The interactive effects of environmental enrichment and extinction interventions in attenuating cue-elicited cocaine-seeking behavior in rats

Kenneth J. Thiel [a], Ben Engelhardt [a], Lauren E. Hood [a], Natalie A. Peartree [a], Janet L. Neisewander [a,b,*]

[a] Department of Psychology, Arizona State University, P.O. Box 871104, Tempe, AZ 85287-1104, United States
[b] The School of Life Sciences, Arizona State University, P.O. Box 874501, Tempe, AZ 85287-4501, United States

ABSTRACT

Cues associated with cocaine can elicit craving and relapse. Attempts have been made to employ extinction therapy, which is aimed at attenuating the incentive motivational effects of cocaine cues, as a treatment for cocaine addiction; however, this approach has been largely unsuccessful perhaps due to the inability to extinguish all cues associated with cocaine use while in a clinic. Recently, environmental enrichment (EE) during abstinence has been proposed as a strategy to attenuate cue-elicited cocaine craving. The present study used an animal model to examine whether the utility of extinction toward attenuating cue-elicited cocaine-seeking behavior could be enhanced by also providing EE. All rats were trained to self-administer cocaine while housed in isolated conditions and then subsequently underwent 17 days of forced abstinence, during which they were either housed in pairs or under EE and they either received daily 1-h extinction sessions or similar handling without exposure to the self-administration environment. Following this intervention period, all rats were tested for cue-elicited cocaine-seeking behavior. To examine whether effects of these interventions persist, all rats were subsequently single-housed for an additional 7-day forced abstinence period, followed by a second test for cue-elicited cocaine-seeking behavior. We found that although daily extinction training and EE each attenuated subsequent cue-elicited cocaine-seeking behavior, the combined treatment of extinction training + EE completely prevented it. However, once these interventions were discontinued, their protective effects diminished. These findings suggest that combining behavioral therapy approaches may improve outcomes; however, future work is needed to improve the longevity of these strategies beyond their implementation.

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1. Introduction

Cocaine addiction is a chronic relapsing disorder for which effective treatments have remained largely elusive. Craving, which can be defined as an intense desire to re-experience drug effects, is thought to drive cocaine taking and relapse (Markou et al., 1993). Craving that is elicited upon exposure to cocaine-related cues (e.g., discrete paraphernalia, cocaine-associated environments) is particularly problematic because it can persist for many months despite abstinence (Gawin and Kleber, 1986; O'Brien, 2008). As an underlying construct of craving, incentive motivation for cocaine is thought to be reflected by cocaine-seeking behavior in animals, which is operationally defined as operant responses previously reinforced by drug that are performed in the absence of drug reinforcement (de Wit and Stewart, 1981; Fuchs et al., 1998). Furthermore, after extinguishing

cocaine-seeking behavior, the incentive motivational effects of discrete cues that had been paired with drug infusions can be measured by their ability to reinstate cocaine-seeking behavior.

A clinical treatment directed at preventing relapse has been to extinguish craving responses elicited by drug stimuli via repeated exposure to these stimuli in the absence of drug reinforcement, similar to preclinical extinction models (Myers and Carlezon, 2010; Taylor et al., 2009). Unfortunately, this strategy has fallen short in preventing relapse when used by itself (Conklin and Tiffany, 2002; Havermans and Jansen, 2003), likely because the therapy takes place in a clinical setting, leaving the motivational effects of situational/contextual components of drug use that take place outside of the clinic largely intact (Bouton, 2002; Childress et al., 1988; Tobena et al., 1993).

Interactions between discrete and contextual drug-associated cues in motivation for cocaine have been demonstrated in animals. Cue-elicited cocaine-seeking behavior is reduced in animals undergoing extinction training in the cocaine-paired context relative to controls that undergo an equivalent period of forced abstinence without exposure to the cocaine-paired context (Di Ciano and Everitt, 2002; Kelamangalath et al., 2007; Kelamangalath and Wagner, 2009). These findings suggest that extinguishing some of an individual's drug-associated stimuli may lessen the impact of other stimuli that have

not undergone extinction. Nevertheless the impact of this approach is limited if extinction occurs in a novel context because the incentive motivational effects of discrete cocaine-paired cues are renewed upon re-exposure to the self-administration context (Crombag et al., 2002; Crombag and Shaham, 2002; Kearns and Weiss, 2007). Furthermore, cue-elicited incentive motivation for cocaine persists over time (Ciccocioppo et al., 2004; Weiss et al., 2001), and actually becomes stronger during the first months of abstinence (Grimm et al., 2001; Neisewander et al., 2000; Tran-Nguyen et al., 1998), whereas the effectiveness of an extinction training intervention within the cocaine-paired context to subsequently reduce cue-elicited cocaine-seeking behavior does not persist once rats are placed into abstinence for an additional length of time (Di Ciano and Everitt, 2002; Kelamangalath and Wagner, 2009). Thus, it is important to explore treatment strategies that may produce longer lasting protection.

Recently, we and others have explored environmental enrichment (EE) as a novel intervention strategy aimed at reducing cue-elicited incentive motivation for cocaine when introduced during forced abstinence (Chauvet et al., 2009; Thiel et al., 2009). Preclinically, EE refers to living conditions in which animals have access to social, physical, and cognitive stimulation (van Praag et al., 2000). This intervention may promote the overall well-being of a recovering drug addict through a major lifestyle change involving social interaction, novelty, and physical exercise, which may in turn reduce cravings and prevent relapse. It is not yet known whether the protective effects of an EE intervention in attenuating cue-elicited incentive motivation will persist beyond the period in which it is implemented.

The purpose of the present study was 2-fold. First, we examined whether extinction training within a drug-paired context in combination with EE during a period of forced abstinence offers additional protective effects beyond either of these treatments given alone. Second, we examined whether the protective effects of these treatments persist beyond their cessation. We predicted that the combination of EE and extinction training would afford the greatest protection against subsequent cue-elicited cocaine-seeking behavior, and that the protective effects would will persist once the rats were returned to non-enriched housing conditions.

2. Method

2.1. Animals and surgery

Adult male Sprague–Dawley rats weighing 225–250 g upon arrival were housed under standard isolated conditions of 1 rat/cage (21.6×45.7×17.8 cm) with only food and water available in a colony room with a 12-h reverse light:dark cycle (lights off at 07:00 h). Care and housing were in adherence to the Guide for the Care and Use of Laboratory Animals (1996). Rats were acclimated to handling for 5 days prior to surgically implanting intravenous (IV) catheters under 2–3% isoflurane anesthesia using procedures described previously (Kufahl et al., 2009). Catheters were flushed daily with 0.1 ml saline containing heparin sodium (70 U/ml; APP Pharmaceuticals, Schaumburg, IL) and Timentin (66.7 mg/ml; GlaxoSmithKline, Research Triangle Park, NC) to maintain patency. Proper catheter function was tested periodically by administering 0.05 ml methohexital sodium (16.7 mg/ml; JHP Pharmaceuticals, Rochester, MI), a dose that produces transient anesthetic effects when administered IV.

2.2. Self-administration

Fig. 1 illustrates the timeline of procedures across the study. It is important to note that all rats remained housed in isolated conditions throughout the self-administration phase of the study. After recovery from surgery, rats underwent 15 consecutive days of cocaine self-administration training in operant conditioning chambers (30×25×25 cm; Med Associates, St. Albans, VT) for 3 h/day during their dark cycle. Initially, sessions began with an FR1 schedule of reinforcement and progressed to a VR5 schedule based on individual performance, with the latter in effect exclusively during the last 5–8 sessions. Schedule completions on a designated lever (i.e., active lever; located on the left side of the chamber) resulted in simultaneous presentation of a tone (500 Hz, 10 db above background), cue light above the lever, and house light, followed one second later by a cocaine infusion (0.75 mg/kg/0.1 ml, IV). Upon completion of the 6-s infusion, the cue light and tone ceased, but the house light remained on for an additional 20-s time-out. Responses on another lever (i.e., inactive lever; located on the right side of the chamber) produced no consequences. The dose of cocaine is based on the salt form of the drug (i.e., cocaine hydrochloride; RTI International Triangle Park, NC), which was dissolved in saline and filtered through a 0.2 μm membrane prior to use. Rats were restricted to 16 g of food/day beginning 2 days before training to facilitate exploration. A rat remained food-restricted until a criterion of ≥ 21 infusions/3 h was achieved on 2 consecutive days, after which food was available *ad libitum* in the home cage throughout the remainder of the experiment. All rats had reached this criterion by the 10th session.
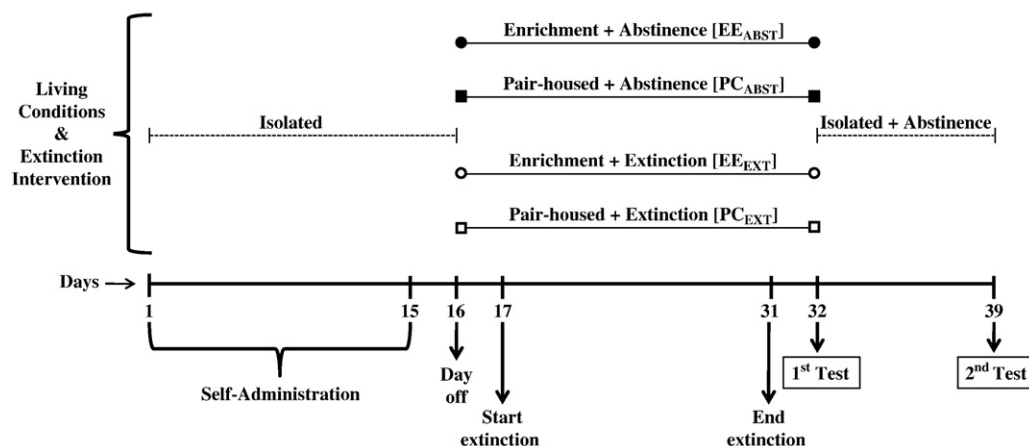

Fig. 1. Experimental timeline. On Day 16, the rats were placed into their assigned intervention living conditions, where they remained until the first test day. The daily extinction intervention (or transportation to alternate environment) began on Day 17 and continued through Day 31. To examine persistence of intervention effects, all rats were again placed into isolated housing immediately after the test on Day 32, where they remained until the 2nd test day. On the test days, all groups were tested under extinction conditions for 2 h, followed by a 1-h reinstatement test phase that began with a programmed presentation of the cocaine-paired stimulus complex, which was then available response-contingently on an FR1 schedule thereafter.

2.3. Intervention conditions

Following self-administration training, all rats were given a day off to allow for cocaine clearance from the last self-administration session. During this day off, rats were placed into new living conditions (see Day 16 in Fig. 1). Rats were assigned to 1 of the following 4 groups (n = 10–11/group) that were either housed under EE or a Pair-housed condition (PC) during a forced abstinence period, and either underwent Extinction training (EXT) or similar handling without extinction during Abstinence (ABST): Enriched/Extinction (EE$_{EXT}$), Enriched/Abstinent (EE$_{ABST}$), Pair-housed/Extinction (PC$_{EXT}$), Pair-housed/Abstinent (PC$_{ABST}$). EE consisted of large plastic tubs (74 × 91 × 36 cm) that housed 5 rats and contained bedding, nesting material, 3 PVC pipes, 2 running wheels, 2 water bottles, 2 food dishes, and 2 small plastic toys. Toys were continually changed 3 times/week to maintain novelty. The pair-housed condition, rather than continued isolated housing, was used in order to avoid the possibility of potential isolation stress affecting subsequent incentive motivation elicited by the cocaine-paired context and cues on the first test day.

To prevent the catheter from being chewed while under social housing, aluminum thread spacers (6/32") were screwed onto the plastic threaded connecter. Group assignment was matched to the extent possible for total cocaine intake and active lever pressing behavior during self-administration. Extinction groups underwent 15 daily 1-h extinction sessions within the self-administration chambers (i.e., context extinction). During these sessions, active and inactive lever presses were recorded but produced no scheduled consequences. To control for handling and time away from their assigned living conditions, Abstinence groups were transported to an alternate environment and were placed into gray plastic holding cages of similar size as the self-administration chambers, but with different bedding and visual cues, and with no levers available. Abstinence rats were transported at the same time of day as the Extinction rats, but the alternate environments were located in a different room from where self-administration had taken place.

2.4. Tests for cocaine-seeking behavior

On the first test day (i.e., Day 32 in Fig. 1), all rats were transported to their self-administration chamber and tested under extinction conditions for 2 h (i.e., lever presses produced no scheduled consequences). Subsequently, all rats were passively presented with the light/tone cue complex previously associated with cocaine, and then for the next 60 min, active lever presses resulted in FR1 presentation of this cue complex (i.e., cue reinstatement).

After this first test, all rats were placed into isolated living conditions for the next 7 days of forced abstinence in order to test for persistence of the effects of the previous interventions (i.e., EE and/or extinction training). Isolated housing for all rats, rather than pair-housing, was chosen for this phase for two reasons: 1. We wanted the previously pair-housed rats to also experience some degree of change in living conditions in order to control for whatever novelty effect this manipulation, *per se*, may have on incentive motivation; and 2. We have examined aggressive behavior among adult rats taken out of enriched conditions and placed into smaller cages with a partner (unpublished observation), and so we wanted to avoid the possible confound of excessive in-fighting affecting incentive motivation. Rats subsequently were given a 2nd test day (i.e., Day 39 in Fig. 1) whereby they underwent the same testing procedure as given 7 days earlier (i.e., 2-h extinction phase followed by a 1-h cue reinstatement phase).

2.5. Data analysis

Cocaine-seeking behavior was operationally defined as active lever responses in the absence of cocaine reinforcement. During the 15-day extinction intervention training period (i.e., Days 17–31 in Fig. 1),

separate mixed factorial ANOVAs were used to analyze active and inactive lever presses with Living Condition (PC vs. EE) as a between subject variable and Extinction Session (1–15) as a repeated measure.

An additional two factor ANOVA was conducted to analyze time-dependent differences in cocaine-seeking behavior between rats whose first extinction session began with the immediate extinction intervention versus rats whose first extinction session began after the intervention period (i.e., on the first test day) with Living Condition (PC vs. EE) and Initial Abstinence Length before first extinction session (i.e., 2 vs. 17 days) as between subject variables. Importantly, the first extinction session for the rats that did not undergo the daily extinction intervention (i.e., Abstinence rats) was 2-h long whereas the first extinction session for the rats that underwent the daily extinction intervention (i.e., Extinction rats) was only 1-h long; therefore, only the first 1-h of cocaine-seeking behavior for the Abstinence rats was analyzed in this particular ANOVA.

On each of the two test days, separate mixed factorial ANOVAs were used to analyze active and inactive lever responses across each test phase (i.e., extinction and cue reinstatement) and at the transition from one phase to the next, with Living Condition (PC vs. EE) and Extinction History (Extinction vs. Abstinence) as between subject variables and 20-min time interval as a repeated measure. In addition, planned paired-sample t-tests were conducted for each individual group comparing the last 20 min of the extinction phase to the first 20 min of the cue reinstatement phase in order to specifically test our hypothesis that the combined intervention conditions would have the greatest protective effect against cue reinstatement. Total active and inactive lever presses for each test phase were analyzed using separate 2-way ANOVAs with Living Condition and Extinction History as between subject variables. Significant interactions were further analyzed using Newman–Keuls tests.

3. Results

Cocaine intake and response rates were similar across the groups. Infusions and response rates averaged over the last 5 sessions of training are presented in Table 1.

3.1. Extinction

The PC$_{EXT}$ and EE$_{EXT}$ groups underwent 15 consecutive daily 1-h sessions of extinction training to devalue the motivational significance of the self-administration environment. Responses on the active and inactive lever across extinction are presented in Fig. 2. The mixed factor ANOVA for active lever presses across extinction revealed a Living Condition × Extinction Session interaction ($F(14,255) = 3.85, p < 0.001$). Post hoc analyses revealed that PC$_{EXT}$ demonstrated higher response rates on sessions 1–4 and 7–13 than the EE$_{EXT}$ rats (Newman–Keuls tests, $p < 0.05$). Active lever presses significantly declined in both groups evident as a decrease during the last 5 days of extinction (i.e., sessions 11–15) relative to the first day of extinction (Newman–Keuls tests, $p < 0.05$). Responses on the inactive lever throughout extinction did not differ between the groups.

Table 1
Mean (±SEM) reinforcement rates and active and inactive lever presses during self-administration training.

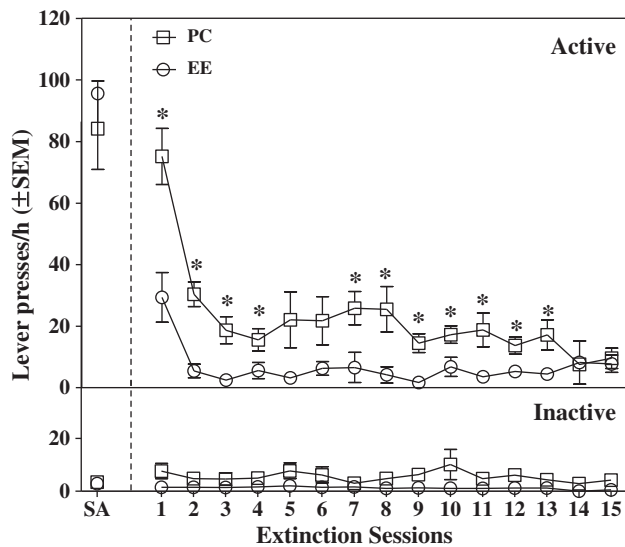| Group (n)	Reinforcement rates		Response rates, last 5 sessions	
	Total infusions	Infusions/session, last 5 sessions	Active lever	Inactive lever
EE$_{EXT}$ (10)	504.8 ± 57.3	42.3 ± 2.4	237.5 ± 37.3	5.4 ± 3.2
EE$_{ABST}$ (10)	526.1 ± 35.9	40.3 ± 2.8	223.8 ± 25.3	6.7 ± 3.3
PC$_{EXT}$ (10)	529.1 ± 28.2	43.1 ± 2.3	249.4 ± 51.2	4.4 ± 1.9
PC$_{ABST}$ (11)	499.1 ± 47.8	42.7 ± 3.2	240.5 ± 42.5	4.5 ± 2.8

Fig. 2. Lever presses ± SEM on the active (top) and inactive (bottom) levers across the 15 sessions of extinction training in groups of rats that either lived in pair-housed (PC) or environmentally enriched (EE) conditions. Lever presses/h on the last day of self-administration training is represented for comparison (i.e., SA). Note that during self-administration, both groups of rats were living in isolated conditions. Both groups demonstrated a decline in active lever pressing across extinction, although PC rats demonstrated greater overall responding. The asterisk (*) indicates greater active lever presses in PC rats relative to EE rats during a particular extinction session (p<0.05, Newman–Keuls). There were no group differences in inactive lever pressing.

Time-dependent differences in cocaine-seeking behavior were observed across groups that were tested for the first time either 2 or 17 days after their last self-administration session (i.e., corresponding to Days 17 and 32 in Fig. 1). The analysis of cocaine-seeking behavior during the first hour of the first extinction session revealed a significant Living Condition × Initial Abstinence Length interaction ($F(1,36) = 5.66$, $p<0.05$). Post hoc analyses revealed higher rates of responding in rats whose first extinction session was 17 days after self-administration ended (i.e., rats in the Abstinence condition during the intervention) than those rats whose first extinction session was 2 days after self-administration ended (i.e., rats that underwent the Extinction intervention), and this was evident in both PC and EE groups (Newman–Keuls tests, $p<0.05$). PC rats given their first extinction session 2 versus 17 days after self-administration exhibited mean (+SEM) active lever responses/h of 75.2 + 9.1 and 185.5 + 12.2, respectively. EE rats given their first extinction session 2 versus 17 days after self-administration exhibited mean (+SEM) active lever responses/h of 29.4 + 8.1 and 83.5 + 16.1, respectively.

3.2. Intervention effects on extinction and cue reinstatement

Cocaine-seeking behavior on the 1st test day (i.e., Day 32 in Fig. 1) across time, as well as totaled for each test phase, is illustrated in Fig. 3A and B, respectively. Context-elicited cocaine-seeking behavior was reduced in the Extinction groups relative to the Abstinence groups during the extinction phase, which was most evident in the first hour of testing. One PC$_{ABST}$ rat was an outlier during the first two 20-min intervals of the extinction phase (>300 lever presses; >5 standard deviations above the group means without him) and was therefore excluded from only the analyses involving this phase, but was included in analyses of the subsequent phases where his behavior conformed to the group mean. There was a Living Condition × Extinction History × Time interaction ($F(5,180) = 7.86$, $p<0.001$) for active lever presses during this phase. The PC$_{ABST}$ group exhibited more cocaine-seeking behavior than all other groups during intervals 1–2 ($p<0.05$, Newman–Keuls). The EE$_{ABST}$ group also exhibited more cocaine-seeking behavior than both Extinction groups during interval
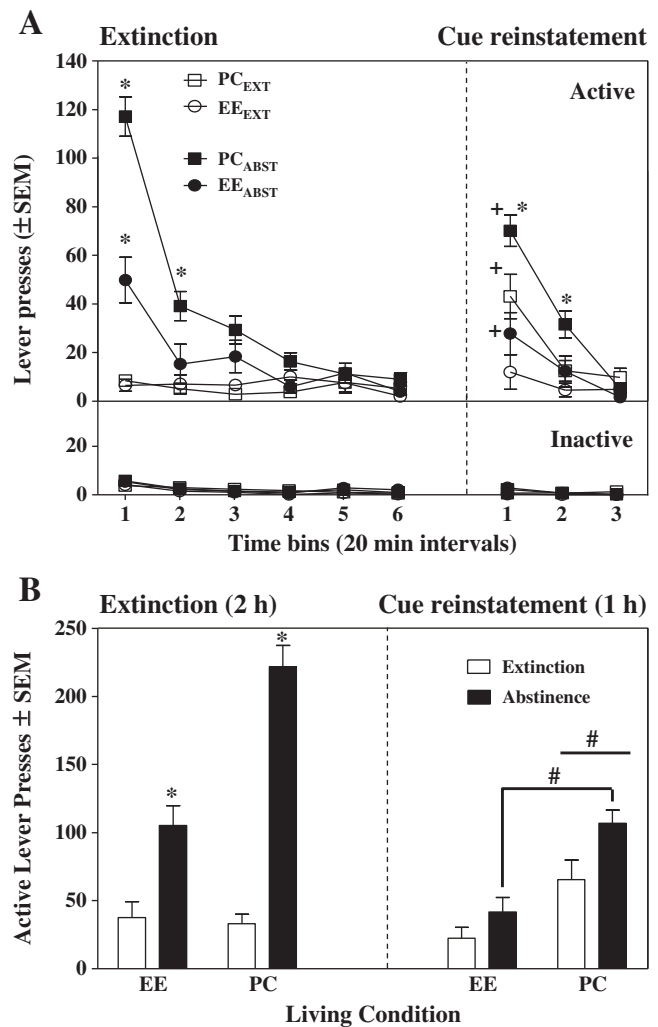

Fig. 3. Context extinction and cue reinstatement on the first test day, which occurred 17 days after self-administration had ended [i.e., the last day of the intervention(s)]: (A) Cocaine-seeking behavior illustrated as active lever presses (± SEM) across 20-min intervals; inactive lever presses are illustrated below for comparison. Group designations indicate whether the animals were living in pair-housed conditions (PC) or environmental enrichment (EE) during extinction (PC$_{EXT}$ or EE$_{EXT}$, respectively) or during abstinence (PC$_{ABST}$ or EE$_{ABST}$, respectively). (B) Total cocaine-seeking behavior (i.e., active lever presses ± SEM) collapsed across time for each test phase (i.e., 2 h extinction and 1 h cue reinstatement). The extinction phase began by placing the rats into the self-administration chambers, and responses produced no consequences during this phase. The cue reinstatement phase began 2 h later by presenting the light/tone cues previously associated with cocaine infusions, and thereafter response-contingent cues were presented on an FR1 schedule for 1 h. The asterisk (*) represents a difference from all other groups ($p<0.05$). The cross (+) represents a difference from previous 20-min interval ($p<0.05$). The pound (#) represents main effects of Living Condition and Extinction History ($p<0.05$).

1 ($p<0.05$, Newman–Keuls). Inactive lever pressing decreased across this phase at a similar rate for all groups [main effect of Time ($F(5,180) = 12.01$, $p<0.01$)]. Total cocaine-seeking behavior during the extinction phase revealed a Living Condition × Extinction History interaction ($F(1,36) = 23.92$, $p<0.001$), with the PC$_{ABST}$ group higher than all other groups, and the EE$_{ABST}$ group higher than both Extinction groups ($p<0.05$, Newman–Keuls; Fig. 3B).

Analyses of cocaine-seeking behavior during the transition from the last 20 min of extinction to the first 20 min of cue reinstatement revealed both Living Condition × Time ($F(1,36) = 13.37$, $p<0.001$) and an Extinction History × Time interactions ($F(1,36) = 4.20$, $p<0.05$). Post hoc analyses revealed that PC groups demonstrated higher initial reinstatement than EE groups, and Abstinence groups demonstrated higher initial reinstatement than Extinction groups

(Newman–Keuls, $p < 0.05$). Subsequent paired-sample t-tests revealed that the PC$_{ABST}$ ($t(9) = 7.4$, $p < 0.001$), PC$_{EXT}$ ($t(9) = 3.8$, $p < 0.01$), and EE$_{ABST}$ ($t(9) = 2.8$, $p < 0.01$) groups demonstrated initial cue reinstatement; importantly, the EE$_{EXT}$ group did not reinstate ($t(9) = 1.4$, $p = 0.17$). Analysis of cue-elicited cocaine-seeking behavior throughout the cue reinstatement phase revealed a Living Condition × Extinction History × Time interaction ($F(2,74) = 6.10$, $p < 0.01$). Post hoc analyses revealed higher active lever responding in the PC$_{ABST}$ group relative to all other groups during intervals 1–2, and higher responding in the PC$_{EXT}$ group relative to EE$_{EXT}$ group during interval 1 (Newman–Keuls, $p < 0.05$). There were no group differences in inactive lever responding during this phase.

Total cocaine-seeking behavior during the cue reinstatement phase is illustrated in Fig. 3B. The ANOVA revealed main effects of both Living Condition ($F(1,37) = 24.47$, $p < 0.001$) and Extinction History ($F(1,37) = 7.76$, $p < 0.01$). Thus, the PC groups demonstrated greater total cue reinstatement than the EE groups, and the Abstinence groups demonstrated greater total cue reinstatement than the Extinction groups. Strong main effects may have masked detection of an interaction; therefore, given the notable graded decrease in total cue reinstatement across the groups, we conducted a trend analysis of these data. The trend analysis revealed a significant linear trend ($F(1,37) = 41.72$, $p < 0.001$), with the PC$_{ABST}$ exhibiting the highest response rates, followed by PC$_{EXT}$, then EE$_{ABST}$, and then finally the EE$_{EXT}$, which exhibited the lowest response rates.

3.3. Extinction and cue reinstatement 7 days post-intervention

"Post" has been added to the group labels in reference to the point that rats were returned to isolated housing for 7 days prior to the 2nd test day (i.e., Day 39 in Fig. 1), which was done to determine the persistence of effects of the previous interventions. During the 7 days of additional abstinence, one rat from the Post-EE$_{EXT}$ group lost his headpiece and was therefore not tested. Cocaine-seeking behavior on this 2nd test day across time, as well as totaled for each test phase, is illustrated in Fig. 4A and B, respectively. Analyses of cocaine-seeking behavior from the last 20 min of the cue reinstatement phase on the first test day relative to the first 20 min of extinction on this second test day revealed only a strong main effect of Time ($F(1,36) = 35.85$, $p < 0.001$), suggesting an increase in cocaine-seeking behavior across groups. During the extinction phase, cocaine-seeking behavior was reduced in all groups by the end of the phase as evidenced by a main effect of Time ($F(5,180) = 7.86$, $p < 0.001$). Inactive lever pressing also decreased across this phase at a similar rate regardless of group [main effect of Time ($F(5,180) = 11.55$, $p < 0.01$)]. Total cocaine-seeking behavior during the extinction phase revealed only a main effect of previous Living Condition ($F(1,36) = 3.29$, $p < 0.05$), indicating that Post-EE groups exhibited an increase in cocaine-seeking behavior relative to Post-PC groups.

Analysis of cocaine-seeking behavior during the transition from the last 20 min of extinction to the first 20 min of cue reinstatement revealed a main effect of Time ($F(1,36) = 67.35$, $p < 0.001$) and a Living Condition × Time interaction ($F(1,36) = 12.10$, $p < 0.001$). Post hoc analyses revealed that all groups demonstrated significant cue reinstatement, but the Post-PC groups demonstrated greater cue reinstatement than the Post-EE groups (Newman–Keuls, $p < 0.05$). Paired-sample t-tests confirmed that all groups demonstrated some degree of initial cue reinstatement [i.e., Post-PC$_{ABST}$ ($t(10) = 4.7$, $p < 0.001$), Post-PC$_{EXT}$ ($t(9) = 7.8$, $p < 0.001$), Post-EE$_{ABST}$ ($t(9) = 3.1$, $p < 0.01$), and Post-EE$_{EXT}$ ($t(8) = 2.6$, $p < 0.05$)].

Analysis of cue-elicited cocaine-seeking behavior throughout the cue reinstatement phase revealed both Living Condition × Time and Extinction History × Time interactions ($F(2,70) = 4.50$, $p < 0.05$; $F(2,70) = 2.95$, $p < 0.05$, respectively). Post hoc analyses again revealed that during interval 1, the Post-PC groups demonstrated greater cue reinstatement behavior than the Post-EE groups. In addition, during interval 1, the Post-
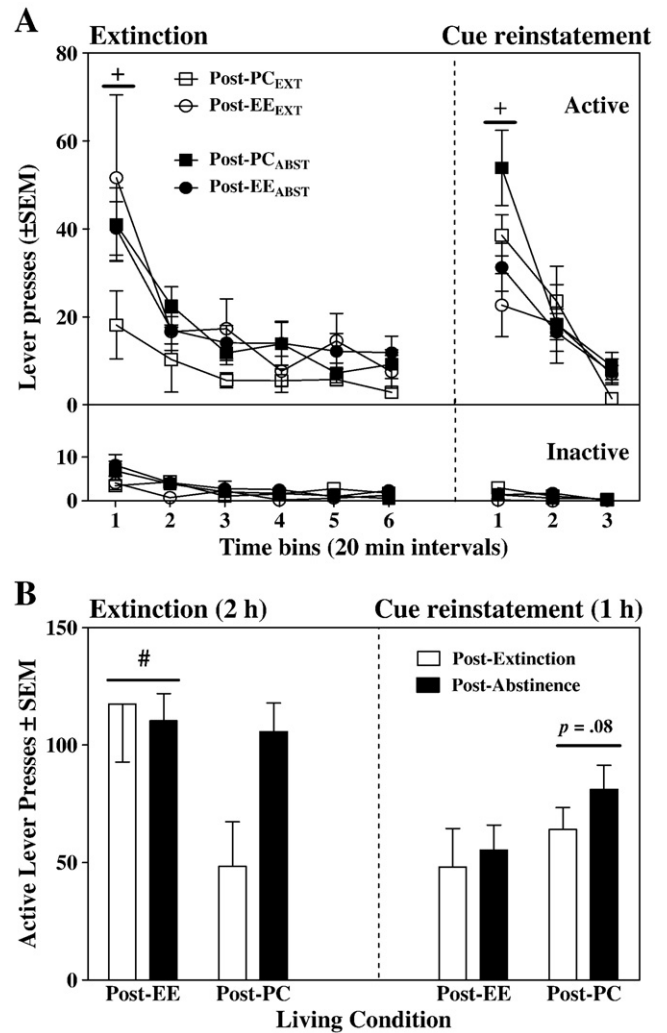

Fig. 4. Context extinction and cue reinstatement on the second test day, which occurred 7 days after the intervention(s) had ended: (A) Cocaine-seeking behavior illustrated as active lever presses ($\pm$ SEM) across 20-min intervals; inactive lever presses are illustrated below for comparison. (B) Total cocaine-seeking behavior (i.e., active lever presses $\pm$ SEM) collapsed across time for each test phase (i.e., 2 h extinction and 1 h cue reinstatement); phase descriptions are provided in the caption of Fig. 3). Note that all rats were living in isolated, abstinent conditions at the time of this test. Therefore, the group designations refer to the intervention conditions that the rats had undergone following self-administration, hence the qualifier "Post" designates that the intervention(s) had ended. Cocaine-seeking behavior declined across the context extinction test phase (i.e., main effect of time) and was then reinstated by response-contingent cue presentation for all previous intervention groups. The cross ($+$) represents a difference from the last 20-min interval of context extinction (main effect, $p < 0.05$). The pound (#) represents main effect of Living Condition ($p < 0.05$).

Abstinence groups demonstrated greater cue reinstatement behavior than the Post-Extinction groups (Newman–Keuls, $p < 0.05$). There were no group differences in inactive lever responding during this phase. Total cocaine-seeking behavior during the cue reinstatement phase is illustrated in Fig. 4B. The analysis revealed only a strong trend toward a main effect of Living Condition ($F(1,36) = 3.18$, $p = 0.08$). In addition, although there was a general pattern of a graded decrease in total cue-elicited cocaine-seeking behavior across groups, there was no significant trend across groups.

4. Discussion

The present findings provide evidence that a combined behavioral intervention that consists of extinguishing the incentive motivation effects of the self-administration environment and providing EE

during abstinence affords strong protection against incentive motivation for cocaine that is elicited by discrete drug-paired cues. Although both intervention approaches alone were effective in attenuating cue reinstatement, only the combined intervention completely prevented initial cue reinstatement. The benefit of the interventions in decreasing overall cue reinstatement exhibited a linear pattern with extinction alone as the least effective, enrichment alone as more effective, and the combination of both as the most effective. However, when the rats were removed from their intervention conditions and placed into isolated forced abstinence for an additional week, rats previously in EE exhibited slightly higher context-elicited cocaine-seeking behavior than rats previously in PC. Furthermore, the subsequent test for discrete cue-elicited reinstatement of cocaine-seeking behavior failed to support our hypothesis that the protective effects of a combined extinction/EE intervention would confer the greatest lasting protection against cue-elicited incentive motivation for cocaine, although there were mild lasting effects of the previous EE and extinction interventions in general. It is important to note that these protective effects had diminished relative to the first test, and that only a trend toward a lasting protective effect of previous enrichment (regardless of extinction history) was observed during the cue reinstatement phase. Overall, the findings are encouraging in terms of support for combined behavioral approaches of EE and extinction therapy as an anti-relapse intervention.

A potential behavioral mechanism for the protective EE effects against cue-elicited cocaine-seeking behavior in the present study is enhanced extinction learning, as suggested previously for EE effects on reducing cue-elicited sucrose-seeking behavior (Grimm et al., 2008). Indeed, EE enhances learning on a number of measures, including spatial learning, reinforcement contingencies, place preference, and discrimination tasks (Bardo et al., 1995; Pham et al., 1999; Rosenzweig and Bennett, 1996; Smith et al., 2005; van Praag et al., 2000). Although we cannot rule out that EE rats may have learned more quickly that cocaine-associated stimuli no longer predicted cocaine reinforcement, it is important to note that both across days and within a given extinction test session, decreases in response rates appear to be similar between PC and EE rats. Thus, rather than differences in extinction rate, there instead appeared to be an initial blunting of cocaine-seeking behavior. We suggest that this pattern is more consistent with EE-induced attenuation of the incentive motivational effects of the cocaine-paired cues. These findings are in line with a previous report demonstrating that EE blunts cocaine-seeking behavior on the first day of extinction, and subsequently throughout training, without appearing to facilitate extinction learning (i.e., rate of extinction across days) *per se* (Chauvet et al., 2009).

An alternative explanation for the EE effects is nonspecific changes in activity or fatigue. This explanation seems unlikely, however, given that Grimm et al. (2008) have demonstrated that there are no differences in locomotion between EE and standard-housed rats when placed into an operant chamber. Furthermore, EE rats exhibit high response rates when engaged in sucrose reinforcement (Green et al., 2010; Grimm et al., 2008; Stairs et al., 2006), suggesting that EE rats are not too tired to respond.

The protective effect of extinction training combined with EE is likely specific to cue-elicited cocaine-seeking behavior, as Chauvet et al. (2009) have demonstrated that a similar EE/extinction combination was unable to provide protection against cocaine-primed drug-seeking behavior. This is not surprising in light of evidence that EE given alone during abstinence fails to protect against cocaine-seeking behavior elicited by cocaine priming (Thiel et al., 2009). Thus the combined treatments in humans would likely be helpful in promoting abstinence, but would not be effective against relapse following a sampling of drug.

A time-dependent increase in cocaine-seeking across the initial period of abstinence was observed for both the PC and EE rats. This finding is evident when comparing the first extinction session of PC_{EXT} and EE_{EXT} rats (Fig. 2) to the first extinction session of PC_{ABST} and EE_{ABST} rats (Fig. 3A), with notably higher cocaine-seeking observed for the latter. This phenomenon has previously been observed in isolated rats and is referred to as the "incubation effect" (Grimm et al., 2001; Neisewander et al., 2000; Tran-Nguyen et al., 1998). This incubation effect within the EE rats is novel and noteworthy for several reasons. For one, these findings mitigate the contribution of rodent isolation stress as a potential cause of incubation, thus supporting the model's validity as a measure of increased drug craving over time that is observed in humans (Gawin and Kleber, 1986). Secondly, these findings are surprising given a report by Grimm et al. (2008) demonstrating that enrichment prevents incubation of sucrose-seeking behavior. Collectively, these findings suggest that perhaps the latter effect is influenced by living conditions more than the incubation of cocaine-seeking. Finally, our results suggest that the protective effects of enrichment are immediate (i.e., observed after just 1 day) and do not involve prevention of the incubation effect, which in turn may partially explain the transient nature of its protective effects once the intervention is discontinued.

The enhanced context-elicited cocaine-seeking behavior in the EE rats compared to the PC rats 7 days after the interventions were terminated was unexpected (see Fig. 4B). This difference could be due to either enhanced incentive motivation or enhanced spontaneous recovery due to less previous extinction learning (i.e., more forgetting) in Post-EE relative to Post-PC rats. The latter explanation seems unlikely given that EE generally enhances learning (van Praag et al., 2000), as well as the fact that the EE rats had demonstrated extinction curves, suggesting learning had occurred. The former explanation is more plausible since the return to isolated housing conditions prior to the second test was likely more stressful for the EE group compared to the PC group. Indeed, social isolation is a known stressor (Hall, 1998), and this stress may have been magnified by the contrast with previous EE experience relative to PC. Regardless of the explanation for group differences on the post-intervention test, the results strongly suggest that the beneficial effects of the interventions did not persist long after the interventions were terminated.

The lack of lasting effects of the interventions against cue-elicited cocaine-seeking behavior in the present study may have been due to methodological limitations. For instance, repeatedly testing each rat at both time points may have reduced sensitivity to detect a lasting effect. However, this seems unlikely given that others utilizing a between-subject design whereby different rats are tested after different amounts of time have elapsed following the intervention have also failed to demonstrate lasting protective effects of an extinction intervention (Di Ciano and Everitt, 2002; Kelamangalath and Wagner, 2009). Furthermore, other intervention approaches are capable of producing lasting inhibitory effects on drug-seeking behavior within the same subject. For instance, Zhou and Kalivas (2008) used a within subjects design to show that a combination of chronic N-acetylcysteine treatment with daily context extinction sessions reduced subsequent cue-induced reinstatement of heroin-seeking behavior both immediately following this treatment, as well as 40 days later.

An alternative explanation for the lack of persistent intervention effects is that the intervention period may have been too short. It is possible that a longer intervention period would have produced longer lasting effects given previous research showing that 4–5 weeks of EE results in persistent synaptogenesis (Briones et al., 2004) and induction of hippocampal long-term potentiation and long-term depression (Artola et al., 2006) for up to a month when rats are placed back into isolation. Thus, future studies will be needed to address whether a longer EE intervention might result in subsequently longer lasting protective effects against drug-seeking.

Although relatively few preclinical studies have examined chronic pharmacological treatments during cocaine abstinence, parallels can be drawn between those that have and the possible mechanisms

underlying EE's protective effects. For instance, similar to EE effects, chronic administration of the antidepressant, fluoxetine, or the opiates, methadone and buprenorphine, during abstinence attenuates subsequent cue-elicited cocaine-seeking behavior (Baker et al., 2001; Leri et al., 2004; Sorge et al., 2005). Thus, EE may produce antidepressant effects or relieve withdrawal-related discomfort and craving similar to opiates. Also similar to EE, Ward et al. (2009) recently demonstrated that chronic treatment with the cannabinoid antagonist, Rimonabant, attenuates context-elicited cocaine-seeking behavior on the first day of extinction, as well as subsequent cue reinstatement, suggesting a possible endocannabinoid mechanism mediating EE's effects throughout extinction and reinstatement. Finally, Zhou and Kalivas (2008) recently demonstrated that restoration of the cysteine–glutamate exchange system via chronic administration of N-acetylcysteine reduces heroin extinction responding. Future studies will be necessary to determine if EE's protective effects as an intervention are due to similar restoration of drug-induced neuroadaptations and/or mediation of particular neurotransmitter systems.

The beneficial effects of the interventions examined in the present study may be linked to important neuroadaptations that occur as a result of their implementation. Indeed, both environmental enrichment (Green et al., 2010; Stairs and Bardo, 2009) and extinction training (Ghasemzadeh et al., 2009a,b; Self et al., 2004) produce a number of cellular and molecular alterations throughout the brain that may counter or reverse the neuroadaptions that contribute to craving and relapse. Importantly, cue reinstatement is thought to be regulated by a neurocircuitry that includes the prefrontal cortex (PFC), nucleus accumbens, and basolateral amygdala (Fuchs et al., 2005; Kalivas and O'Brien, 2008; McLaughlin and See, 2003). Extinction training, compared to forced abstinence, reduces functional activation in response to drug-paired context and cues within these regions, as evidenced through the use of Fos protein expression (Neisewander et al., 2000; Zavala et al., 2007; Zavala et al., 2008). Additionally, we recently found that EE reduces Fos protein expression in response to cocaine-paired context and discrete cues throughout the PFC, striatum, VTA, and amygdala (Thiel et al., 2010). Thus, extinction and EE interventions may attenuate functional activation within circuitries involved in the compulsive, habitual control over drug-seeking behavior elicited by conditioned drug stimuli.

In conclusion, an acute enrichment/extinction intervention provides robust protection against cue-elicited cocaine-seeking behavior. However, it remains unclear whether these protective effects can last beyond the time period in which the intervention takes place, as the present study did not observe robust lasting effects of these treatments. The findings are consistent with an emerging literature suggesting EE as an effective intervention strategy to reduce incentive motivational effects of drug cues (Chauvet et al., 2009; Grimm et al., 2008; Solinas et al., 2008; Thiel et al., 2009). Previous human and animal studies have demonstrated that various components of EE, including physical exercise, social support, and exposure to alternate reinforcers, are protective against addiction-related behaviors (Carroll et al., 2009; Kearns and Weiss, 2007; Smith et al., 2008); however, it is likely that their combination provides the best outcome (van Praag et al., 2000). The present results also compliment evidence that extinguishing drug-seeking behavior within the drug context can subsequently lessen incentive motivation elicited by discrete drug-paired cues (Di Ciano and Everitt, 2002; Kelamangalath et al., 2007; Kelamangalath and Wagner, 2009). The finding that EE and extinction together reduce cocaine-seeking behavior more than either treatment alone is exciting because it suggests that added benefit may be gained by combining these treatment strategies.

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