg/kg cocaine and were immediately reintroduced to the entire conditioning apparatus for a 30 min test for reinstatement of conditioned drug effects (Sanchez et al., 2003, Brenhouse and Andersen, 2008). Notably, reinstatement was defined in these studies as the significant return of conditioned place preferences for a formerly cocaine-associated context, *after* those preferences had been extinguished (Aguilar et al., 2009). Therefore, only individual subjects that had reached extinction upon retest were included in these analyses. Several adolescent subjects (mostly vehicle controls) did not reach extinction criterion after seven days of UP as predicted, and thus were excluded from reinstatement testing to avoid marked differences in extinction training duration between UP

and EP subjects (e.g., 7 vs 4 days, respectively). Due to the small percentage of adolescent vehicle-treated UP subjects that reached extinction in these studies (6 animals of 20), reinstatement data was pooled across all control adolescent UP subjects in Experiments 1-3. Two EP subjects did not reach extinction upon retest, and were also excluded from reinstatement testing. In order to determine whether EP and UP differentially affected reinstatement in adolescents and adults, a three-way ANOVA with repeated measures (Age $\times$ Paradigm $\times$ Test) compared preference scores during the last extinction test to preference scores during the reinstatement test between training groups and ages.

Experiment 2: Effects of atomoxetine on adolescent extinction in the UP paradigm

In order to determine whether administration of atomoxetine during UP would facilitate extinction in adolescents, rats were conditioned and subjected to UP as described above. During the first four days of UP, subjects were administered atomoxetine (Sigma-Aldrich, St Louis, MO; 2 mg/kg, i.p.) or vehicle (1 ml/kg i.p.) 25 min prior to being placed into the apparatus (n=8). This dose of atomoxetine was chosen based on previous studies examining the effects of atomoxetine on nicotine generalization (Reichel et al., 2007) and reinstatement of drug-seeking in rats (Economidou et al., 2009). On the fifth day of RT, rats were tested in a drug-free state for a final assessment of extinction. Two-way analysis of variance (ANOVA) with repeated measures compared baseline, initial preference scores, and scores after extinction training between vehicle controls and atomoxetine-treated rats.

The day following the last UP session, rats that extinguished their initial preferences were tested for drug-primed reinstatement, as described above. Two-way ANOVA with repeated measures compared preference scores during the last extinction test to preference scores during the reinstatement test between as a function of drug treatment.

Experiment 3: Effects of pIPFC microinjections of a D1 dopamine receptor agonist on adolescent extinction in the UP paradigm

Surgery—On P35-36, 17 rats were surgically implanted with guide cannulae aimed at the pIPFC, as previously described (Brenhouse et al., 2008). Rats were anesthetized with ketamine/xylazine and were implanted with bilateral 26-gauge stainless steel guide cannulae (Plastics One, Roanoke VA) above the pIPFC [AP: +3.3; ML: $\pm$ 0.6; DV: 1.6 (Sherwood and Timeras, 1970)].

Experimental Procedure—Subjects were given 2 full days after surgery to recover before experimental procedures commenced. Conditioning to 20 mg/kg cocaine was performed as described above. Twenty-four hours following the initial place preference test, UP extinction trials began. During the first four days of UP, rats were infused bilaterally with vehicle or the partial D1R agonist SKF38393 (1.0 μ g/0.3 μ l/side; Sigma-Aldrich; n=4), using an injector cut to protrude 1mm beyond the guide cannula (Plastics One). This dose of agonist was based on our previous study showing that 1.0 μ l was the most effective dose to invoke place preferences when infused into the pIPFC of juvenile rats (Brenhouse et al., 2008). Injections were performed 10 min prior to introduction into the chamber. With the animal gently restrained, infusions were made at a rate of 0.2 μ l/min, followed by one additional minute to allow full diffusion of the drug. On the fifth day of UP, rats were tested in a drug-free state for a final assessment of extinction. Two-way analysis of variance (ANOVA) with repeated measures compared baseline, initial preference scores, and scores after extinction training between vehicle- and SKF38393-infused groups. Rats were then tested for drug-primed reinstatement as described above.

Cannula placement verification—Following testing, rats were infused bilaterally with 1% toluidine blue (Sigma-Aldrich, 0.3 μ l/side) and 10 minutes later were deeply

anesthetized and intracardially perfused with PBS and then 4% paraformaldehyde. Brains were cut into 40- μ m sections and stained with cresyl violet. The placement and spread of infused dye was observed with a light microscope (see Figure 5 for microinjection placement). Behavioral data from one animal with a misplaced injections site was excluded from analyses.

Results

Experiment 1: Differential effects of two training strategies on adolescent extinction

EP training facilitated extinction to a greater degree than UP training in adolescent rats. Following a significant paradigm $\times$ test interaction ($F[2,34]=5.3$; $p=0.01$), individual t-test analyses indicated that preference scores upon retest after four days of EP were significantly lower than after four days of UP (Figure 2; $t[17]=3.4$; $p=0.004$). Our previous study demonstrated that adult rats extinguished after four days of UP conditioning trials (Brenhouse and Andersen, 2008). To confirm that EP would be just as efficient for extinguishing preferences, we ran an $n=15$ adults and tested them as described for the adolescent rats. Both EP and UP training were equally efficient paradigms for extinguishing conditioned preferences (Figure 2; paradigm $\times$ test interaction $p=0.843$). Extinction-training paradigms did not differentially affect drug-primed reinstatement of conditioned preferences at either age (Figure 3; $p=0.189$ for age $\times$ paradigm $\times$ test interaction). However, independent t-tests revealed a trend-level difference between paradigms in adolescence, since EP adolescents did not display significant reinstatement after extinction ($p=0.089$), while UP adolescents ($p=0.001$) and both groups of adults (UP: $p=0.01$; EP: $p=0.0004$) did display significant reinstatement after extinction.

Experiment 2: Effects of atomoxetine on adolescent extinction in the UP paradigm

As shown in Figure 4, preference scores for cocaine-associated environments were not different at baseline or at test, but differed in the extinction phase following atomoxetine ($F(2,24)=4.0$; $p=0.03$ drug $\times$ testing interaction). Atomoxetine, administered during the first four days of UP, facilitated extinction in adolescent rats when tested on day 5 and significantly decreased preference scores after four days of UP compared to vehicle controls (Figure 4; $t[12]=2.48$; $p=0.029$). Drug-primed reinstatement was also significantly reduced in animals treated with atomoxetine during extinction, when compared to vehicle controls (Figure 4; $F[1,8]=10.67$; $p=0.02$ drug $\times$ testing interaction).

Experiment 3: Effects of PFC microinjections of a D1 dopamine receptor agonist on adolescent extinction in the UP paradigm

Direct infusion of the D1 agonist SKF38393 into the plPFC also facilitated extinction in adolescents, as microinjection placement was verified within plPFC, with minimal spread to Cg1, but not the infralimbic PFC (Figure 5). SKF38393, administered directly to the plPFC during the first four days of UP, resulted in a significant interaction between drug treatment and testing (Figure 5; $F[2,18]=5.08$; $p=0.018$ for drug $\times$ testing interaction). While baseline and initial preference tests yielded similar preference scores between treatment groups, animals administered SKF38393 displayed significantly decreased preference scores after four days of UP compared to vehicle controls (Figure 5; $t[12]=2.48$; $p=0.029$). Exposure to the D1R agonist during extinction also reduced drug-primed reinstatement; animals administered SKF38393 during extinction training displayed lower preference scores upon drug-primed reinstatement, compared to vehicle controls (Figure 5; $F[1,7]=10.68$; $p=0.014$).

Discussion

Here we report that treatment strategies targeted to a vulnerable population that readily forms strong drug-cue associations can facilitate their extinction. To our knowledge, this is the first investigation of the effectiveness of different extinction strategies in adolescents, which appear to be more resistant to extinction of cocaine-conditioned associations than adults (Waylen and Wolke, 2004, Brenhouse and Andersen, 2008). Both UP and EP paradigms have been used to successfully extinguish conditioned place preferences for cocaine, morphine and ethanol in adults (Mueller and Stewart, 2000, Mueller et al., 2002, Kreibich and Blendy, 2004, Ventura et al., 2005, Font et al., 2008, Aguilar et al., 2009). However, we have observed that UP is less effective in adolescents, compared to adults (Brenhouse and Andersen, 2008). The results of the current study show that EP of a cocaine-associated context with the absence of drug extinguishes conditioned preferences for that context more efficiently in adolescents than UP. These data suggest that human adolescents may also be less likely to abstain from drugs when there is a lack of explicit pairings of drug-cues in the absence of the drug (e.g., peers in other contexts).

We hypothesized that the adolescent pLPFC is primed to respond to strong stimuli. During adolescence, D1R-mediated excitatory output of the pLPFC produces a high motivational salience of drug-paired cues (Brenhouse et al., 2008) that may normally override the inhibitory effect of extinction memories. Under addiction states, D1R receptor activity in the pLPFC is dominant (Kalivas et al., 2005), and therefore the enhanced activity of D1R-mediated responses in adolescents renders these animals (and possibly humans) more susceptible to drug-seeking initially and to be more resistant to extinction. Our data suggests that the creation of an explicit new memory competed more effectively than passive re-exposure to the drug-paired context, since such exposure increases dopamine efflux in the medial PFC (including the pLPFC) (Lin et al., 2007). Administration of atomoxetine, as well as direct pLPFC microinjection of a D1R agonist, *during extinction training* targeted this D1R mechanism more specifically. Activating the over-expressed D1R receptor in adolescents kindled salience attribution to the *absence* of drug (i.e., relative dullness) thereby providing new associations that competed with conditioned memories to facilitate extinction.

There is evidence that PFC norepinephrine transmission is necessary for motivational salience attribution to both reward- and aversion-related stimuli through modulation of dopamine in the NAc (Ventura et al., 2007). To address this hypothesis, we administered atomoxetine during the extinction phase. Notably, atomoxetine increases extracellular dopamine in the PFC to the same magnitude as norepinephrine at the dose used in the current study (Bymaster et al., 2002). While earlier studies have shown that atomoxetine reduces reinstatement in rats, due in part to noradrenergic actions on impulsivity (Economidou et al., 2009), we believe that it is through its actions at the D1R that atomoxetine reduces drug-seeking. Here, we observed that atomoxetine given during extinction training reduced conditioned preferences for a drug-associated environment when the subjects were tested off-drug. If impulsivity were to play a role, then atomoxetine would have to be on-board at the time of testing, not during extinction training. To further support this hypothesis, prior atomoxetine treatment also prevented subsequent relapse to a priming dose of cocaine. We believe it is because adolescents treated with atomoxetine created a new, more salient association that endured to reduce conditioned associations for drug-related cues. In this regard, the cognitive enhancing effects of atomoxetine (Sofuoglu, 2010) would serve to facilitate this new learning. The effect of atomoxetine on extinction was mimicked by direct administration of a D1R agonist to the pLPFC, further suggesting that atomoxetine exerted its effects through a dopaminergic mechanism.

Several neurotransmitter systems and brain areas have been implicated in the extinction and reinstatement of place preferences. Much of the current knowledge of the neurobiological substrates of extinction derives from research on extinction of fear conditioning, since many parallels exist between the two conditioned responses. The plPFC does not appear to be necessary for extinction of fear conditioning, and rather is believed to be more important in the expression of both fear conditioning and drug-seeking (Peters et al., 2009). The ventromedial infralimbic region of the PFC (ilPFC) has been traditionally understood to control the suppression of these behaviors in the extinction process (Peters et al., 2009). The ilPFC coordinates with the basolateral (BLA) and central nuclei of the amygdala to detect the omission of the US and suppress conditioned responses (Berretta et al., 2005, Peters et al., 2009). Lesions to (Fuchs et al., 2002), or enhanced activity of (Schroeder and Packard, 2003, Schroeder and Packard, 2004) the BLA impairs and facilitates extinction of conditioned preferences, respectively, when performed prior to or immediately after non-reinforced confinements in a previously drug-paired environment. Manipulations of the ilPFC also affect extinction (Quirk et al., 2000), though have not been extensively studied for extinction of drug-seeking (reviewed by (Quirk and Mueller, 2007, Myers and Carlezon). However, it is important to note that the ilPFC plays a role in the *consolidation* and *expression* of extinction memories (Quirk and Mueller, 2007). Here, we establish that adolescents benefit from a strengthened *acquisition* of extinction memories, which may require heightened motivational salience assigned to a new, drug-free association. While the plPFC may not directly control or be necessary for the suppression of drug-seeking behaviors, we believe that this region plays an important role in directing attention to a new extinction memory, in order to facilitate its acquisition.

Strengthening extinction memories to reduce both fear conditioning and drug-seeking in animals (Walker et al., 2002, Paolone et al., 2009) and humans (Ressler et al., 2004) has been pharmacologically facilitated using glutamatergic agonists such as d-cycloserine. Such treatments are targeted to the amygdala, which is critical in the incorporation of CS-US associations. This has been extended to clinical treatment of addiction—with and without supplemental pharmacological intervention—whereby cue-exposure sessions are presented in the absence of drug (Saladin et al., 2006, Lee et al., 2007, Price et al., 2009). However, the efficacy of trials in adults has been disappointing (reviewed by (Myers and Carlezon, 2010). Our finding that extinction in adults did not differ between EP training and UP training is consistent with these observations.

The EP paradigm retains many of the same concurrent cues present during conditioning, such as i.p. injections and confinement to one chamber. Associative learning studies have shown that concurrent cues interact with conditioning cues to affect the consequences of a conditioning trial (e.g., Rescorla, 1988). Importantly, concurrent stimuli interact in controlling change during extinction in much the same way as they do during acquisition (Rescorla, 2000). Therefore, EP training would be expected to enhance extinction, however it did not appear to facilitate extinction in adults. Since adults sufficiently achieved extinction after four days of UP training, a floor effect may have prohibited the assessment of any further facilitation of the EP paradigm. Alternatively, adolescents may be more sensitive to the associative summation provided by EP than adults. Indeed, D1R activity in the plPFC has been shown to influence behavioral responses to compound stimuli (Haddon and Killcross, 2007). Adolescents may therefore distinctly benefit from EP, and may particularly benefit from manipulation of plPFC activity.

Extinction of drug seeking is most valuable if relapse is also prevented. The high recidivism rate in human addicts that initiated drug use during adolescence (O'Brien and Anthony, 2005) is modeled by adolescent rats, which are more likely than adults to reinstate conditioned place preferences after extinction with UP (Brenhouse and Andersen, 2008).

That said, extinction efficacy can influence reinstatement (Neisewander et al., 2000, Kelamangalath et al., 2007). It was therefore important to investigate whether more effective extinction strategies will decrease reinstatement in adolescents. Adults and adolescents both have shown reinstatement to conditioned place preference for 20 mg/kg cocaine after UP extinction training (Mueller and Stewart, 2000, Brenhouse and Andersen, 2008). Following a priming injection of cocaine after extinction, subjects once again approach and remain in the presence of the drug-related stimuli, suggesting that the incentive salience of these stimuli are renewed (Mueller and Stewart, 2000). While we observed that EP training facilitated extinction in adolescent subjects, reinstatement was only marginally affected; no interaction between paradigm (UP vs. EP) and test (extinction score vs. reinstatement score) was found. Anecdotally, adolescents subjected to EP did not display significant reinstatement, although the variability was high (in 9 subjects) compared to pharmacological treatments. It is possible that EP-assisted learning is linked to a separate mechanism than that required to block reinstatement. However, extinction that was accompanied by pharmacological intervention with atomoxetine or a D1R agonist resulted in significantly attenuated reinstatement in adolescents.

A distinct circuit for drug-primed reinstatement involves the dopamine projection from the VTA directly to the anterior cingulate (including the pLPFC); projections from the pLPFC to the NAc are modified by VTA input to initiate a behavioral response. Normally, adolescents display a D1R-dominant profile in the pLPFC (Brenhouse et al., 2008), whereby activation of pLPFC projections to the NAc by a priming stimulus would readily recruit drug-cue information, even after extinction training. However, pLPFC D1R activation during extinction training appears to re-program the motivational salience circuitry of adolescents such that a new strong association of the context *not* paired with drug has taken the place of the initial context-drug pairing. In other words, with pharmacological intervention, adolescent subjects ‘focused’ on a new association, where normally less salient associations would have been ignored.

In conclusion, exploiting the adolescent's unique ability to learn about what is important should characterize new approaches toward the treatment of adolescent addiction. Whether adults could also benefit from the strategies we have shown to be effective in younger rats is certainly an important question—however, given the transient developmental profile of the adolescent pLPFC, it is likely these treatments will be selectively useful in the adolescent population. We have observed that while adult rats readily extinguish conditioned place preferences regardless of training paradigm, adolescents require specialized training to acquire strong enough extinction memories to compete with existing drug-context associations. Effects of pharmacological intervention with atomoxetine and SKF38393 suggest that this acquisition can occur via mechanisms targeted to PFC D1R. Since the developing brain responds differently to drugs of abuse (Spear, 2000, Andersen, 2003, Crews et al., 2007, Schramm-Sapota et al., 2009), treatments that target addiction during vulnerable developmental stages are crucial. Specifically, treatment of addiction during adolescence could reverse otherwise lifelong alterations in motivational processing (Andersen, 2003). Here we establish a potential direction for distinct strategies to treat this vulnerable population.

List of Abbreviations

BLA	basolateral amygdala
CS	conditioned stimulus
D1R	D1 dopamine receptor

EP	explicitly paired training
NAc	nucleus accumbens
plPFC	prelimbic prefrontal cortex
ilPFC	infralimbic prefrontal cortex
UP	un-explicitly paired training
US	unconditioned stimulus
VTA	ventral tegmental area

References

- Aguilar MA, Rodriguez-Arias M, Minarro J. Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev.* 2009; 59:253–277. [PubMed: 18762212]
- Alexander GE, Goldman PS. Functional development of the dorsolateral prefrontal cortex: an analysis utilizing reversible cryogenic depression. *Brain Res.* 1978; 143:233–249. [PubMed: 415802]
- Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev.* 2003; 27:3–18. [PubMed: 12732219]
- Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse.* 2000; 37:167–169. [PubMed: 10881038]
- Badanich KA, Adler KJ, Kirstein CL. Adolescents differ from adults in cocaine conditioned place preference and cocaine-induced dopamine in the nucleus accumbens septi. *Eur J Pharmacol.* 2006; 550:95–106. [PubMed: 17011546]
- Berretta S, Pantazopoulos H, Caldera M, Pantazopoulos P, Pare D. Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience.* 2005; 132:943–953. [PubMed: 15857700]
- Brenhouse HC, Andersen SL. Delayed extinction and stronger reinstatement of cocaine conditioned place preference in adolescent rats, compared to adults. *Behav Neurosci.* 2008; 122:460–465. [PubMed: 18410184]
- Brenhouse HC, Sonntag KC, Andersen SL. Transient D1 dopamine receptor expression on prefrontal cortex projection neurons: relationship to enhanced motivational salience of drug cues in adolescence. *J Neurosci.* 2008; 28:2375–2382. [PubMed: 18322084]
- Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH, Morin SM, Gehlert DR, Perry KW. Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology.* 2002; 27:699–711. [PubMed: 12431845]
- Carlezon WA Jr. Place conditioning to study drug reward and aversion. *Methods Mol Med.* 2003; 84:243–249. [PubMed: 12703329]
- Crews F, He J, Hodge C. Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav.* 2007; 86:189–199. [PubMed: 17222895]
- Economidou D, Pelloux Y, Robbins TW, Dalley JW, Everitt BJ. High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence. *Biol Psychiatry.* 2009; 65:851–856. [PubMed: 19181308]
- Everitt BJ, Wolf ME. Psychomotor stimulant addiction: a neural systems perspective. *J Neurosci.* 2002; 22:3312–3320. [PubMed: 11978805]
- Foltin RW, Haney M. Conditioned effects of environmental stimuli paired with smoked cocaine in humans. *Psychopharmacology (Berl).* 2000; 149:24–33. [PubMed: 10789879]
- Font L, Miquel M, Aragon CM. Involvement of brain catalase activity in the acquisition of ethanol-induced conditioned place preference. *Physiol Behav.* 2008; 93:733–741. [PubMed: 18155096]

- Fuchs RA, Weber SM, Rice HJ, Neisewander JL. Effects of excitotoxic lesions of the basolateral amygdala on cocaine-seeking behavior and cocaine conditioned place preference in rats. *Brain Res.* 2002; 929:15–25. [PubMed: 11852027]
- Fuster, J. *The Prefrontal Cortex: Anatomy, Physiology, and Neuropsychology of the Frontal Lobe.* Raven Press; New York: 1980.
- Graham DL, Hoppenot R, Hendryx A, Self DW. Differential ability of D1 and D2 dopamine receptor agonists to induce and modulate expression and reinstatement of cocaine place preference in rats. *Psychopharmacology (Berl).* 2006
- Haddon JE, Killcross S. Contextual control of choice performance: behavioral, neurobiological, and neurochemical influences. *Ann N Y Acad Sci.* 2007; 1104:250–269. [PubMed: 17344528]
- Hsu E, Packard MG. Medial prefrontal cortex infusions of bupivacaine or AP-5 block extinction of amphetamine conditioned place preference. *Neurobiol Learn Mem.* 2008; 89:504–512. [PubMed: 17905604]
- Jupp B, Lawrence AJ. New horizons for therapeutics in drug and alcohol abuse. *Pharmacol Ther.* 2010; 125:138–168. [PubMed: 19917308]
- Kalivas PW, Duffy P. Dopamine regulation of extracellular glutamate in the nucleus accumbens. *Brain Res.* 1997; 761:173–177. [PubMed: 9247082]
- Kalivas PW, Volkow N, Seamans J. Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *Neuron.* 2005; 45:647–650. [PubMed: 15748840]
- Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB. Development of the dopaminergic innervation in the prefrontal cortex of the rat. *J Comp Neurol.* 1988; 269:58–72. [PubMed: 3361004]
- Kelamangalath L, Swant J, Stramiello M, Wagner JJ. The effects of extinction training in reducing the reinstatement of drug-seeking behavior: involvement of NMDA receptors. *Behav Brain Res.* 2007; 185:119–128. [PubMed: 17826849]
- Kreibich AS, Blendy JA. cAMP response element-binding protein is required for stress but not cocaine-induced reinstatement. *J Neurosci.* 2004; 24:6686–6692. [PubMed: 15282271]
- Lee JH, Kwon H, Choi J, Yang BH. Cue-exposure therapy to decrease alcohol craving in virtual environment. *Cyberpsychol Behav.* 2007; 10:617–623. [PubMed: 17927528]
- Lin SK, Pan WH, Yeh PH. Prefrontal dopamine efflux during exposure to drug-associated contextual cues in rats with prior repeated methamphetamine. *Brain Res Bull.* 2007; 71:365–371. [PubMed: 17208653]
- Mueller D, Perdikaris D, Stewart J. Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. *Behav Brain Res.* 2002; 136:389–397. [PubMed: 12429400]
- Mueller D, Stewart J. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. *Behav Brain Res.* 2000; 115:39–47. [PubMed: 10996406]
- Myers KM, Carlezon WA Jr. Extinction of drug- and withdrawal-paired cues in animal models: Relevance to the treatment of addiction. *Neurosci Biobehav Rev.* 2010
- Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF. Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *J Neurosci.* 2000; 20:798–805. [PubMed: 10632609]
- O'Brien CP, Childress AR, McLellan T, Ehrman R. Integrating systemic cue exposure with standard treatment in recovering drug dependent patients. *Addict Behav.* 1990; 15:355–365. [PubMed: 2248109]
- O'Brien MS, Anthony JC. Risk of becoming cocaine dependent: epidemiological estimates for the United States, 2000-2001. *Neuropsychopharmacology.* 2005; 30:1006–1018. [PubMed: 15785780]
- Paolone G, Botreau F, Stewart J. The facilitative effects of D-cycloserine on extinction of a cocaine-induced conditioned place preference can be long lasting and resistant to reinstatement. *Psychopharmacology (Berl).* 2009; 202:403–409. [PubMed: 18695929]
- Peters J, Kalivas PW, Quirk GJ. Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn Mem.* 2009; 16:279–288. [PubMed: 19380710]
- Price KL, McRae-Clark AL, Saladin ME, Maria MM, DeSantis SM, Back SE, Brady KT. D-cycloserine and cocaine cue reactivity: preliminary findings. *Am J Drug Alcohol Abuse.* 2009; 35:434–438. [PubMed: 20014913]

- Quirk GJ, Mueller D. Neural Mechanisms of Extinction Learning and Retrieval. *Neuropsychopharmacology*. 2007
- Quirk GJ, Russo GK, Barron JL, Lebron K. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J Neurosci*. 2000; 20:6225–6231. [PubMed: 10934272]
- Reichel CM, Linkugel JD, Bevins RA. Nicotine as a conditioned stimulus: impact of attention-deficit/hyperactivity disorder medications. *Exp Clin Psychopharmacol*. 2007; 15:501–509. [PubMed: 17924784]
- Rescorla RA. Behavioral studies of Pavlovian conditioning. *Annu Rev Neurosci*. 1988; 11:329–352. [PubMed: 3284445]
- Rescorla RA. Extinction can be enhanced by a concurrent excitator. *J Exp Psychol Anim Behav Process*. 2000; 26:251–260. [PubMed: 10913990]
- Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, Davis M. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Arch Gen Psychiatry*. 2004; 61:1136–1144. [PubMed: 15520361]
- Saladin ME, Brady KT, Graap K, Rothbaum BO. A preliminary report on the use of virtual reality technology to elicit craving and cue reactivity in cocaine dependent individuals. *Addict Behav*. 2006; 31:1881–1894. [PubMed: 16516397]
- Sanchez CJ, Bailie TM, Wu WR, Li N, Sorg BA. Manipulation of dopamine d1-like receptor activation in the rat medial prefrontal cortex alters stress- and cocaine-induced reinstatement of conditioned place preference behavior. *Neuroscience*. 2003; 119:497–505. [PubMed: 12770563]
- Schell TL, Orlando M, Morral AR. Dynamic effects among patients' treatment needs, beliefs, and utilization: a prospective study of adolescents in drug treatment. *Health Serv Res*. 2005; 40:1128–1147. [PubMed: 16033496]
- Schramm-Sapota NL, Walker QD, Caster JM, Levin ED, Kuhn CM. Are adolescents more vulnerable to drug addiction than adults? Evidence from animal models. *Psychopharmacology (Berl)*. 2009; 206:1–21. [PubMed: 19547960]
- Schroeder JP, Packard MG. Systemic or intra-amygdala injections of glucose facilitate memory consolidation for extinction of drug-induced conditioned reward. *Eur J Neurosci*. 2003; 17:1482–1488. [PubMed: 12713651]
- Schroeder JP, Packard MG. Facilitation of memory for extinction of drug-induced conditioned reward: role of amygdala and acetylcholine. *Learn Mem*. 2004; 11:641–647. [PubMed: 15466320]
- Seamans JK, Yang CR. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol*. 2004; 74:1–58. [PubMed: 15381316]
- Sherwood, N.; Timeras, P. *A Stereotaxic Atlas of the Developing Rat Brain*. University of California Press; Los Angeles: 1970.
- Sofuoglu M. Cognitive enhancement as a pharmacotherapy target for stimulant addiction. *Addiction*. 2010; 105:38–48. [PubMed: 20078461]
- Spear L. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev*. 2000; 24:417–463. [PubMed: 10817843]
- Taylor JR, Olsson P, Quinn JJ, Torregrossa MM. Targeting extinction and reconsolidation mechanisms to combat the impact of drug cues on addiction. *Neuropharmacology*. 2009; 56(Suppl 1):186–195. [PubMed: 18708077]
- Ventura R, Alcaro A, Puglisi-Allegra S. Prefrontal cortical norepinephrine release is critical for morphine-induced reward, reinstatement and dopamine release in the nucleus accumbens. *Cereb Cortex*. 2005; 15:1877–1886. [PubMed: 15728739]
- Ventura R, Morrone C, Puglisi-Allegra S. Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. *Proc Natl Acad Sci U S A*. 2007; 104:5181–5186. [PubMed: 17360372]
- Walker DL, Ressler KJ, Lu KT, Davis M. Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *J Neurosci*. 2002; 22:2343–2351. [PubMed: 11896173]
- Waylen A, Wolke D. Sex 'n' drugs 'n' rock 'n' roll: the meaning and social consequences of pubertal timing. *Eur J Endocrinol*. 2004; 151(Suppl 3):U151–159. [PubMed: 15554900]

Figure 1.
Timeline illustrating testing and training procedures in Experiment 1; UP: un-explicit paired training; EP: explicitly-paired training

Figure 2. Effect of un-explicitly paired (UP) and explicitly paired (EP) training on extinction of cocaine-conditioned place preferences after four days of training in adolescents and adults. *p<0.05 difference between paradigms.

Figure 3.

Effect of un-explicitly paired (UP) and explicitly paired (EP) extinction training on subsequent drug-primed reinstatement in adolescents and adults. No interaction between training paradigm, test, and age was found. # signifies $p < 0.05$ between extinction score and reinstatement score within each training group.

Figure 4. Effect of atomoxetine treatment during un-explicitly paired (UP) extinction training on extinction (left) and reinstatement (right) in adolescents. * $p < 0.05$ difference between treatment groups.

Figure 5.

Effect of plPFC microinjections of D1 dopamine receptor agonist SKF38393 during un-explicit (UP) extinction training on extinction (middle) and reinstatement (right) in adolescents. * $p < 0.05$ difference between treatment groups. Left panel: verified placements of SKF38393 microinjections; numbers next to atlas plates depict mm from bregma; shaded areas depict total area within which all placements were found.