Published in final edited form as:

Behav Pharmacol. 2009 March ; 20(2): 195–203. doi:10.1097/FBP.0b013e32832a8f78.

Effects of Abstinence or Extinction on Cocaine-Seeking as a Function of Withdrawal Duration

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Abstract

The resumption of drug seeking behavior following abstinence or extinction are commonly studied models for relapse in addiction. For the benefits of extinction training over a given withdrawal period to be determined, it is necessary to discriminate between the potentially overlapping occurrence of incubation with that of spontaneous recovery. This comparison has been assessed using a between-subjects design in groups of abstinent and extinguished rats tested at various periods of withdrawal following cocaine self-administration. Multiple forms of priming were employed to evoke the resumption of drug seeking, as different priming stimuli have been reported to utilize distinct neurobiological mechanisms and therefore may exhibit different temporal characteristics. In abstinent animals (30 days), neither the non-contingent CS-primed nor the non-contingent cocaine-primed drug seeking displayed incubation, whereas the drug seeking provoked by exposure to the contextual cues of the operant chamber significantly increased. In extinguished animals, evidence of spontaneous recovery of responding was observed following priming with exposure to either contextual or cocaine priming stimuli. Lastly, extinction training remained effective in reducing the reinstatement response levels following contextual or cocaine priming even if such training was initiated after an extended period (24 days) of abstinence. These findings provide further insight into the time dependent effects of abstinence and extinction on the resumption of drug seeking behavior.

Keywords

rat; addiction; spontaneous recovery; incubation

Introduction

The reinstatement of drug-seeking behavior is a commonly utilized rodent model for aspects of human addiction such as craving and relapse (Shaham et al., 2003); however several issues of interest concerning the characterization of the model remain to be investigated. For example, although the general effectiveness of extinction training is well accepted, the protocols employed have varied widely in terms of number of training sessions, the interval of abstinence following self-administration, the timing of reinstatement test sessions, etc. One item of interest as it may relate to the treatment of human addicts concerns the persistence of extinction to reduce subsequent drug-seeking behavior. It would be necessary to know what the expected time course of effectiveness would be in order to be able to determine an appropriate treatment

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maintenance schedule for patient management. A second area of interest is the timing of the initiation of the extinction training protocol with respect to the last drug exposure. Due to a variety of circumstances, the timing for the initiation of extinction-based therapy in human addicts could vary considerably with respect to the length of abstinence experienced before treatment was begun. The determination of whether a critical window of time exists following the cessation of drug use that alters the effectiveness of treatment intervention is therefore of great practical importance.

As compared with extinction training, enforced abstinence is not an effective means to reduce reinstatement of drug-seeking, as increasing periods of withdrawal have been shown to enhance drug-seeking behavior under some conditions (Grimm et al., 2001; Tran Nguyen et al., 1998). This “incubation effect” has been suggested to be an important component of the persisting susceptibility for relapse in humans (Grimm et al., 2001). In order to determine the persistence of extinction effectiveness, it is therefore necessary to have time-matched abstinence control groups to account for any confounding influence of concomitant incubation. Using a second-order schedule of reinforcement and reinstatement of cocaine-seeking, one published study has assessed the persistence of the effectiveness of extinction training vs. abstinence (Di Ciano & Everitt, 2002). In that report, the authors concluded that spontaneous recovery observed following extinction was possibly due to incubation, although importantly, incubation of responding was not evident among their abstinent groups. Thus regarding the potential interaction between spontaneous recovery (the resumption of extinguished drug-seeking with the passage of time) and incubation (the time-dependent increase in drug-seeking following withdrawal from self-administration), further characterization is needed using a protocol in which both phenomena can be observed.

As argued above, the potentially confounding effects of both spontaneous recovery and incubation must be differentiated in the experimental protocol in order to allow for an effective interpretation of the results. It is also evident that the time dependency of any such changes is potentially unique for the specific form of reinstatement being assessed. For example, Lu et al. (2004) have reported that incubation is observed when cocaine-experienced rats are allowed contingent access to lever light/tone cues, but time-dependent changes were not observed if a non-contingent i.p. administration of cocaine itself was used to evoke reinstatement behavior. Thus monitoring drug-seeking responses following priming with multiple types of stimuli is another important consideration for incorporation into the experimental design. In this report, reinstatement results from both abstinent control and extinguished groups are described for three different forms of primed resumption of drug-seeking behavior, following exposure to diffuse contextual cues (operant environment), proximal CS cues (lever light/tone), or drug infusion (i.v. cocaine), using a protocol employing the noncontingent presentation of these cues (Kelamangalath et al., 2007).

The matter of delay in the initiation of extinction training is another topic that has not been explored systematically by evaluating time-matched abstinent and extinguished groups of animals using the rodent reinstatement model. The temporal component for the initiation of extinction training following drug exposure has been explored here by assessing the effectiveness of an “early” extinction protocol (initiated 1-day following the last cocaine self-administration session) as compared with a “late” extinction protocol (initiated 24-days following the last cocaine self-administration session). Taken together, the results indicate that the effectiveness of extinction training to significantly reduce reinstatement can persist for a period of more than three weeks, depending on the form of reinstatement being assessed. In addition, a three-week delay in the initiation of extinction training does not prevent reductions in reinstatement behaviour, depending on the form of reinstatement being assessed. These preclinical findings using the rodent self-administration/reinstatement model demonstrate that behavioral therapies based on extinction principles can exhibit both the persistence and

temporal characteristics consistent with practical applications for the treatment of addiction in humans.

METHODS

Subjects

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 free access to food and water 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 vein catheterization—The animals were anesthetized using a combination of ketamine (75 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 and cleaned. 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.

Apparatus—The experiment was performed in an operant chamber 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 then 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 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.

Procedure—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 and 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. 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. In order to increase active lever responding, 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.

After the 15 days of self-administration (SA) training, the animals were divided into 5 groups (balanced for cocaine intake): 1) extinguished starting the day after SA, continued for five days, and then tested one day after this “early” extinction (EET1). 2) extinguished starting the day after SA, continued for five days, and then tested twenty four days after early extinction (EET24). 3) extinguished starting twenty four days after SA, continued for five days, and then tested one day after this “late” extinction (LET1). 4) abstinent for six days after SA and tested on this day with out any prior extinction training experience (A6). 5) abstinent for thirty days and tested on this day with out any prior extinction training experience (A30). During extinction training, animals were placed back in the self-administration environment (attached to the drug tether, house light and ventilation fan on) and were exposed to the environment stimuli (i.e. diffuse, contextual cues) of the operant chamber. Responses on the active lever had no programmed consequences during extinction training. During the extinction sessions, responses on both active and inactive levers were counted by the software (although as stated above, syringe pumps were not activated during this phase of training). Extinction training proceeded over a period of 5 days, with one 90 minute session each day during which the animals in the extinction training groups (EET1, EET24 and LET1) 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. Thus extinction training was started either “early”-one day after the self-administration phase (EET1 and EET24) or “late”-after waiting 24 days after the last SA day (LET1). As previously mentioned, the abstinent group animals remained in their home cages until their test day, except that the A30 rats were moved to an alternate environment for 90 minutes during the last 5 days of abstinence (days 25–29) before being tested on day 30. On each group’s reinstatement test day, the 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 placed in the operant chamber environment (attached to the drug tether, house light and ventilation fan on) and any responses on the active lever were not reinforced by the contingent availability of either CS or drug.

On the first reinstatement test day, drug-seeking in response following reintroduction to the operant chamber environment was assessed from active lever presses during the first 10 minutes of the session. We have previously characterized this measure as a reinstatement response to the diffuse contextual cues of the drug taking environment (Kelamangalath et al., 2007). Later during the same test session, lever presses evoked in response to a CS presentation were then assessed. A single, non-contingent presentation of the CS 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 was delivered. The response to the non-contingent CS was quantified as the number of responses on the active lever over ten minutes following the priming event ($t = 40\text{--}50$ min), and compared with those from the 10 minutes prior to the priming event ($t = 30\text{--}40$ min). Although modest in terms of the absolute response magnitude, the rate of responding among the abstinent rats for this 10 minute period is comparable to the number of active lever presses observed for an equivalent period during the final day of self-administration (c.f. Kelamangalath et al., 2007).

Response to the drug prime stimulus was assessed on the second test day. We tested the reinstatement of drug seeking behavior using the same dose as the self-administered dose (0.5 mg/kg) of cocaine. A single, non-contingent cocaine infusion was programmed to be delivered intravenously by the syringe pump at the 40th minute of the 90 minute session on this drug primed reinstatement test day. Again, the initial 40 minutes served as an extinction period which allowed lever presses initiated by exposure to contextual stimuli to subside before the reinstatement test. Drug seeking behavior elicited by the cocaine prime was quantified as the number of responses on the active lever over 30 minutes following the drug prime ($t = 40\text{--}70$ min), and compared with those from the 30 minutes prior to the priming event ($t = 10\text{--}40$ min). The cocaine dose-response and the absence of response to saline vehicle i.v. priming has previously been reported (Kelamangalath et al., 2007).

Abstinent groups of rats were tested for their responses to the contextual stimuli, non-contingent cue prime and the non-contingent drug prime as a function of the withdrawal period (A6 vs. A30). Also note that, as described above, these abstinent animals experienced extinction conditions during the first 40 min of the reinstatement test sessions. In extinguished groups of rats, both the persistence of the effects of extinction (EET1 vs. EET24) and the effectiveness of delayed extinction training (EET1 vs. LET1) to reduce the reinstatement responses were assessed.

Drugs—Cocaine hydrochloride was a gift from the National Institute on Drug Abuse (Research Triangle Institute).

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. One-way ANOVA was applied to the data in Figure 1, Figure 2A and Figure 3A. For Figure 2B and C, the two factors taken into consideration for the 2-way ANOVA were: 1) trial (pre vs. post priming responses) and 2) the duration of abstinence (A6 vs. A30). For Figure 3B and C, the two factors taken into consideration for the 2-way ANOVA were: 1) trial (pre vs. post priming responses) and 2) either the persistence of extinction (EET1 vs. EET24) or the delay in initiating the extinction training (EET1 vs. LET1). A value of $p < .05$ was taken as significant, being determined either from the Holm-Sidak post hoc test method or from Students unpaired t-tests for planned comparisons. All the statistics were done using SigmaStat, version 3.1 software.

RESULTS

Self-administration training

All groups of rats exhibited similar acquisition of cocaine self administration over 12 days of 90 min sessions on an FR-1 schedule of reinforcement (Figure 1A). Following transition to an FR-3 schedule on day 13, the mean $\pm$ SEM of the infusions during the last 3 days of cocaine self-administration training for the five study groups (A6, A30, EET1, EET24, LET1; Figure 1B) was 27.7 ± 1.7 . There was no significant difference in the mean number of infusions self administered among the five different groups of rats ($p > .05$, one-way ANOVA). A saline self-administration group from a previous study averaged fewer than 3 infusions/day (Kelamangalath et al, 2007).

Drug-Seeking Responses in Abstinent Rats

Three different groups of animals kept abstinent for different withdrawal periods (1, 6, 30 days-A1, A6, A30 groups, respectively) were tested for their extinction response following reintroduction to the drug taking environment (Figure 2A). Two of these groups of abstinent animals were also tested for their reinstatement response to a single, non-contingent

presentation of either the compound CS (Figure 2B) or an intravenous drug infusion (Figure 2C), as described previously (Kelamangalath et al., 2007). None of the abstinent animals tested in this experiment were exposed to extinction conditions until their respective reinstatement test sessions were initiated.

Response in the drug taking environment (contextual cues)—Drug seeking behavior in response in the environmental context of the self-administration chamber was assessed on the initial reinstatement test by quantifying the active lever responses during the first 10 minutes of the session. Figure 2A illustrates the non-reinforced responses on the active and inactive levers following different periods of withdrawal in the groups tested. The A1 group was tested after one day of abstinence (derived by combining the EET1 & EET24 responses from their 1st day of extinction training). The A6 group was tested on the 6th day of abstinence and the A30 group was tested on the 30th day of abstinence. A significant effect was evident for withdrawal period on active lever responding in the A30 group as compared to the A1 ($t=4.78$, $p<.05$, one-way ANOVA, Holm-Sidak) and A6 ($t=3.04$, $p<.05$, one-way ANOVA, Holm-Sidak) groups. Responses on the inactive lever were not significantly different among these abstinence groups. These results indicate that the drug seeking behavior in response to the diffuse contextual drug cues present in the operant chamber environment can incubate over time in abstinent animals, in agreement with previous reports (Tran-Ngyuen et al, 1998; Grimm et al, 2001).

Response to the non-contingent CS—The A6 and A30 groups were also tested for their response to the non-contingent CS (light/tone) during the first reinstatement test session. Figure 2B illustrates the active and inactive lever presses for both the 10 minutes before (“pre”) and 10 minutes after (“post”) delivery of the compound CS for the A6 and A30 groups. Analysis by two-way ANOVA confirmed an effect of trial [$F(1,42)=13.56$, $p<0.001$] but not condition between the groups. In the A6 group, the post-prime response on the active lever was significantly greater than that of the pre-prime response ($p<.05$, paired t-test). Similarly, in the A30 group, the post-prime response was significantly greater as compared to the pre-prime response ($p<.01$, paired t-test). Comparison of the post CS prime responses between the A6 and A30 groups was not significantly different ($p>.05$, two-way ANOVA) and therefore demonstrates that the drug seeking behavior in response to the non-contingent CS prime did not incubate as a function of the additional 24 day withdrawal period under these conditions. This is in contrast to the findings in 2A above concerning the diffuse contextual cues.

Reinstatement response to the noncontingent drug prime—The A6 and A30 groups were also tested for their response to a single, noncontingent drug prime (i.v. cocaine, 0.5mg/kg) on the second reinstatement test day. Figure 2C illustrates the active and inactive lever presses for 30 minutes before (“pre”) and after the drug prime (“post”). Analysis by two-way ANOVA confirmed a significant effect of trial [$F(1,42)=14.4$, $p<0.001$] but not condition between the groups. The post-prime responses on the active lever in both the A6 and A30 groups were significantly greater than their respective pre-prime responses ($p<.05$ for A6 and $p<.01$ for A30, paired t-tests). Similar to the results with the CS primed reinstatement test, a comparison of drug prime response between the A6 and A30 groups indicates that the drug-seeking behavior in response to the noncontingent cocaine prime does not incubate over the additional 24 day withdrawal period in these abstinent animals, as they did not significantly differ in their reinstatement response ($p>.05$, two-way ANOVA). This finding is consistent with that of Lu et al. (2004) concerning i.p. cocaine-induced drug seeking.

Drug-Seeking Response in Extinguished Rats

Three different groups of animals were given extinction training for 90 minute sessions for a period of consecutive 5 days and then tested for reinstatement of drug-seeking behavior at

different time points following their last extinction session (Figure 3). One group of animals were extinguished beginning 1 day after their last self-administration session; this group was then tested for reinstatement responses 1 day after the last extinction session. Thus, this “early extinction” group “tested 1” day later for reinstatement was designated group “EET1”. A second group was also extinguished beginning 1 day after self-administration, but reinstatement testing was not initiated until 24 days after the last extinction session. This “early extinction” group was “tested 24” days later for reinstatement and was designated as group “EET24”. A third group was not extinguished until 24 days after the last self-administration session; this group was then tested for reinstatement responses 1 day after their last extinction session. This “late extinction” group was “tested 1” day later for reinstatement and was designated as group “LET1”. Both the rate of extinction over the 5-day extinction training period and active lever responding on the fifth day of extinction training was not significantly different among the three groups (2-way RM ANOVA, data not shown).

Response to the drug taking environment (contextual cues)—All three extinguished groups were tested for their drug seeking behavior in response to the environmental context of the self-administration chamber (Figure 3A) as described above. The EET1 and LET1 groups were tested on the first day after their last extinction session. Regardless of whether extinction was initiated 1 day after the last self-administration session (EET1) or whether the extinction was initiated 24 days after the last self-administration session (LET1), these animals showed a similar level of responding during the first ten minutes of the test session. However, we observed an increase in the contextual response from the animals extinguished 1 day after the last self-administration session, but not tested for reinstatement until 24 days since last extinction session (EET24 vs. EET1). A significantly higher responding on the active lever was evident in this EET24 group ($\dagger p < .001$, one-way ANOVA, Holm-Sidak) as compared to the EET1 group tested on the first day after their last extinction session. These results indicate that the drug seeking behavior in response to the diffuse contextual cues may spontaneously recover over time in extinguished animals.

Response to the non-contingent CS—The extinguished animals were also tested for their response to the non-contingent CS during the first reinstatement test session. Figure 3B illustrates the active and inactive lever presses for both the 10 minutes before (“pre”) and 10 minutes after (“post”) delivery of the compound CS for the EET1, LET1 and the EET24 groups. Analysis by two-way ANOVA confirmed an effect of trial [$F(1,74) = 23.3$, $p < 0.001$] but not of condition among all three groups. The post-prime responses on the active lever were significantly greater compared to their respective pre-prime responses ($**p < .01$, $*p < .05$, paired t-tests). The planned comparisons of the post-prime responses among these three extinguished groups showed no significant differences ($p > .05$, unpaired t-tests).

Response to the non-contingent drug prime—The three extinguished groups were also tested for their response to a single, non-contingent drug prime (i.v. cocaine, 0.5mg/kg) on the second reinstatement test day. Figure 3C illustrates the active and inactive lever presses for 30 minutes before (“pre”) and after the drug prime (“post”). Analysis by two-way ANOVA confirmed an effect of trial [$F(1,68) = 30.5$, $p < 0.001$] and condition [$F(2,68) = 3.3$, $p < 0.05$], but no significant trial x condition interaction. The post-prime responses on the active lever in the EET1, EET24 and the LET1 groups were significantly greater than their respective pre-prime responses ($**p < .01$, paired t-tests). The planned comparisons of the post-prime responses between these extinguished groups showed a significantly greater reinstatement response for EET24 as compared to EET1 ($\dagger t = 2.8$, $p < .05$, unpaired t-tests), suggesting that the drug seeking behavior in response to the non-contingent cocaine prime may spontaneously recover over time in extinguished animals.

Comparison between the extinguished and abstinent animals

In this study, we were interested in characterizing both the persistence of the effects of extinction training as well as exploring the temporal component for the initiation of such training following the end of drug self-administration. To fully evaluate these parameters, we needed to compare the effects of extinction training to those of equivalent periods of abstinence in separate groups of animals. We have previously reported that extinction training is effective in reducing reinstatement responding to contextual cues, non contingent CS, and non contingent drug prime events (Kelamangalath et al., 2007), as illustrated again in Figure 2 and Figure 3 (compare A6 vs. EET1). In the current study, regarding the reinstatement response elicited by exposure to the contextual cues of the operant environment (Figure 2A and Figure 3A), we have found that both the EET24 and LET1 groups still significantly reduced reinstatement responding ($p < .001$, one-way ANOVA, Holm-Sidak) compared with their abstinent time-matched control group (A30). These findings demonstrate that the effects of extinction training can persist for more than 3 weeks (EET24) concerning this form of reinstatement, and that such a delay in initiating extinction training does not prevent a significant reduction in responding (LET1).

With respect to the reinstatement response elicited by non contingent exposure to CS cues (Figure 2B and Figure 3B), we found that compared with their respective time-matched abstinent control (A30), neither the EET24 group nor the LET1 group significantly reduced reinstatement responding ($p > .05$, unpaired t-tests). These results suggest that in the case of the EET24 group, the effectiveness of extinction training to reduce CS reinstatement does not persist over this time period. In the case of the LET1 group, the effectiveness of extinction training to reduce CS reinstatement is diminished if delayed following the final day of self-administration. Finally, when the reinstatement response was elicited by non contingent exposure to i.v. drug prime (Fig 2C & Fig 3C), we found that the LET1 group significantly ($t = 2.9$, $p < .05$, unpaired t-test) reduced reinstatement responding as compared with the A30 group whereas the EET24 group did not. These results suggest that in the case of the EET24 group, the effectiveness of extinction training to reduce drug primed reinstatement spontaneously recovers over this 24 day period following the final day of self-administration. A 24 day delay in initiating extinction training did not preclude a significant reduction of responding to this form of priming in the LET1 group.

DISCUSSION

In this study we investigated the time dependent increase in the drug-seeking behavior induced by the different withdrawal periods in animals having previously self-administered cocaine. A between-subjects design was utilized in which different groups of animals were tested at different time points. We found that the drug-seeking behavior in response to the diffuse contextual cues of the self-administration chamber increase in a time-dependent manner, such that animals tested after a withdrawal period of 30 days exhibited a greater drug-seeking response than those tested after a withdrawal period of 6 days. In contrast, we did not observe a time-dependent increase in the non-contingent cue-induced reinstatement response nor the non-contingent drug-induced reinstatement response in the animals tested at these same withdrawal time points. In the second part of this study our aim was to investigate two aspects of practical importance regarding the effects of extinction training on the reinstatement of drug-seeking behavior: The first was to assess the persistence of the effects of extinction on reinstatement. The second was to determine whether there was evidence for a critical time period within which extinction training should be initiated for it to be effective in reducing the reinstatement response. Our previous findings indicate that the extinction training protocol we have employed is effective in reducing the reinstatement response to diffuse contextual cues, non-contingent cue prime and non-contingent drug prime (Kelamangalath, et al., 2007). Those

results were obtained from initiating the extinction training the next day following 15 days of cocaine self-administration (illustrated herein as the EET1 group). In comparison, the current data from the reinstatement tests for the EET24 group suggest that the effects of extinction to reduce the reinstatement response to certain stimuli such as the non-contingent cue prime or a drug prime do not persist over >3 weeks of abstinence (although a decreased reinstatement response to the contextual cues of the operant chamber was still present). Importantly, the reinstatement data from the LET1 group suggest that extinction can still reduce the reinstatement response even if there is a delay in the initiation of such training, demonstrating that a >3 week's time delay does not render extinction training ineffective. Details concerning these results and their implications are outlined below.

The data obtained using our experimental protocol demonstrate a time-dependent increase in drug-seeking behavior in response to the contextual cues of the drug-taking environment among the abstinent animals as a function of the withdrawal period. We observed an incubation-like effect in response to these contextual cues when the abstinent animals were reintroduced to the operant chamber environment for the first time after 30 days of withdrawal. These results agree with the findings of other investigators reporting a resistance to extinction among abstinent animals held for longer withdrawal periods (Tran-Nguyen et al, 1998; Grimm et al, 2001, 2003). Concerning cue-induced reinstatement studies, prior investigations have utilized a contingent method where the discrete cues associated with the drug availability were presented in a response contingent manner. These reports have observed either modest time-dependent increases (Tran-Nguyen et al, 1998; Neiswander et al, 2000) or no increase (Di Ciano and Everitt, 2002; Deroche-Gamonet et al, 2003 and Marinelli et al, 2003) in cue-induced reinstatement. In contrast, others have found robust time-dependent increases in drug-seeking behavior using a contingent paradigm of cued reinstatement (Grimm et al, 2001, 2003; Lu et al, 2004). In these previous studies, cocaine-seeking behavior is operationally defined as the non-reinforced lever pressing response; however the typical method of cued reinstatement assessment also contains some aspect of reinforcement in that the animal's lever pressing behavior initiates a response-contingent presentation of the CS, which itself can exhibit incentive salience (Uslaner et al, 2006). Since self-reported craving in humans can be induced via the non-contingent presentation of these cues (Childress et al, 1999), it has been suggested that a non-contingent CS reinstatement protocol in the rodent model should also employ this form of priming (Katz and Higgins, 2003). In the attempt to model the human condition of relapse better, we have utilized a single non-contingent passive presentation of the CS to evoke resumption of drug seeking. Using this approach, we did not observe an incubation of the cued reinstatement response over >3 weeks, suggesting that contingency may be an important consideration for the observance of incubation of CS primed responding in the rodent self-administration model.

Several studies have also investigated the incubation-like effect on the drug-induced resumption of drug-seeking behavior. It is common for addicts to experience lapses in abstinence, and such re-exposures to drug can increase wanting and craving (Jaffe et al, 1989). Hence it is important to study the degree of vulnerability to drug-induced resumption of drug seeking among abstinent animals for longer withdrawal periods, to determine whether the expected incubation pattern occurs. Three studies have assessed the time-dependent changes in drug-seeking behavior induced by cocaine priming and found that the drug-seeking behavior either increases over the first month of withdrawal (Tran-Nguyen et al, 1998 cocaine 15mg/kg i.p.), decreases (Deroche-Gamonet et al, 2003 using a range of doses (0.2–1.6mg/kg i.v., using a within subject design) or does not change (Lu et al, 2004 cocaine 2.5, 5 and 15mg/kg i.p.). Thus it is not clear whether the cocaine-induced drug seeking behavior follows a similar pattern as previously observed for the contextual reinstatement. In our experiments we have used a between-subjects design and primed with a single i.v. infusion of cocaine at a dose of 0.5mg/kg (same as the self-administered dose and route) and these results demonstrated that

the cocaine-induced drug-seeking behavior does not increase over the one month withdrawal period.

With respect to characterizing the effectiveness of extinction to reduce drug-seeking behavior, we had two primary objectives: The first was to assess the persistence of the effects of extinction and the second was to determine whether extinction is effective in reducing the reinstatement of drug-seeking behavior even when the initiation of extinction training is delayed. Both of these aspects of the effects of extinction on the reinstatement of drug seeking behavior are important considerations when it comes to the potential use of extinction-based behavioral therapy in the treatment of addiction. The effects of extinction to reduce conditioned responding have been observed to diminish over time, a phenomenon referred to as spontaneous recovery (see Bouton et al., 2006 for review). There has not been a clear discrimination of this effect from that of incubation in the rodent self-administration literature. Our current results, using either a non-contingent CS prime, or a non-contingent drug prime, support the conclusion that spontaneous recovery can occur in the absence of incubation in a time-matched abstinent group (c.f. Figure 2B&C and Figure 3B&C). A second objective was to examine the timing for beginning extinction training following drug exposure. If the initiation of extinction was delayed for a period of >3 weeks, we found that the extinction training was still effective in reducing the drug seeking behavior induced by either exposure to the diffuse contextual cues of the operant environment (Figure 3A) or by exposure to drug itself (Figure 3C). These findings are promising in that they suggest that extinction therapy can be effective in reducing drug-seeking behavior even if there is a delay in starting the treatment protocol.

In summary, we have noted that the effectiveness of extinction to reduce drug-seeking behavior does not persist indefinitely, as the effectiveness of extinction decreases with the passage of time. These findings of spontaneous recovery support the concept of needing “booster” extinction trials or spaced extinction trials to maintain decreased primed responding. Rescorla (1997) found that when animals are extinguished in the presence of cues, the extinguished response returned in one group of rats tested 8 days post-extinction, compared to those tested immediately after extinction. Bouton (1993) has shown that spontaneous recovery among extinguished animals will occur if extinction trials are relatively isolated. In human addiction therapy, the treatment sessions are often conducted for several consecutive sessions within a specified period, and subsequently cease. Our results reinforce the idea of having ongoing, spaced extinction trials as reminder sessions in order to attenuate the influence of spontaneous recovery. Preclinical findings such as these should be considered in the design of effective protocol for the treatment of addiction in humans (Conklin and Tiffany, 2002).

Acknowledgments

This work was supported by the National Institutes of Health (DA016302)

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Figure 1.

A) Cocaine self-administration training: The results illustrate the average number of cocaine infusions earned daily during the 90 minute sessions of self-administration phase (days 1–15). Transition to FR3 schedule on day 13 did not significantly alter the number of earned infusions, and the treatment groups tested in this study did not significantly differ in the mean number of cocaine infusions earned per session nor the total amount of cocaine ingested (not shown).

B) An experimental outline for the five experimental conditions in this study. Each respective group "" began reinstatement testing either six days (A6, EET1) or 30 days (A30, EET24, LET1) following the last day of self-administration.

Figure 2.

Exposure to contextual cues or priming with either CS or cocaine evoked drug seeking behavior among abstinent animals. **A)** Incubation effect in the enforced abstinent animals shown as a response to the diffuse contextual cues on the first reinstatement test day: Data illustrate the mean $\pm$ SEM of the lever presses for the initial 10 minutes of the first reinstatement test session in 1 day (A1, $n=32$), 6 day (A6, $n=14$) and 30 day (A30, $n=10$) groups of abstinent animals. Filled bars represent the active lever presses and open bars represent the inactive lever presses for each group. Drug seeking behavior in the A30 abstinent animals in response to the diffuse contextual cues was significantly higher as compared to the A1 and A6 abstinent groups ($\dagger p < .01$, one-way ANOVA, Holm-Sidak). **B)** Data illustrate the mean $\pm$ SEM of the active (filled

bars) and inactive (open bars) lever presses 10 minutes before and after the single non-contingent CS prime for the A6 (n=14) and A30 (n=10) groups. The filled bar on the left of A6 and A30 groups represents the pre prime response (30–40min) and the filled right bar represents the post prime response (40–50min) on the active lever. The empty bars represent the corresponding inactive lever presses for each group and the post prime response on the active lever was significantly greater than the pre prime response (* $p < .05$, ** $p < .01$, paired t-tests). C) Data illustrate the mean + SEM of the active (filled bars) and inactive (open bars) lever presses 30 minutes before and after the delivery of a single, non-contingent intravenous drug prime (0.5mg/kg) at time = 40 minutes on the second day of reinstatement testing. The filled bars on the left of A6 and A30 groups represents the pre-prime response (10–40min) and the filled right bar represents the post-prime response (40–70min) on the active lever. The empty bars represent the corresponding inactive lever presses for each group. For both the A6 (n=14) and A30 (n=10) groups, the post prime response on the active lever was significantly greater than the pre prime response (* $p < .05$, ** $p < .01$, paired t-tests).

Figure 3.

Exposure to contextual cues or priming with either CS or cocaine evoked drug seeking behavior among the extinguished animals. **A)** Data show the mean + SEM of the lever presses for the initial 10 minutes on the first reinstatement test day in the group EET1 (n=22), EET24 (n=10) and LET1 (n=9) when they were exposed to the diffuse contextual cues of the operant chamber environment. Patterned bars represent the active lever responding and the empty bars represent the inactive lever responding. The EET24 group exhibited spontaneous recovery of contextual responding on the active lever when tested 24 days after their last extinction experience compared with the EET1 animals tested 1 day post extinction ($\dagger p < .001$, one-way ANOVA, Holm Sidak). **B)** Data show the mean + SEM of the active (patterned bars) and inactive (empty

bars) lever presses 10 minutes before and after the single non-contingent CS prime for the groups EET1 (n=22), EET24 (n=9) and LET1 (n=9). Patterned bars on the left represent the pre-prime response (30–40min) and on the right represent the post-prime response (40–50min) on the active lever for each group. The empty bars represent the corresponding inactive lever presses for each group and the post prime response on the active lever was significantly greater than the pre prime response for all groups (**p<.001, *p<.01, paired t-tests). C) Data represent the mean + SEM of the lever presses for the 30 minute window before and after the delivery of the single non-contingent intravenous drug prime at a dose of 0.5mg/kg at time = 40 minutes of the 90-minute test session on the second test day. Patterned bars represent the active and the empty bars represent the inactive lever presses. In each group, the filled bars on the left show the active lever presses for the pre-prime period (10–40 min) and those on the right show the same for the post-prime period (40–70min). The empty bars represent the corresponding inactive lever presses, and the post prime response on the active lever was significantly higher for the all groups (EET1, n=20; EET24, n=8; and LET1, n=9) as compared to the pre prime response (**p<.001, *p<.01, paired t-tests). The post-prime response of group EET24 was significantly greater (†p<.05, unpaired t-test) than the post-prime response of group EET1.