Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 80 (2005) 621–630

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Reconditioning of drug-related cues: A potential contributor to relapse after drug reexposure

Francesco Leri*, Zoe Rizos

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

Received 26 September 2004; received in revised form 27 January 2005; accepted 28 January 2005
Available online 2 March 2005

Abstract

To investigate the process of relapse to drug seeking caused by reexposure to drugs, we studied the consequences of recurring instances of stimuli–drug associations using heroin conditioned place preference (CPP) in rats. After original conditioning and extinction, rats received either a single compartment–heroin pairing (reconditioning) or were primed with heroin and tested for reinstatement of CPP. It was found that the session of reconditioning, but not the session of reinstatement, caused the reappearance of a preference for the heroin-paired compartment on a test given 24 h later, in drug-free conditions. The effect of reconditioning was found to be dependent on heroin doses, and was not seen when heroin injections were given outside the conditioning environment. Furthermore, a single session of reconditioning elevated heroin seeking even on a test given 96 h later. Finally, heroin seeking was found to be significantly elevated on a test given 28 days after the last extinction session whether animals received 1 or 3 reconditioning sessions. These results suggest that the motivational value of cues associated with heroin is not eliminated by extinction and, importantly, that these cues can rapidly regain their ability to promote drug seeking behavior if they are re-associated with the effect of heroin.

Keywords: Heroin; Reconditioning; Relapse; Conditioned place preference; Extinction; Drug seeking; Spontaneous recovery; Rat

1. Introduction

It is well known that exposure to drug conditioned cues can precipitate drug craving in humans and drug seeking behavior in animal models of relapse (Childress et al., 1992, 1999; Ciccocioppo et al., 2001; Foltin and Haney, 2000; See, 2002; Shaham et al., 2002; Carter and Tiffany, 1999). Possibly in combination with other factors such as the experience of stress and negative affect (Shaham et al., 2002; Bradley, 1989; Bradley et al., 1989; Sinha et al., 2000), drug cravings and seeking may lead to instances of renewed drug use, which result in the reexposure of the organism to the effects of the drug. Typically, such reexposure precipitates a cyclical process whereby drug seeking progressively grows and promotes further drug use, eventually resulting in full-blown relapse (Witkiewitz and Marlatt, 2004; Marlatt and Gordon, 1985; Chornock et al., 1992; Curry et al., 1988; Shiffman et al., 2000; Hall et al., 1990; Gossop et al., 1989; 2002; Moore and Budney, 2003).

If drug conditioned cues are re-paired with drug exposure after long periods of abstinence and/or extinction of responding to the cues, the conditions exist for these cues to reacquire their incentive properties leading to increased likelihood of approach and interaction with them. Thus, studies of how sporadic drug use/exposure in the presence of drug-related cues after periods of abstinence promotes the reacquisition of drug seeking can increase our understanding of the process that leads to full-blown relapse. Animal models appear useful to explore psychopharmacological factors involved in this process (Leri and Stewart, 2002; Lu et al., 2002).

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

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.01.013

In the present experiments, we studied reacquisition of an extinguished heroin conditioned place preference (CPP). Rats were initially trained to associate an environment with heroin administration (i.e., conditioning). Subsequently, the resulting heroin CPP was extinguished by pairing vehicle administration with both the previous vehicle- and heroin-paired compartments (Bardo et al., 1984; Calcagnetti and Schechter, 1993; Mueller and Stewart, 2000; Mueller et al., 2002; Lu et al., 2002). Rats were then reconditioned by re-pairing heroin with the heroin-paired compartment on a single occasion. Finally, a test of CPP was given at least 24 h after reconditioning, in drug-free conditions.

Four separate experiments were performed to expand in several ways a previous study of reacquisition of heroin seeking using the intravenous self-administration model (Leri and Stewart, 2002). More specifically, in this previous study, it was shown that one short session of heroin self-administration following a period of extinction induced the reappearance of heroin seeking when animals were tested 24 h later in drug-free conditions. In this study, it was also found that passive heroin infusions given in the self-administration chamber, but in the absence of cues normally associated with heroin self-administration (i.e., lever), did not induce the reappearance of drug seeking on the subsequent test. However, in Leri and Stewart (2002), there was no evaluation of the dose–response nature of reconditioning, there was no attempt to distinguish between the effect of reconditioning and the effect of reinstatement on subsequent drug seeking assessed in a drug-free state, and there was no investigation of the permanence of the effect of reconditioning over time.

Thus, Experiment 1 investigated whether an injection of heroin that would reinstate heroin CPP following a period of extinction (Mueller et al., 2002), would also lead to elevated heroin seeking on a second test given 24 h after reinstatement, in drug-free conditions. Experiment 2 explored whether a single session of reconditioning during which animals received a heroin–compartment pairing would induce heroin seeking on a test given 24 h later in drug-free conditions. Experiment 2 also investigated whether the effect of reconditioning on reacquisition of heroin seeking is dose-dependent. Experiment 3 was designed to further explore the issue of dose-dependence, and to determine whether heroin injections given outside the conditioning environment would be effective in inducing reacquisition. Experiment 3 also assessed whether the effect of reconditioning is short-lived, and whether a history of heroin exposure not associated with the conditioning environment would contribute to the effect of 'reconditioning.' Finally, Experiment 4 investigated whether the permanence of the effect of reconditioning over longer periods of time (7 and 21 days) would be affected by the number of reconditioning sessions.

2. Methods

2.1. Subjects

Subjects were adult male Sprague–Dawley rats (Charles River, Quebec) weighing 225–250 g at the beginning of the experiments. Rats were paired 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 testing, which occurred during their 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. Apparatus

Six, custom made (University of Guelph, Ontario, Canada), place conditioning boxes were used in these experiments. The boxes were located in the center on the floor of a laboratory room. Each place conditioning box was made of dark gray PVC, and comprised of three compartments: two large ($30 \times 40 \times 26$ cm) and one smaller, middle ($23 \times 30 \times 26$ cm) compartment. Removable inserts, with or without small archway openings (10×10 cm) formed the center compartment. The two large compartments differed primarily in visual cues; one large compartment was dark gray while the other had a white wall and a 10 cm white stripe painted along the top of the other walls. In addition, there were cues that provided spatial information external to the compartments, such as posters on walls, benches, door and lights. In this apparatus, rats do not display a significant spontaneous preference for any of the compartments (i.e., the apparatus is balanced). The entire apparatus was covered by black wire mesh to allow video tracking of the rats during testing. The tracking software employed was EthoVision (version 3, Noldus Information Technology, The Netherlands). This system was used to automatically record two dependent variables: time (seconds) spent in each compartment during tests for place preference, and locomotor activity (total centimeters) during conditioning and reconditioning. However, to promote conciseness, only the locomotor activity results for Experiment 3 are reported because of their implications for reconditioning.

2.3. General procedures

Rats were allowed 6 days to habituate to the animal facility and were handled twice for approximately 10 min before the beginning of the experiments. The experiments consisted of six phases: habituation, conditioning, Test I, extinction, reinstatement (Experiment 1 only) or reconditioning (Experiments 2 to 4), and Test II (but see below for variations). Table 1 includes a list of experiments,

Table 1
Experiments, experimental phases, sample size, treatment groups figures and tables where the results are represented

Conditioning and Test I			Reinstatement and Test II		
Sample size	H dose/group (mg/kg)	CPP	Sample size	H dose/group (mg/kg)	CPP
Experiment 1					
26	1.0	Fig. 1A	9	0.0—Table 2	Fig. 1B
			9	0.3—Table 2	
			8	1.0—Table 2	
Conditioning and Test I			Reconditioning and Test II		
Sample size	H dose/group (mg/kg)	CPP	Sample size	H dose/group (mg/kg)	CPP
Experiment 2					
36	1.0	Fig. 2A	9	0.0	Fig. 2B
			9	0.3	
			9	1.0	
			9	3.0	
Experiment 3					
45	0.3	Fig. 3A	9	0.3	Fig. 3B
			9	1.0	
			9	0.3 Unpaired R	Fig. 3C
			9	0.0 Delay	Fig. 3D
			9	0.3 Delay	
9	0.0	No figure	9	0.3—Fig. 4A	Fig. 4B
8	0.3 Unpaired C	No figure	8	0.3—Fig. 4A	
Experiment 4					
48	0.3	Fig. 5A	16	0.0 H	Fig. 5B
			16	0.3 H × 1	Fig. 5C
			16	0.3 H × 3	Fig. 5D

treatment groups and sample size. Table 1 also indicates the number of the figure in which the results of each experiment and phase are presented.

Habituation (all experiments): On this day, the inserts with openings were used, and the rats had free access to the 3 compartments for 20 min. The main purpose of habituation was to allow the rats to become accustomed to the apparatus, and to measure level of spontaneous preference for each large compartment.

Conditioning (all experiments): The day after habituation, place conditioning began. For this phase, the inserts with openings were replaced with solid inserts to fully separate the compartments. Rats underwent 4 days of conditioning, and each day received two 30-min conditioning sessions, one in the morning and one in the afternoon (morning session: between 9:00 am and 12:00 pm; afternoon session: between 2:00 and 5:00 pm; minimum time between the two sessions for a given subject: 4 1/2–5 h). Each day, rats received one conditioning session with heroin (see doses below) and the other with vehicle. The specific compartment chosen to be associated with heroin was counterbalanced across rats. In addition, the time of heroin sessions (am or pm) was counterbalanced across rats and, for each rat, across days of conditioning. Injections were administered immediately before confinement in one of the two large compartments (but see exception in Experiment 3).

Test I (all experiments): On the day after conditioning, the solid inserts were replaced by those with openings, and a preference test was given to measure the effect of place conditioning. For this test, the rats were placed in the middle compartment and given 20 min of free access to all compartments. Rats were not given injections before this test.

Extinction (all experiments): After Test I, rats were left undisturbed in the colony room for one day before the beginning of extinction. Extinction was similar to conditioning in that it was carried out over 4 days, with two 30-min conditioning sessions each day. However, during extinction, rats received vehicle injections before confinement in both compartments.

Reinstatement (Experiment 1 only): Following the last day of extinction, the solid inserts were replaced by those with openings, and rats received a priming injection of heroin (see doses below) and were immediately tested for reinstatement of CPP. For this test, the rats were placed in the middle compartment and given 30 min of free access to all compartments.

Reconditioning (Experiments 2–4): Following the last day of extinction, rats were given a single day of reconditioning (except in Experiment 4) during which they received one session with heroin and the other with vehicle, in the compartments that were previously paired with heroin and vehicle, respectively. The occurrence of the heroin

session (am or pm) was counterbalanced across rats and, as for conditioning, reconditioning sessions lasted 30 min. Injections were administered immediately before confinement in one of the two large compartments (except in Experiment 3).

Test II (all experiments): On the day following reinstatement or reconditioning, the inserts with openings were used, and rats received a second preference test that lasted 20 min (but see Experiments 3 and 4 for variations). Rats were not given injections before this test.

2.3.1. Experiment 1

This experiment tested whether priming injections that reinstate CPP (Mueller et al., 2002; Lu et al., 2002; Parker and Mcdonald, 2000; Zavala et al., 2003; Itzhak and Martin, 2002; Mueller and Stewart, 2000) have delayed consequences on drug seeking when rats are tested again, but in a drug-free state.

A dose of 1.0 mg/kg heroin was chosen for conditioning because it is known to produce a reliable CPP in rats (Bardo et al., 1995). A total of 27 rats were initially conditioned with 1.0 mg/kg heroin and then randomly assigned to 3 groups ($n=9$) following extinction, each receiving a different priming dose of heroin: 0.0, 0.3, and 1.0 mg/kg (see Table 1). One animal in the 1.0 mg/kg group had to be excluded from the study because of health-related problems that arose during the extinction phase.

2.3.2. Experiment 2

This experiment tested whether one reconditioning session is sufficient to induce reacquisition of an extinguished place preference, and whether the effect of reconditioning is dose dependent. A total of 36 rats were initially conditioned with 1.0 mg/kg heroin and then randomly assigned to 4 groups ($n=9$) following extinction, each receiving a different heroin dose on reconditioning: 0.0, 0.3, 1.0 and 3.0 mg/kg (see Table 1).

2.3.3. Experiment 3

This experiment further analyzed the issue of dose dependence by employing a lower dose of heroin for conditioning (0.3 mg/kg) and then using the same dose, or a higher dose (1.0 mg/kg) for reconditioning. This experiment also included a group ($n=9$) that, on the reconditioning, received heroin (0.3 mg/kg) after confinement in the compartment. This "Unpaired R" group (see Table 1) was introduced to determine whether heroin reexposure alone would produce the same effect of reconditioning. Furthermore, in order to determine whether the effect of reconditioning was short-lived, two additional groups of rats were conditioned with 0.3 mg/kg ($n=9$ in each) and then reconditioned with 0.0 or 0.3 mg/kg heroin. These latter two groups were given Test II 96 h after reconditioning. Finally, in order to ascertain whether the effect of reconditioning depended on heroin pairings given during original conditioning, two additional groups were run. One ($n=9$) was confined to the compartments during the conditioning period, but never received heroin (0.0 group). The other group ($n=8$) was treated similarly, but was exposed to heroin (0.3 mg/kg) after confinement to the "heroin-paired" compartment, and hence the suffix "Unpaired C" (Table 1). In other words, the "Unpaired C" group received a total of four injections of heroin, but heroin was not paired with the compartment. Following "extinction," both groups received a single "reconditioning" session with 0.3 mg/kg heroin.

2.3.4. Experiment 4

This final experiment was designed to investigate whether the permanence of the effect of reconditioning over longer periods of time (7 and 21 days) would be affected by the number of reconditioning sessions. It was hypothesized that multiple reconditioning sessions would be necessary in order to induce long-lasting reacquisition of the CPP. To test this hypothesis, 48 rats where conditioned with 0.3 mg/kg heroin, tested once (Test I) and extinguished as described above. Two days after the last day of extinction, rats were randomly assigned to 3 reconditioning groups (see Table 1): "0.0 H" group received three days of 'reconditioning' but never received heroin (extended extinction training); "0.3 H × 1" group received two days of reconditioning with vehicle and one with 0.3 mg/kg heroin; finally, "0.3 H × 3" group received three days of reconditioning with heroin (0.3 mg/kg). Reconditioning sessions were given on alternate days over a 6-day period. The first test for place preference following reconditioning (Test IIa) was given 7 days later, followed by a second test (Test IIb) given 21 days after Test IIa.

2.4. Drugs

Diacetylmorphine HCl (heroin) was obtained from Almat Pharmachem (Concord, Ontario, Canada), dissolved in 0.9% physiological saline, and injected subcutaneously, SC, at volume of 1.0 ml/kg. Vehicle (0.9% physiological saline) was injected at the same volume and by the same route.

2.5. Statistical analyses

In our laboratory, place preference typically results from opposite shifts in times spent in the vehicle- and drug-paired compartments. Thus, our statistical analysis involves the comparison between these times within each group; a method used by a number of other laboratories employing the unbiased CPP procedure (Bardo et al., 1995; van der Kooy, 1987; Parker and Mcdonald, 2000; Mueller et al., 2002; Bossert and Franklin, 2001; Leri and Franklin, 2000; Everitt et al., 1991; Hoffman, 1989). Analyses of time spent in the middle compartment are not reported because we did not notice significant effects in these heroin CPP studies.

Relative preferences for the two large compartments at baseline habituation and on Test I were evaluated using separate paired *t*-tests. Preference during Test II was evaluated using 2-way mixed design ANOVAs with Heroin dose/group as the between factor and Compartment (vehicle- and heroin-paired) as the within factor. In Experiment 3, locomotion activity measured during reconditioning was analyzed using the same statistical design. 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. The alpha level was set to <0.05. The specific values of negative findings are not reported. All statistical analyses were performed using SigmaStat (version 3.0.1 for Windows, SPSS Chicago, USA).

3. Results

3.1. Experiment 1

Rats showed no significant spontaneous preference on the baseline habituation test day but, after conditioning, they displayed a significant CPP for the heroin-paired compartment (Fig. 1A; $[t(25)=-3.38, p<0.005]$). This finding confirms that the conditioning parameters used (number of pairings, duration of conditioning sessions, timing of conditioning injections, and heroin dose) were effective in inducing a heroin CPP.

Priming injections of heroin reinstated heroin CPP dose-dependently (see data in Table 2): there was a significant main effect of Heroin dose/group $[F(2, 23)=3.9, p<0.05]$ and of Compartment $[F(1, 23)=2.8, p<0.05]$. However, when the same rats were tested again, in drug-free conditions, 24 h after this reinstatement session, no group differences were found (see Fig. 1B). In other words, the heroin prime that significantly reinstated CPP (1.0 mg/kg, Table 2), had no delayed effect on heroin seeking assessed in the same animals when tested again, but in a drug-free state. The lack of preference in rats primed with 0.0 mg/kg on both the reinstatement test and Test II indicates that our extinction protocol was appropriate in eliminating heroin CPP.

Table 2
Reinstatement of heroin conditioned place preference in Experiment 1

H dose/group (mg/kg)	Vehicle-paired compartment mean (SEM) seconds	Heroin-paired compartment mean (SEM) seconds
0.0	469.9 (45.8)	560.1 (88.3)
0.3	398.6 (51.5)	511.3 (80.1)
1.0	321.3 (160.8)	1013.8.1 (212.9)*

* Represents a significant difference between seconds in vehicle- and heroin-paired compartments ($p=0.003$).

3.2. Experiment 2

Rats showed no significant spontaneous preference on the baseline habituation test day but, after conditioning, they displayed a significant CPP for the heroin-paired compartment (Fig. 2A; $[t(35)=-5.16, p<0.001]$).

After extinction, rats were randomly assigned to 4 different groups ($n=9$), each receiving a different dose of

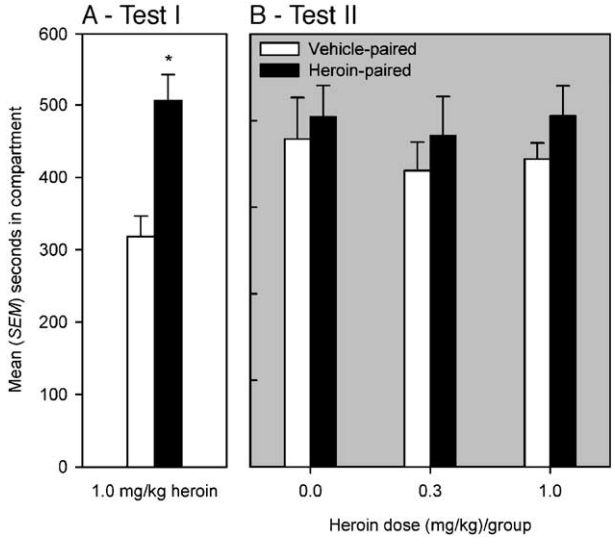

Fig. 1. Mean (SEM) seconds spent in vehicle- and heroin-paired compartments on: Panel A—Test I in animals conditioned with 1.0 mg/kg heroin ($n=26$); Panel B—Test II in animals that, on the day previous to this test, were primed with 0.0 mg/kg ($n=9$), 0.3 mg/kg ($n=9$) and 1.0 mg/kg ($n=8$) heroin and tested for reinstatement. The asterisk indicates a significant difference between vehicle- and heroin-paired compartments (i.e., significant CPP).

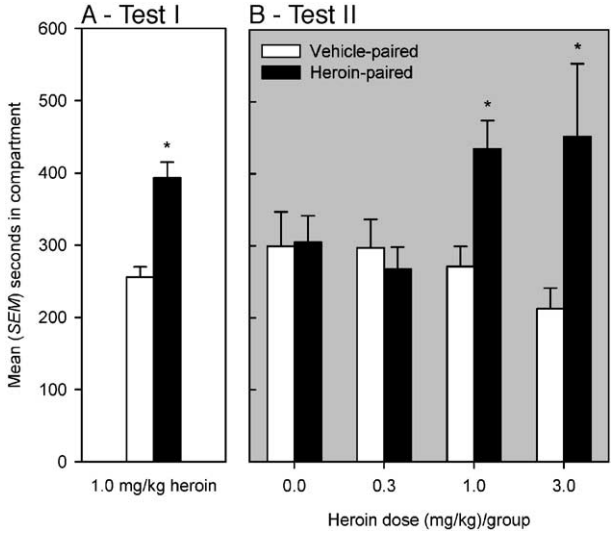

Fig. 2. Mean (SEM) seconds spent in vehicle- and heroin-paired compartments on: Panel A—Test I in animals conditioned with 1.0 mg/kg heroin ($n=36$); Panel B—Test II in animals reconditioned with 0.0, 0.3, 1.0 and 3.0 mg/kg heroin ($n=9$ each dose). The asterisk indicates a significant difference between vehicle- and heroin-paired compartments.

heroin on the day of reconditioning (Table 1). Fig. 2B displays the results of Test II given 24 h following reconditioning. The ANOVA identified the presence of a significant main effect of Compartment [$F(1, 32)=5.95$, $p<0.05$] and multiple comparisons isolated significant heroin CPPs only in rats that were reconditioned with 1.0 mg/kg ($p=0.04$) and with 3.0 mg/kg ($p=0.004$).

3.3. Experiment 3

In this experiment, as in Experiment 2, rats exhibited no spontaneous preference for any compartment on the baseline habituation test, but after conditioning with 0.3 mg/kg heroin, they displayed a significant heroin CPP on Test I (Fig. 3A; [$t(44)=-6.14$, $p<0.001$]).

Fig. 3B/C/D show the results CPP Test II given after reconditioning in the different groups. Rats originally conditioned with 0.3 mg/kg heroin displayed significant reacquisition when reconditioned with either 0.3 or 1.0 mg/kg heroin (Fig. 3B; main effect of Compartment: [$F(1, 16)=11.77$, $p<0.05$]; multiple comparisons: 0.3 mg/kg, $p=0.04$; 1.0 mg/kg, $p=0.014$). Fig. 3C shows that reacquisition did not occur if rats received heroin (0.3 mg/kg) after the session of reconditioning, outside the conditioning compartment. Finally, as shown in Fig. 3D, reacquisition of the CPP was observed in the group that received 0.3 mg/kg (but not 0.0 mg/kg) on the reconditioning day even if a delay of 96 h was imposed between reconditioning and CPP Test II (Heroin dose/group by Compartment interaction: [$F(1, 16)=5.11$, $p<0.05$]; main effect of Compartment: [$F(1, 16)=8.91$, $p<0.05$]; multiple comparison: $p=0.002$). From Fig. 3D it is also evident that there was no spontaneous recovery of CPP after 96 h in rats reconditioned with 0.0 mg/kg heroin.

Not surprisingly, rats that received 0.0 mg/kg heroin during conditioning or that received heroin after each conditioning session (0.3 Unpaired C) did not display a significant CPP on Test I (no figure; mean (SEM) seconds spent in compartment: 0.0 group, vehicle-paired=298.7 (31.7) and heroin-paired=279.2 (35.5); 0.3 Unpaired C group, vehicle-paired=301.1 (40.0) and heroin-paired=261.4 (33.1)). However, these rats showed a differential locomotor response to heroin during the 'reconditioning trial.' In fact, as shown in Fig. 4A, only the 0.3 Unpaired C group displayed a significant elevation in locomotion when injected with heroin (0.3 mg/kg) as compared to their activity when injected with vehicle (main effect of injection [$F(1, 15)=9.7$, $p<0.01$]; multiple comparison: $p=0.005$). Interestingly, on Test II given after the 'reconditioning' trial (see Fig. 4B), in contrast to the 0.0 group, rats given repeated exposure to heroin outside the compartment during conditioning (0.3 Unpaired C group) displayed a significant CPP (Heroin dose/group by Compartment interaction: [$F(1, 15)=8.93$, $p<0.05$]; multiple comparisons: $p=0.009$).

3.4. Experiment 4

There was no spontaneous preference for any compartment on the baseline habituation test, but after conditioning rats displayed the expected preference for the heroin-paired compartment on Test I (Fig. 5A; [$t(47)=-9.81$, $p<0.001$]).

Fig. 5 B/C/D shows the results of Tests IIa (7 days after last day of reconditioning) and IIb (21 days later) in the three groups. The group that received no heroin during 'reconditioning' (0.0 H group), showed no preference on the first test for reacquisition (Test IIa), but did show a significant preference on Test IIb (Fig. 5B—main effect of

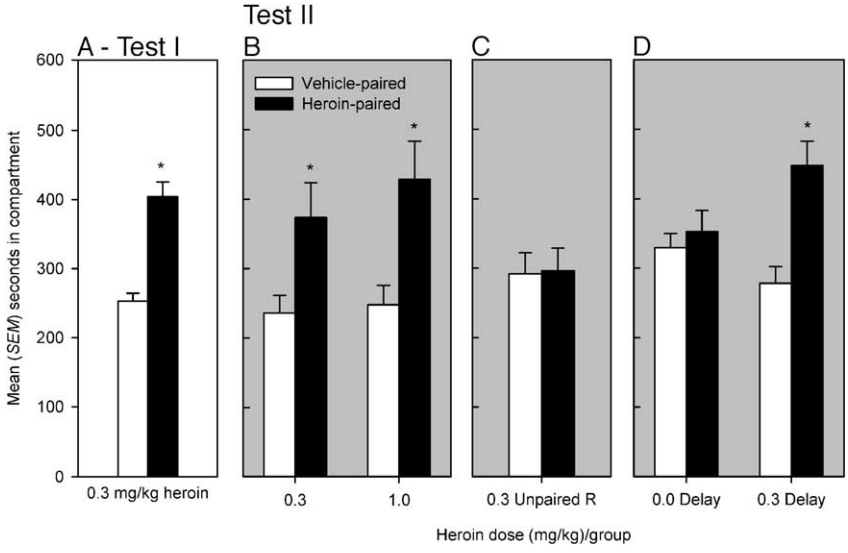

Fig. 3. Mean (SEM) seconds spent in vehicle- and heroin-paired compartments on: Panel A—Test I in animals conditioned with 0.3 mg/kg heroin ($n=45$); Panel B—Test II in animals reconditioned with 0.3 and 1.0 mg/kg heroin ($n=9$ each dose); Panel C—Test II in animals that received heroin (0.3 mg/kg) after reconditioning ($n=9$); Panel D—Test II in animals reconditioned with 0.3 and 1.0 mg/kg heroin ($n=9$ each dose) and tested 96 h following reconditioning. The asterisk indicates a significant difference between vehicle- and heroin-paired compartments.

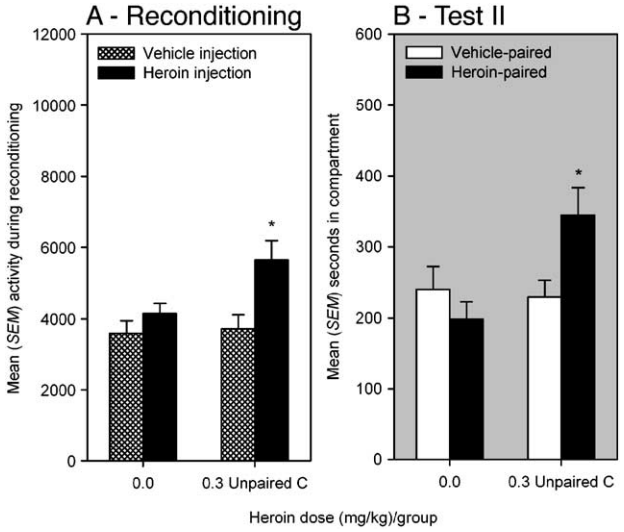

Fig. 4. Panel A—mean (SEM) locomotor activity after the vehicle and the heroin (0.3 mg/kg) injections given for 'reconditioning' in animals that received vehicle (0.0 group, $n=9$) or 0.3 mg/kg heroin (0.3 Unpaired C group, $n=9$) after each conditioning session. Panel B—mean (SEM) seconds spent in vehicle- and heroin-paired compartments on Test II. The asterisk indicates a significant difference between vehicle and heroin injections (Panel A) and between vehicle- and heroin-paired compartments (Panel B).

Compartment: [$F(1, 15)=5.23$, $p<0.05$]; multiple comparisons: Test IIb, $p=0.01$). In contrast, the groups that received one (0.3 H × 1 group) or three (0.3 H × 3 group) compartment–heroin pairings during reconditioning displayed a significant preference on both Tests IIa and IIb (Fig. 5C—main effect of Compartment: [$F(1, 15)=35.30$, $p<0.001$: multiple comparisons: Test IIa, $p=0.001$; Test IIb, $p=0.001$]; Fig. 5D—main effect of Compartment: [$F(1, 15)=10.44$, $p<0.001$]: multiple comparisons: Test IIa, $p=0.01$; Test IIb, $p=0.01$), and the size of the CPPs displayed by these two groups appeared very similar at every test. These latter results do not support the hypothesis that greater numbers of reconditioning sessions are required to promote maintenance of the place preference over longer periods of time.

4. Discussion

The results of Experiments 2 and 3 show that one session of reconditioning is sufficient to induce reacquisition of an extinguished heroin CPP, and that this effect is dependent on the relationship between the dose of heroin used during reconditioning and the dose used during original conditioning. In Experiment 3, it was also found that elevated drug seeking could be measured up to 96 h after reconditioning. This result is important because it rules out drug priming as an explanation for these effects. It is well known that acute drug exposure can prime drug seeking measured by place preference (Mueller et al., 2002; Lu et al., 2002; Parker and Mcdonald, 2000; Zavala et al., 2003; Itzhak and Martin, 2002; Mueller and Stewart, 2000). However, heroin is deacetylated within minutes from its administration (Cohn et al., 1973) and the half-life of its metabolites ranges between 2–5 h in rats (Barjavel et al., 1995; Aasmundstad et al., 1995; Mullis et al., 1979), and therefore, our rats were almost certainly heroin-free when tested 96 h following reconditioning. Furthermore, in Experiment 1, we explicitly investigated the effect of drug primes on reinstatement and on subsequent drug seeking behavior assessed when the acute effect of heroin had subsided (i.e., 24 h later). In this experiment, we found a significant reinstatement of CPP, but no evidence of drug seeking the following day. Taken together, these experiments indicate that reinstatement is not the primary cause

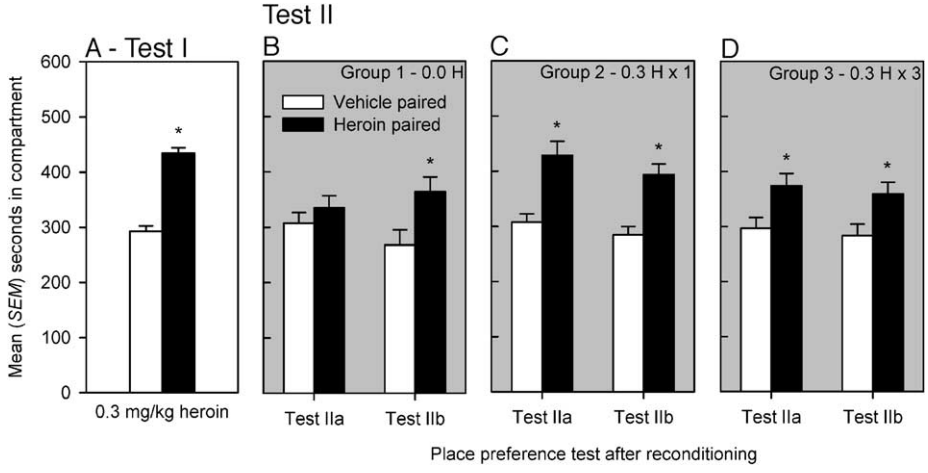

Fig. 5. Mean (SEM) seconds spent in vehicle- and heroin-paired compartments on: Panel A—Test I in animals conditioned with 0.3 mg/kg heroin ($n=48$); Panel B—Test IIa (7 days following last reconditioning session) and Test IIb (21 days following Test IIa) in animals that received 3 reconditioning sessions with vehicle (0.0 H group, $n=16$); Panel C—Tests IIa and IIb in animals that received 2 reconditioning sessions with vehicle and one with 0.3 mg/kg heroin (0.3 H × 1 group, $n=16$); Panel D—Tests IIa and IIb in animals that received 3 reconditioning sessions with 0.3 mg/kg heroin (0.3 H × 3 group, $n=16$). The asterisk indicates a significant difference between vehicle- and heroin-paired compartments.

of reconditioning, and that the reconditioning procedure may be particularly appropriate to investigate the effect that renewed drug exposure has on the reacquisition of drug seeking behavior.

Our present findings of rapid reacquisition of heroin seeking employing place conditioning are in agreement with those of a previous study where it was observed that a single, short period of heroin self-administration was sufficient to elevate lever-pressing in extinction conditions when animals were tested 24 h following reconditioning (Leri and Stewart, 2002). Our findings are also consistent with a morphine CPP experiment in which a single session of reconditioning with morphine, heroin and cocaine given after intense extinction training (21 days) was sufficient to reactivate the original preference (Lu et al., 2002).

Interestingly, Lu et al. (2002) also found that a single session of reconditioning during which the drug was administered in the previous vehicle-paired compartment was effective in inducing a preference for this compartment. We observed a comparable effect in animals that were exposed to heroin (i.e., 0.3 Unpaired C group; Fig. 4B), but that were never conditioned. In these animals, a single pairing of heroin with a compartment during "reconditioning" was effective in establishing a CPP. In contrast, one "reconditioning" session was not sufficient to induce a CPP in animals that never received heroin. These results are in line with demonstrations that a history of morphine exposure facilitates the acquisition of morphine CPP (Lett, 1989; Shippenberg et al., 1996; Harris and Aston-Jones, 2003). Furthermore, we also found that the locomotor response of the 0.3 Unpaired C group to the "reconditioning" heroin injection was significantly enhanced in comparison to the response to the same heroin dose administered to animals that were never exposed to heroin (see Fig. 4A). This result indicates that the former group displayed some degree of locomotor sensitization to the stimulatory effect of heroin (Robinson and Berridge, 2003; Stewart and Badiani, 1993; Pierre and Vezina, 1997) and suggests that sensitization may play a role in the rapid reacquisition of drug seeking behavior.

However, increased speed of acquisition in new learning situations where a familiar reinforcer is used ("learning to learn" Kehoe and Macrae (1997)), and rapid reacquisition of previously extinguished behaviors (Napier et al., 1992; Rescorla, 2003; Rescorla, 2001b), are phenomena that are observed in classical and operant conditioning experiments where drugs of abuse are not the primary reinforcers/unconditioned stimuli. This suggests that rapid reacquisition of heroin CPP may not be entirely attributable to higher sensitivity to the motivational properties of heroin. A possible additional factor involved in reconditioning, therefore, may be savings of the original compartment (CS)–heroin (US) association. Supporting this conclusion, in Experiment 4, we observed significant spontaneous recovery (Pavlov, 1927; Bouton and Swartzentruber, 1991; Di Ciano and Everitt, 2002) of the extinguished CPP in animals that were tested 28 days after reconditioning with vehicle (Fig. 5B). Furthermore, in Experiment 3, we found that mere heroin reexposure was not effective in elevating subsequent drug seeking (Fig. 3C). The finding that reacquisition is triggered only by contiguous reexposure to the extinguished CS and to the drug US replicates the results of Leri and Stewart (2002), and lends support to the interpretation that rapid reacquisition may be the product of competing CS–US associations acquired during conditioning, extinction, and reconditioning (Rescorla, 2001b).

In designing Experiment 4, we hypothesized that increasing the number of reconditioning sessions would produce more robust reacquisition which, in turn, would promote the maintenance of the place preference over longer periods of time. We found little support for this idea; 1 and 3 reacquisition sessions yielded CPPs of similar magnitude when assessed 7 and 21 days following the last reacquisition session. This observation is consistent with the idea that the savings of the original compartment (CS)–heroin (US) association was substantial and that reacquisition can be very rapid.

Our observation of rapid reacquisition and of spontaneous recovery support the argument that extinction does not "erase" original conditioning, but rather it involves the acquisition of new information which compete with original conditioning to control behavior (Rescorla, 2001a; Bouton and Swartzentruber, 1991). Konorski (1948), Grice (1972) and Rescorla and Wagner (1972) suggested the mapping of CS–US associations into behavioral performance is modulated by a "threshold" of excitation of the US representation by the CS. Extinction supposedly changes this threshold, superimposing an inhibitory process that reduces the "excitability of the US center" (Konorski, 1948), or that "degrades the US representation" (Rescorla and Cunningham, 1977). It is not clear what is the exact nature of this inhibitory process, although it has been argued that it results from the emotional consequence of omitting an anticipated US during extinction (Rescorla, 2001b; Bolles, 1972; Tolman, 1948; Solomon and Corbit, 1974; Wagner, 1981; Amsel, 1958). According to this interpretation, therefore, reconditioning may re-establish drug seeking behavior because of an interference with an inhibitory process acquired during extinction that actively suppresses responses to drug conditioned stimuli.

In conclusion, cognitive behavioral views of relapse emphasize the role of sporadic drug use following abstinence in the progressive enhancement of drug seeking and drug taking which potentially culminates in full-blown relapse (Marlatt and Gordon, 1985; Witkiewitz and Marlatt, 2004). Here, we presented an animal model of reacquisition of drug seeking behavior which captures the evolving aspect of drug relapse. Such model may be useful to investigate the neurobiological mechanisms involved in the relapse process, as well as pharmacological interventions that may interfere with its progression.

Acknowledgements

This research was funded by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC). The authors wish to thank Dr. Jane Stewart for the helpful comments on the manuscript. The authors also wish to thank Ms. Jelena Ovari and Ms. Amanda Healy for the technical assistance.

References

Aasmundstad TA, Morland J, Paulsen RE. Distribution of morphine 6-glucuronide and morphine across the blood–brain barrier in awake, freely moving rats investigated by in vivo microdialysis sampling. J Pharmacol Exp Ther 1995;275:435–41.

Amsel A. The role of frustrative nonreward in noncontinuous reward situations. Psychol Bull 1958;55:102–19.

Bardo MT, Miller JS, Neisewander JL. Conditioned place preference with morphine: the effect of extinction training on the reinforcing CR. Pharmacol Biochem Behav 1984;21:545–9.

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995;19:39–51.

Barjavel MJ, Scherrmann JM, Bhargava HN. Relationship between morphine analgesia and cortical extracellular fluid levels of morphine and its metabolites in the rat: a microdialysis study. Br J Pharmacol 1995;116:3205–10.

Bolles RC. Reinforcement, expectancy and learning. Psychol Rev 1972;79:394–409.

Bossert JM, Franklin KB. Pentobarbital-induced place preference in rats is blocked by GABA, dopamine, and opioid antagonists. Psychopharmacology 2001;157:115–22.

Bouton ME, Swartzentruber D. Sources of relapse after extinction in Pavlovian and instrumental learning. Clin Psychol Rev 1991; 11:123–40.

Bradley BP. Heroin and the opiates. In: Gossop M, editor. Relapse and Addictive Behaviour. London and New York: Tavistock/Routledge; 1989. p. 73–85.

Bradley BP, Phillips G, Green L, Gossop M. Circumstances surrounding the initial lapse to opiate use following detoxification. Br J Psychiatry 1989;154:354–9.

Calcagnetti DJ, Schechter MD. Extinction of cocaine-induced place approach in rats: a validation of the "biased" conditioning procedure. Brain Res Bull 1993;30:695–700.

Carter BL, Tiffany ST. Meta-analysis of cue-reactivity in addiction research. Addiction 1999;94:327–40.

Childress AR, Ehrman R, Rohsenow DJ, Robbins SJ, O'Brien CP. Classically conditioned factors in drug dependence. In: Lowinson JL, Ruiz P, Millman RB, editors. Substance Abuse: A Comprehensive Textbook. Baltimore: Williams and Wilkins; 1992. p. 56–69.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP. Limbic activation during cue-induced cocaine craving. Am J Psychiatry 1999;156:11–8.

Chornock WM, Stitzer ML, Gross J, Leischow S. Experimental model of smoking re-exposure: effects on relapse. Psychopharmacology 1992;108:495–500.

Ciccocioppo R, Sanna PP, Weiss F. Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D(1) antagonists. Proc Natl Acad Sci U S A 2001;98:1976–81.

Cohn GL, Cramer JA, Kleber HD. Heroin metabolism in the rat. Proc Soc Exp Biol Med 1973;144:351–5.

Curry SJ, Marlatt GA, Gordon J, Baer JS. A comparison of alternative theoretical approaches to smoking cessation and relapse. Health Psychol 1988;7:545–56.

Di Ciano P, Everitt BJ. Reinstatement and spontaneous recovery of cocaine-seeking following extinction and different durations of withdrawal. Behav Pharmacol 2002;13:397–405.

Everitt BJ, Morris KA, O'Brien A, Robbins TW. The basolateral amygdala–ventral striatal system and conditioned place preference: further evidence of limbic–striatal interactions underlying reward-related processes. Neuroscience 1991;42:1–18.

Foltin RW, Haney M. Conditioned effects of environmental stimuli paired with smoked cocaine in humans. Psychopharmacology 2000;149:24–33.

Gossop M, Green L, Phillips G, Bradley B. Lapse, relapse and survival among opiate addicts after treatment: a prospective follow-up study. Br J Psychiatry 1989;154:348–53.

Gossop M, Stewart D, Browne N, Marsden J. Factors associated with abstinence, lapse or relapse to heroin use after residential treatment: protective effect of coping responses. Addiction 2002;97:1259–67.

Grice GR. Conditioning and a decision theory of response evocation. In: Bower GH, editor. Psychol Learn Motiv: Advances in Research and Theory, vol. 5. New York: Academic Press; 1972.

Hall SM, Havassy BE, Wasserman DA. Commitment to abstinence and acute stress in relapse to alcohol, opiates, and nicotine. J Consult Clin Psychol 1990;58:175–81.

Harris GC, Aston-Jones G. Enhanced morphine preference following prolonged abstinence: association with increased Fos expression in the extended amygdala. Neuropsychopharmacology 2003;28:292–9.

Hoffman DC. The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Res Bull 1989;23:373–87.

Itzhak Y, Martin JL. Cocaine-induced conditioned place preference in mice: induction, extinction and reinstatement by related psychostimulants. Neuropsychopharmacology 2002;26:130–4.

Kehoe EJ, Macrae M. Savings in animal learning: implications for relapse and maintenance after therapy. Behav Ther 1997;28:141–55.

Konorski J. Conditioned Reflexes and Neuron Organization. Chicago: University of Chicago Press; 1948.

Leri F, Franklin KB. Effects of diazepam on conditioned place preference induced by morphine or amphetamine in the rat. Psychopharmacology 2000;150:351–60.

Leri F, Stewart J. The consequences of different lapses on relapse to heroin-seeking in rats. Exp Clin Psychopharmacol 2002;10:339–49.

Lett BT. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology 1989;98:357–62.

Lu L, Xu NJ, Ge X, Yue W, Su WJ, Pei G, et al. Reactivation of morphine conditioned place preference by drug priming: role of environmental cues and sensitization. Psychopharmacology 2002;159:125–32.

Marlatt GA, Gordon JR. Relapse Prevention: Maintenance Strategies in the Treatment of Addictive Behavior. New York: Guilford Press; 1985.

Moore BA, Budney AJ. Relapse in outpatient treatment for marijuana dependence. J Subst Abuse Treat 2003;25:85–9.

Mueller D, Stewart J. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behav Brain Res 2000;115:39–47.

Mueller D, Perdikaris D, Stewart J. Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. Behav Brain Res 2002;136:389–97.

Mullis KB, Perry DC, Finn AM, Stafford B, Sadee W. Morphine persistence in rat brain and serum after single doses. J Pharmacol Exp Ther 1979;208:228–31.

Napier RM, Macrae M, Kehoe EJ. Rapid reacquisition in conditioning of the rabbit's nictitating membrane response. J Exp Psychol, Anim Behav Process 1992;18:182–92.

Parker LA, Mcdonald RV. Reinstatement of both a conditioned place preference and a conditioned place aversion with drug primes. Pharmacol Biochem Behav 2000;66:559–61.

Pavlov IP. Conditioned Reflexes. Cambridge: Cambridge University Press; 1927.

Pierre PJ, Vezina P. Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology 1997;129:277–84.

Rescorla RA. Experimental extinction. In: Mowrer OH, Klein SB, editors. Handbook of Contemporary Learning Theory. New Jersey: Lawrence Erlbaum Associates; 2001. p. 119–54.

Rescorla RA. Retraining of extinguished Pavlovian stimuli. J Exp Psychol, Anim Behav Process 2001;27:115–24.

Rescorla RA. More rapid associative change with retraining than with initial training. J Exp Psychol, Anim Behav Process 2003; 29:251–60.

Rescorla RA, Cunningham CL. The erasure of reinstated fear. Anim Learn Behav 1977;5:386–94.

Rescorla RA, Wagner AR. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In: Black A, Prokasy WF, editors. Classical Conditioning II. New York: Appleton Century Crofts; 1972. p. 64–99.

Robinson TE, Berridge KC. Addiction. Annu Rev Psychol 2003;13:155–62.

See RE. Neural substrates of conditioned–cued relapse to drug-seeking behavior. Pharmacol Biochem Behav 2002;71:517–29.

Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology 2002;168:3–20.

Shiffman S, Balabanis MH, Paty JA, Engberg J, Gwaltney CJ, Liu KS, et al. Dynamic effects of self-efficacy on smoking lapse and relapse. Health Psychol 2000;19:315–23.

Shippenberg TS, Heidbreder C, LeFevour A. Sensitization to the conditioned rewarding effects of morphine: pharmacology and temporal characteristics. Eur J Pharmacol 1996;299:33–9.

Sinha R, Fuse T, Aubin LR, O'Malley SS. Psychological stress, drug-related cues and cocaine craving. Psychopharmacology 2000;152: 140–8.

Solomon RL, Corbit JD. An opponent-process theory of motivation I Temporal dynamics of affect. Psychol Rev 1974;81:119–45.

Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 1993;4:289–312.

Tolman EC. Cognitive maps in rats and men. Psychol Rev 1948; 55:189–208.

van der Kooy D. Place conditioning: a simple and effective method for assessing the motivational properties of drugs. In: Bozarth MA, editor. Methods of Assessing the Reinforcing Properties of Abused Drugs. New York: Springer-Verlag; 1987. p. 229–40.

Wagner AR. SOP: a model of automatic memory processing in animal behavior. In: Spear NE, Miller RR, editors. Information Processing in Animals: Memory Mechanisms. Hillsdale: Lawrence Erlbaum Associates; 1981. p. 5–47.

Witkiewitz K, Marlatt GA. Relapse prevention for alcohol and drug problems: that was Zen, this is Tao. Am Psychol 2004;59:224–35.

Zavala AR, Weber SM, Rice HJ, Alleweireldt AT, Neisewander JL. Role of the prelimbic subregion of the medial prefrontal cortex in acquisition, extinction, and reinstatement of cocaine-conditioned place preference. Brain Res 2003;990:157–64.