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ORIGINAL INVESTIGATION

Social reinstatement: a rat model of peer-induced relapse

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Abstract

Background An important factor that can lead to drug relapse is to re-associate with drug-using social peers, but there is little literature on the effect of social peers on relapse in animal models.

Methods The current study used a dual-compartment operant conditioning apparatus that allowed adult male rats to respond for cocaine in the presence of a conspecific. In experiment 1, rats were trained to self-administer cocaine in the presence of a social peer that was separated by a wire screen partition and then that peer was used as a reinstatement cue following a period of extinction. In the next experiments, rats were trained on alternating sessions to self-administer cocaine in the presence of one peer and to self-administer saline in the presence of a different peer using either a single-active lever procedure (experiment 2) or a double-active lever procedure (experiment 3). Following extinction of responding in the absence of the peers, the effect of re-exposure to the cocaine- and saline-associated peers on reinstatement of drug seeking was determined. This was tested using both single- and double-active lever procedures.

Results In experiment 1, a peer that was present throughout cocaine self-administration was able to reinstate cocaine seeking following a period of extinction. In experiments 2 and 3, drug seeking was reinstated by the cocaine-associated peer (S+), but not the saline-associated peer (S−). This discrimination occurred when using either the single-active lever procedure or double-active lever procedure.

Conclusion These results indicate that a social peer can be used as a discriminative stimulus to signal cocaine availability and that re-introduction of a peer previously paired with cocaine can reinstate cocaine seeking, confirming clinical reports that peer affiliation among abstinent cocaine users is an important determinant of relapse.

Keywords Cocaine · Self-administration · Social · Reinstatement

Introduction

Following treatment for a substance use disorder, evidence indicates that maintaining abstinence is best achieved when the patient avoids the environment previously associated with drug use (Kelly et al. 2014). This stems from evidence showing that drug cues play a role in craving and relapse (Kosten et al. 2006; O'Brien et al. 1992). These cues can be both contextual (neighborhood) and discrete (drug paraphernalia). In addition to these inanimate cues, considerable evidence indicates that re-associating with former drug-using social peers plays an important role in relapse (Beattie 2001; Brewer et al. 1998; Brown et al. 1989; Sun 2007). Unfortunately, those latter studies are primarily retrospective self-reports, and thus it is unclear if re-associating with drug-using peers is a cause or consequence of relapse. It could be that encountering a drug-using peer directly triggers relapse or, conversely, that a relapse initiates re-association with drug-using peers to gain drug access and social acceptance among users. In the current preclinical report, we sought to determine if a drug-associated peer could serve as a discriminative stimulus that signals drug availability leading to relapse.

A commonly used preclinical model to study relapse in laboratory animals is the reinstatement model (Shaham et al. 2003; Shalev et al. 2002). To date, however, there has been only one published study that investigated whether a social peer can reinstate cocaine seeking in rats (Smith et al. 2016). The apparatus used in that study consisted of two standard operant conditioning chambers that were connected by removing one side on each chamber and replacing it with a wire

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screen partition (Smith 2012). This allowed the animals to have limited contact with a social peer, but prevented any direct interference of operant responding in either chamber. Using this apparatus, Smith et al. (2016) trained rats in a discrimination procedure in which cocaine was self-administered in the presence of a peer that also self-administered cocaine in the adjacent chamber; on alternate sessions, saline was self-administered in the presence of a peer that also self-administered saline. After extinction of the cocaine-reinforced responding in the absence of any peer, reinstatement responding was assessed by re-introducing either the cocaine- or saline-associated peers on separate sessions. Results showed that non-reinforced responding on a reinstatement test was greater in the presence of the cocaine-associated peer than in the presence of the saline-associated peer; however, the cocaine-associated peer did not significantly reinstate responding relative to the last day of extinction. The difference in responding observed in the presence of the cocaine- and saline-associated peers was interpreted to reflect a combined weak excitatory effect of the cocaine peer and weak inhibitory effect of the saline peer.

One limitation in the study reported by Smith et al. (2016) is that it was not clear if the social peer per se controlled responding or whether the behavior of the peer controlled responding. This is an important distinction because the cocaine self-administering peer pressed the lever more than the saline self-administering peer, and thus, in addition to the mere presence of the peer, there were additional non-social discriminative cues (e.g., lever extension/retraction, sound of infusion pump). As a result, the discriminative stimulus was actually a compound stimulus (social peer + non-social cues). To address this issue, in the current study, we used a procedure in which the discriminative stimulus was merely the presence of a passive social peer that did not undergo any training in the operant conditioning chamber.

In the current study, three experiments were conducted using a peer discrimination procedure. In experiment 1, using a wire screen partition, we determined if a peer present throughout acquisition of cocaine self-administration would reinstate cocaine seeking following a period of extinction (no cocaine and no peer). In experiments 2 and 3, we determined if two different peers could be used as discriminative stimuli to signal the availability of cocaine; in these studies, one peer was paired with cocaine availability (S+) and a different peer was paired with saline availability (S−) across training sessions. Following a period of extinction (no peers or cocaine), we hypothesized that reinstatement of cocaine seeking would occur when the cocaine-paired peer (S+) was re-introduced, but not when the saline-paired peer (S−) was re-introduced. This latter hypothesis was tested using either a single-active lever training procedure in which the same lever was active for both cocaine and saline sessions (experiment 2) or a double-active lever procedure in which one lever was active on cocaine sessions and the opposite lever was active on saline sessions (experiment 3).

Materials and methods

Animals

A total of 66 adult male Sprague Dawley rats (200–250 g) were obtained from Envigo (Indianapolis, IN). Males were used in these initial experiments because they find social interaction more rewarding than females (Douglas et al. 2004) and testing was conducted in the light cycle because most social interaction studies use lighted conditions for the purpose of video taping of social behaviors (van Kerkhof et al. 2014). A total of 49 rats were used as responders, and 17 rats were used as social peers. They were acclimated to a colony room and handled for 7 days prior to each experiment. Rats were housed individually in a colony room held at constant temperature. Light and dark phases were on a 12:12-h cycle, and all experimental sessions occurred in the light phase. During initial food-reinforced training sessions for experiments 1 and 2, rats were food restricted (85% of free feed body weight with 10–20 g of food delivered at the end of the session), but had unlimited access to water in their home cage. For all experiments, all rats had unlimited access to food and water in their home cage during the self-administration, extinction, and reinstatement phases. All procedures were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council 2010) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

The apparatus consisted of two identically built operant conditioning chambers that were adjacent to one another, with a partition in between that confined a rat to each chamber, but allowed some social contact (custom ordered; MED Associates, St. Albans, VT; see Gipson et al. 2011 for a schematic). Each dual-chamber apparatus was located inside of a sound attenuation compartment with an exhaust fan that supplied ambient background noise. The chambers were separated by a wire screen (1.27 cm, 19 gauge) partition. Each of the adjacent operant conditioning compartments was made of aluminum, and the sidewalls were made of clear Plexiglas. The entire apparatus measured 69.8 cm × 53.3 cm × 60.9 cm (length × width × height), and each individual compartment measured 34.9 cm long. In the right compartment of each chamber (responder compartment), there was a food tray (5 × 4.2 cm) located in the bottom-center of the front wall and a retractable response lever was located on each side of the recessed food tray on the front wall. A 28-V cue light was

located 6 cm above each response lever and a house light was mounted in the back wall of each compartment. In the left compartment of each chamber (peer compartment), the recessed food tray, response levers, and cue lights were removed.

Experiment 1: peer-induced reinstatement of cocaine seeking

The purpose of experiment 1 was to determine if presence of a peer during acquisition of cocaine self-administration would serve as a cue to reinstate cocaine seeking in a responder rat following a period of extinction in which no cocaine or peer was available.

Procedure

Phase 1: pretraining Responder rats ($n = 6$) received two daily 60-min sessions of magazine training followed by eight daily sessions of lever press training. During lever press training, both levers were present, but only responses on one lever (active lever) resulted in the delivery of a palatable food pellet (45 mg; F0021 dustless precision pellet; Bio-Serv, Frenchtown, NJ). Responses on the other lever (inactive lever) had no programed consequences. Rats were required to earn 100 pellets in 60 min. The position of the active and inactive levers was counterbalanced across responder rats.

Surgery Following food pretraining, rats underwent surgery to implant a chronic indwelling jugular catheter. Rats were treated with carprofen (5 mg/kg, s.c.) the day before surgery, the day of surgery, and 2 days after surgery. For surgery, rats were anesthetized (75 mg/kg ketamine, 7.5 mg/kg xylazine, and 0.75 mg/kg acepromazine mixture) and a catheter was inserted into the right jugular vein, which exited through a port (Plastics One, Roanoke, VA) that was stabilized to a headmount made of dental acrylic. A silastic leash was used to attach an infusion pump to the headmount port during the self-administration sessions. Rats recovered from surgery for 1 week prior to beginning self-administration sessions. During this time, catheters were flushed daily with a mixture of gentamicin (0.2 ml), heparin (0.6 ml), and saline (2.0 ml).

Phase 2: cocaine self-administration Following recovery from surgery, responder rats were trained to self-administer cocaine. The response requirement increased from an FR 1 to a terminal FR 5. Sessions began with the presentation of the house light and extension of both the active and inactive levers in the responder compartment. Responses on the active lever resulted in the illumination of both cue lights and a 0.1-ml infusion of cocaine (0.5 mg/kg/infusion) delivered across 5.9 s, followed by a 20-s time-out period in which both cue lights were illuminated. A response on the active lever during the time-out had no programmed consequence. During all cocaine self-administration sessions, a male peer (similar age and weight) was present in the adjacent chamber. Responder rats were exposed to the same peer during every cocaine self-administration session. Responder rats underwent 14 consecutive daily 1-h sessions of cocaine self-administration.

Phase 3: extinction Following acquisition of cocaine self-administration, responder rats underwent 14 sessions of extinction with no peer present. The house light was on and both levers were extended; however, there was no programmed consequence for responding on either lever. Extinction sessions were 1 h in length, and responses on both levers were recorded.

Phase 4: reinstatement tests Following extinction, responder rats were re-introduced for a single session to the same peer that had been present throughout acquisition training and responses on both the previously active and inactive levers were recorded. During this reinstatement test, the house light was illuminated and both levers were extended; however, there was no programmed consequence for responding on either lever. Responder rats then underwent three more extinction sessions, and the same peer was re-introduced for a second test. Finally, after three more extinction sessions, a novel male peer (similar age and weight) was introduced on the test day. Reinstatement tests were 1 h long.

Experiment 2: peers as discriminative stimuli for reinstatement of cocaine seeking using a single-active lever procedure

The purpose of experiment 2 was to determine if two different peers would serve as discriminative stimuli capable of controlling the reinstatement of cocaine seeking after a period of extinction. After initial acquisition, cocaine self-administration sessions alternated pseudo-randomly with saline self-administration sessions, with one peer paired with cocaine and a different peer paired with saline. The number of training sessions was either 12 (experiment 2A; $n = 8$) or 24 (experiment 2B; $n = 17$). In these experiments, the same active lever was used across both cocaine and saline self-administration sessions so that only the cocaine-associated peer, and not lever position, served as the consistent predictor of cocaine availability when responding on the active lever.

Procedure

Phase 1: pretraining, acquisition, and training Initial pretraining, surgery, and cocaine self-administration were performed as described in experiment 1. On each of the first 14 daily sessions of cocaine self-administration, the responder rat was exposed to a peer (similar age and weight); the same peer

was used on each session. Following the 14 days of cocaine self-administration acquisition, the procedure was adjusted such that saline was substituted for cocaine on intermittent daily FR 5 sessions. The presentation order of cocaine or saline self-administration was pseudo-randomized, such that neither was available for more than three consecutive sessions. On both cocaine and saline sessions, the active lever and programmed consequences (activation of syringe pump and cue light illumination) were the same. However, on the cocaine self-administration sessions, responder rats were exposed to the same male peer that was present during the initial 14 days of cocaine self-administration; on the saline self-administration sessions, responder rats were exposed to a different male peer (similar age and weight), which remained the same throughout the experiment. Separate groups of rats were given either 12 training sessions (6 cocaine and 6 saline; experiment 2A) or 24 training sessions (12 cocaine and 12 saline; experiment 2B).

Phase 2: extinction The procedure was identical to that used in experiment 1, with no peers present.

Phase 3: reinstatement tests Following extinction, responder rats were re-introduced to the cocaine- and saline-associated peers. There were 3 days in between each reinstatement test; the order of peer presentations was counterbalanced across responder rats. During testing, the house light was illuminated and both levers were extended, but responses on either lever had no programmed consequence.

Experiment 3: peers as discriminative stimuli for reinstatement of cocaine seeking using a double-active lever procedure

This experiment was similar to experiment 2, except that a double-active lever procedure was used rather than a single-active lever procedure. With the double-active lever procedure, in addition to having different peers present on cocaine or saline sessions, one lever was active on cocaine self-administration sessions and the opposite lever was active on saline self-administration sessions, thus enhancing the discriminability between cocaine and saline sessions.

Procedure

Phase 1: acquisition and training Compared to experiment 2, several procedural changes were implemented: (1) no food pretraining was used in order to enhance the association of the lever specifically to cocaine; (2) a double-active lever design was implemented, where one lever was active for cocaine self-infusions and the other lever was active for saline self-infusions (counterbalanced); (3) each infusion on cocaine self-administration sessions produced a 20-s time-out signaled by illumination of the cue light above the cocaine lever and infusions on saline self-administration sessions produced a 20-s time-out signaled by illumination of the cue light above the saline lever; (4) both cocaine and saline self-administration sessions were initiated from the beginning of self-administration, rather than after the initial 14 cocaine self-administration sessions; and (5) presentation order of cocaine or saline self-administration sessions was alternated daily rather than pseudo-randomly; however, session 1 was always a cocaine self-administration session. There were a total of 44 daily self-administration sessions, with 22 being with cocaine and 22 being with saline. As in experiment 2, two different peers (same age and weight) were paired with either cocaine or saline sessions. During training sessions ($n = 18$), fixed ratio values increased from a FR 1 to a terminal FR 5, as in experiments 1 and 2 (sessions 1–20). Once they reached the FR 5 schedule, rats received 24 discrimination sessions on the FR 5 schedule, as with experiment 2B (sessions 21–44).

Phase 2: extinction Following acquisition of cocaine self-administration, responder rats underwent 14 sessions of extinction, which were similar to self-administration sessions, except that social peers were no longer present during extinction and both levers were active (i.e., five responses on either lever led to activation of the syringe pump and illumination of the cue light above the corresponding lever). Rats received no drug during extinction sessions.

Phase 3: reinstatement tests Reinstatement tests were identical to extinction sessions, except that the cocaine- and saline-associated peers were re-introduced on separate sessions. There were three additional extinction sessions (no peers) between each reinstatement test; the order of peer presentations was counterbalanced across responder rats.

Statistical analysis

Data from experiment 1 reinstatement sessions were log transformed prior to conducting analyses because the number of lever presses was not normally distributed. A Bonferroni correction was used when appropriate. Data from experiment 2 drug discrimination sessions were analyzed using repeated measures ANOVA, with drug as a within-subjects factor. During the duration of experiment 2, seven rats were lost due to catheters/headmount failures. Data for these rats are included in all phases that they completed. Two headmount failures from rats in experiment 2B occurred during the final self-administration session, and therefore the data from these rats were included in self-administration analyses. Data from experiment 3 drug discrimination sessions were analyzed using repeated measures ANOVA, with drug and lever as within-subjects factors. During the duration of experiment 3, five rats were lost due to faulty catheters and headmounts.

Data for these rats are included in all phases that they completed. Across each experiment, data from reinstatement tests were analyzed using paired samples *t* tests. All results were considered statistically significant at $p < 0.05$. Cohen's *d* and partial eta squared were used to provide measures of effect size for *t* tests and ANOVAs, respectively.

Results

Experiment 1: peer-induced reinstatement of cocaine seeking

Figure 1 shows responding during the training phase in the presence of a peer in experiment 1. Across the five FR 5 sessions, a 2 × 5 ANOVA revealed a main effect of lever ($F(1, 5) = 8.76$, $p < 0.05$, $\eta_p^2 = 0.64$), but no significant main effect of session or lever × session interaction. It is important to note that one rat responded 203 (± 42.29) times on the inactive lever during the final five FR 5 sessions (data included in analyses). As expected, when the extinction phase was implemented, there was a decrease in responding on the active lever across sessions, with a significant difference between the average of the last three sessions of cocaine self-administration and the last three sessions of extinction ($t(4) = 5.96$, $p < 0.05$, $d = 3.92$; results not shown).

Figure 2 shows responding during the three reinstatement sessions (peer presentation) relative to the three extinction sessions preceding each reinstatement test. There was a significant difference between the average of the last 3 days of extinction and the first reinstatement test ($t(4) = 2.87$, $p < 0.05$, $d = -1.54$). This difference was not significant following a second reinstatement test with the same peer, or following the presentation of a novel peer.

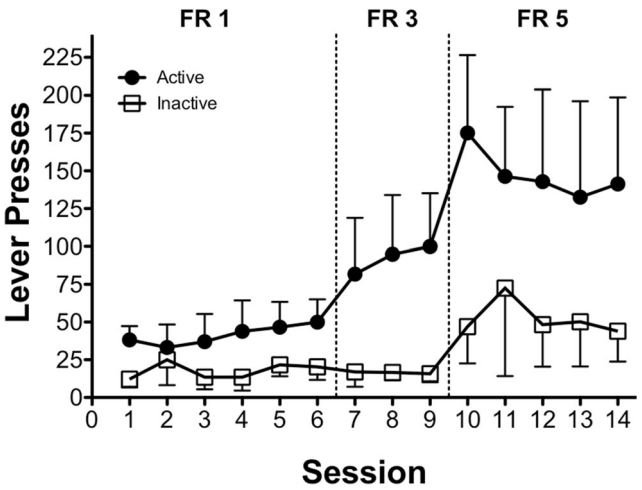

Fig. 1 Acquisition of cocaine self-administration in experiment 1. Mean (± SEM) number of active and inactive lever presses during acquisition of cocaine self-administration across incrementing FR requirements in the presence of a peer in experiment 1. $N = 6$

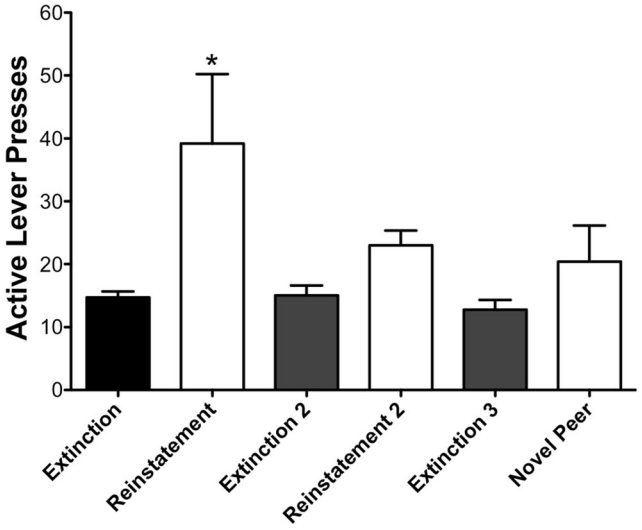

Fig. 2 Reinstatement of responding in experiment 1. Mean (± SEM) number of lever presses during reinstatement tests. Each extinction bar (1–3) represents the 3 days prior to each reinstatement test; reinstatement bar represents first presentation of peer used in acquisition; reinstatement 2 bar represents second presentation of peer used in acquisition; and novel peer bar represents presentation of a novel peer. Values were log transformed for analysis because responses during cocaine self-administration were not normally distributed. *$p < 0.05$. $N = 6$

Experiment 2: peers as discriminative stimuli for reinstatement of cocaine seeking using a single-active lever procedure

During the final cocaine acquisition session, rats made an average of 110.67 (± 42.09) responses on the active lever, and 14.90 (± 24.42) responses on the inactive lever (data not shown). Figure 3 represents the mean number of responses made on the active and inactive levers across the 12 discrimination sessions in experiment 2A (Fig. 3a) and 24 discrimination sessions in experiment 2B (Fig. 3b). In experiment 2A, a mixed factor ANOVA (drug × session) revealed no significant effects. In experiment 2B, a mixed factor ANOVA revealed a significant main effect of session ($F(11, 88) = 2.05$, $p < 0.05$, $\eta_p^2 = 0.20$); a main effect of drug reached near significance ($F(1, 8) = 4.99$, $p = 0.056$, $\eta_p^2 = 0.38$), but there was no drug × session interaction. Subsequent analysis revealed that responder rats in experiment 2B earned significantly more cocaine infusions than saline infusions across all 24 discrimination trials (218.5 ± 20.64 vs. 169.28 ± 21.37; $t(13) = 3.58$, $p < 0.01$, $d = 1.98$). As expected, when the extinction phase was implemented, there was a decrease in responding on the active lever across sessions (results not shown); on the final extinction session, rats made an average of 12.35 (± 11.9) responses on the active lever and 6.10 (± 8.48) responses on the inactive lever.

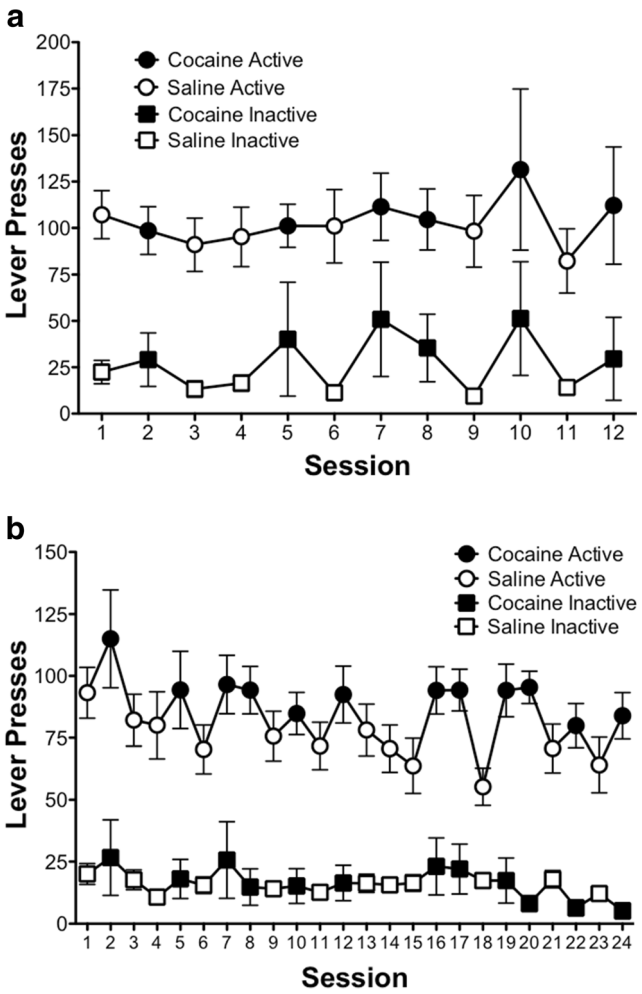

Fig. 3 Discrimination training on cocaine and saline self-administration sessions in experiment 2. Mean (± SEM) number of active and inactive lever presses across 12 training sessions for experiment 2A (**a**) and across 24 training sessions for experiment 2B (**b**) using a single-active lever procedure. In each panel, the closed symbols represent lever presses for cocaine in the presence of one peer (S+) and open symbols represent lever presses for saline in the presence of a different peer (S−). $N = 7$ and $N = 13$ per experiment

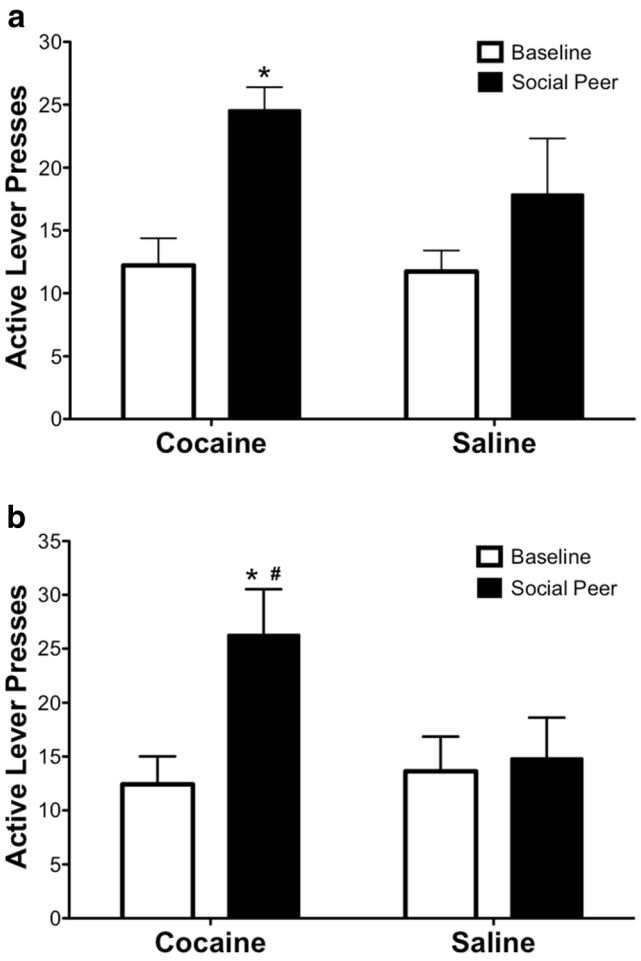

Fig. 4 Reinstatement following 12 discrimination training sessions in experiment 2. Mean (± SEM) number of active lever presses during baseline (averaged across previous 3 extinction sessions) and reinstatement tests for experiment 2A (**a**), in which 12 training sessions (6 cocaine and 6 saline), and experiment 2B (**b**), in which 24 training sessions (12 cocaine and 12 saline), were used with a single-active lever procedure. Baseline values represent the average of 3 extinction sessions immediately prior to the reinstatement test. $^*p < 0.05$ compared to baseline. $N = 7$ and $N = 11$ per experiment

Figure 4 shows the results of reinstatement sessions from responder rats receiving 12 or 24 sessions of discrimination training. Following 12 discrimination sessions (Fig. 4a), pairwise comparisons showed that there was a significant increase in the number of presses on the previously active lever in responder rats when the cocaine-associated peer was present compared to the average number of presses on the 3 days of extinction immediately prior to the reinstatement test ($t(6) = 7.10$, $p < 0.05$, $d = -2.75$). In contrast, there was no significant difference in responding when the saline-associated peer was present compared to the average of the 3 days of extinction immediately prior to the reinstatement test. However, a paired samples t test revealed no significant difference in responding between reinstatement tests when the cocaine-associated peer was present compared to when the saline-associated peer was present. Following 24 discrimination sessions (Fig. 4b), pairwise comparisons revealed a significant increase in the number of presses on the previously active lever in responder rats when the cocaine-associated peer was present compared to the average number of presses on the 3 days of extinction immediately prior to the reinstatement test ($t(10) = 7.803$, $p < 0.01$, $d = 4.94$). In contrast, there was no significant difference in responding when the saline-associated peer was present compared to the average of the 3 days of extinction immediately prior the reinstatement test. A paired samples t test also revealed that rats responded significantly more on the previously active lever when the cocaine-associated peer was present compared to when the saline-associated peer was present ($t(10) = 3.145$, $p < 0.05$, $d = 1.99$).

Experiment 3: peers as discriminative stimuli for reinstatement of cocaine seeking using a double-active lever procedure

Figure 5 shows the results from responder rats during discrimination training using the double-active lever procedure. During FR 1 training sessions (Fig. 5a), a 2 × 2 × 7 ANOVA (drug × lever × session) revealed that there was a significant main effect of lever ($F(1, 11) = 7.59$, $p < 0.05$, $\eta_p^2 = 0.41$), such that rats responded more on the cocaine-associated lever (also the saline-inactive lever) than the saline-associated lever (also the cocaine-inactive lever). There was also a main effect of session ($F(6, 66) = 2.67$, $p < 0.05$, $\eta_p^2 = 0.19$). During FR 3 training sessions (Fig. 5a), a 2 × 2 × 3 ANOVA (drug × lever × session) revealed that there was a drug × lever interaction ($F(1, 13) = 9.13$, $p = 0.01$, $\eta_p^2 = 0.41$). As shown in Fig. 5b, this preference for the cocaine-active lever position continued during FR 5 training. During the 24 FR 5 sessions, a 2 × 2 × 12 ANOVA (drug × lever × session) revealed that there was a significant drug × lever interaction ($F(1, 6) = 7.11$, $p < 0.05$, $\eta_p^2 = 0.54$). Overall, rats responded significantly more on the active lever during cocaine sessions than during saline sessions ($t(15) = 5.83$, $p < 0.001$, $d = 3.01$). As expected, when the extinction phase was implemented, there was a decrease in responding on the active lever across sessions (results not shown), with rats making an average of 14.31 (± 7.54) responses on the cocaine-associated lever, and 15.00 (± 8.00) responses on the saline-associated lever on the final extinction session.

Figure 6 shows the results of reinstatement sessions from responder rats trained with the double-active lever procedure. Pairwise comparisons revealed a significant increase in lever pressing when the cocaine-associated peer was present compared to the average of the 3 days of extinction immediately prior to the reinstatement test ($t(16) = 6.84$, $p < 0.001$, $d = 3.42$). In contrast, there was no significant change in lever pressing when the saline-associated peer was present compared to the average of the 3 days of extinction immediately prior to the reinstatement test. However, a paired samples t test revealed no significant difference in responding between reinstatement tests when the cocaine-associated peer was present compared to when the saline-associated peer was present.

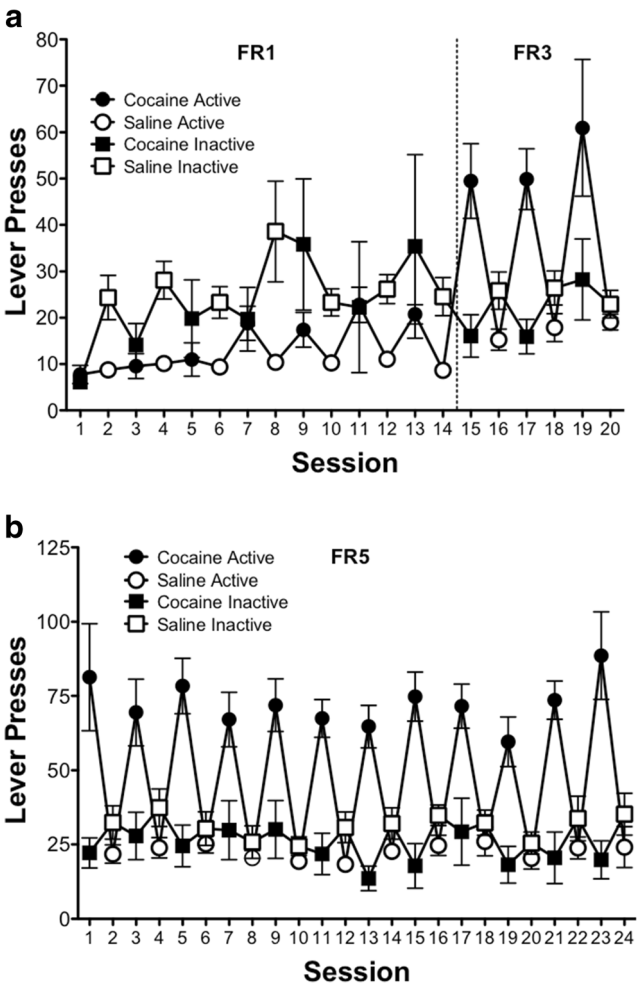

Fig. 5 Discrimination training on cocaine and saline self-administration sessions in experiment 3. Mean (± SEM) number of active and inactive lever responses during discrimination training sessions across incrementing FR 1 and FR 3 sessions (**a**) and FR 5 sessions (**b**) for experiment 3 using a double-active lever procedure. The closed symbols represent presses on the active and inactive levers during cocaine sessions in the presence of one peer (S+) and open symbols represent presses on the active and inactive levers during saline sessions in the presence of a different peer (S−). $N = 13$

Discussion

This study provides evidence that a conspecific can be used as a social cue to reinstate cocaine seeking in rats under the

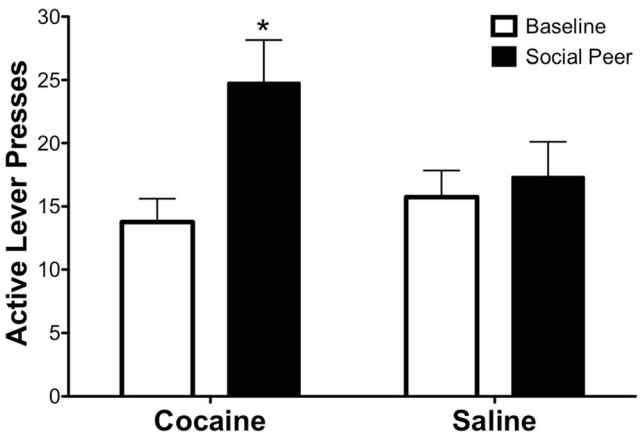

Fig. 6 Reinstatement following 44 discrimination training sessions in experiment 3. Mean (± SEM) number of active lever presses during baseline (averaged across previous 3 extinction sessions) and reinstatement tests for experiment 3, in which 44 training sessions (22 cocaine and 22 saline) were used with a double-active lever procedure. Baseline values represent the average of 3 extinction sessions immediately prior to the reinstatement test. *$p < 0.05$ compared to baseline. $N = 13$

appropriate social procedures. Experiment 1 sought to determine if a social peer could serve as a cue for cocaine availability by pairing a social peer with cocaine during every self-administration session and then measuring peer-induced reinstatement following extinction in the absence of the peer. Results revealed that responder rats reinstated cocaine seeking when the social peer was re-introduced. However, social-induced reinstatement of cocaine seeking was transient, being evident only on the first test. The weakening of social-induced reinstatement following repeated presentations of the reinstating cue is consistent with other forms of reinstatement, including reinstatement induced by discrete non-social cues (Buffalari et al. 2013; Liu et al. 2008) and contextual cues (Crombag et al. 2008). Results from this experiment provide evidence that a social peer can be used as a cue for reinstatement of cocaine seeking. However, since the same peer was used on both training and reinstatement tests, it is not possible to determine from this experiment if reinstatement was specific to the social peer paired with cocaine or to the mere presence of any peer.

After repeated reinstatement testing with the cocaine-associated peer in experiment 1, presentation of a novel peer not previously associated with cocaine availability during training did not reinstate cocaine seeking. Previous work has shown that the mere presentation of the novel peer can non-associatively increase ongoing responding of a well-learned operant response (Gipson et al. 2011; Zajonc 1965; Zentall and Levine 1972), a phenomenon known as social facilitation. Since a novel peer did not increase lever pressing in this study, it seems unlikely that the transient increase in responding observed in the presence of the cocaine-associated peer reflected an example of social facilitation. Instead, a more likely explanation is that the cocaine-associated peer was able to induce reinstatement due to its previous association with cocaine availability. However, since the novel peer was tested after the cocaine-associated peer (no counterbalancing), we cannot rule out the possibility that different results would have been obtained if rats were tested with the novel peer prior to re-exposure to the cocaine-associated peer.

Results from experiments 2A and 2B indicate that two different peers serve as discriminative stimuli for differential reinstatement of cocaine seeking. In these experiments, rats were allowed to self-administer cocaine with one peer (S+) and allowed to self-administer saline with a different peer (S−). Following a period of extinction (neither peer nor cocaine present), results revealed that responder rats reinstated cocaine seeking in the presence of the S+ peer, but not in the presence of the S− peer. Furthermore, experiment 2B shows that, with sufficient discrimination training (24 sessions), rats respond significantly more in the presence of the S+ peer compared to the S− peer. These results provide evidence that social peers can be used as discriminative stimuli to control responding for cocaine. These results are in keeping with previous literature on social recognition memory, which has shown that rats are capable of remembering another rat (Gheusi et al. 1994). These results are also in keeping with, and expand upon, previous literature on discriminative stimuli, which show that traditional cues (e.g., lights, sounds, odors) can also be used as discriminative stimuli to reinstate cocaine seeking (Martin-Fardon et al. 2008; Matzeu et al. 2015).

A notable finding from experiments 2A and 2B is that operant behavior in responder rats across discrimination training was not dramatically different between cocaine and saline self-administration sessions. That is, with only 12 discrimination sessions (experiment 2A), response rates did not differ across cocaine and saline training sessions in responder rats. By increasing the number of discrimination training sessions to 24 (experiment 2B), overall response rates were increased significantly on cocaine sessions compared to saline sessions. While these results suggest that cocaine infusions per se had some control over responding following extended training, this effect was relatively weak using the current training procedures. While enhancing differences between cocaine and saline sessions may be achieved by increasing either the cocaine unit dose or the number of training sessions, the training procedures used here were sufficient to demonstrate social reinstatement of cocaine seeking.

The purpose of experiment 3 was to adjust the training procedures in order to increase discriminative responding between cocaine and saline self-administration sessions. This was accomplished by using a double-active lever procedure in which the position of the active lever was alternated between cocaine and saline self-administration sessions, along with the social peer (S+ or S−). As expected, rats responded at higher rates during cocaine sessions, compared to saline sessions, beginning with the FR 3 schedule of reinforcement. This response difference persisted throughout the rest of training. In this phase of training, rats responded more on the active lever during cocaine sessions, whereas they responded *less* on the active lever on saline sessions. Since the active lever was different on cocaine and saline sessions, this latter finding indicates that rats simply developed a preference for the cocaine-associated lever, regardless of the session type. Although the 20-s cue light illumination used to signal infusions on saline sessions would be expected to have some weak reinforcing strength on its own (Cain et al. 2006; Marx et al. 1955), any weak reinforcing effect of the light cue on saline sessions clearly did not overcome the strong conditioned reinforcing strength that accrued to the cocaine-active lever position.

Despite the differential performance on cocaine and saline sessions during training in experiment 3, peer-induced reinstatement of cocaine seeking was not enhanced beyond what was observed in the single-active lever procedure in experiment 2. In fact, this double-active lever procedure did not produce a significant difference in responding between reinstatement sessions when the S+ and S− peers were re-introduced; whereas, rats in experiment 2B that received less

discrimination trials showed significantly higher responding during reinstatement testing with the S+ peer, compared to the S− peer. One possible explanation for the failure to enhance social-induced reinstatement in experiment 3 is that the double-active lever procedure may not have established robust discriminative stimulus control by the social peer. That is, in contrast to experiments 2A and 2B where the discriminative stimulus consisted of a single element (social peer), the discriminative stimulus for cocaine availability in experiment 3 consisted of two elements (social peer + signaled active lever position). By having two elements making up the compound discriminative stimulus, it is possible that the signaled active lever element weakened or overshadowed the social peer element, as has been described previously when two punctate stimulus elements are combined to make a compound Pavlovian CS or operant discriminative stimulus (Miller and Escobar 2002; Rescorla 2000; Thein et al. 2008). One way to test this possibility in future work is to test each element of the compound discriminative stimulus alone (social peer vs. signaled active lever position) compared to the compound, as has been described previously (Kearns and Weiss 2005).

There are at least two limitations of the study, i.e., the exclusive use of males and the use of only a single dose of cocaine. First, males were chosen for this study because male rats find social interaction more rewarding than females (Douglas et al. 2004). Even though rats were not allowed to fully interact socially because of the wire mesh partition, limited social interaction through a wire screen partition produces conditioned place preferences in males (Kummer et al. 2011; Peartree et al. 2012), thus demonstrating that the limited visual, olfactory, and tactile interaction in the current study is socially relevant, at least among males. Second, a single high unit dose of cocaine (0.5 mg/kg/infusion) was selected because it is on the descending limb of the inverted U-shaped dose response curve (Piazza et al. 2000), even when tested in the presence of social peers (Smith 2012), and thus was expected to be more discriminable than lower unit doses. However, since lower doses would be expected to increase the number of infusions earned per session, it is also possible that a stronger association between cocaine and the S+ peer could be achieved at other unit doses.

Despite these limitations, the current results together demonstrate that a social peer can serve as a contextual discriminative stimulus to reinstate cocaine seeking, an effect that is similar to results obtained with other discriminative stimuli such as olfactory, auditory, and visual cues (Bossert et al. 2013; Matzeu et al. 2015). Future research should investigate what specific characteristic of a social peer is necessary and sufficient to reinstate drug seeking or whether the strength of the social peer to serve as a discriminative stimulus relies on the combination of multiple characteristics (i.e., olfactory, visual, and auditory). In any case, these results contrast with a previous study by Smith and colleagues (Smith et al. 2016). In that study, discrimination training consisted of presenting a cocaine self-administering peer (S+) on some sessions and a saline self-administering peer (S−) on other sessions. Following a period of extinction, lever pressing was greater in the presence of the S+ peer than in the presence of the S− peer; however, neither peer alone altered responding compared to the last day of extinction. The difference in responding in the presence of the S+ and S− peers was interpreted to reflect the combined effect of both a weak excitatory effect of the S+ peer and a weak inhibitory effect of the S− peer, while the lack of effect of the S+ peer compared to the last day of extinction was interpreted to reflect a failure to observe reinstatement of cocaine seeking. One reason why we observed reinstatement to the S+ peer, whereas Smith et al. (2016) did not, might be due to differences in the behavior of the S+ peer used between studies. That is, Smith et al. (2016) used a cocaine self-administering peer as the S+ and a saline self-administering peer as the S−. Since operant responding was greater with the cocaine S+ peer compared to the saline S− peer, the discriminative stimulus used in that study may be characterized as a compound stimulus in which the presence of the peer was combined with the presence of non-social discriminative cues (i.e., lever extension/retraction, sound of infusion pump). In contrast, in the current study, the presence of a passive non-using peer was the only element making up the S+ and S−, which likely increased the associative strength between discriminative stimulus (e.g., social peer) and drug. In any case, these results suggest that re-association with drug-associated peers may be an important event for triggering relapse.

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Compliance with ethical standards

All procedures were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council 2010) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Conflict of interest The authors declare that they have no conflicts of interest.

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