Published in final edited form as:

Behav Brain Res. 2007 December 28; 185(2): 119–128.

The effects of extinction training in reducing the reinstatement of drug-seeking behavior: Involvement of NMDA receptors

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Abstract

Although the process of extinction has been well documented for various forms of behavioral responses, the effects of extinction on the reinstatement of drug seeking behavior are relatively understudied. In this report, the effectiveness of an extinction training protocol to reduce primed reinstatement responses was compared with the effectiveness of an equivalent period of enforced abstinence. We found that extinction training performed in the drug taking environment significantly reduced reinstatement behavior subsequently primed by either contextual cues, conditioned cues, or cocaine infusion. The ability of extinction to reduce cocaine primed reinstatement was blocked by the systemic administration of the competitive NMDAR antagonist ((±)CPP, 5 mg/kg i.p.) administered prior to each extinction training session. Interestingly, this pharmacological intervention had no impact on the effectiveness of extinction to reduce drug-seeking behavior primed by either contextual cues or conditioned cues. These results suggest that an extinction training experience involves multiple mechanisms that can be dissociated into nonNMDAR and NMDAR dependent components with respect to the type of reinstatement (i.e. context-, CS-, or drug-induced) being assessed.

Keywords

self-administration; cocaine; extinction; reinstatement; NMDA receptor; (±)CPP; D-serine

Introduction

The reinstatement of drug seeking behavior in rodents as a model for relapse in humans has taken a prominent position in the preclinical field of addiction research [26]. In this model, exposure to various modes of priming stimuli (e.g. environmental context, conditioned cues, addictive drugs, stressors) following either abstinence or extinction can evoke, or reinstate, instrumental behaviors previously associated with the self-administration of an addictive substance [27]. An extinction training phase is typically incorporated into such protocols, in order to reduce instrumental responding to a low, stable baseline level from which the effectiveness of primed reinstatement can be assessed, although the resumption of drug-seeking behavior evoked by priming can also be assessed following periods of prolonged abstinence

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[28]. Much effort has been directed toward determining the neural substrates involved in the mechanisms of priming itself induced by various stimuli [24], however surprisingly little information is available concerning the mechanisms underlying the effectiveness of an extinction training experience to reduce instrumental responding following a priming event.

Using other behavioral models, evidence indicates that new learning is occurring during the extinction training experience [4,20,21]. This new learning may be dependent upon the activation of n-methyl-d-aspartate receptors (NMDARs), and either blocking NMDARs with antagonists or enhancing NMDAR activity with coagonists would be expected to affect the ability of an extinction training experience to alter the response to primed reinstatement. For example, conditioned fear has been used to demonstrate that NMDAR antagonists administered prior to extinction sessions can significantly inhibit extinction [2,10,22], and recent reports have indicated that treatment with D-cycloserine (an NMDAR coagonist) can facilitate extinction of conditioned fear [17,35]. Together, these findings indicate an involvement of NMDARs in the learning process that occurs during an extinction training experience.

With respect to the various methods employed in previous studies to prime reinstatement of drug seeking behavior, both diffuse (environmental context; [6]) and discrete (conditioned stimuli; [7,27]) cues have been evaluated, as well as the administration of the unconditioned stimulus (drug itself; [7,27]). In addition, stress and anxiety have been suggested to be effective inducers of reinstatement behavior [8,29]. Interestingly, different neural mechanisms appear to underlie the reinstatement induced by these priming stimuli, and several specific brain regions are involved in some of these events. For example, one early report identified that the basolateral region of the amygdala as being critical for the reinstatement response to conditioned stimuli (CS) priming [19]. Subsequently, Grimm & See [13] demonstrated that inactivation of the nucleus accumbens was effective in preventing drug (cocaine) induced drug seeking, but had no effect on CS induced priming of reinstatement. The opposite relationship was found to exist for inactivation of the basolateral amygdala, as tetrodotoxin infusion did not affect drug primed reinstatement. This study established a basis for multiple, discrete neuronal mechanisms in mediating the primed reinstatement of drug-seeking behavior. Additional work has found that the dorsomedial prefrontal cortex is also involved in CS induced reinstatement [18], and that inactivation of either the dorsomedial prefrontal cortex, the basolateral amygdala or the dorsal hippocampus can inhibit context induced reinstatement [11].

In this report we have tested the hypothesis that NMDARs may also be involved in the extinction of drug-seeking behavior. The efficacy of extinction was directly compared with the responses measured in another group of abstinent rats that remained in their home cage environments for an equivalent amount of time. The results demonstrate that extinction training is effective in reducing the reinstatement of drug seeking behavior elicited by noncontingent exposure to contextual cues, conditioned stimuli, or cocaine infusion. Facilitating the activation of NMDARs during extinction training did not significantly affect the subsequent reinstatement, but inhibiting NMDAR activation resulted in the selective blockade of the extinction effects on drug primed reinstatement. These results indicate that diverse mechanisms participate in mediating the effects of extinction training on the expression of reinstatement of drug seeking behavior.

Materials and Methods

Animals

Male Sprague-Dawley rats (Harlan) weighed approximately 300 g at the beginning of the experiment and were housed individually in a temperature and humidity controlled vivarium having a 12 hour light/dark cycle (lights off at 7:00 P.M.). They were given access to food and

water *ad libitum* and were handled daily for 5 days prior to the surgery in order to diminish stress associated with handling. The housing and experimental procedures followed the *Guide for the Care and Use of Laboratory Animals* and were approved by the local ACUC at the University of Georgia.

Jugular catheterization protocol

The animals were anesthetized using a combination of ketamine (70 mg/kg), xylazine (10 mg/kg) and acepromazine (1 mg/kg) administered i.p. Depth of anaesthesia was assessed by monitoring respiration rate and palpebral and pedal withdrawal reflexes. Under anesthesia, the right jugular vein was isolated. The catheter was exteriorized by passing it subcutaneously to the base of the skull, where it was connected to a modified 22 gauge cannula. A silastic catheter (Dow Corning) was then inserted into the vein (4–5 cm) and secured in position with silk sutures (6/0). The animal was then placed in a stereotaxic frame (Stoelting), where the right-angled cannula (Plastics One) was mounted to the top of the skull using dental cement and 4 screws. Immediately after surgery and once daily for 5 days, the animals were treated with gentamicin at a dose of 5 mg/kg, i.v. The catheters were flushed every day with saline prior to each self-administration session and with heparin (10 USP/ml) after the session to maintain the patency of the catheter. Catheter patency was verified daily by drawing blood from the catheter.

Self-administration environment

The operant chambers (Med associates) were equipped with 2 levers, one “active” and another “inactive” with lights positioned above each lever. The chambers had a rod grid floor, a house light, a speaker/tone generator (2.9 kHz, 10 dB above ambient) and were housed inside enclosures equipped with ventilation fans. A syringe pump was located outside the enclosure. The method for delivering a cocaine infusion was as follows: The modified 22 gauge cannula mounted on the rat’s skull was connected to a liquid swivel with PE-50 tubing protected by a metal spring. The swivel was connected with tygon tubing to the syringe mounted in the infusion pump. Infusion volumes were calculated according to the animal’s weight. For cocaine animals, the syringes mounted in the infusion pump contained cocaine hydrochloride (NIDA) dissolved in normal saline at 4 mg cocaine/ml of solution. Each infusion delivered an infusion volume of 0.125 ml/kg body weight, hence the dose of cocaine self-administered was 0.5 mg/kg/infusion. The MED-PC software program recorded the number of active lever presses, inactive lever presses and the number of infusions.

Self-administration protocol (days 1–15)

The animals having patent indwelling catheters were subjected to self-administration training for a period of 15 days with one session each day. Self-administration training sessions were 90 minutes in duration. Upon entry into the self-administration environment, the house light and the ventilation fan were on. In addition to triggering an infusion, active lever presses had the following programmed consequences: the house light was turned off, and the active lever light/tone (i.e. the CS) was turned on for a period of 30 seconds. Additional responses on the active lever during this 30 second period had no programmed consequences, although the program continued to count the number of active/inactive lever presses and infusions. This “timeout” period protected the animals from cocaine overdose. After this 30 second period the lever light and tone were terminated and the house light came back on. Rats were initially trained for 12 days on an FR-1 (fixed ratio schedule-1) schedule in which each active lever press outside the timeout period triggered the programmed consequences. For the last 3 days of self-administration training, an FR-3 schedule was imposed where 3 active lever presses outside the time out period were required to trigger an infusion and the CS. Each rat was placed in the same operant conditioning chamber throughout the course of the experiment.

Extinction protocol (days 16–20)

After the 15 days of self-administration training, the animals were divided into 4 groups (balanced for cocaine intake): 1) extinguished (saline), 2) extinguished ((±)CPP), 3) extinguished (D-serine), 4) abstinent (saline). All groups received i.p. injections of their respective treatments in their home cage environment. Both the extinguished group 1 and the abstinent group 4 received injections of saline (1ml/kg). Group 2 received an injection of (±) CPP (5 mg/kg) and group 3 received an injection of D-serine (100 mg/kg). Groups 1–3 underwent extinction training 4–5 hours following their respective daily pharmacological treatments, whereas group 4 rats remained in their home cages. During their extinction training sessions, the animals in the operant chambers were attached to the drug tether but exposed only to the environment stimuli (i.e. diffuse, contextual cues). Responses on the active lever had no programmed consequences during the extinction training phase. For protocol days 16–20, responses on both active and inactive levers, as well as the equivalent “number of infusions” were counted by the software (although as stated above, syringe pumps were not activated during this phase of training). Extinction proceeded for a period of 5 days, with one 90 minute session each day during which the animals in the extinction training groups 1–3 were taken to the operant chambers. Under these conditions, the animals extinguished their lever pressing behavior to less than 20% of their former activity during self-administration. As previously mentioned, group 4 abstinent animals remained in their home cages throughout days 16–20.

Reinstatement tests (protocol days 21–24)

On days 21–24, all the animals (including the home cage abstinent animals), were placed back in the operant chambers for reinstatement tests. The reinstatement test session conditions were similar to an extinction session in that the animals were exposed only to the contextual cues of the operant chamber environment and the active lever responding were not reinforced by the contingent availability of either CS or US.

Reinstatement to the contextual drug stimuli—On test day 21, response to the contextual prime was assessed from active lever presses during the first 10 minutes in which the animals were exposed only to the contextual cues of the drug environment.

Reinstatement to the CS cues—Later during the same test session on day 21, lever presses evoked in response to a CS presentation were then assessed. A single, noncontingent presentation of the CS was delivered at the 40th minute of the 90 minute test session. Thus the initial 40 minutes of the 90 minute session served as an extinction period to allow lever presses initiated by exposure to contextual stimuli to subside before the CS reinstatement test. As the CS was expected to evoke an immediate response from animals, the noncontingent CS was quantified as the number of lever presses during the subsequent 10 minutes following the priming event (t=40–50 min).

Reinstatement to the drug prime—Response to the drug prime stimulus was assessed on days 22–24. We tested the reinstatement of drug seeking behavior using 3 different doses (0.25 mg/kg, 0.5 mg/kg and 1 mg/kg) of cocaine on three consecutive days (22nd, 23rd and 24th day), respectively. A single, noncontingent drug prime was programmed to be infused intravenously by the syringe pump at the 40th minute of the 90 minute session on these 3 US reinstatement test days. Again, the initial 40 minutes of the 90 minute session served as an extinction period which allowed lever presses initiated by exposure to contextual stimuli to subside before the US reinstatement test. Drug seeking behavior elicited by the different doses of cocaine was quantified from the number of responses on the active lever following the drug prime for 30 minutes immediately after the priming event (t=40–70 min).

Drugs & Dosage Justification

Cocaine hydrochloride was a gift from NIDA (RTI). The NMDA receptor antagonist 3-(2-carboxipiperazin-4-yl)propyl-1-phosphonic acid ((±)CPP) and the coagonist D-serine were obtained from Sigma (St. Louis). D-serine and (±)CPP were administered in the home cage environment approximately 4 hours prior to the extinction sessions on protocol days 16–20. These compounds have long-lived effects when administered i.p. at the indicated doses [14, 15], and therefore any state-dependent or acute locomotor effects were minimized by this advance treatment in the home cage environment. The D-serine dose of 100 mg/kg was chosen in order to avoid possible nephrotoxic effects at higher doses [37], while still affecting learning [31]. The (±)CPP dose of 5mg/kg has been shown to disrupt synaptic plasticity processes in the hippocampal formation 12–24 hours following i.p. administration [34].

Statistics

The number of active lever presses, infusions and inactive lever presses were recorded for each session. These data were used to calculate the responses during each experimental session. A repeated measures ANOVA was applied to the data in figure 1B, and a 2-way repeated measures ANOVA was applied to figure 4. A 2-way ANOVA was used for the other data analysis. The two factors taken into consideration for the 2-way ANOVA were: 1) either trial (pre vs. post priming responses) or days and 2) either condition (extinction vs. abstinence) or treatment, as the case may be. A value of $p < .05$ was taken as significant, being determined from the Holm-Sidak post hoc test method. All the statistics were done using SigmaStat software.

Results

Cocaine self-administration and extinction of the drug seeking behavior

Animals having indwelling jugular catheters were trained to self-administer cocaine in an operant chamber environment for 15 consecutive days. During the daily 90-minute sessions, rats were initially trained on an FR-1 schedule for first 12 days and switched to an FR-3 schedule for the last 3 days of self-administration training. The transition to the FR-3 schedule was done to increase the number of active lever pressing responses. Animals typically achieved stable self-administration by day 10 of training, and the FR-3 schedule did not significantly alter the number of earned infusions per session (Figure 1). Over the entire fifteen day self-administration training phase the average total number of infusions earned was 334 ± 12 , or the equivalent of approximately 11 mg/kg/day of cocaine per animal. There was no significant difference in the average number of infusions earned per animal among the four different groups of self-administration rats utilized for the reinstatement studies described in this report (data not shown).

After 15 days of SA, the animals were subjected to either an extinction training phase (extinction groups) in the same operant chamber environment for 90 minute sessions in the absence of both CS and US (i.e. the active lever had no programmed consequences) for a period of 5 days (Figure 1B, protocol days 16–20), or they were kept forcibly abstinent in their home cage environment (abstinent group). Active and inactive lever presses were monitored during the extinction sessions and it was found that the animals extinguish their drug seeking behavior under these conditions. Evaluation of drug seeking behavior between extinction sessions demonstrates that lever pressing activity observed on second-fifth days of extinction is significantly decreased as compared with the first day of extinction (Figure 1B). Conversely, the second-fifth days' activities were not significantly different from each other.

Reinstatement of drug seeking behavior

Evaluation of drug seeking behavior within an extinction session illustrates that the majority of lever pressing activity occurs during the initial ten minutes in the operant chamber environment, suggesting that environmental contextual cues are priming this response. Following this initial burst of activity, active lever pressing diminishes rapidly. As illustrated during the fifth extinction session on protocol day 20 (Figure 2A), active lever responses are minimal (< 1) by 20–30 minutes and remain low for the remainder of the 90 minute session. This within session response pattern was observed during all extinction days (data not shown). Therefore, during the reinstatement experiments involving the noncontingent presentation of either CS or US stimuli, the priming event was delivered at time=40 min of the test session. A temporal distinction can thus be made between the drug-seeking activity induced by introduction to the operant chamber environment (i.e. activity during the first ten minutes) versus the subsequent activity induced via noncontingent presentation of priming events delivered later within the same test session.

The effects of extinction training versus enforced abstinence on drug-seeking behavior

The resumption of lever-pressing activity was induced using three forms of priming stimuli: contextual cues, conditioned cues, and drug prime. Once the animals underwent either extinction training or enforced abstinence for a period of 5 days (days 16–20), they were tested for the resumption of drug seeking behavior following exposure to the contextual stimuli and the conditioned stimuli on day 21, and to cocaine on days 22–24.

The resumption of drug seeking induced by diffuse environmental cues was assessed during the first ten minutes of the test session conducted on day 21 (Figure 2B). The level of responding on the active lever in the extinguished (saline) group of rats during this period was significantly decreased ($p < 0.01$, unpaired t-test) compared with responding during the same period in the abstinent (saline) group kept in the home cage environment for 5 days (6.4 ± 0.9 vs. 41 ± 5.3 , respectively). These results suggest that the 5-day extinction training experience decreased the efficacy of the contextual cues present in the operant chamber environment to provoke drug seeking behavior.

Until time=40 min of the day 21 reinstatement test session, the animals experienced extinction conditions. At this point, a single, noncontingent presentation of the discrete CS complex was delivered for a period of 30 seconds, and active lever responding for the next 10 min was measured as an indication of the reinstatement of drug seeking behavior evoked by the CS (Figure 3A). As previously described for extinction conditions, active lever presses had no programmed consequences at any time during these test sessions. In addition to the active lever presses, the inactive lever presses were also monitored, so as to ensure that the noncontingent CS prime was in fact inducing activity previously associated with cocaine infusion. In order to confirm that any responding due to contextual cues had subsided by the time of the CS reinstatement test, active lever pressing during a “pre-prime” ten minute period of time ($t=30-40$) was also assessed and compared with the cue-induced “post-prime” level of reinstatement ($t=40-50$). Results for both the extinguished (saline) and abstinent (saline) groups of rats tested in this manner are illustrated for 10 min bins of time before and following the CS priming event. A 2-way ANOVA on the data of figure 3A confirmed an effect of trial, $F_{\text{trial}(1,68)} = 15.398$ and condition, $F_{\text{condition}(1,68)} = 7.452$. The post hoc analysis showed that the 10 minute post priming response was significantly greater than the 10 minute pre priming response for both the extinguished (saline), ($t=2.276$, $p=.026$) and the abstinent (saline), ($t=3.204$, $p=.002$) group. The magnitude of post priming response of the abstinent group was significantly greater than that of the extinguished group, ($t=2.698$, $p=.009$), indicating that an extinction training experience decreases the efficacy of noncontingent CS cues to trigger responses on the active lever. Importantly, the level of responding during the same period of time (i.e. 40–

50 min) during the final day of self-administration (day 15) in these animals was 9.3 ± 0.8 and 8.1 ± 0.8 for the extinguished (saline) and the abstinent (saline) groups, respectively. By comparison, the close agreement in terms of the magnitude of the response in the abstinent animals (i.e. 8.1 ± 2.2) demonstrates the effectiveness of this noncontingent priming protocol to evoking drug seeking behavior and further indicates that a genuine reinstatement of drug seeking had occurred in response to the CS event.

Cocaine-induced reinstatement was tested on the next 3 consecutive days (protocol days 22–24) at 3 different priming doses (0.25 mg/kg, 0.5 mg/kg, 1 mg/kg) with a single, noncontingent intravenous infusion of drug delivered at time=40 minute of the session at the respective dose on each day. The active lever responding for the next 30 minutes was then measured as an indication of the reinstatement of drug seeking behavior evoked by the US stimuli. The dose-response results are plotted to demonstrate the shift in the sensitivity to cocaine-induced drug seeking activity (Figure 3B). At the 0.25mg/kg dose of cocaine, 2-way ANOVA confirmed an effect of trial, $F_{\text{trial}}(1,66)=4.046$ and condition, $F_{\text{condition}}(1,66)=5.864$. The post hoc analysis showed an increase in the 30 minute post priming response for the extinguished group, ($t=2.486$, $p=0.015$) as compared to the 30 minute pre prime response (this comparison was not significant for the abstinent group due to their high baseline response on day 22). At the 0.5mg/kg priming dose of cocaine the 2-way ANOVA showed an effect of trial, $F_{\text{trial}}(1,63)=20.336$ and condition, $F_{\text{condition}}(1,63)=10.89$. The post hoc analysis showed that this priming dose of cocaine significantly reinstated the drug seeking behavior among the abstinent group, ($t=4.324$, $p<0.001$) but not among the extinguished group. The comparison of the 30 minute post priming response levels between the extinguished and the abstinent group showed that the reinstatement in the abstinent group was significantly greater than that of the extinguished group, ($t=3.828$, $p=0.000$). A similar distinction was observed at the priming dose of 1mg/kg for which $F_{\text{trial}}(1,61)=35.548$ and $F_{\text{condition}}(1,61)=5.816$ was obtained. Pair-wise comparisons showed a significant effect of priming for both the extinguished ($t=3.444$, $p=0.001$) and the abstinent group ($t=4.869$, $p<0.001$). The post prime response of the abstinent group was significantly higher than the extinguished group at this priming dose ($t=2.984$, $p=0.004$). Also, in smaller groups of these extinguished (saline) and the abstinent (saline) conditions, the animals were tested on day 25 with a saline prime to test for any conditioned effects of the noncontingent cocaine primes that might accumulate over days 22–24. No evidence for such an effect was observed, as there was no change in active lever pressing following the saline prime, as responding was 2.3 ± 1.0 ($n=8$) and 1.6 ± 0.8 ($n=4$) for the extinguished (saline) and the abstinent (saline) groups, respectively).

These drug prime reinstatement results illustrate the effectiveness of extinction training in reducing sensitivity to cocaine-induced drug seeking as compared to an equivalent period of enforced abstinence. The level of responding during the same period of time (i.e. 40–70 min) during the final day of self-administration (day 15) in these animals was 27.6 ± 1.9 and 24.0 ± 1.0 for the extinguished (saline) and the abstinent (saline) groups, respectively. In comparison, at the 0.5mg/kg priming dose, the extinguished (saline) group response on the active lever was 8.7 ± 2.4 , whereas abstinent (saline) group responses were significantly greater (26.5 ± 6.9). A similar distinction was observed for the 1mg/kg priming dose, as the responses from the extinguished (saline) and abstinent (saline) groups were 12.9 ± 2.5 and 25.5 ± 6.6 , respectively. As mentioned previously with the CS priming test, the close agreement in the magnitude of the response in the abstinent animals indicates that a genuine reinstatement of drug seeking had occurred during this period of time in response to the noncontingent US priming events.

NMDAR involvement in the effects of extinction training on reinstatement behavior

The role of NMDARs in the extinction process was also evaluated in two additional groups of extinguished rats treated with either D-serine (a coagonist of NMDAR at the glycine site), or ($\pm$)CPP (a competitive antagonist of NMDAR). These compounds were administered (D-serine: 100 mg/kg i.p., ($\pm$)CPP: 5 mg/kg i.p.) in the home cage environment prior to the extinction sessions (see Methods). Over the course of the 5-day extinction phase carried out on days 16–20, it was observed that the D-serine treatment significantly facilitated extinction as compared to the saline group. A 2-way RM ANOVA ($F_{\text{treatment}(2,204)}=3.106$ and $F_{\text{day}(4,204)}=110.912$) showed this facilitation of extinction to be statistically significant on days 1 ($t=2.956$, $p=.004$) and 3 ($t=2.138$, $p=.034$). Treatment with ($\pm$)CPP had no discernable effect on the progression of extinction, as active lever responding was not significantly affected on any day of the extinction protocol. By protocol day 20, the final extinction day, all groups exhibited similar levels of drug-seeking behavior during the 90 minute session.

The resumption of lever-pressing activity induced by diffuse environmental cues is illustrated in Figure 5A, and was again assessed during the first ten minutes of the test session conducted on protocol day 21 (as described previously for Figure 2B). The level of responding on the active lever in the saline group of rats during this period was 6.4 ± 0.9 and both the D-serine and ($\pm$)CPP treated animals showed comparable levels of responding (6.4 ± 1.3 and 8.7 ± 1.8 , respectively). Thus, neither positive modulation (w/D-serine) nor competitive antagonism (w/ ($\pm$)CPP) of NMDAR activity during extinction training had significant impact on the subsequently measured contextual reinstatement as compared with the saline treated control group following a five-day extinction protocol.

In order to investigate the effects of NMDAR activity during extinction on the discrete CS-induced reinstatement, active lever pressing was again measured during a “pre-prime” ten minute period of time as well as the “post prime” period (Figure 5B). Analysis via 2-way ANOVA confirmed an effect of trial, $F_{\text{trial}(1,100)}=27.003$, but not an effect of treatment. Pairwise comparisons showed that the post priming activity following the CS prime was significantly greater than the pre priming activity for the control ($t=3.328$, $p=.001$), the D-serine ($t=2.816$, $p=.006$) and the ($\pm$)CPP ($t=2.940$, $p=.004$) groups. Thus although active lever pressing activity was significantly increased in the ten minute period immediately following the CS prime in both the D-serine (0.0 ± 0.0 , 3.4 ± 1.2 , $n=15$) and ($\pm$)CPP (0.2 ± 0.1 , 3.6 ± 1.1 , $n=17$) treatment groups, since the magnitude of post prime responding was not significantly different from that of the saline treated extinguished group (3.7 ± 1.0 , $n=22$), no role for NMDAR activity during the extinction training experience is indicated for the CS-induced reinstatement outcome.

Finally, cocaine-induced reinstatement was tested on the next 3 consecutive days (protocol days 22–24) at 3 different priming doses (0.25mg/kg, 0.5mg/kg, 1mg/kg) with a single, noncontingent intravenous infusion of drug delivered at time=40 min of the session. The active lever responding during the next 30 minutes was measured as an indication of the reinstatement of drug seeking behavior evoked by the US stimuli. The dose-response results are plotted to demonstrate the shift in the sensitivity to cocaine-induced drug seeking activity (Figure 5C). Treatment with D-serine during extinction did not alter reinstatement at any of the priming doses tested as compared with the saline treated group. At the 0.25mg/kg priming dose of cocaine, the 2-way ANOVA confirmed an effect of trial, $F_{\text{trial}(1,93)}=24.037$ but not an effect of treatment. The post hoc analysis of this data showed that the 30 minute post priming response was significantly greater than the pre priming response for the saline control ($t=3.383$, $p=.001$) and the ($\pm$)CPP ($t=3.409$, $p=.001$) groups. The analysis of the data at the priming dose of 0.5mg/kg showed an effect of trial, ($F_{\text{trial}(1,84)}=35.075$), treatment, ($F_{\text{treatment}(2,84)}=4.204$) and an effect of interaction between these two factors ($F_{\text{interaction}(2,84)}=4.749$). The post priming responses were significantly greater than the pre priming responses for both the control

($t=2.738, p=.008$) and the ($\pm$)CPP group ($t=5.720, p=0.000$). Pairwise comparison between the post prime responses of the different treatments showed that the ($\pm$)CPP treated group responded significantly greater than the saline group ($t=3.753, p=0.000$). At the 1mg/kg priming dose of cocaine, the 2- way ANOVA showed an effect of trial, $F_{\text{trial}(1,86)}=37.21$, but did not show an effect of the treatment and interaction. The post hoc analysis showed that with in the treatment groups, the post priming responses were significantly greater than the pre priming responses (control($t=3.150, p=.002$), D-serine($t=2.756, p=.007$) and ($\pm$) CPP ($t=4.681, p=0.000$)). Even though the main effect of treatment was not observed at this dose, planned comparisons of the post priming responses of the treatment groups showed a significantly greater response for the ($\pm$)CPP group compared to the control group ($t=2.439, p=.017$). These results indicate that the ability of extinction to reduce drug primed reinstatement (c.f. Fig. 3C) is dependent upon the activation of NMDARs during the training protocol.

In sum, altering NMDAR activity during extinction training did not affect the resumption of lever pressing activity induced by diffuse contextual or discrete CS cues. However, the blockade of NMDARs during the extinction protocol did affect the US (cocaine) primed reinstatement, and the magnitude of responding in this group was similar to that of the abstinent group. This suggests that the effectiveness of a prior extinction training experience to reduce the reinstatement response to drug priming involves an NMDAR dependent mechanism, and is therefore distinguishable from the mechanisms underlying the effects of extinction on priming via diffuse contextual or discrete CS cues.

Discussion

In this report we have directly assessed the effectiveness of an extinction training protocol to reduce primed reinstatement as compared with an equivalent period of enforced abstinence. This was a prerequisite step for us in pursuing our intent to investigate the mechanisms underlying the effectiveness of extinction training to reduce reinstatement. We have found that in addition to reducing active lever responding when placed back into the drug paired environment (Figure 2B), extinction training can also significantly reduce the primed drug seeking response following exposure to either CS or US cues (Figure 3).

In the rat reinstatement model of craving and/or relapse, the presentation of discrete, drug associated stimuli can be used to prime the reinstatement of drug seeking behavior [24]. Since an early study that employed the use of CS cues presented in a noncontingent manner [7], the majority of the subsequent work in rats has used a contingent method of assessing CS induced reinstatement [26]. This issue has been a point of contention concerning the validity of the reinstatement protocol for modeling the human condition in which self-reported craving can be triggered by passive exposure to drug associated cues. As craving in humans can be induced via noncontingent presentation of these cues, it has been argued that the CS reinstatement protocol should employ this form of priming as well [16]. In our study, we have determined conditions under which a single, noncontingent presentation of the CS or US results in a significant increase in drug seeking behavior. In order for this effect to be observed, it was important to utilize a within session design and to select an appropriate period of time for the analysis of the response. By waiting until the immediate, context stimulated responding had dissipated (Figure 2A), the relatively low remaining level of activity could be effectively compared with the responses on the active lever triggered by the noncontingent CS or US presented at time = 40 minutes into the test session.

The neural circuitry underlying reinstatement has been the subject of several studies, recently reviewed by See [24]. Evidence suggests that the basolateral amygdala, the dorsal medial prefrontal cortex, and the lateral orbitofrontal cortex participate in the expression of reinstatement following exposure to CS cues. In addition, the dorsal hippocampus is selectively

involved in reinstatement following exposure to contextual cues [11] and both the nucleus accumbens [13] and the ventral hippocampus [30] are involved in reinstatement following exposure to drug prime. Stress induced via foot shock can evoke reinstatement [8], and this effect may involve the bed nucleus of the stria terminalis [9] and the ventral tegmental area [36]. Finally, the participation of the dorsolateral caudate putamen region is required for either context or CS cued reinstatement following periods of either abstinence or extinction [12], whereas the participation of the basolateral amygdala or the dorsomedial prefrontal cortex is not required for reinstatement following a period of abstinence (as opposed to a period of extinction, as in the earlier studies cited above). In sum, it is evident that the reinstatement evoked via these different types of priming stimuli can differentially involve distinct regions of the brain, and therefore may also recruit different mechanisms at the synaptic level for the processing, storage, and recall of such information.

In contrast to the self-administration behavioral model, the mechanisms of extinction training have been extensively investigated in other paradigms such as fear conditioning, inhibitory avoidance, spatial navigation and conditioned taste aversion [5]. Extinction likely involves new learning [4,20,21], and at the molecular level, both NMDAR and nonNMDAR dependent forms of synaptic plasticity are thought to contribute to this type of learning. Although some studies have indicated that extinction learning in conditioned taste aversion [3] or conditioned fear [1] does not require the activation of NMDARs, other reports have indicated a critical role for NMDARs [2,10,22]. Our results with cocaine self-administration have demonstrated that antagonism of NMDARs during extinction had no acute effect on responding within a session, nor was there any effect on the progression of extinction over the five day protocol. However, there was a trend in the NMDAR coagonist treated group for enhanced extinction within each daily session (particularly evident at days 16 & 18), but again there was no significant difference in the level of responding by the end of the extinction training protocol (Figure 4). Thus although the ($\pm$)CPP results demonstrate that NMDAR activation is not required for the normal progression of extinction, the D-serine results suggest that NMDAR activation may facilitate extinction, at least over the initial days of training.

As far as we are aware, the mechanism(s) underlying the effects of extinction to reduce the reinstatement of drug seeking behavior have not been described. As mentioned previously, since different types of priming stimuli have been shown to involve different neural substrates, it is reasonable to propose that the effects of extinction training on these various forms of reinstatement might also be linked to different mechanisms during the extinction learning process. Our results demonstrate that although extinction training is effective in reducing the reinstatement evoked by context, CS, and cocaine priming (Figures 2B & 3), the underlying mechanism involves both NMDAR dependent and NMDAR independent components. This was illustrated in Figure 5, where only the effect of extinction on the reinstatement primed via cocaine administration was sensitive to NMDAR blockade during extinction training (Figure 5C), whereas extinction was equally effective despite NMDAR antagonist treatment in the case of either context or CS primed reinstatement (Figures 5A & B). Based on these findings, we expected that treatment with the NMDAR coagonist D-serine would enhance the effects of extinction training such that the magnitude of cocaine primed reinstatement responses would be decreased relative to the saline treated group. This expectation was not met (Figure 5C), and we suggest that over training is likely to occur by the end of our five day protocol such that the effects of extinction are maximal and cannot be significantly enhanced. Future studies that assess the efficacy of a shorter extinction training period will directly test this hypothesis, and indicate whether enhancing the activity of NMDARs during extinction training might be an effective means by which to significantly decrease the duration and/or number of extinction sessions required to reduce the magnitude of the drug primed reinstatement response. In any case, the demonstration of the participation of NMDARs in the effects of extinction to reduce cocaine primed reinstatement indicates that pharmacotherapy targeting these receptors may be

useful, and it is tempting to speculate that just as different brain regions are involved in mediating the various forms of primed reinstatement of drug-seeking behavior, different synaptic mechanisms (i.e. both NMDAR dependent and NMDAR independent synaptic plasticity) may also be involved in mediating the effects of extinction on primed reinstatement.

The mechanisms involved with the resulting effects of extinction training as they may relate to the self-administration of addictive substances have been investigated at the molecular level in the ventral tegmental area and nucleus accumbens regions of the brain [25]. These studies have demonstrated that an extinction training experience can restore tyrosine hydroxylase to predrug levels in the nucleus accumbens shell, whereas an equivalent abstinent period showed a persistent deficit in tyrosine hydroxylase protein levels [23]. Similarly, a down regulation of NMDA receptor NR1 subunit during abstinence is reversed by extinction [25]. Finally, an extinction induced upregulation of GluR1 AMPA receptors occurs in the nucleus accumbens shell [32], and an upregulation of tyrosine hydroxylase also has been observed in the ventral tegmental area following extinction training [23]. All of these extinction induced neuroadaptations would be expected to act in a compensatory manner in order to counteract the drug induced depression of the synapses in the nucleus accumbens shell [33]. However, it is important to consider that these changes have been measured as a result from the animal having undergone extinction, and are not necessarily direct contributors to the learning mechanisms at work during the extinction training process itself that underlie the subsequent effects on the expression of primed reinstatement.

In conclusion, we have provided a clear measure of the effectiveness of extinction training in reducing the resumption of drug seeking behavior primed by noncontingent exposure to context, CS, or drug primes as compared to an equivalent period of enforced abstinence. In addition, the effectiveness of extinction was differentially sensitive to pharmacological intervention with an NMDAR antagonist administered during the training protocol, suggesting that multiple forms of synaptic plasticity may be utilized during this form of new learning that are specific to the different types of priming and the associated neural circuitry involved in mediating the resumption of drug seeking behavior. If this is the case, addiction treatment strategies will need to take this into account when the effectiveness of any one approach is being evaluated, and suggests that significant gains may only be achieved when several therapeutic approaches are combined.

Acknowledgements

We thank R.E. See for helpful discussion and B.A. Gosnell for critiquing an earlier draft of the manuscript. This work was supported by National Institutes of Health Grant DA016302 to J.J.W.

Literature references

1. Akirav I, Khatsrinov V, Vouimba R-M, Merhav M, Ferreira G, Rosenblum K, Maroun M. Extinction of conditioned taste aversion depends on functional protein synthesis but not on NMDA receptor activation in the ventromedial prefrontal cortex. *Learn Mem* 2006;13:254–258. [PubMed: 16741279]
2. Baker JD, Azorlosa JL. The NMDA antagonist MK-801 blocks the extinction of pavlovian fear conditioning. *Behav Neurosci* 1996;110:618–620. [PubMed: 8889007]
3. Berman DE, Dudai Y. Memory extinction, learning anew, and learning the new: Dissociations in the molecular machinery of learning in cortex. *Science* 2001;291:2417–2419. [PubMed: 11264539]
4. Bouton ME, Westbrook RF, Corcoran KA, Maren S. Contextual and Temporal Modulation of Extinction: Behavioral and Biological Mechanisms. *Biol Psychiat* 2006;60:352–60. [PubMed: 16616731]
5. Cammarota M, Bevilaqua LRM, Barros DM, Vianna MRM, Izquierdo LA, Medina JH, Izquierdo I. Retrieval and the Extinction of Memory. *Cellular and Molecular Neurobiology* 2005;25:465–474. [PubMed: 16075375]

6. Crombag HS, Shaham Y. Renewal of drug seeking by contextual cues after prolonged extinction in rats. *Behav Neurosci* 2002;116:169–173. [PubMed: 11895178]
7. de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl)* 1981;75:134–143. [PubMed: 6798603]
8. Erb S, Shaham Y, Stewart J. Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. *Psychopharmacology* 1996;128:408–412. [PubMed: 8986011]
9. Erb S, Stewart J. A Role for the Bed Nucleus of the Stria Terminalis, But Not the Amygdala, in the Effects of Corticotropin-Releasing Factor on Stress-Induced Reinstatement of Cocaine Seeking. *J Neurosci* 1999;19:1–6. [PubMed: 9870932]
10. Falls WA, Miserendino MJ, Davis M. Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. *J Neurosci* 1992;12:854–863. [PubMed: 1347562]
11. Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE. The Role of the Dorsomedial Prefrontal Cortex, Basolateral Amygdala, and Dorsal Hippocampus in Contextual Reinstatement of Cocaine Seeking in Rats. *Neuropsychopharmacology* 2005;30:296–309. [PubMed: 15483559]
12. Fuchs RA, Branham RK, See RE. Different Neural substrates mediate cocaine seeking after abstinence versus extinction: A critical role for the dorsolateral caudate-putamen. *J Neurosci* 2006;26:3584–3588. [PubMed: 16571766]
13. Grimm JW, See RE. Dissociation of Primary and Secondary Reward-Relevant Limbic Nuclei in an Animal Model of Relapse. *Neuropsychopharmacology* 2000;22:473–479. [PubMed: 10731622]
14. Hashimoto A, Chiba Y. Effect of administration of D-serine on the levels of D- and L-serine in several brain areas and periphery of rat. *Eur J Pharmacol* 2004;495:153–158. [PubMed: 15249164]
15. Hernandez RV, Derrick BE, Rodriguez WA, Martinez JL. (±) CPP, an NMDA receptor antagonist, blocks the induction of commissural-CA3 LTP in the anesthetized rat. *Brain Res* 1994;656:215–219. [PubMed: 7804841]
16. Katz JL, Higgins ST. The validity of the reinstatement model of craving and relapse to drug use. *Psychopharmacology* 2003;168:21–30. [PubMed: 12695875]
17. Ledgerwood L, Richardson R, Cranney J. Effects of D-Cycloserine on extinction of conditioned freezing. *Behav Neurosci* 2003;117:341–349. [PubMed: 12708530]
18. McLaughlin J, See RE. Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology* 2003;168:57–65. [PubMed: 12845418]
19. Meil WM, See RE. Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. *Behav Brain Res* 1997;87:139–148. [PubMed: 9331482]
20. Pavlov, IP. Conditioned reflexes. Oxford University Press; London: 1927.
21. Rescorla RA, Heth CD. Reinstatement of fear to an extinguished conditioned stimulus. *J Exp Psychol Anim Behav Process* 1975;1:88–96. [PubMed: 1151290]
22. Santini E, Muller RU, Quirk GJ. Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. *J Neurosci* 2001;21:9009–9017. [PubMed: 11698611]
23. Schmidt EF, Sutton MA, Schad CA, Karanian DA, Brodtkin ES, Self DW. Extinction training regulates tyrosine hydroxylase during withdrawal from cocaine self-administration. *J Neurosci* 2001;21:RC137. [PubMed: 11264329]
24. See RE. Neural substrates of cocaine-cue associations that trigger relapse. *Eur J Pharmacol* 2005;526:140–146. [PubMed: 16253228]
25. Self DW, Choi K-H, Simmons D, Walker JR, Smagula CS. Extinction training regulates neuroadaptive responses to withdrawal from chronic cocaine self-Administration. *Learn Mem* 2004;11:648–657. [PubMed: 15466321]
26. Shaham Y, Miczek KA. Reinstatement-toward a model of relapse. *Psychopharmacology* 2003;168:1–2.
27. Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl)* 2003;168:3–20. [PubMed: 12402102]

28. Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: A review. *Pharmacol Rev* 2002;54:1–42. [PubMed: 11870259]
29. Shepard JD, Bossert JM, Liu SY, Shaham Y. The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. *Biol Psychiat* 2004;55:1082–1089. [PubMed: 15158427]
30. Sun W, Rebec GV. Lidocaine inactivation of ventral subiculum attenuates cocaine-seeking behavior in rats. *J Neurosci* 2003;23:10258–10264. [PubMed: 14614084]
31. Stouffer EM, Petri HL, Devan BD. Effect of D-serine on a delayed match-to-place task for the water maze. *Behav Brain Res* 2004;152:447–452. [PubMed: 15196813]
32. Sutton MA, Schmidt EF, Choi K-H, Schad CA, Whisler K, Simmons D, Karanian DA, Monteggia LM, Neve RL, Self DW. Extinction-induced upregulation in AMPA receptors reduces cocaine-seeking behaviour. *Nature* 2003;421:70–75. [PubMed: 12511956]
33. Thomas MJ, Beurrier C, Bonci A, Malenka RC. Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat Neurosci* 2001;4:1217–1223. [PubMed: 11694884]
34. Villarreal DM, Do V, Haddad E, Derrick BE. NMDA receptor antagonists sustain LTP and spatial memory: active processes mediate LTP decay. *Nat Neurosci* 2002;5:48–52. [PubMed: 11740500]
35. Walker DL, Ressler KJ, Lu K-T, 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]
36. Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You Z-B. Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: A role in stress-induced relapse to drug seeking. *J Neurosci* 2005;25:5389–5396. [PubMed: 15930388]
37. Williams RE, Jacobsen M, Lock EA. ¹H NMR pattern recognition and ³¹P NMR studies with C-serine in rat urine and kidney, time- and dose-related metabolic effects. *Chem Res Toxicol* 2003;16:1207–1216. [PubMed: 14565762]

Figure 1. Self-administration of cocaine and extinction training

A) The results from one cocaine group of rats (open boxes, $n=22$) illustrate the average number cocaine infusions earned daily during each 90 minute session during the SA phase (days 1–15). Transition to the FR3 schedule on day 13 did not significantly alter the number of earned infusions. A saline control group of rats (filled circles, $n=10$) is illustrated for comparison. B) Active and inactive lever presses of a cocaine extinguished group ($n=22$) are illustrated (mean $\pm$ SEM) for the 90 minute sessions on each day of extinction (days 16–20). In the absence of both CS and US the animals extinguished their drug seeking behavior over a period of 5 days. Active lever presses on extinction days 2–5 were significantly decreased (** $p<.001$, RM

ANOVA, Holm-Sidak) from those measured on the first day of extinction, (i.e. protocol days 17–20 vs. 16).

Figure 2. Extinction training reduces the initial response to diffuse contextual cues

A) Extinction behavior within a session on the fifth day of extinction (protocol day 20) is illustrated using 10 minute bins. Data are the mean $\pm$ SEM of lever presses throughout the 90 minute extinction session. B) Data show the mean $\pm$ SEM of lever presses for the first 10 minute bin of the initial reinstatement test day (day 21) when the animals were placed back into the operant environment after either 5 days of extinction training (n=22) or 5 days of enforced abstinence (n=14). Drug seeking behavior on day 21 in response to diffuse contextual cues was significantly decreased in the extinguished group as compared to the behavior of the abstinent group (** $p < .01$, unpaired t-test).

Figure 3. Priming with either CS or US evoked drug seeking behavior

A) Data show the mean $\pm$ SEM of active and inactive lever presses in 10 minute bins for 30–70 minutes of the CS prime test session on the first reinstatement test day (day 21) for the extinguished ($n=22$) and the abstinent groups ($n=14$). Lever pressing responses during the “pre priming” period (30–40 min) were minimal. The delivery of a single, noncontingent CS prime (light and tone) at time=40 minutes reinstated the response specifically on the active lever, whereas inactive lever responses remained low throughout the session. Post prime responses on the active lever were significantly higher for both groups when compared to their respective pre prime responses (2-way ANOVA, Holm-Sidak). In addition, the abstinent group showed significantly higher responding to the single noncontingent CS prime as compared to the extinguished group (* $p<.05$, 2-way ANOVA, Holm-Sidak). B) Data represent the mean $\pm$ SEM of lever presses for a 30 minute window after the single, noncontingent intravenous delivery of a drug prime at the indicated dose was administered at time=40 minutes on each of the reinstatement test days (protocol days 22–24). For both extinguished and abstinent

groups, the post prime response was significantly greater than the pre prime response at all of the cocaine doses tested (data not shown) and the drug seeking responses of the abstinent animals (n=14 for 0.5 mg/kg and n=11 for 1 mg/kg) were significantly higher than those of the extinguished animals (n=21 for 0.5 mg/kg and 1 mg/kg) at priming doses of 0.5 mg/kg and 1 mg/kg (* $p < .05$, 2-way ANOVA, Holm-Sidak).

Figure 4. Influence of NMDAR activity on the progression of extinction

Data shows the mean $\pm$ SEM of active lever presses for the entire 90 minute extinction sessions on protocol days 16–20. The saline treated control group data is replotted from Fig. 1B for comparison. Active lever pressing behavior was extinguished across the 5 daily extinction sessions and responding on extinction days 2–5 was significantly decreased ($p < .001$, 2-way RM ANOVA, Holm-Sidak) from that measured on the first day of extinction (i.e. protocol days 17–20 vs. 16) within each treatment group. A trend was evident in the D-serine treated group ($n=15$) for an enhanced rate of extinction as compared to the animals treated with ($\pm$) CPP ($n=17$) or saline, and the drug seeking responses of the D-serine group were significantly lower (* $p < .05$, 2-way RM ANOVA, Holm-Sidak) on the first and third days of extinction (protocol days 16 & 18).

Figure 5. Altering NMDAR activity during extinction had no effect on the resumption of drug-seeking following either exposure to contextual cues or the CS priming, but did effect the response to US priming

The saline group data depicted in panels A–C. was previously designated as the “extinguished group” in Figs. 2 & 3 and is replotted here for comparison. A) Data show the mean $\pm$ SEM of lever presses for the first 10 minute bin of time on the first reinstatement test day (protocol day 21). Neither D-serine (100 mg/kg i.p., n=15) nor (±)CPP (5 mg/kg i.p., n=17) treatment during extinction training had a significant effect on responding as compared with the saline treated control group. B) Data show the mean $\pm$ SEM of active and inactive lever presses in 10 minute bins for 30–70 minutes of the CS prime test session on the first reinstatement test day (day 21)

for the D-serine (n=15) and the ($\pm$)CPP (n=14) groups. Post prime responses on the active lever were significantly higher for both groups when compared to their respective pre prime responses (2-way ANOVA, Holm-Sidak). Neither treatment affected the magnitude of the post priming response as compared with the saline control groups. C) Data represent the mean $\pm$ SEM of lever presses for a 30 minute window after the single, noncontingent intravenous delivery of a drug prime at the indicated dose was administered at time=40 minutes on each of the reinstatement test days (protocol days 22–24). For both D-serine and ($\pm$)CPP groups, the post prime response was significantly greater than the pre prime response at all of the cocaine doses tested (data not shown) and the drug seeking responses of the ($\pm$)CPP animals (n=13 for 0.5 mg/kg and 1 mg/k) were significantly higher than those of the saline animals (* $p < .05$, 2-way ANOVA, Holm-Sidak).