ELSEVIER

Behavioural Brain Research 115 (2000) 39–47

BEHAVIOURAL BRAIN RESEARCH

www.elsevier.com/locate/bbr

Research report

Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction

Devin Mueller, Jane Stewart *

Department of Psychology, Center for Studies in Behavioral Neurobiology, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Québec, Canada H3G 1M8

Received 3 April 2000; accepted 1 May 2000

Abstract

To explore the way in which drugs act to reinstate drug-seeking behavior, we studied drug-induced reinstatement of a cocaine-induced conditioned place preference (CPP). In a series of experiments, we studied the establishment, maintenance, extinction and reinstatement of a cocaine-induced CPP in a three-chamber 'unbiased' apparatus. Groups of rats were given four 20-min pairings of one chamber with cocaine (10.0 mg/kg, i.p.) and four of the other with saline on alternate days. In 15-min tests for CPP, drug-free rats were placed in the center choice chamber with access to the entire apparatus. Experiments were designed to study the expression of the CPP, the maintenance of the CPP in tests given at 2, 4, and 6 weeks after training, and the reinstatement of the CPP by cocaine (5.0 mg/kg) after extinction by 12 repeated tests in the non-drugged state, and after extinction by pairing each chamber, on alternate days, with saline on four occasions. Robust CPPs were obtained that endured for 4 weeks and were maintained for up to 6 weeks when tests were given at 2-week intervals. Both extinction procedures led to the loss of the CPP that was in turn reinstated by priming injections of cocaine. These results indicate that a cocaine-induced CPP, once developed, endures for several weeks, and is maintained by occasional testing even in the absence of additional drug experience. The fact that the CPP is easily reinstated when testing is preceded by a priming injection of cocaine suggests that drugs may induce relapse by renewing the incentive value of drug-associated cues.

Keywords: Cocaine; Conditioned place preference; Extinction; Relapse; Reinstatement; Reactivation

1. Introduction

Relapse to drug use after long periods of abstinence is a common feature of drug addiction [16]. Traditionally, the animal model used to study relapse to drug seeking has been the reinstatement procedure. After training to make a response to self-administer a drug, and the subsequent extinction of that response, an acute, non-contingent injection results in the reinstatement of responding [32]. Such drug-induced reinstatement of drug seeking, or relapse, has been reported in animals trained to self-administer cocaine [8,11,14], heroin [9,12,27], nicotine [26] and alcohol [17] (for a recent review see Ref. [7]).

One question that can be asked about the reinstatement of a drug-reinforced response following the non-contingent drug injection is how does the drug act to induce this behavior. Some have argued that the drug acts as a stimulus to elicit the well-learned lever pressing response (for discussions of this issue see Refs. [3,7,10]), and that the renewed behavior may not represent drug seeking in any real sense. We have argued, on the other hand, that the priming injection of the drug acts to renew the significance or salience of the drug-related environmental stimuli drawing the animal to approach the lever and to engage in lever pressing [31,33] (see also Ref. [23]). Thus, after extinction, a priming injection of the previously self-administered drug could be said to renew the salience of the lever and surrounding stimuli. The place conditioning procedure provides

* Corresponding author. Tel.: +1-514-8482193; fax: +1-514-8482817.

E-mail address: stewart@csbn.concordia.ca (J. Stewart).

0166-4328/00/$ - see front matter
PII: S0166-4328(00)00239-4

a way to explore this hypothesis. In this procedure a particular stimulus complex, or environment, is paired with the effects of the drug, without the animal having to learn to make a response to obtain the drug, and a second environment is explicitly paired with the absence of the drug. On the test trial, the animal is allowed for the first time to move freely between an area previously paired with drug and a non-drug environment. If the animal stays longer in the presence of stimuli previously paired with the drug, these stimuli can be said to have acquired secondary or conditioned incentive properties through pairings with the rewarding effects of the drug. We propose that this learning paradigm can be used to test the idea that a priming injection of the drug used to develop the conditioned place preference (CPP), given after extinction conditions, acts to restore the salience or attractiveness of the environment previously paired with drug. We argue that if, after extinction of the CPP, the animal is given a test trial following a priming injection of the drug, and if the animal stays longer in the presence of the drug-associated cues, the priming injection can be said to increase the salience, attractiveness, or positive valence of those cues.

In the present experiments, we used a place conditioning procedure, pairing cocaine, the unconditioned stimulus (UCS), with a previously neutral environment, the conditioned stimulus (CS), to test these ideas. The place conditioning procedure has been used extensively to assess the rewarding properties of cocaine [24,25].

We first established the CPP and explored the time course of the expression of the cocaine-induced CPP as well as the movement of the animal between chambers (transitions) over the test session. Further experiments were designed to determine: (a) whether, once established, the cocaine-induced CPP would endure over time, with and without occasional testing; (b) whether the CPP could be extinguished by repeated test trials or by repeated pairings of both environments with saline [4]; and (c) whether, following extinction, the CPP could be reinstated by priming injections of cocaine given before a test session.

2. Materials and methods

2.1. Subjects

In total, 96 male Long–Evans rats (Charles River Canada) served as subjects in these experiments. All rats were naïve, and were used in a single experiment only. They were housed individually in hanging wire cages ($18 \times 24 \times 18$ cm) upon arrival and maintained on a 12-h light/12-h dark normal cycle (lights on at 08:00 h) with food and water available at all times. Housing was located in a temperature- and humidity-controlled environment. Animals were conditioned and tested during the light phase of the cycle. All experimental procedures were approved by the Animal Care Committee of Concordia University, in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Apparatus

Conditioning was conducted in four identical gray PVC plastic rectangular boxes ($71.5 \times 36.5 \times 30$ cm), each containing three chambers separated by guillotine doors. The two large end chambers (24×35 cm) were separated by a smaller center choice chamber (15.5×19.5 cm), which was used on the pre-exposure and test days. The center choice chamber (middle chamber) had punched aluminum flooring (0.4 cm diameter holes) and was separated from the two main chambers by gray walls that had 12.5×16 cm doorways cut in them that could be occluded by removable guillotine doors. One of the main chambers had gray walls and a wire screen floor (0.63×0.63 cm squares); the other had a white wall located across from the guillotine door and a stainless steel mesh floor (1.3×1.3 cm squares). All floors were raised 5 cm to reduce the accumulation of urine and feces. Through a computer interface, time spent in each chamber was recorded by means of infrared beam crossings. In each of the two end chambers, there were two beams separated by 8 cm. A rat was said to be in an end chamber if the beam furthest from the door was broken. If only the beam closest to the door was broken, the rat was said to be in the center choice chamber. This criterion tended to err on the side of exaggerating the overall time spent in the center chamber, but avoided counting partial entries as time spent in the end chambers. Preliminary data indicated that naive animals showed no preference for either end chamber, although they spent the least amount of time in the center choice chamber. During conditioning and testing, the room was kept in semi-darkness with only a single lamp reflecting light off one wall of the room.

2.3. Procedures

The place conditioning procedure consisted of three phases: pre-exposure, conditioning, and CPP test. All animals were allowed to habituate to the colony room for 1 week upon arrival. Subsequently, each animal was habituated to handling for 3 days before the start of the experiment. Rats were weighed daily and then transported to the testing room in groups of four.

Following habituation, animals received a single pre-exposure test in which they were placed in the center choice chamber with the guillotine doors removed to allow access to the entire apparatus for 15 min. The amount of time spent in each chamber was monitored and used to assess unconditioned preferences.

During the following conditioning phase (8 days), rats were assigned to receive drug pairings with one of the two end chambers in a counterbalanced fashion (the 'unbiased' procedure). As well, half of each group began the experiment on the drug-paired side and half on the saline-paired side. With the exception of Experiment 2 in which three doses were used, cocaine was administered in a dose of 10.0 mg/kg, i.p., once every other day immediately before the rats were placed into the assigned chamber for 20 min. On alternate days, rats received saline injections (1.0 ml/kg) before being placed in the other chamber. Half of each treatment group received drug injections on the first, third, fifth, and seventh day; the remaining subjects received drug injections on the second, fourth, sixth, and eighth days. The center choice chamber was never used during conditioning and was blocked by guillotine doors.

Two days after the last conditioning trial, a test for CPP was given. Animals were placed in the center choice chamber with the guillotine doors removed and allowed free access to the entire apparatus for 15 min. The amount of time spent in each chamber was recorded to assess individual preferences. No injections were given during the CPP test, maintaining the same procedure as that used during the pre-exposure test.

2.3.1. Experiment 1: CPP

Twenty-four rats, weighing 310–360 g at the start and 400–470 g at the end of the experiment, served as subjects. In both the pre-exposure and the CPP tests, time spent in each of the three chambers of the apparatus was collected in 3-min bins. In addition, the number of complete transitions from one chamber to another was recorded.

2.3.2. Experiment 2: maintenance of the CPP

Thirty-six rats, weighing 360–400 g at the start and 470–660 g at the end of the experiment, were used as subjects. Prior to conditioning, the rats were divided into three groups ($n = 12$ per group) corresponding to the three doses of cocaine (5.0, 10.0, and 20.0 mg/kg, i.p.) used for conditioning.

Because there were no effects of dose on the magnitude of the CPP, the rats were reassigned to three new groups ($n = 12$ per group), one for each of the three delay periods of 2, 4, or 6 weeks. These groups were matched according to the amount of time spent on the cocaine-paired side of the apparatus during the first CPP test and each contained four rats from each training dose. (A one-way ANOVA for time spent on the cocaine-paired side revealed no differences between these new groups; $F(2,33) = 0.00$, $P =$ ns). Group 1 ($n = 12$) was retested 2, 4, and 6 weeks after the first CPP test. Group 2 ($n = 12$) was retested 4 and 6 weeks after, and group 3 ($n = 12$) was retested only at 6 weeks.

2.3.3. Experiment 3: extinction by repeated testing and reinstatement

Twelve rats, weighing 320–390 g at the start and 432–534 g at the end of the experiment, served as subjects. After conditioning and following the initial CPP test, rats were given 15-min tests daily for 12 days. No injections were given during this extinction period. The day following the last extinction trial, all rats received a priming injection of cocaine (5.0 mg/kg, i.p.) and were placed in the center choice chamber with access to the entire apparatus for 15 min.

2.3.4. Experiment 4A and B: extinction by saline pairings and reinstatement

Two experiments were conducted using 12 animals each (Exp. 4A: animals weighed 320–400 g at the start and 430–560 at the end of the experiment; Exp. 4B: animals weighed 300–330 g at the start and 340–400 g at the end of the experiment). In each experiment, following conditioning and the initial CPP test, animals were given pairings of saline with each chamber, one per day, for 8 days. The animals did not receive cocaine during this period. Following this period of extinction, the animals were given a test for CPP. The next day, all animals received a priming injection of cocaine (5.0 mg/kg i.p.) immediately before the final test for CPP. Exp. 4B served as a replication of the drug-induced reinstatement effect found in Exp. 4A and allowed us to assess the time course of the expression of the reinstated CPP.

2.4. Drugs

The cocaine hydrochloride used in these studies was obtained from BDH Chemicals (Toronto, Canada). All doses are expressed as the salt. The drug was dissolved in 0.9% saline and injected in a volume of 1.0 ml/kg.

2.5. Statistical analysis

Pre-exposure and CPP test outcomes were determined by the time spent in each chamber. For each test, a within-subjects repeated measures ANOVA was used to assess the effect of Chamber. A statistically significant Chamber effect was followed by Student–Newman–Keuls post-hoc comparisons. Analyses specific to each experiment are outlined in the appropriate results section. All follow-up analyses were performed using the Student–Newman–Keuls test for post-hoc comparisons (acceptable significance level, $P < 0.05$).

3. Results

3.1. Experiment 1: CPP

The pre-exposure test showed that animals spent an equal amount of time (mean ± S.E.M. seconds) in the two outer chambers (wire: 326.0 ± 12.7; steel: 316.4 ± 14.7) and less time in the smaller center choice chamber (256.2 ± 10.0). The repeated measures ANOVA for Chamber (wire, middle, steel) revealed a significant effect ($F(2,46) = 6.00$, $P < 0.01$). Post hoc comparisons confirmed that animals spent more time in the end chambers than in the center ($P < 0.05$), but no differences were found in time spent in the end chambers. Thus, the test boxes were truly unbiased in terms of chamber preferences of untreated rats.

Fig. 1(A) shows the results of the CPP test. It can be seen that rats given free access to the apparatus spent more time in the chamber previously paired with cocaine. The repeated measures ANOVA for Chamber (cocaine-paired, middle, saline-paired) revealed a significant effect ($F(2,46) = 32.53$, $P < 0.0001$). Post hoc pair-wise comparisons revealed that the effect was attributable to a greater amount of time spent in the cocaine-paired chamber than in either the saline-paired or the middle chamber (Ps < 0.05). Fig. 1(B) shows the time course of the expression of the CPP as measured in 3-min bins. It can be seen that the time spent in the cocaine-paired chamber increased gradually over the course of the test. The repeated measures ANOVA for Chamber by Time revealed a significant effect of Chamber ($F(2,46) = 30.42$, $P < 0.0001$) and a Chamber by Time interaction ($F(8,184) = 5.10$, $P < 0.0001$). Animals spent more time in the cocaine-paired chamber than in the saline-paired chamber at each time point (Ps < 0.05). As seen in Fig. 1(C), the number of transitions from one chamber to another decreased over the course of the test ($F(4,92) = 17.82$, $P < 0.0001$). The mean number of transitions decreased significantly from a mean of 29.1 ± 1.7 to a mean of 14.9 ± 1.5 from the start of the test to the end ($P < 0.05$).

3.2. Experiment 2: maintenance of the CPP

The results of the initial CPP test showed that, as in the first study, animals spent a greater amount of time in the cocaine-paired chamber than in the saline-paired or middle chamber at all doses used for conditioning. This was confirmed by a mixed ANOVA for Chamber and Dose (5.0, 10.0, 20.0 mg/kg), which revealed only an effect of Chamber ($F(2,66) = 50.40$, $P < 0.0001$). Animals spent significantly more time in the cocaine-paired chamber than in the saline-paired or middle chamber regardless of the conditioning dose (Ps < 0.05).

Fig. 2 shows the results from the three groups tested at either 2, 4, or 6 weeks after the initial CPP test (time 0). Fig. 2(A) shows the expression of the CPP for the group tested at 0, 2, 4, and 6 weeks. It can be seen that rats spent more time in the cocaine-paired chamber at all time points. A repeated measures ANOVA for Chamber by Week (0, 2, 4, 6) revealed only a significant effect of Chamber ($F(2,22) = 16.89$, $P < 0.0001$). In all cases, animals spent more time in the cocaine-paired chamber than in either the saline-paired or middle chamber (Ps < 0.05).

Similar analyses were conducted for the second group tested first at 4 and again at 6 weeks following the initial test (time 0). Again, only the effect of Chamber was significant ($F(2,22) = 23.76$, $P < 0.0001$; Fig. 2(B)). In all cases, animals spent more time in the

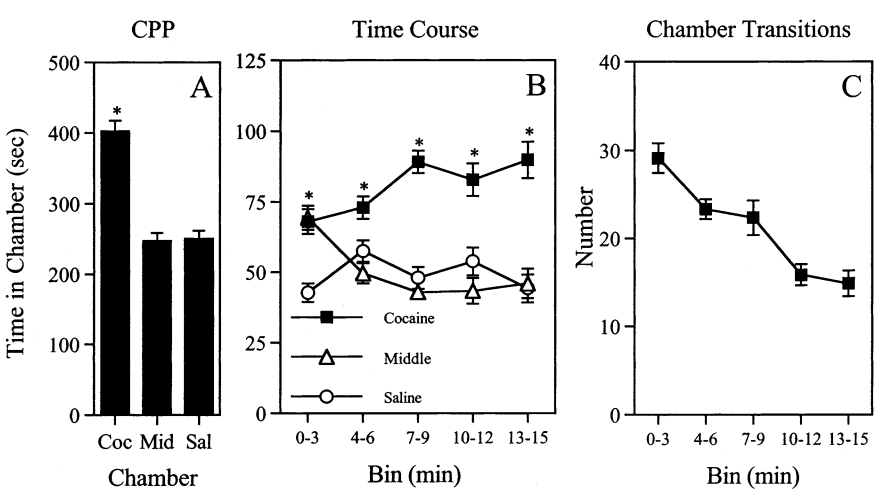

Fig. 1. Expression of a cocaine-induced CPP. (A) CPP: mean (± S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in the 15-min test for CPP. (B) Time course: mean (± S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in 3-min bins over the 15-min test for CPP. (C) Chamber transitions: mean (± S.E.M.) number of discrete chamber transitions in 3-min bins over the 15-min test for CPP. *Different from the saline-paired side, $P < 0.05$.

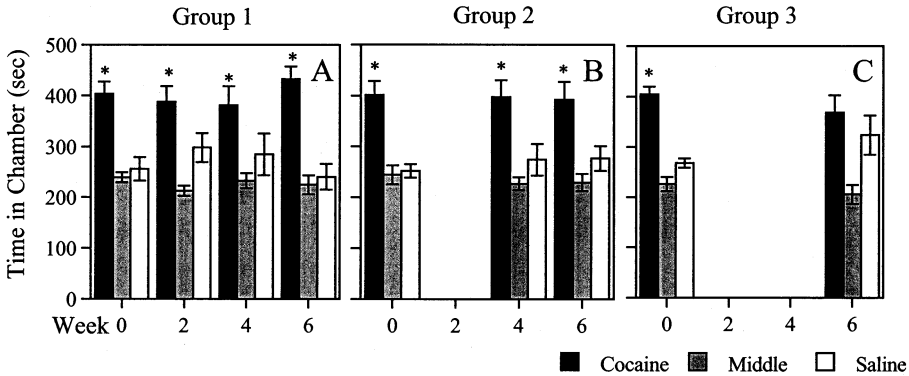

Fig. 2. Maintenance of a cocaine-induced CPP. (A) Group 1: mean (±S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in 15-min tests for CPP at 0, 2, 4, and 6 weeks post-conditioning. (B) Group 2: mean (±S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in 15-min tests for CPP at 0, 4, and 6 weeks post-conditioning. (C) Group 3: mean (±S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in 15-min tests for CPP at 0 and 6 weeks post-conditioning. *Different from the saline-paired side, $P < 0.05$.

cocaine-paired chamber than in either the saline-paired or middle chamber (Ps < 0.05).

Fig. 2(C) shows the expression of the CPP for the group tested for the first time 6 weeks after the initial test (time 0). It can be seen that these animals preferred the cocaine-paired chamber to the saline-paired chamber on the first test, but not when tested again 6 weeks later. The repeated measures ANOVA for Chamber by Week revealed only a significant effect of Chamber ($F(2,22) = 18.64$, $P < 0.0001$). At week 0, the animals spent more time in the cocaine-paired chamber than in either the saline-paired or middle chamber (Ps < 0.05), but at week 6 there was no difference in time spent between the cocaine- and the saline-paired chamber.

3.3. Experiment 3: extinction by repeated testing and reinstatement

Fig. 3(A) shows the results of the experiment in which animals were given 12 daily extinction trials following the establishment of the CPP. It can be seen that time spent in the cocaine-paired chamber gradually diminished over days and did not differ from the time spent in the saline-paired chamber by day 8. For simplicity of analysis, the data were collapsed into four 3-day blocks. The repeated measures ANOVA for Chamber by Block revealed a significant effect of Chamber ($F(2,22) = 37.73$, $P < 0.0001$), and a significant Chamber by Block interaction ($F(6,66) = 4.50$, $P < 0.001$), reflecting the fact that the time spent in the cocaine-paired chamber was decreasing, whereas the time in the saline-paired chamber was increasing over trials. Animals spent more time in the cocaine-paired chamber than in the saline-paired chamber on days 1 through 7, and on day 10 (Ps < 0.05). On days 8, 9, 11, and 12, the animals spent a similar amount of time in the cocaine-paired chamber as in the saline-paired chamber.

As shown in Fig. 3(B), the priming injection of cocaine given before the test resulted in a partial reinstatement of the CPP. There was a significant effect of Chamber ($F(2,22) = 8.31$, $P < 0.01$) as a result of animals spending more time in the cocaine-paired chamber than in either the saline-paired or middle chamber (Ps < 0.05).

3.4. Experiment 4: extinction by saline pairings and reinstatement

Fig. 4 shows the results from the first set of animals given repeated pairings of the two chambers with saline to induce extinction of the CPP (Exp. 4A). During the initial test for CPP given before extinction, animals

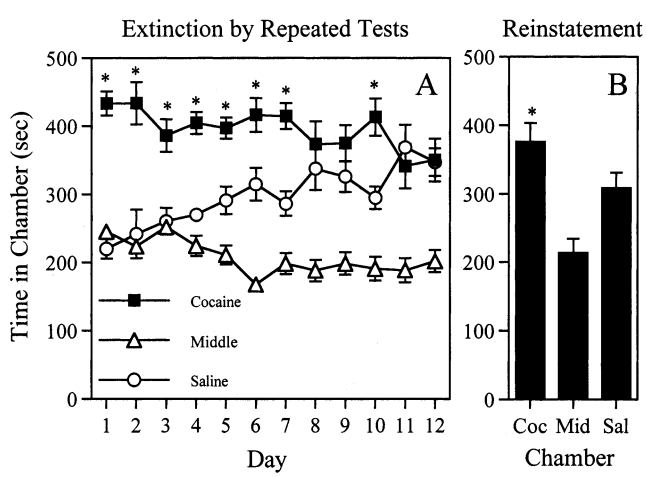

Fig. 3. Extinction by repeated tests and reinstatement. (A) Extinction: mean (±S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in 12 daily 15-min tests for CPP. (B) Reinstatement: mean (±S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in a 15-min test for CPP following a priming injection of cocaine (5 mg/kg, i.p.). *Different from the saline-paired side, $P < 0.05$.

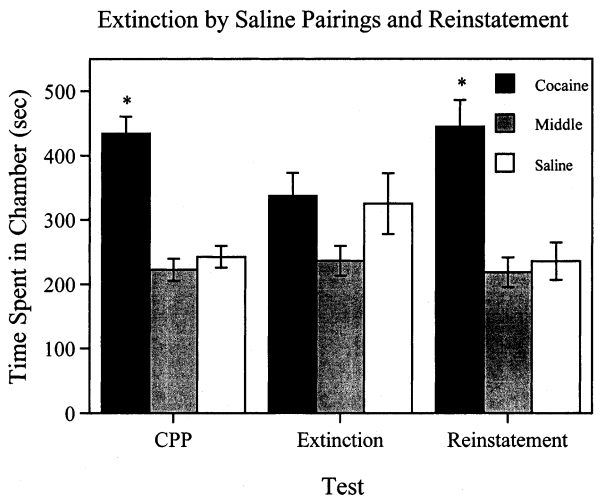

Fig. 4. Extinction by saline pairings and reinstatement. Mean (± S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in 15-min tests for the initial CPP and extinction. Reinstatement of the CPP following a priming injection of cocaine (5.0 mg/kg, i.p.) is shown in the right panel. *Different from the saline-paired side, $P < 0.05$.

spent more time in the chamber previously paired with cocaine, whereas following extinction there was no chamber preference. It can be seen, however, that in this experiment the priming injection of cocaine completely reinstated the CPP. The repeated measures ANOVA for Chamber by Test (CPP, extinction, reinstatement) revealed a significant effect of Chamber ($F(2,22) = 11.23$, $P < 0.001$) and a Chamber by Test interaction ($F(4,44) = 3.31$, $P < 0.05$). Animals spent more time in the cocaine-paired chamber than in either the saline-paired or middle chamber during the initial CPP and the reinstatement test ($Ps < 0.05$), but not during the extinction test.

A similar set of findings was obtained from the second group of animals following conditioning and extinction by repeated pairing of the chambers with saline (Exp. 4B). Again animals showed a strong initial CPP (cocaine: 440.9 ± 26.8 s; saline: 273.9 ± 20.3 s) and extinction of the CPP following saline pairings (cocaine: 371.6 ± 25.0 s; saline: 307.3 ± 28.8 s). The repeated measures ANOVA for Chamber by Test (CPP, extinction) revealed an effect of Chamber ($F(2,22) = 17.94$, $P < 0.0001$) and a Chamber by Test interaction ($F(2,22) = 4.27$, $P < 0.05$). Post hoc comparisons confirmed that animals spent more time in the cocaine-paired chamber than in the saline-paired chamber before extinction ($P < 0.05$), but not following the period of extinction. Fig. 5 shows the results of the reinstatement test and time course of the expression of the reinstated CPP from Exp. 4B. It can be seen in Fig. 5(A) that the priming injection of cocaine administered to animals before the test completely reinstated the CPP (effect of Chamber, $F(2,22) = 8.35$, $P < 0.01$). Fig. 5(B) shows the time course of the reinstated CPP measured in 3-min bins. It can be seen that the time spent in the cocaine-paired chamber increased over the course of the test as it had in the test for CPP in the initial experiment (Fig. 1). The repeated measures ANOVA for Chamber by Time revealed a significant effect of Chamber ($F(2,22) = 8.35$, $P < 0.01$) and a Chamber by Time interaction ($F(8,88) = 6.28$, $P < 0.0001$). Post hoc comparisons revealed that rats spent more time in the cocaine-paired chamber than in the saline-paired chamber during all but the first time bin, i.e. from 4 to 15 min ($P < 0.05$). As seen in Fig. 5(C), the number of transitions from one chamber to another decreased

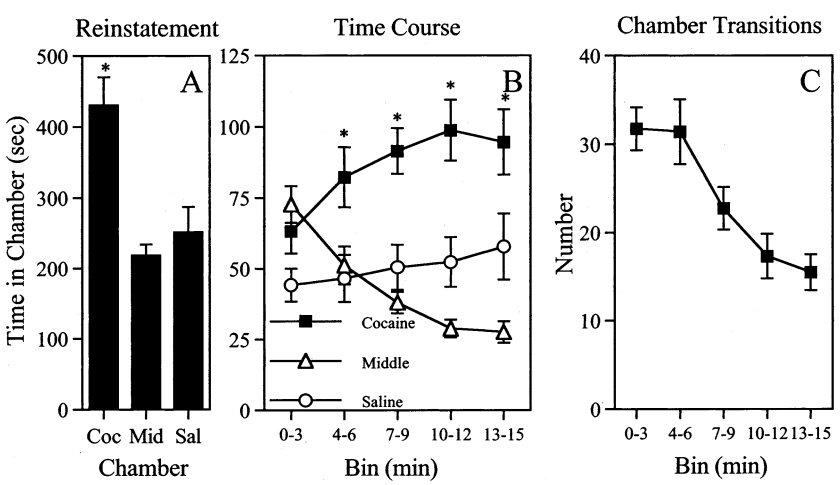

Fig. 5. Expression of the reinstated CPP following extinction by saline pairings. (A) Reinstatement: mean (± S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in a 15-min test for CPP following a priming injection of cocaine (5.0 mg/kg, i.p.). (B) Time course: mean (± S.E.M.) time spent in the cocaine-paired, middle, and saline-paired chambers in 3-min bins over a 15-min test for CPP following a priming injection of cocaine. (C) Chamber transitions: mean (± S.E.M.) number of discrete chamber transitions in 3-min bins over the 15-min test for CPP following a priming injection of cocaine. *Different from the saline-paired side, $P < 0.05$.

over the course of the test ($F(4,44) = 11.50$, $P < 0.0001$). The mean number of transitions decreased significantly from a mean of 31.8 ± 2.4 to a mean of 15.5 ± 2.0 from the start of the test to the end ($P < 0.05$).

4. Discussion

Three major findings arise from these experiments. First, it was found that a cocaine-induced CPP, once established, was maintained for at least 4 weeks and for longer when animals were given occasional tests. Second, it was found that the CPP could be extinguished by either repeated testing for the place preference without any drug exposure, or by explicit pairing of the two environments with injections of saline only. Finally, and most importantly, it was found that, after extinction, a priming injection of cocaine reinstated the cocaine-induced CPP; after a period of extinction, animals given an injection of cocaine before the test session spent more time in the environment originally paired with cocaine. This latter finding will be discussed first.

4.1. Reinstatement

The reinstatement of the CPP by the priming injection of cocaine is similar to that reported in studies of drug self-administration in which, following extinction of lever pressing, priming injections increase responding on a lever that previously delivered the drug [7,32]. Importantly, the fact that the priming injection is effective in the CPP procedure may help answer the question of how priming injections influence behavior. It must be noted that in this procedure the drug is paired with a stimulus complex, but the animal does not have to learn to make a response to obtain the drug. Thus, if after training the animal chooses to spend more time in the presence of stimuli previously paired with the rewarding effects of the drug, these stimuli can be said to have acquired conditioned incentive properties. Here we show that after a period of extinction no preference for the drug-related environment was exhibited. Following a priming injection of the drug, however, the animal once again, approached and remained in the presence of the drug-related stimuli. This finding leads us to conclude that the incentive salience and attractiveness of these stimuli were renewed by the presence of the drug (see also Ref. [23]).

An interesting aspect of the behavior of the animals, both in the original test for the CPP and in the test for reinstatement, is that they spent more and more time in the presence of the drug-related stimuli as time passed during the 15-min test, making fewer and fewer transitions from chamber to chamber. This would seem to imply that the animals initially explore the apparatus and then gradually settle down to spend more time in the presence of the drug-related cues. These findings effectively demonstrate that the behavior of the animal following the priming injection of the drug is determined by the drug-related stimuli. Thus, in studies using the self-administration procedure, it can be argued that after a period of extinction, a priming injection of the previously self-administered drug reinstates lever pressing by increasing the attractiveness, salience, or positive valence of the drug-related lever (see also Refs. [34,35]).

A similar idea was previously put forward in the context of avoidance learning. Spear and colleagues argued that the presentation of the UCS could serve as a 'reminder' of the former significance of the CS. They found, for example, that 9 days after the last pairing of a light CS with footshock (UCS), the latency to cross into a 'safe' compartment in response to the CS was significantly reduced by an unsignaled presentation of the UCS given 24 h before test [28]. In a similar study, a conditioned avoidance response to an extinguished CS was reinstated by the presentation of the UCS (footshock) or by an unconditional loud noise that induced a similar affective state given 24 h before the test trial [22]. This finding led these authors to conclude that the affective responses to the UCS are important for reinstatement, i.e. the internal state induced by the fear-eliciting UCS. Because a CPP is said to develop from the pairing of the rewarding or affective properties of the drug with the distinctive environment [6], it might be argued that, in a manner similar to that seen in reinstatement of avoidance behavior, the priming injection 'reminds' the animal of, or renews, the former significance or attractiveness of environmental stimuli previously paired with the drug.

4.2. Expression, maintenance and extinction

We turn now to the findings concerning the expression, maintenance and extinction of the CPP. In the present study, a temporal analysis of the expression of a cocaine-induced CPP revealed that the relative magnitude of the CPP increased over the course of the test. Parallel results have been reported wherein a morphine-induced CPP was found to increase from the start of the test to the end [21,36]. Another result from the present experiments was the lack of an effect of dose of cocaine administered intraperitoneally on the magnitude of the CPP. This finding is similar to the findings of several researchers [2,20,30] and is consistent with the results of a meta-analysis [1].

The finding that the CPP, once established, was maintained over time is interesting on two accounts. First, it was seen that, in the absence of any intervening tests, the cocaine-induced CPP was maintained up to 4 weeks. This finding demonstrates that the drug-related cues maintain their effectiveness over considerable time.

The conditioned emotional significance of the drug-paired environment (the CS) persists in the absence of any opportunity for extinction. Similar findings have been reported before. For example, conditioned place preferences or aversions based on pairing an environment with either morphine or naloxone were expressed 1 month following the last conditioning trial [18]. Thus, the passage of time alone is not sufficient to disrupt the salience of drug-related environmental cues.

The second interesting finding from this experiment is that intermittent testing at long delays appeared to facilitate the maintenance of the CPP. These results suggest that tests given at sufficiently long intervals serve as reminders to maintain the significance of the drug-related stimuli. This finding might be relevant for former cocaine users trying to remain drug free. Unlike what would be expected to happen if an addict were given repeated massed exposure to drug-related stimuli, occasional infrequent exposures to such stimuli might serve to reactivate the emotional significance of the stimuli and to reinforce their attractiveness. Parallel findings have been reported with avoidance conditioning. In those studies, following a single pairing of an aversive stimulus (e.g. footshock) with a CS (e.g. a light), the CS, as expected, led to a conditioned emotional response as measured by autonomic responses. Most interesting, however, was the fact that subsequent exposures to the CS alone resulted in enhanced autonomic responses [13,19]. This finding led Eysenck [15] to propose a theory to explain why avoidance behavior is maintained and even enhanced after presentations of the CS alone when theoretically extinction might be expected to occur. The idea proposed was that the reactivation of the conditioned autonomic response served to strengthen the association between the CS and the autonomic response. In other studies of conditioned avoidance responses, it was found that after conditioning the presentation of a CS given after relatively long retention intervals was sufficient to maintain the avoidance response [5,29]. These findings support the argument that the occasional exposure to a CS is sufficient to maintain behavior. Thus, the present finding that testing at 2-week intervals maintained the CPP suggests that, for cocaine users, occasional encounters with environments previously associated with drug taking would be sufficient to maintain their potential for inducing drug seeking.

The results of the present study indicate that both extinction procedures, repeated daily testing and repeated pairings of the two environments with saline alone, led to the reduction of the CPP (see also Ref. [4]). This suggests that repeated exposure to the previously drug-paired environment (CS) in the absence of the drug (UCS) led to a decline in the significance of the drug-paired stimuli.

In conclusion, the CPP procedure is amenable to studies of drug-induced reinstatement and may help to provide insight into the mechanisms controlling relapse. It was found that following extinction training, a priming injection of cocaine given before a test for preference reinstated the CPP. It should be noted, however, that in these initial experiments relatively large groups of animals were used and were tested only once for reinstatement. Thus we cannot say whether smaller groups of animals could be used or whether repeated tests for reinstatement would yield useful data. Albeit, use of the CPP technique has a number of advantages over the self-administration procedure; although both are labor intensive, the CPP technique is relatively inexpensive, non-invasive, and simple to use. It may serve, therefore, as an alternative to the traditional intravenous self-administration method as an animal model of relapse.

Acknowledgements

Supported by grants from NIDA, MRC (Canada), and FCAR (Quebec). D. Mueller was supported by a graduate fellowship from NSERC (Canada).

References

[1] Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995;32:683–9.
[2] Bell SM, Stewart RB, Thompson SC, Meisch RA. Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. Psychopharmacology 1997;131:1–8.
[3] Bickel WK, Kelley TH. The relationship of stimulus control to the treatment of substance abuse. In: Ray BA, editor. Learning Factors in Substance Abuse. Washington, DC: US Government Printing Office, 1988:122–40.
[4] 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.
[5] Campbell B, Jaynes J. Reinstatement. Psychol Rev 1966;73:478–80.
[6] Carr GD, Fibiger HC, Phillips AG. Conditioned place preference as a measure of drug reward. In: Liebman JM, Cooper SJ, editors. The Neuropharmacological Basis of Reward. Oxford: Clarendon Press, 1989:264–319.
[7] Carroll ME, Comer SD. Animal models of relapse. Exp Clin Psychopharmacol 1996;4:11–8.
[8] De Vries TJ, Schoffelmeer AN, Binnekade R, Mulder AH, Vanderschuren LJ. Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. Eur J Neurosci 1998;10:3563–71.
[9] De Vries TJ, Schoffelmeer AN, Binnekade R, Vanderschuren LJ. Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal of IV drug self-administration. Psychopharmacology 1999;143:254–60.
[10] de Wit H. Priming effects with drugs and other reinforcers. Exp Clin Psychopharmacol 1996;4:5–10.

[11] de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 1981;75:134–43.
[12] de Wit H, Stewart J. Drug reinstatement of heroin-reinforced responding in the rat. Psychopharmacology 1983;79:29–31.
[13] Dykman R, Mack R, Ackerman P. The evaluation of autonomic and motor components of the unavoidance conditioned response in the dog. Psychophysiology 1965;1:209–30.
[14] Erb S, Shaham Y, Stewart J. Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology 1996;128:408–12.
[15] Eysenck H. A theory of the incubation of anxiety/fear responses. Behav Res Ther 1968;6:309–21.
[16] Jaffe JH. Drug addiction and drug abuse. In: Gilman AG, Rall TW, Nies AS, Taylor P, editors. Goodman & Gilman's the Pharmacological Basis of Therapeutics. New York: Pergamon, 1990:522–73.
[17] Lê AD, Quan B, Juzystch W, Fletcher PJ, Joharchi N, Shaham Y. Reinstatement of alcohol-seeking by priming injections of alcohol and exposure to stress in rats. Psychopharmacology 1998;135:169–74.
[18] Mucha RF, Iversen SD. Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. Psychopharmacology 1984;82:241–7.
[19] Napalkov A. Information process of the brain. In: Weiner N, Sefade JC, editors. Progress of Brain Research: Vol. 2. Nerve, Brain and Memory Models. Amsterdam: Elsevier, 1963:59–69.
[20] O'Dell LE, Khroyan TV, Neisewander JL. Dose-dependent characterization of the rewarding and stimulant properties of cocaine following intraperitoneal and intravenous administration in rats. Psychopharmacology 1996;123:144–53.
[21] Reid LD, Marglin SH, Mattie ME, Hubbell CL. Measuring morphine's capacity to establish a place preference. Pharmacol Biochem Behav 1989;33:765–75.
[22] Rescorla RA, Heth CD. Reinstatement of fear to an extinguished conditioned stimulus. J Exp Psychol Anim Behav Proc 1975;1:88–96.
[23] Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 1993;18:247–91.
[24] Schechter MD, Calcagnetti DJ. Trends in place conditioning with a cross-indexed bibliography; 1957–1991. Neurosci Biobehav Rev 1993;17:21–41.
[25] Schechter MD, Calcagnetti DJ. Continued trends in the conditioned place preference literature from 1992 to 1996, inclusive, with a cross-indexed bibliography. Neurosci Biobehav Rev 1998;22:827–46.
[26] Shaham Y, Adamson LK, Grocki S, Corrigall WA. Reinstatement and spontaneous recovery of nicotine seeking in rats. Psychopharmacology 1997;130:396–403.
[27] Shaham Y, Stewart J. Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology 1995;119:334–41.
[28] Smith GJ, Spear NE. The analysis of treatments to alleviate forgetting in rats. Am J Psychol 1984;97:475–91.
[29] Spear NE, Parsons P. Alleviation of forgetting by reactivation treatment: a preliminary analysis of the ontogeny of memory processing. In: Medin D, Roberts W, Davis R, editors. Processes in Animal Memory. Hillsdale, NJ: Erlbaum, 1976:19–38.
[30] Spyraki C, Fibiger HC, Phillips AG. Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. Brain Res 1982;253:195–203.
[31] Stewart J. Neurobiology of conditioning to drugs of abuse. In: Kalivas PW, Samson HH, editors. The Neurobiology of Drug and Alcohol Addiction. Annals of the New York Academy of Sciences. New York: New York Academy of Sciences, 1992:335–46.
[32] Stewart J, de Wit H. Reinstatement of drug-taking behavior as a method of assessing incentive motivational properties of drugs. In: Bozarth MA, editor. Methods of Assessing the Reinforcing Properties of Abused Drugs. New York: Springer–Verlag, 1987:211–27.
[33] Stewart J, de Wit H, Eikelboom R. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 1984;91:251–68.
[34] Tomie A. Locating reward cue at response manipulandum (CAM) induces symptoms of drug abuse. Neurosci Biobehav Rev 1996;20:505–35.
[35] Vezina P, Pierre PJ, Lorrain DS. The effect of previous exposure to amphetamine on drug-induced locomotion and self-administration of a low dose of the drug. Psychopharmacology 1999;147:125–34.
[36] Vezina P, Stewart J. Conditioned locomotion and place preference elicited by tactile cues paired exclusively with morphine in an open field. Psychopharmacology 1987;91:375–80.