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Reinstatement of conditioned reinforcing properties of cocaine-conditioned stimuli

Benjamin Goddard, Francesco Leri *

Department of Psychology, University of Guelph, Guelph (Ontario), Canada N1G 2W1

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Abstract

The aim of the present experiment was to investigate the effects of cocaine primes and exposure to foot shock stress on reinstatement of operant responding maintained by a cocaine-conditioned stimulus in rats never trained to actively self-administer cocaine. Following a baseline session of responding for a light-buzzer compound stimulus, rats underwent classical conditioning whereby the compound stimulus was paired with passive intravenous infusions of cocaine (vehicle, 0.5 or 1.0 mg/kg/inf). On subsequent test sessions, operant responding for the compound stimulus was re-assessed in the absence of cocaine. Finally, rats received a cocaine prime (20 mg/kg, i.p.) and foot shock stress prior to two separate test sessions assessing lever pressing for the cocaine-conditioned stimulus. It was found that the animals conditioned with cocaine displayed sustained responding on the lever activating the cocaine-conditioned stimulus. In addition, priming injections of cocaine reinstated responding for the light-buzzer compound stimulus, and this effect was proportional to the dose of cocaine received during classical conditioning. Foot shock stress also reinstated responding, but its effect was smaller and observed only in animals conditioned with the highest dose of cocaine. These findings suggest that cocaine primes and stress can induce reinstatement by reactivating the motivational value of cocaine-conditioned cues.

Keywords: Cocaine; Classical conditioning; Conditioned reinforcement; Reinstatement; Relapse; Stress; Rat

1. Introduction

Conditioned reinforcement is the process whereby a previously conditioned stimulus acts as the reinforcer for an instrumental action (Mackintosh, 1974). Davis and Smith (1976, 1987) described an interesting procedure to explore the conditioned reinforcing properties of drug-conditioned cues. Rats implanted with intravenous catheters received infusions of morphine or amphetamine paired with the presentation of a discrete buzzer stimulus. After this period of classical conditioning, it was found that rats emitted vigorous operant responding for the activation of the buzzer stimulus, in the absence of any drug. These results indicated that repeated intravenous infusions of morphine or amphetamine imparted conditioned reinforcing properties to the stimulus, thus making it effective in reinforcing operant behavior.

This procedure appears particularly interesting because of two reasons. Firstly, it is suitable to explore the effect of drug-conditioned cues on drug-seeking behavior. Currently, there are several animal models exploring how drug-conditioned cues initiate (Stewart et al., 1984; McFarland and Ettenberg, 1997; Fuchs et al., 1998; Grimm et al., 2001; Weiss et al., 2001; Di Ciano and Everitt, 2002; Cervo et al., 2003) maintain (See et al., 1999; Everitt and Robbins, 2000; Schindler et al., 2002; Di Ciano and Everitt, 2003) and enhance (Parkinson et al., 1999; Wyvell and Berridge, 2001; Leri and Stewart, 2002; Di Ciano and Everitt, 2004; Glasner et al., 2005) operant behavior in the absence of drug. All of these models, however, employ active drug self-administration as a method to impart motivational properties to discrete drug-associated cues. A different approach has been used by Kruzich et al. (2001), who demonstrated that cues previously paired with passive intravenous infusions of cocaine can significantly elevate lever pressing. However, even in these experiments, rats received previous operant training reinforced by intravenous infusions of cocaine. The procedure described by Davis and Smith (1976, 1987) does not require

* Corresponding author. Tel.: +1 519 824 4120x58264; fax: +1 519 837 8629.
E-mail address: fleri@uoguelph.ca (F. Leri).

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active self-administration training and, therefore, reduces the requirement of regular and frequent access to the venous system of the animal. In addition, because subjects receive drug infusions passively, all animals in a given experimental group are exposed to identical quantities of drug. This is particularly important for studies investigating the effect of passive drug conditioning on brain neurochemistry.

Secondly, the procedure described by Davis and Smith (1976, 1987) appears to be well suited to explore specific psychobiological processes involved in relapse to drug-seeking behavior. It is known that drug priming and exposure to acute stressors precipitate reinstatement of drug-seeking behavior in rats trained to actively self-administer drugs (Shalev et al., 2002). It has been postulated that these manipulations induce reinstatement because they enhance the motivational value of cues present during previous self-administration training (Stewart, 2000; Leri and Stewart, 2001). However, current theories of addiction also suggest that with repeated self-administration, drug-seeking behavior can become a prepotent response habit (Robbins and Everitt, 1999; Everitt et al., 2001; Everitt and Wolf, 2002). Thus, in most reinstatement studies where rats previously self-administered a drug in the presence of drug-predictive or drug-associated cues, drug primes or stressors can induce reinstatement because of reactivation of the incentive value of these cues, and/or because of reactivation of response habits directed toward these cues. The adaptation of the procedure described by Davis and Smith (1976, 1987) to the study of reinstatement appears particularly useful to selectively investigate one of these relapse mechanisms as rats are not trained to actively press a lever to receive drug infusions and hence cannot acquire a drug response habit.

The present experiment had two primary objectives. The first objective was to investigate whether discrete environmental stimuli associated with passive intravenous infusions of cocaine could subsequently maintain operant behavior in the absence of the drug. The second objective was to determine whether cocaine primes and exposure to foot shock stress could reinstate extinguished operant responding solely reinforced by cocaine-conditioned cues.

2. Methods

2.1. Subjects

Fifty-five male Sprague–Dawley rats (Charles River, Qc) weighing 300–325 g were used in this experiment. They were singly housed and maintained on a reverse light/dark cycle (8:00 am lights off; 8:00 pm lights on) with free access to food and water except during behavioral testing, which always occurred during the dark cycle. All experiments were approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

2.2. Intravenous catheterization surgery

Rats were surgically implanted with intravenous silastic catheters (Dow Corning, Midland, MI) in the right jugular vein, under general anesthesia induced by a combination of sodium pentobarbital (18.5 mg/kg IP, MTC Pharmaceutical, Cambridge, ON), morphine (5 mg/kg SC, Ontario Veterinary College, Guelph, ON) and diazepam (1 mg/kg SC, Sabex Inc., Boucherville, QC). Rats were given atropine sulfate (4.5 mg/kg SC, Ontario Veterinary College, Guelph, ON) just before surgery and Depocillin (300,000 IU, 0.1 ml/rat IM, Intervet Canada, Whitby, ON) immediately following surgery. The catheter was secured to the vein with silk sutures and was passed subcutaneously to the top of the skull where it exited into a connector (a modified 22 gauge cannula; Plastics One, Roanoke, VA) mounted to the skull with jeweler's screws and dental cement. A plastic blocker was placed over the opening of the connector when not in use. Catheters were flushed prior to and during classical conditioning with 0.1 ml of a saline–heparin solution (0.2 mg/ml Hepalean 1.000 IU, Organon, Toronto, ON).

2.3. Apparatus

Twenty-six Plexiglas operant chambers (model ENV-008CT, Med Associates, Georgia, VT) were each enclosed in larger sound-attenuating plywood chambers (model ENV-018M, Med Associates). Each operant box had a house light (28 V), and two levers, one retractable and one stationary, located 10 cm apart and 8 cm above the floor of the box. Presses on the retractable lever (active lever) activated a white light (28 V) and a 65 dB buzzer located 3 and 8 cm above the lever, respectively. The stationary lever served to control for non-specific lever responding; pressing this lever had no consequence (inactive lever), but all presses were recorded. Infusion pumps (Razel Scientific Instruments, Stamford, CT) for the delivery of drug solutions during the period of classical conditioning were positioned outside the sound-attenuating chamber. Each operant chamber was fitted to deliver constant-current, intermittent, inescapable, electric foot shock through a scrambler to the grid floor (model ENV-414, Med Associates).

2.4. Procedure

Prior to surgery rats were allowed 4 days to habituate to the animal facility and were handled twice for approximately 10 min. After surgery, rats recovered for a period of 7 days prior to beginning the experiment, which had four phases.

2.4.1. Phase 1: baseline

Rats were placed in the chambers, and following a delay of 5 min, the 3-h session started with the activation of the house light, and after 10 s, the entry of the active lever and the activation of the light-buzzer compound stimulus for 45 s, or until the rat made the first response. Subsequent presses on this lever led to the activation of the light-buzzer compound stimulus for 10 s. This baseline session was given in order to assess the spontaneous tendency of rats to respond for novel sensory stimulation which, as suggested by previous pilot studies, shows large individual variability. Responses emitted during this session were used to assign rats to three equivalent conditioning groups.

2.4.2. Phase 2: classical conditioning

On three classical conditioning sessions (one 2-h and two 4-h sessions) given over 3 consecutive days, rats received passive intravenous infusions of cocaine (or saline) accompanied by the presentation of the light-buzzer compound stimulus. The duration of the first session was shorter in order to reduce possible aversive effects of intense cocaine exposure in cocaine-naïve rats. During all conditioning sessions, the stimulus compound was activated 5 s before, and during the 10 s intravenous infusion (300:l). Rats received one stimulus-infusion pairing every 4 min. This time period was selected to approximate the inter-infusion interval observed in well trained rats that actively self-administer 0.5 mg/kg/inf cocaine (Leri et al., 2005a). Seventeen rats received saline (Vehicle group), while 19 rats received 0.5 mg/kg/infusion cocaine and an additional 19 rats received 1.0 mg/kg/infusion. Three sessions of classical conditioning were given in order to obtain conditioning while minimizing access to the venous system, and to expose rats to quantities of cocaine similar to levels achieved in rats that actively self-administered cocaine in previous reinstatement studies (Leri and Stewart, 2001; Leri et al., 2002, 2004). It is important to emphasize that, in the present experiment, the active lever was never introduced during the period of classical conditioning, and thus rats never learned to press it to receive cocaine.

2.4.3. Phase 3: testing

Two days following conditioning, lever pressing for the compound stimulus was re-assessed during four 3-h test sessions given over 4 consecutive days. Because cocaine was not infused, these sessions also served to extinguish the conditioned reinforcing property of the cocaine-conditioned stimulus.

2.4.4. Phase 4: reinstatement

The day following the fourth test session, half the rats in each group received a cocaine prime (20 mg/kg, i.p.) and the other half was exposed to foot shocks (15 min period, 0.5 mA, 0.5 s ON, a mean OFF period of 40 s), 10 min prior to the beginning of a 3-h session. The following day, rats received the alternative treatment and were tested for a final 3 h. During both reinstatement sessions, presses on the active lever led to the activation of the compound stimulus but not to cocaine infusions. The priming dose of cocaine and the intensity/duration of foot shock were based on our previous studies of reinstatement in rats that actively self-administered cocaine (Leri and Stewart, 2001; Leri et al., 2002, 2004).

2.5. Drugs

Cocaine HCL (Dumex, Toronto, On) was dissolved in 0.9% physiological saline solution.

2.6. Statistical analyses

A three-way repeated measures ANOVA was conducted to analyze the effect of classical conditioning with different doses of cocaine (Group; independent factor) on active/inactive lever pressing (Lever; repeated factor) over the baseline and subsequent tests (Test sessions; repeated factor).

In order to further characterize lever pressing behavior maintained by the cocaine-conditioned compound stimulus, a two-way repeated measures ANOVA was conducted to compare responses on the active lever displayed by the different groups (Group; independent factor) during the first hour of Test 1 (Time period; repeated factor).

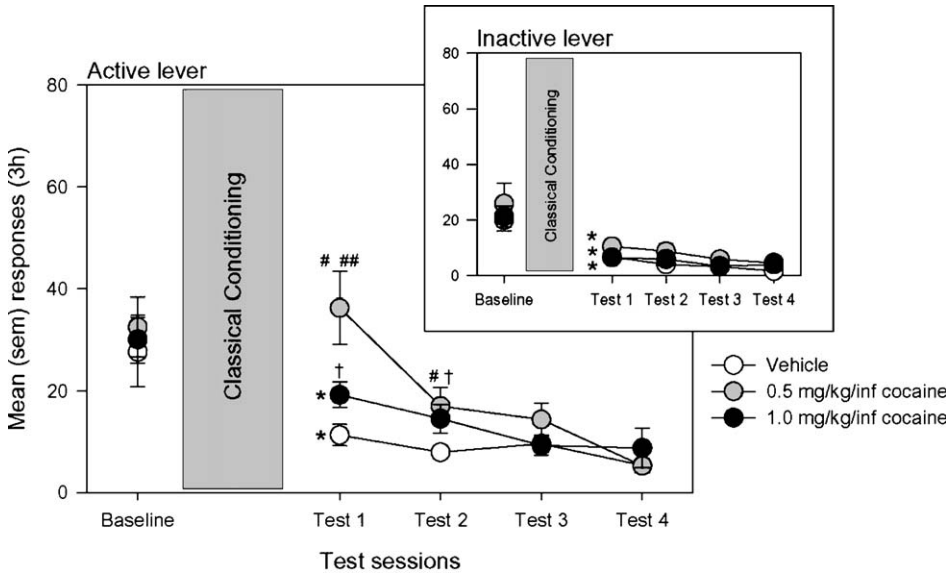

Fig. 1. Mean (sem) responses on the active (larger panel) and inactive levers (smaller panel) during the Baseline test, and four tests given following classical conditioning with saline (Vehicle group, $n=17$), 0.5 mg/kg/infusion cocaine ($n=19$) or 1.0 mg/kg/infusion cocaine ($n=19$). Presses on the active lever activated a light-buzzer compound stimulus that was associated with passive drug infusions during the period of classical conditioning. Presses on the inactive lever had no scheduled consequences. The * denotes a significant decrease in responding from Baseline to Test 1 within a given group. The $^{\#}$ denotes a significant difference between the Vehicle and the 0.5 mg/kg/inf cocaine groups. The $^{\#\#}$ denotes a significant difference between the two cocaine-conditioned groups. The † denotes a significant difference between the Vehicle and the 1.0 mg/kg/inf cocaine groups.

Finally, a three-way repeated measures ANOVA was employed to assess whether the cocaine prime and the foot shock (Reinstatement; repeated factor) resulted in differential active/inactive lever pressing (Lever; repeated factor) in the different groups (Group; independent factor). In case of a significant interaction or a significant main effect, multiple comparisons were performed using the Holm-Sidak method in order to identify individual mean differences ($\alpha = 0.05$). The specific values of negative findings are not reported. All statistical analyses were performed using SigmaStat (version 3.0 for Windows, SPSS Inc).

3. Results

Fig. 1 represents responses on the active (larger panel) and inactive (smaller panel) levers across the baseline session, and the four test sessions given following classical conditioning with vehicle, 0.5 or 1.0 mg/kg/inf cocaine. The ANOVA yielded a significant Group by Test sessions by Lever interaction [$F(8, 208) = 2.89$, $p < 0.01$], as well as a significant main effect of Test sessions [$F(4, 208) = 39.30$, $p < 0.001$] and Lever [$F(1, 52) = 47.04$, $p < 0.001$]. Multiple comparisons isolated significant mean differences indicating that classical conditioning with cocaine promoted responding for the cocaine-conditioned compound stimulus. In fact, while no group differences in responding on the active lever were noted during the Baseline test, on Tests 1 and 2 both groups conditioned with cocaine responded significantly more than animals in the Vehicle group. Furthermore, at Baseline, all groups responded equivalently on the active and on the inactive levers. However, on Tests 1 and 2, animals conditioned with cocaine displayed a preference of responding for the lever that activated the compound stimulus. Interestingly, by the last test session (i.e., Test 4), there were no group differences in responding on the active lever, and all groups responded equally on the two levers.

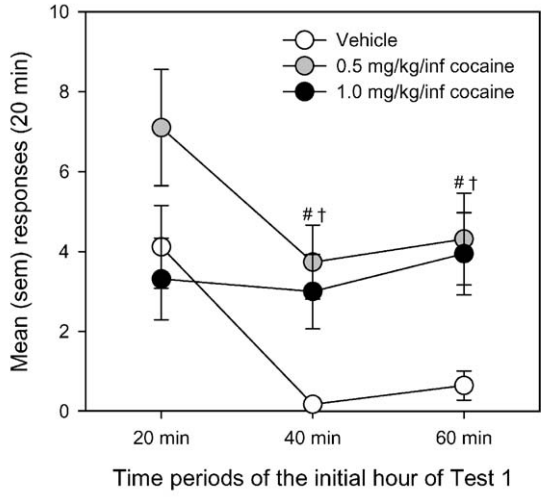

Fig. 2. Mean (sem) responses emitted on the active lever across 20 min periods of the initial hour of Test 1. The # denotes a significant difference between the Vehicle and the 0.5 mg/kg/inf cocaine groups. The † denotes a significant difference between the Vehicle and the 1.0 mg/kg/inf cocaine groups.

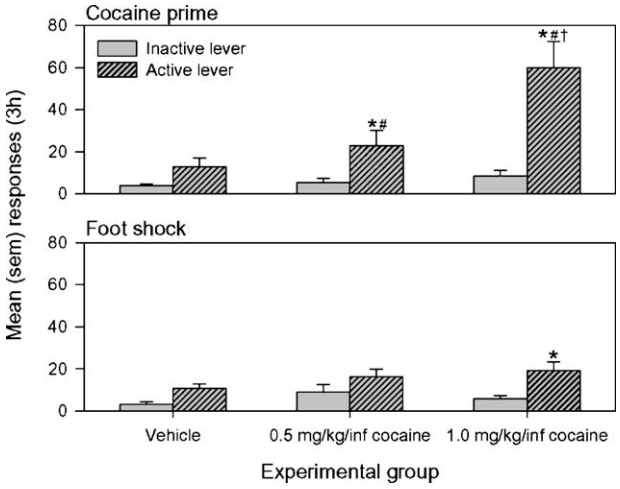

Fig. 3. Mean (sem) responses on the inactive and active levers during the tests of reinstatement induced by priming injections of cocaine (upper panel) and by foot shock (lower panel). The * denotes a significant difference in responding on the inactive and active levers within a given group. The # denotes a significant difference in responding on the active lever between cocaine groups and the Vehicle group. The † denotes a significant difference in responding on the active lever between the cocaine groups.

In order to further characterize lever pressing behavior maintained by the cocaine-paired compound stimulus, we analyzed responding on the active lever during the initial hour of Test 1 (Fig. 2). The ANOVA revealed significant main effects of Group [$F(2, 52) = 5.64$, $p < 0.01$] and of Time period [$F(2, 104) = 7.18$, $p < 0.01$]. During the initial 20 min of the session, levels of responding displayed by vehicle- and cocaine-conditioned rats did not differ significantly. However, rats conditioned with cocaine responded for an additional 40 min at rates significantly higher than the Vehicle group, which showed virtually no responding after 30 min from the beginning of the session.

Fig. 3 represents the effects of a cocaine (20 mg/kg) prime (upper panel) and foot shock (lower panel) on active and inactive lever responding in the different groups. The findings of primary interest yielded by the ANOVA were a significant Group by Reinstatement by Lever interaction [$F(2, 52) = 4.09$, $p < 0.05$], and significant main effects of Group [$F(2, 52) = 9.18$, $p < 0.001$], Reinstatement [$F(1, 52) = 6.87$, $p < 0.05$] and Lever [$F(1, 52) = 54.62$, $p < 0.0001$]. Multiple comparisons indicated that the cocaine prime elevated responding selectively on the active lever in rats conditioned with cocaine, and this effect was larger in animals previously conditioned with the highest cocaine dose (i.e., 1.0 mg/kg/infusion). Similarly, foot shock stress produced statistically significant increases of responding on the active lever in the group previously conditioned with 1.0 mg/kg/infusion cocaine, although this effect was smaller than the effect of the cocaine prime.

4. Discussion

The results of the present experiments demonstrate that cocaine primes and exposure to foot shock stress can reinstate operant responding maintained by a cocaine-conditioned stimulus in rats never trained to actively self-administer cocaine.

Rats underwent a period of classical conditioning whereby a compound stimulus (light and buzzer) was paired with passive intravenous infusions of vehicle, 0.5 or 1.0 mg/kg/infusion cocaine. Subsequently, operant responding on an active lever eliciting the compound stimulus and on an inactive lever with no scheduled consequences was assessed. When compared to the rats that received vehicle during conditioning, it was found that animals conditioned with cocaine displayed sustained responding selective to the active lever. Through repeated testing in the absence of cocaine, group differences in responding on the active lever and selectivity of responding on this lever dissipated. Importantly, in the cocaine-conditioned groups, priming injections of cocaine effectively reinstated responding selectively on the active lever. Foot shock stress had a weaker effect on reinstatement, which was significant only in the group previously conditioned with the highest dose of cocaine.

Although our findings are in general agreement with those reported by Davis and Smith (1976, 1987), we did not observe increases in responding from the baseline test to the first test given following classical conditioning. Rather, it was found that animals conditioned with cocaine maintained responding for the compound stimulus longer than animals conditioned with vehicle. Four possible reasons may account for this discrepancy between studies. Firstly, it is possible that the period of baseline testing employed in our study induced some conditioned inhibition (Lubow, 1997) which attenuated the effect of classical conditioning. However, we would argue that this procedural step is necessary in order to create groups equated for their level of operant responding for a novel sensory stimulus (Marx et al., 1955), a behavioral tendency known to display large individual variability (Deminiere et al., 1989; Piazza et al., 1989; Bardo et al., 1996; Bevins et al., 1997). Secondly, differently from Davis and Smith (1987) who employed morphine and amphetamine, we used cocaine, a drug that can produce variable effects in models based on classical conditioning (Bardo et al., 1995). Thus, it is possible that longer conditioning sessions would have been required to obtain more robust conditioning with cocaine. Thirdly, the time period imposed between the end of conditioning and the beginning of testing (i.e., 2 days) may not have been long enough to detect larger effects of conditioning on lever pressing for the drug-conditioned stimulus. In fact, it is known that long withdrawal periods (7 days and more) are required to obtain robust cocaine-seeking behavior as indexed by levels of operant responding for a drug-conditioned cue (Grimm et al., 2001; Lu et al., 2005), although this has only been demonstrated in rats trained to actively self-administer cocaine. Furthermore, it is possible that shortly after intense cocaine exposure, rats experienced anhedonia associated with cocaine withdrawal (Ahmed et al., 2002; Kenny et al., 2003) which interfered with responding for the cocaine-conditioned cue (Barr and Markou, 2005). Our finding that, on Test 1, animals conditioned with 1.0 mg/kg/infusion cocaine actually showed less responding on the active lever than rats conditioned with 0.5 mg/kg/infusion is certainly consistent with this interpretation. Finally, it is possible that our initial baseline session was too short to induce sufficient habituation to the novelty of the compound stimulus, which was necessary to detect subsequent increases in responding attributable to conditioning.

Arguably, however, the most interesting finding of this experiment was that priming injections of cocaine reinstated operant responding only in animals previously conditioned with cocaine, and selectively on the lever that activated the cocaine-conditioned compound stimulus. This is notable because rats in this experiment never learned to press the active lever to obtain cocaine infusions. This finding parallels findings of drug-induced reinstatement using the conditioned place preference procedure (Mueller and Stewart, 2000; Mueller et al., 2002; Leri and Rizos, 2005), another model of precipitated drug-seeking in which animals are not trained to perform specific responses to obtain drugs. Taken together, therefore, these experiments suggest that cocaine primes can induce reinstatement by reactivating the incentive value of cocaine-conditioned cues (Stewart, 2000).

It should be noted that the increase in responding on the active lever caused by the cocaine prime may be a phenomenon related, but not homologous, to amphetamine-enhanced sensitivity to conditioned reinforcers (Taylor and Robbins, 1984; Cador et al., 1989; Taylor and Horger, 1999; Parkinson et al., 1999). In fact, the procedure employed in the current study differs from those used in these latter experiments in that the conditioned reinforcing value of the cocaine-conditioned cue was extinguished before tests of reinstatement. Furthermore, we observed that the magnitude of reinstatement was dependent upon the dose of cocaine used during classical conditioning. This suggests that cocaine-enhanced responding on the active lever during the reinstatement test was dependent on previous conditioning and did not reflect a general effect of cocaine on sensitivity to conditioned reinforcers. It should also be noted that, although in this experiment we did not administer saline control injections, it is unlikely that injection-associated stress was the primary cause of cocaine reinstatement because in other studies we have found that IP administration of saline had no effect on operant behavior in rats similarly conditioned (Leri et al., 2005b).

Finally, we also observed reinstatement of responding on the lever activating the cocaine-conditioned cue following acute exposure to foot shock stress, although this effect was less robust than the cocaine-induced reinstatement. At this point, it is not clear why reinstatement produced by stress was not as substantial as typically seen in animals that actively self-administer cocaine (Shaham et al., 2000). A possible explanation based on known neurobiological differences between drug- and stress-induced relapse (Shaham and Stewart, 1996; Shaham et al., 1997; Erb et al., 1998; Kalivas and McFarland, 2003; Stewart, 2003; McFarland et al., 2004) may be that stress-induced reinstatement is less dependent on reactivation of drug-conditioned cues and more so on the reactivation of response habits to these cues.

In conclusion, our present report describes the application of the conditioned reinforcement procedure described by Davis and Smith (1976, 1987) to the study of reinstatement of drug-seeking in rats. In addition to its theoretical interest, this procedure offers three experimental advantages: 1) individual differences in drug intake are eliminated as all animals in a

given group are exposed to identical quantities of a drug; 2) because the current procedure requires only limited access to the venous system of the animal, the chances of losing experimental animals to systemic infections or blocked intravenous catheters are reduced; and 3) animals can be conditioned using drug doses or drug classes that would not be readily self-administered.

Acknowledgments

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