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Cocaine Conditioned Place Preference:

Expression, Endurance, Extinction, and Drug-Induced Reinstatement

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A Thesis

in

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Abstract

Cocaine Conditioned Place Preference:

Expression, Endurance, Extinction, and Drug-Induced Reinstatement

Devin Mueller

The expression, maintenance, and extinction of a cocaine-induced conditioned place preference was studied using a three-chamber 'unbiased' apparatus. During training, rats were given four 20 minute pairings of one chamber with cocaine (Experiment 1, 3 and 4: 10 mg/kg, IP; Experiment 2: 5, 10, and 20 mg/kg, IP) and four of the other with saline on alternate days. In 15 minute tests for preference rats were placed in the centre choice region drug-free with access to the entire apparatus. In Experiment 1, rats demonstrated a conditioned place preference for the cocaine-paired side, accompanied by a decrease in chamber transitions over the duration of the test. In Experiment 2, after training at all doses, strong preferences for the cocaine-paired chamber were evident. The preference was maintained in tests given at 2, 4, and 6 weeks. In Experiment 3, after training, rats were given repeated tests in a non-drugged state (extinction). In Experiment 4, extinction training consisted of pairing each chamber with saline on four occasions. Both methods led to the abolition of the conditioned place preference. Following extinction in both experiments rats were given a priming injection of cocaine (5 mg/kg, IP) which reinstated the conditioned place preference. These results indicate that the development, maintenance, and extinction of a conditioned place preference follow the principles of associative learning and suggest that this procedure can be used to study drug-induced reinstatement.

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Cocaine Conditioned Place Preference:
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Cocaine produces a strong conditioned place preference (e.g., Cramer, Hubbell, & Reid, 1998; Kosten & Miserendino, 1998; Nomikos & Spyraki, 1988). The present set of experiments is concerned with an analysis of the rewarding effects of cocaine as determined through place conditioning. The expression, endurance, extinction, and cocaine-induced reinstatement of a cocaine conditioned place preference are explored. The primary purpose of this study is to establish that a cocaine-induced place preference follows the principles of associative learning. While several studies have sought answers regarding particular rules of learning, few studies have addressed these issues systematically. Further, the ability of a priming injection of cocaine to induce reinstatement of a conditioned place preference was tested.

Drugs as Rewarding Stimuli

Stimuli with rewarding properties have been studied extensively in animals, including food, saccharin solution, and abused drugs. A number of methodologies have been developed to assess rewarding stimuli and the effects they have on behaviour. The most common methods used by behavioural neuroscientists to study rewarding events are instrumental learning and classical conditioning. These methods are used by researchers to study whether certain stimuli can serve as reinforcers and how effectively (i.e., the magnitude).

The study of the rewarding properties of drugs is a major area of research in behavioural pharmacology. Each of the methods currently used in these studies have, however, their own short-comings making it necessary to use more than one to address any particular question. Reward itself is a subjective experience, but the objectivity of

science requires it to be related to elicited behaviour (e.g., White, Messier, & Carr, 1987). Behaviour is considered to be organized by the effects of reward in two ways. First, stimuli that are said to be rewarding are able to elicit approach responses and maintenance of contact (e.g., Schneirla, 1959). This feature is termed the incentive salience of rewarding stimuli. Second, rewards have the capacity to increase the probability of responses which preceed them (e.g., Thorndike, 1933). This is the reinforcing feature of rewards.

These two behavioral effects of rewarding stimuli can be used to study the rewarding properties of drugs. The self-administration procedure requires the animal to make a response, such as pressing a lever, to receive a drug infusion. Under these circumstances, the rewarding properties of the drug are inferred from the animal's willingness to repeat the lever pressing. Furthermore, using various schedules of drug delivery, it is possible to gain information about the magnitude of the rewarding effects by studying the amount of effort an animal will make to obtain the injection.

The place conditioning method differs from the self-administration method in that it is based on the observation that an animal will approach stimuli that have previously been paired with rewarding stimuli. Thus, this method utilizes a classical conditioning procedure, in which a neutral stimulus environment becomes associated with the rewarding effects of a drug. When the place conditioning method is used to study drugs as rewarding stimuli, it is the state induced by the drug that is paired with the external neutral stimuli of an environment. If at test the animal approaches and maintains contact with the stimuli in that environment, the drug can then be deemed rewarding.

The Place Conditioning Method

In place conditioning studies, animals are usually given an injection of a drug prior to confinement to one chamber of a two-chamber apparatus and are confined to the

alternate chamber following a vehicle injection (e.g., van der Kooy, 1987). The two chambers may or may not be separated by a central choice area. During the subsequent place preference test, the animals are allowed to explore the entire apparatus in a drug-free state. A conditioned place preference or a conditioned place aversion is revealed by comparing the amount of time spent in the drug-paired chamber with the amount of time spent in the vehicle-paired chamber, or to a previously established baseline.

Place conditioning was first used to explore the aversive effects of radiation on rats, resulting in a conditioned place aversion for the radiation-paired chamber (Garcia, Kimeldorf, & Hunt, 1957). Shortly after, the first report of drug-induced conditioned place preference was conducted using a Y-maze apparatus to assess the rewarding effects of morphine in rats (Beach, 1957). Since then, a vast amount of literature has been produced using the place conditioning method (see reviews by Carr, Fibiger, & Phillips, 1989; Hoffman, 1989; Schechter & Calcagnetti, 1993, 1998; Tzschentke, 1998). Many drugs with reinforcing qualities have been shown to produce a conditioned place preference, such as morphine (e.g., Vezina & Stewart, 1987; Will, Watkins, & Maier, 1998), amphetamine (e.g., Carr, Phillips, & Fibiger, 1988; Parker, 1992), methylphenidate (e.g., Clarke & Fibiger, 1987; Mithani, Martin-Iverson, Phillips, & Fibiger, 1986), apomorphine (e.g., Parker, 1992; van der Kooy, Swerdlow, & Koob, 1983), and nicotine (e.g., Fudala, Teoh, & Iwamoto, 1985). Further, a variety of animal species has been subjected to the place conditioning procedure, including mice (e.g., Laviola & Adriani, 1998), hamsters (e.g., Schnur & Morrell, 1990), primates (e.g., Pomerantz, Wertz, Hepner, Walso, & Piazza, 1992), birds (e.g., Hughes, Baker, & Rettig, 1995), and most commonly rats (e.g., Nomikos & Spyraki, 1988; see also Schechter & Calcagnetti, 1993, 1998).

The Pre-exposure Phase

Animals are normally given pre-exposure to the apparatus prior to conditioning. This pre-exposure is similar to the preference test. Animals are given free-choice access to the entire apparatus in a drug-free state. The pretest serves two purposes: 1) to reduce the novelty of the centre choice chamber (if present) and the testing procedure itself, and 2) to determine whether or not there are unconditional preferences for either end chamber. Place conditioning without a pretest introduces possible problems due to the influence of novelty. For example, in one study it was found that in the absence of a pretest conditioning with cocaine resulted in a lack of side preference at test (Nomikos & Spyraki, 1988). As the animals had not experienced free access to the apparatus until the preference test, the lack of a conditioned place preference may have been the result of neophobia. That is, the novel nature of the test may have induced anxiety in the animals such that approach towards the drug-paired chamber may have been masked. Another explanation comes from studies showing that a novel environment can produce a place preference (Bardo, Neisewander, & Pierce, 1989; Laviola & Adriani, 1998; Parker, 1992; Scoles & Siegel, 1986), which may then interfere with drug-seeking behaviour. The first interpretation suggests that novelty-induced fear interferes with the expression of a place preference at time of test. The second one suggests that an animal is more likely to approach a novel environment, specifically the centre choice area. In both cases, the novel nature of the test can attenuate or prevent the expression of a conditioned place preference for the previously drug-paired chamber. Carr et al. (1988) explored the role of novelty in place conditioning. Rats that were given six exposures to one chamber and none to the other subsequently displayed an initial neophobia for the novel chamber for the first 5 minutes of testing, which shifted to a clear preference for the novel side after 10 minutes had elapsed. If one chamber was relatively novel, i.e., if rats experienced a single exposure in one chamber and six in the other, the animals immediately displayed a

preference for the relatively novel side. Mucha and Iversen (1984) failed to demonstrate an effect of novelty when rats were given 4 exposures to one side and none to the other. However, the authors looked at total time spent in the chambers for the entire test whereas Carr et al. (1988) explored the time course of the effects of novelty in 5-minute bins.

A disadvantage of pre-exposing animals to the apparatus is that pre-exposure to the apparatus prior to conditioning introduces the potential for latent inhibition to occur. Latent inhibition refers to the fact that prior exposure to the to-be-conditioned stimulus in the absence of the unconditioned stimulus can delay or attenuate subsequent conditioning (Mackintosh, 1974). Carr et al. (1988) demonstrated that three 30 minute pre-exposure sessions slightly weakened the conditioned place preference produced by amphetamine relative to that produced with only one 10 minute pre-exposure. Thus, a pretest can reduce the influence of novelty, but also introduces the problem of latent inhibition. Both factors must be considered when determining the amount of pre-exposure animals should receive.

A second use of the pretest is to determine whether either end chamber is preferred prior to conditioning. Two distinct procedures have been used in studying conditioned place preference. First, many researchers have opted to use the 'biased' technique (e.g., Calcagnetti & Schechter, 1993; Heinrichs & Martinez, 1986; Nomikos & Spyraki, 1988), wherein drug injections are paired with the least preferred side as determined by a baseline free run of the entire apparatus. A second procedure is known as the 'unbiased' technique, in which animals are given drug pairings in both chambers of the apparatus in a randomly assigned counterbalanced fashion (e.g., Cunningham, Henderson, & Bormann, 1998; Hemby, Jones, Hubert, Neill, & Justice, 1994; Parker, 1992; Shippenberg & Heidbreder, 1995; Will et al., 1998). This type of totally balanced experimental design, in which the mean amount of time spent in the two main chambers is

equated for the group before drug pairing, has been argued (Carr et al., 1989) to allow a clearer measure of the rewarding properties of a drug than 'biased' designs in which the drug is paired with the least preferred chamber.

When animals are presented with a choice between two chambers, they often show a consistent unconditioned preference for one chamber over the other. Some researchers have chosen to incorporate this bias into their procedures (e.g., Calcagnetti & Schechter, 1993) whereas others have attempted to eliminate the bias by varying the stimuli in the boxes (e.g., Mucha & Iversen, 1984). Although data obtained using a 'biased' procedure may be a valid reflection of reward, the possibility always exists that unpredicted interactions may occur between an unconditioned side preference and some other effect of a drug, and result in a shift in preference due to a factor other than reward. Currently there are several discrepant results between studies using biased versus equally preferred chambers to assess the rewarding properties of drugs. With the use of biased chambers, there is a greater chance that if a drug is paired with a chamber that the animal initially avoids, the resulting place preference could be due to fear-reducing rather than rewarding effects of the drug. Although this has yet to be confirmed empirically, it represents a concern for the use of the 'biased' procedure. A closer examination of the place conditioning literature reveals some results that caution against the use of the 'biased' method. Using a 'biased' design, Schechter (1995) demonstrated that cocaethylene produces a conditioned place preference only when paired with the initially non-preferred side, but not when paired with the initially preferred side. Similar results were found for intraperitoneally administered (IP) cocaine [but not cocaine administered intravenously (IV) (Nomikos & Spyraki, 1988)], subcutaneously administered (SC) heroin (Schenk, Ellison, Hunt, & Amit, 1985) and for IP administered clonidine (Cervo, Rossi, & Samanin, 1993). Using [Leu]enkephalin, administered IP, it was reported that a conditioned place preference was produced when paired with the initially non-preferred

chamber, but a conditioned place aversion when paired with the initially preferred chamber (Heinrichs & Martinez, 1986). A dependence of the magnitude of a conditioned place preference on the baseline preference of the animals was also reported for amphetamine (Costello, Carlson, Glick, & Bryda, 1989). In light of these findings, results arising from the use of the 'biased' procedure in place conditioning should be treated with caution. In fact, a recent review (Schechter & Calcagnetti, 1998) showed that more researchers are now incorporating the 'unbiased' design in their studies in order to circumvent the interpretational problems.

Little criticism has been raised over the use of the 'unbiased' design in place conditioning. However, equally preferred end chambers do not necessarily mean that they serve as neutral stimulus environments. In fact, the chambers may be equally aversive to the animals, resulting in a lack of baseline side preference. Thus, a conditioned place preference for the drug-paired chamber seen at time of test could in theory be due to effects of the drug that alleviate the aversion. There is, however, no evidence at present to support this possibility. Thus, the 'unbiased' design appears to allow for a better assessment of the rewarding effects of drugs.

The Conditioning Phase

The standard procedure for conditioning with a drug using the place conditioning method is to pair one distinct chamber with a drug injection for one session, and pair a second chamber with vehicle the next. Depending on the drug used for conditioning, the dose used, and the route of administration, the number of pairings required may vary from one to six. A single pairing has been shown to produce a conditioned place preference with morphine (Bardo & Neisewander, 1986), heroin (Bozarth & Wise, 1982), or β-endorphin (Amalric, Cline, Martinez, Bloom, & Koob, 1987). The most common number of pairings is four, and the most common route of administration is IP (see Bardo,

Rowlett, & Harris, 1995). The magnitude of a morphine-induced conditioned place preference has been shown to increase by increasing the number of pairings from two through four (Mucha & Iversen, 1984). Although the most common duration of the pairings is 30 minutes, the duration has been varied from four (Reid, Hunter, Beaman, & Hubbell, 1985) to 120 minutes (Parker, Tomlinson, Horn, & Erb, 1994). It was demonstrated that a morphine conditioned place preference was not affected by varying the pairing duration from 10 to 90 minutes (Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982). However, Parker et al. (1994), using a three-choice apparatus, demonstrated that the relative place preference for morphine- and cocaine-paired chambers varied depending on the length of the conditioning session; rats showed a stronger preference for the morphine-paired chamber when the pairing duration was 120 minutes, whereas the cocaine-paired chamber was preferred when pairings lasted only 15 minutes. The authors argued that this shift in relative preference was the result of the pharmacokinetic properties of the drugs. Cocaine has a faster onset of action and the peak effects occur sooner than those of morphine such that manipulations of the pairing duration can shift the relative place preference.

The Preference Test Phase

During the test for preference, animals are given free-choice access to the entire apparatus, with all barriers removed. Side preferences are assessed by recording the time spent in each chamber, either by an observer (e.g., Carr & White, 1983; Katz & Gormezano, 1979) or by an automated system (e.g., Iwamoto, 1986; Martin-Iverson, Reimer, & Sharma, 1997). The test duration ranges from a period of 10 (e.g., Swerdlow & Koob, 1984) to 45 minutes (e.g., Barr, Paredes, & Bridger, 1985), with 15 minutes being the most common.

Animals are generally in a drug-free state during the test for preference. One

variation of the test day procedure involves testing the animals in a drugged state. This variation will be discussed below under priming and drug-induced reinstatement of drug-seeking behaviour.

Associative Learning in "Place Conditioning Studies"

In classical conditioning procedures, a neutral conditional stimulus is typically paired with a stimulus with rewarding or aversive properties, resulting in a learned association or relationship. The conditional stimulus comes to elicit behavioural and physiological responses similar to those induced by the original stimulus. This type of learning is assumed to occur in place conditioning, in which a neutral environmental stimulus is paired with the effects of a drug. Thus, approach towards an environment previously paired with a drug with rewarding properties would be expected.

There is considerable evidence that the mechanisms underlying the development of a conditioned place preference (or aversion) follow the principles of classical (Pavlovian) conditioning. This has been demonstrated, for example, in context-dependent blocking/unblocking effects (McKee, Hinson, & Baxter, 1994), and in the fact that conditioned place preference can show extinction when the animals are repeatedly exposed to the CS in absence of the UCS (Calcagnetti & Schechter, 1993; Cunningham et al., 1998; Hinson, McKee, Lovenjak, & Wall, 1993; Hughes et al., 1995; Tzschentke & Schmidt, 1995). Mithani et al. (1986) demonstrated a systematic reduction in the size of a methylphenidate conditioned place preference over three tests. A similar effect was demonstrated by Clarke and Fibiger (1987) with amphetamine, but extinction of a methylphenidate conditioned place preference was not clear in that study. Bardo et al. (1986) demonstrated that a conditioned place preference produced by cocaine extinguished over repeated tests, even when additional drug pairings were given between tests. The conditioned place aversion produced by scopolamine, an anticholinergic drug,

was also extinguished over repeated tests (MacMahon, Blampied, & Hughes, 1981). Cunningham (1981) found an ethanol-induced conditioned place aversion that was apparent on the second and third, but not the first test. No explanation was given and this result remains inexplicable. Cunningham et al. (1998) showed only a small decrease in magnitude of an ethanol-induced conditioned place preference (pre-CS injection), and a somewhat larger attenuation of an ethanol conditioned place aversion (post-CS injection) over repeated tests. In this study the authors noted the remarkable degree of resistance to extinction which was greatest with the established conditioned place preference. Finally, a gradual reduction in the difference in time spent in the drug- and saline-paired chambers after the establishment of an amphetamine-induced conditioned place preference was shown across repeated tests (Lin, Wu, Hsu, & Liang, 1998). The majority of the studies cited failed to show a complete abolishment of a conditioned place preference or aversion, likely a result of too few extinction trials.

Mucha and Iversen (1984) demonstrated a conditioned place preference in rats tested one month after their last morphine pairing. Vezina and Stewart (1987), using an open field with removable textured quadrants, demonstrated that a morphine conditioned place preference was evident more than two weeks after the initial preference test. Retention of a conditioned place preference induced by IP injections of cocaine was shown using repeated tests at 1, 4, 7, and 30 days postconditioning (Nomikos & Spyraki, 1988). Fudala and Iwamoto (1986) demonstrated that a conditioned place preference produced by nicotine pairings was obtained even if the rats received 14 unpaired exposures to the drug alone between the last pairing and the test day. These studies, although needing replication with other drugs, suggest that time alone or exposure to the UCS (drug) alone does not produce extinction, but rather it depends on drug-free exposure to the pairing chambers.

Cocaine Conditioned Place Preference

Cocaine is classified as a psychostimulant and is known to block reuptake of several neurotransmitters, including serotonin, norepinephrine and dopamine. This drug is widely abused in the human population, and can be shown to produce habitual seeking behaviour in animals. Cocaine can induce a conditioned place preference, as has been shown by a number of researchers (e.g., Cramer et al., 1998; Kosten & Miserendino, 1998; Nomikos & Spyraki, 1988), and has led to over 100 publications in the place conditioning literature (Schechter & Calcagnetti, 1993, 1998). The route of administration during conditioning does not appear to produce differing results; cocaine-induced conditioned place preferences have been produced using intravenous (IV; O'Dell, Khroyan, & Neisewander, 1996), intraperitoneal (IP; Cramer et al., 1998), subcutaneous (SC; Durazzo, Gauvin, Goulden, Briscoe, & Holloway, 1994), and intracranial (IC; Gong, Neill, & Justice, 1996) administration. According to a recent meta-analysis (Bardo et al., 1995), no dose response characterization could be established for cocaine when results from studies using all routes of administration were pooled. On a study by study basis, some researchers were successful in finding significant effects of dose according to the route of administration on the establishment of a cocaine-induced conditioned place preference (Mayer & Parker, 1993; Nomikos & Spyraki, 1988; O'Dell et al., 1996). In a study exploring conditioned place preferences produced by either IP or SC routes of adminsistration, SC cocaine was found to produce a conditioned place preference at 0.32 mg/kg whereas IP cocaine did not until a dose of 10 mg/kg was used (Durazzo et al., 1994). Some researchers have reported optimal doses for use in their studies, such as 15 mg/kg IP (Mayer & Parker, 1993). The most common dose chosen for conditioning with IP cocaine has been 5 mg/kg (Bardo, Neisewander, & Miller, 1986; Bilsky, Montegut, Nichols, & Reid, 1998; Cramer, Hubbell, & Reid, 1998; Mackey & van der Kooy, 1985; Morency & Benninger, 1986; Nomikos & Spyraki, 1988; Spyraki, Fibiger, & Phillips,

1982).

When administered IP, cocaine has a much shorter latency of onset and duration of action than when administered SC, with peak blood levels lasting considerably longer for SC than for IP cocaine (Nayak et al., 1976); the plasma half-life for IP cocaine is approximately 0.3 h and the plasma half-life for SC cocaine is approximately 0.8 h. During place conditioning, the peak effects of IP cocaine may be experienced while the rats are still in the conditioning chamber during the conditioning trial, but the peak effects of SC cocaine may not be experienced until the animals have been returned to their home cages. Nomikos and Spyraki (1988) reported that IV cocaine produces stronger place conditioning than IP cocaine, likely a result of the rapid onset of cocaine's actions following IV administration. Furthermore, de Wit, Bodker and Ambre (1992) reported in humans that the rate of increase of the plasma drug level is directly related to reported "liking" for the drug; the faster the increase in plasma drug level of pentobarbitone, the greater the positive hedonic rating of the drug. The speed of onset of cocaine effects may also play a role in the rewarding properties of this agent.

Priming and Reinstatement of Drug-Seeking Behaviour

To date, researchers have ignored the possibility that a conditioned place preference, once extinguished, might be reinstated by a priming injection of the drug administered during conditioning. The reinstatement of drug-seeking behaviour following extinction was first reported by Stretch and Gerber (1973) in squirrel monkeys (see also Gerber & Stretch, 1975). In fact, studies using the animal model of self-administration, where rats are trained to lever press for drug, have incorporated the use of priming injections of the training drug (Comer, Lac, Curtis, & Caroll, 1993; de Wit & Stewart, 1981; Erb, Shaham, & Stewart, 1996). A non-contingent injection of a self-administered drug serves as a potent stimulus for relapse following extinction (see Stewart & de Wit,

1987), and has been shown to elicit craving in human cocaine addicts (Jaffe, Cascella, Kumor, & Schere, 1989).

Few researchers have explored the effects of a priming injection on conditioned place preference. Bozarth (1987) showed that an injection of morphine at time of test potentiated the conditioned place preference compared to drug-free animals. More recently, Cramer and associates (Cramer, Gardell, Boedeker, Harris, Hubbell, & Reid, 1998; Cramer et al., 1998) showed that a similar procedure using cocaine also potentiated conditioned place preference using an 'unbiased' procedure. However, it should be noted that Nomikos and Spyraki (1988) were unable to show a similar effect of a priming injection on cocaine conditioned place preference using a 'biased' design. Laviola and Adriani (1998) studied place conditioning in mice using amphetamine (2 or 10 mg/kg) as the conditioning drug. During conditioning, the mice were exposed only to the drug-paired side without exposure to the opposite side of a shuttle box. At test, a preference for the novel side was revealed. When given a priming injection of amphetamine (2 mg/kg), they showed a place preference for the previously drug-paired side if the training dose was 2 mg/kg, but not 10 mg/kg. The authors explanation was that the higher training dose was an aversive one, and therefore could not overcome the novelty preference. However, the lower dose (2 mg/kg) was considered an appetitive dose, with the priming injection of amphetamine leading to a state-dependent preference for the previously drug-paired side. Each of the above studies incorporated priming injections at time of test, but no one has reported the use of priming injections following extinction in the place conditioning literature.

If a priming injection of the drug employed during conditioning does reinstate a conditioned place preference, this would provide further results comparable to those seen in studies using the self-administration method. Critics of the place conditioning method argue that any discrepancies between findings with this method and the self-

administration method should not be ignored. For example, Wise (1989) argued that should such an event occur, the legitimacy of the place conditioning methodology should be questioned. Because rats both self-administer cocaine (e.g., de Wit & Stewart, 1981; Erb et al., 1996) and display a cocaine-induced conditioned place preference (e.g., Cramer et al., 1998), evidence for extinction and drug-induced reinstatement would provide stronger support for the continued use of the place conditioning method in studying the rewarding properties of drugs.

Purpose of the Present Experiments

The first experiment was designed to explore the time course of the expression of a cocaine conditioned place preference within a test session and to study the relation of this expression to transitions from one chamber to another. Further experiments were designed to determine a) whether the apparatus used was sensitive to the dose of cocaine used during conditioning, b) whether an established cocaine-induced conditioned place preference would endure over time, c) whether a conditioned place preference could be extinguished by repeated test trials or by repeated pairings of both environments with saline, and d) whether a conditioned place preference could be reinstated by the previously administered drug following extinction. In summary, the experiments were done to determine the extent to which place conditioning follows the principles of associative learning and shows parallels with findings obtained from studies of cocaine self-administration.

General Methods

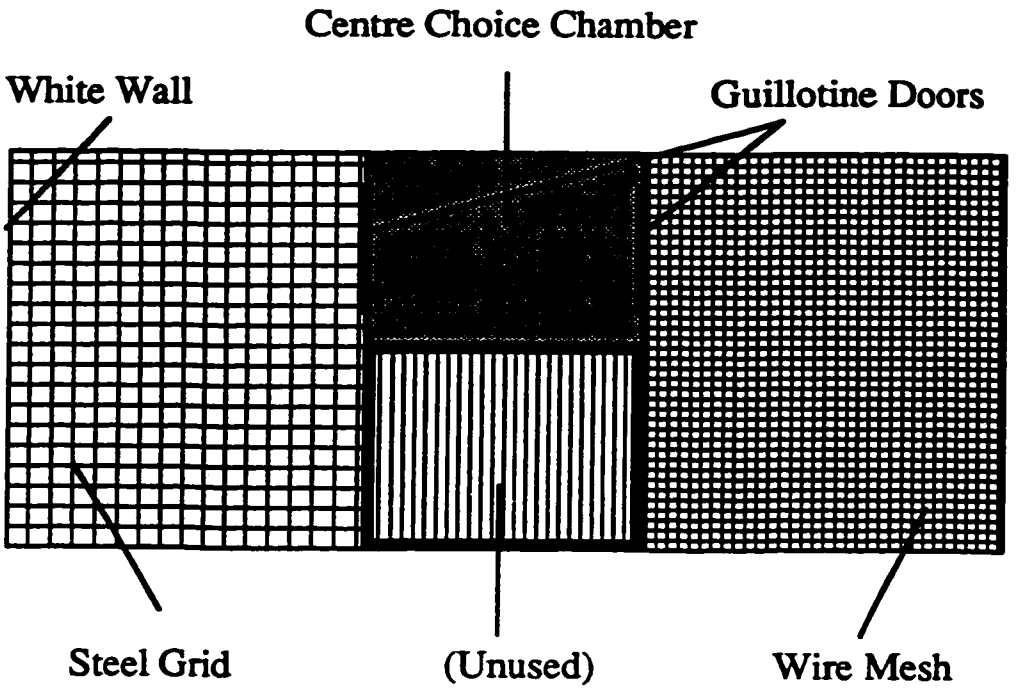
Subjects

A total of 108 male Long-Evans rats (Charles River Canada) were used as subjects. Subjects were housed individually in hanging wire cages (18 x 24 x 18 cm) upon arrival and maintained on a 12 h light/12 h dark normal cycle (lights on at 0800 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.

Apparatus

Conditioning was conducted in four matching grey PVC plastic rectangular boxes (71.5 x 36.5 x 30 cm), each containing three chambers separated by guillotine doors (see Figure 1). The two large end chambers (24 x 35 cm) were separated by a smaller centre choice area (15.5 x 19.5 cm), which was used on the pretest and test days. The centre choice area had punched aluminum flooring (0.4 cm diameter holes) and was separated from the two main chambers by grey walls that had 12.5 x 16 cm passageways cut in them that could be occluded by removable guillotine doors. One of the main chambers had grey walls and a wire screen floor (0.63 x 0.63 cm squares); the other had a white wall located across from the guillotine door and a stainless steel mesh floor (1.3 x 1.3 cm squares). All floors were raised 5 cm to reduce the accumulation of urine and faeces. Through a computer interface, time spent in each chamber was recorded by means of infrared beam crossings. In each of the two end chambers, two beams separated by 8 cm could be recorded from. A rat was said to be in the end chamber if the most peripheral beam was crossed. If the central beam was broken, the rat was determined to be in the centre choice region. Preliminary data indicated that naive animals showed no preference for either end chamber, although all preferred the end chambers over the centre choice

Figure 1. Place conditioning apparatus design.


Centre Choice Chamber
White Wall
Guillotine Doors
Steel Grid
(Unused)
Wire Mesh

region. During conditioning and testing of the animals the room was not illuminated directly (i.e., faint light).

Drug Administration

The cocaine hydrochloride used in these studies was obtained from BDH Chemicals (Toronto, Canada). All doses are expressed as the salt. For intraperitoneal (IP) injections, the drug was dissolved in 0.9% saline and injected in a volume of 1 ml/kg.

General Procedure

The place conditioning procedure consisted of three phases: pre-exposure, conditioning, and conditioned place preference test. All animals were allowed to habituate to the colony room for one week upon arrival. Subsequently, each animal was habituated to handling for three days prior to the start of the experiment. Rats were weighed daily prior to being transported to the testing room. Four rats were transported as a group for each session.

Pre-exposure

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

Conditioning

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 while the other half started on the saline-paired side. Cocaine was administered IP once every other day immediately before the rats were placed into the assigned side for 20 minutes. On alternate days, rats received saline injections (1 mL/kg) prior to being placed in the opposite 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 centre choice region was never used during conditioning and was blocked by guillotine doors.

Conditioned Place Preference Test

Two days following the last conditioning trial, a test for conditioned place preference was given. Animals were placed in the centre choice area with the guillotine doors removed and allowed free access to the entire apparatus for 15 minutes. The amount of time spent in each chamber was recorded to assess individual preferences. No injections were given during the preference test, using the same procedure as during the baseline pretest.

Statistical Analysis

Pretest and preference 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 up by Student-Newman-Keuls post-hoc comparisons, $p<.05$. Analyses specific to each experiment are outlined in the appropriate results section. All follow-up analyses were performed using the Student-Newman-Keuls post-hoc test.

Experiment 1

Numerous studies have been published in which a cocaine-induced conditioned place preference was obtained (e.g., Cramer, Hubbell, & Reid, 1998; Kosten & Miserendino, 1998; Nomikos & Spyraki, 1988). However, a more indepth characterization of the expression of a cocaine-induced conditioned place preference during testing is needed. Several researchers have reported temporal analyses of place preferences. For example, Carr et al. (1988) explored the time course of a novelty-induced place preference, demonstrating an initial avoidance of the novel chamber followed by a robust preference. In another report, heroin-induced conditioned place preference was found to decrease from the first portion of a 15 minute test compared to the last portion of the test (Bozarth, 1987). In another study, however, it was found that a morphine-induced conditioned place preference increased slightly across a 60 minute test session (Reid, Marglin, Mattie, & Hubbell, 1989). Vezina and Stewart (1987) also demonstrated a slight increase in a morphine-induced conditioned place preference over time within a test. These somewhat contradictory findings suggest that it would be worthwhile to determine the time course of the preference for a place associated with cocaine. Thus, the main objective of the present study was to explore the temporal expression of a cocaine-induced conditioned place preference. A secondary objective was to determine whether there were any shifts in the number of discrete transitions from one chamber to another. Reid et al. (1989) reported fewer chamber transitions over time across a 60 minute test for a morphine-induced conditioned place preference. This issue has not yet been explored using cocaine as the drug for conditioning.

Method

Subjects

Twenty-four male Long-Evans rats, weighing 310 to 360 g at the start of the experiment, served as subjects. All animals were treated as outlined in the General Methods.

Procedure

The conditioning procedure was exactly as outlined in the General Methods in the previously described apparatus. The dose used for conditioning was 10 mg/kg cocaine, administered IP. In both the pre-exposure and the conditioned place preference tests, time spent in all three chambers of the apparatus was collected for the entire test as well as in 3-minute bins. Further, the number of discrete transitions from one chamber to another was recorded.

Results

Pre-exposure Test

Figure 2 shows the total time spent in the three chambers over the 15-minute pre-exposure phase. The repeated measures ANOVA for Chamber revealed a significant effect ($F(2,46) = 6.003$, $p<.01$). Post hoc pair-wise comparisons revealed that animals spent less time in the middle chamber than in either end chamber ($ps<.05$). The mean time (±SEM) spent in the wire screen, middle, and steel mesh chambers respectively was 326.0 (±12.7), 256.2 (±10.0), and 316.4 (±14.7) sec.

Conditioned Place Preference Test

Figure 3A shows the results of the conditioned place preference test. It can be seen that rats given free access to the apparatus spent more time in the previously

Figure 2. Pre-exposure phase: Mean (±SEM) time spent in the wire mesh, middle, and steel grid chambers in the 15-minute test for baseline preferences.

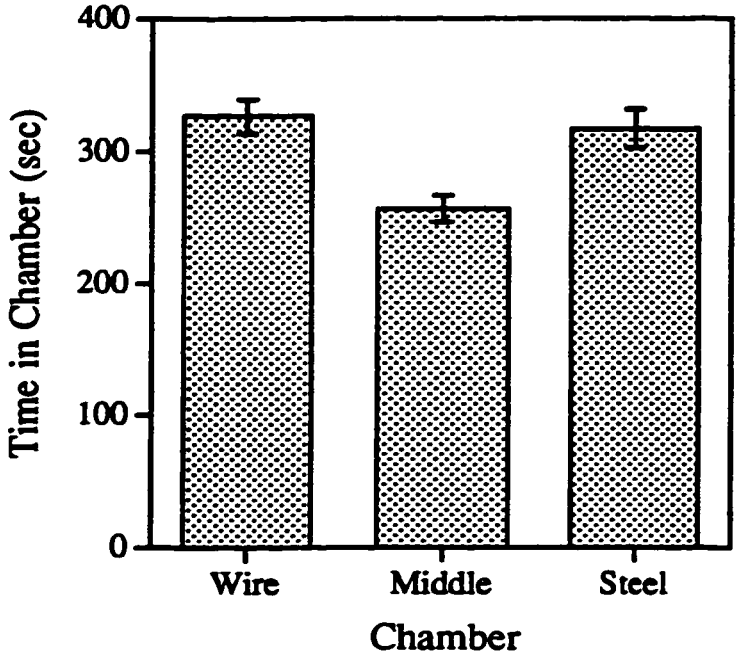
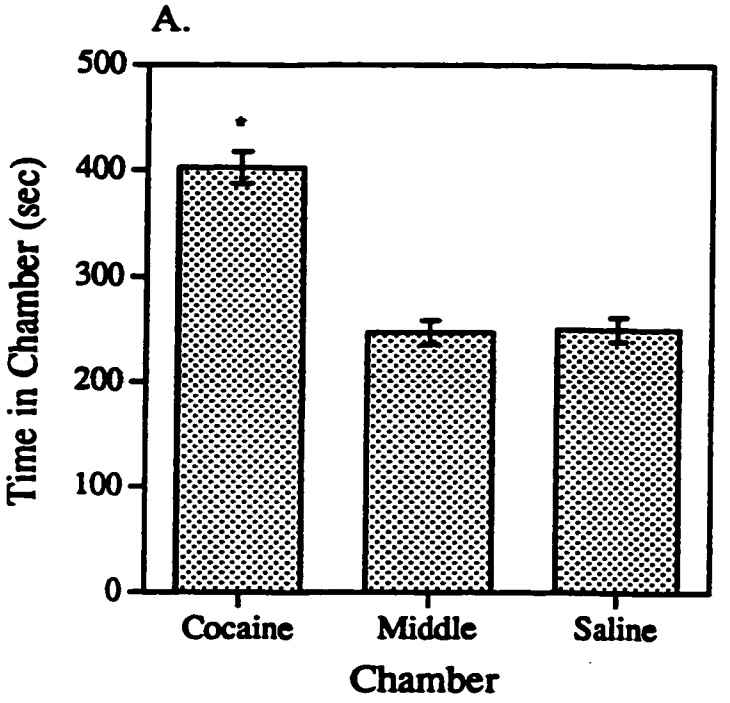
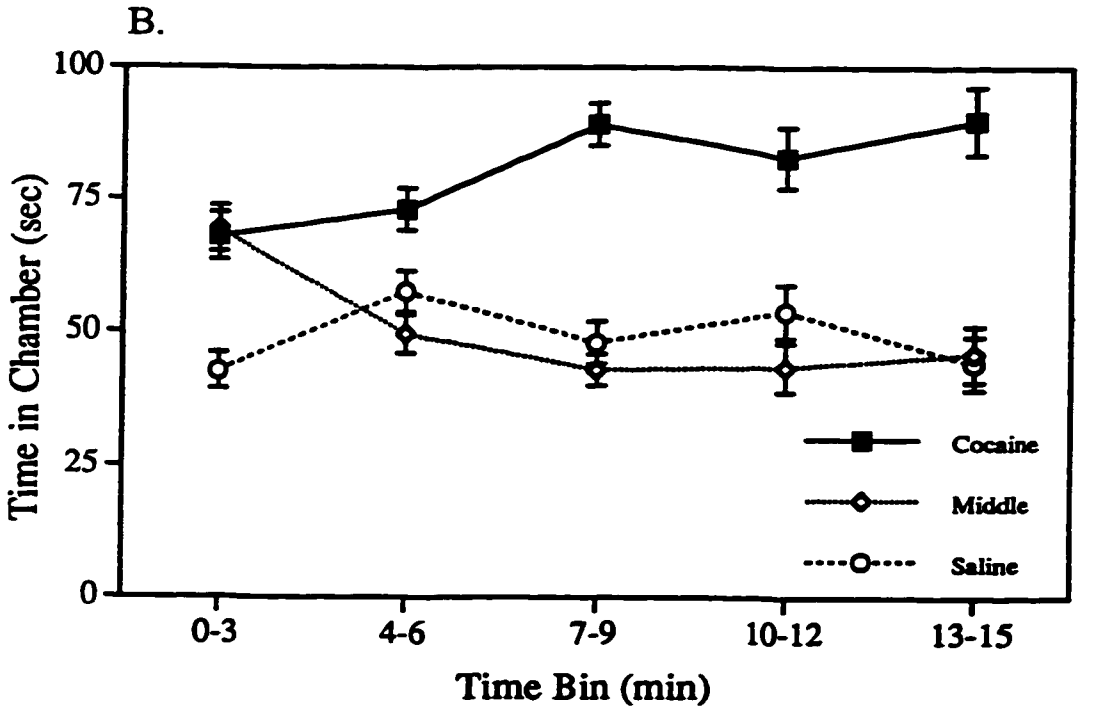

400
300
200
100
0
Time in Chamber (sec)
Wire
Middle
Steel
Chamber

Figure 3. Conditioned place preference test: **A**: Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in the 15-minute test for conditioned place preference. * Different from the Saline side, $p<.05$. **B**: Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in 3-minute bins over the 15-minute test for conditioned place preference.


A.
500
400
300
200
100
0
Time in Chamber (sec)
*
Cocaine
Middle
Saline
Chamber


B.
100
75
50
25
0
Time in Chamber (sec)
0-3
4-6
7-9
10-12
13-15
Time Bin (min)
Cocaine
Middle
Saline

cocaine-paired chamber. The repeated measures ANOVA for Chamber revealed an effect of Chamber ($F(2,46) = 32.526$, $p<.0001$). 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 middle or saline-paired chamber ($ps<.05$). Figure 3B shows a more detailed examination of the data, in which the time course of the conditioned place preference was measured in 3-minute bins. It can be seen that the time spent in the cocaine-paired chamber increased slightly over the course of the test. The repeated measures ANOVAs for Chamber revealed a significant effect of Chamber at all time points (all $Fs(2,46) > 6.5$, $ps<.01$). Post hoc comparisons revealed that rats spent more time in the cocaine-paired chamber than in the saline-paired chamber at each time point ($ps<.05$).

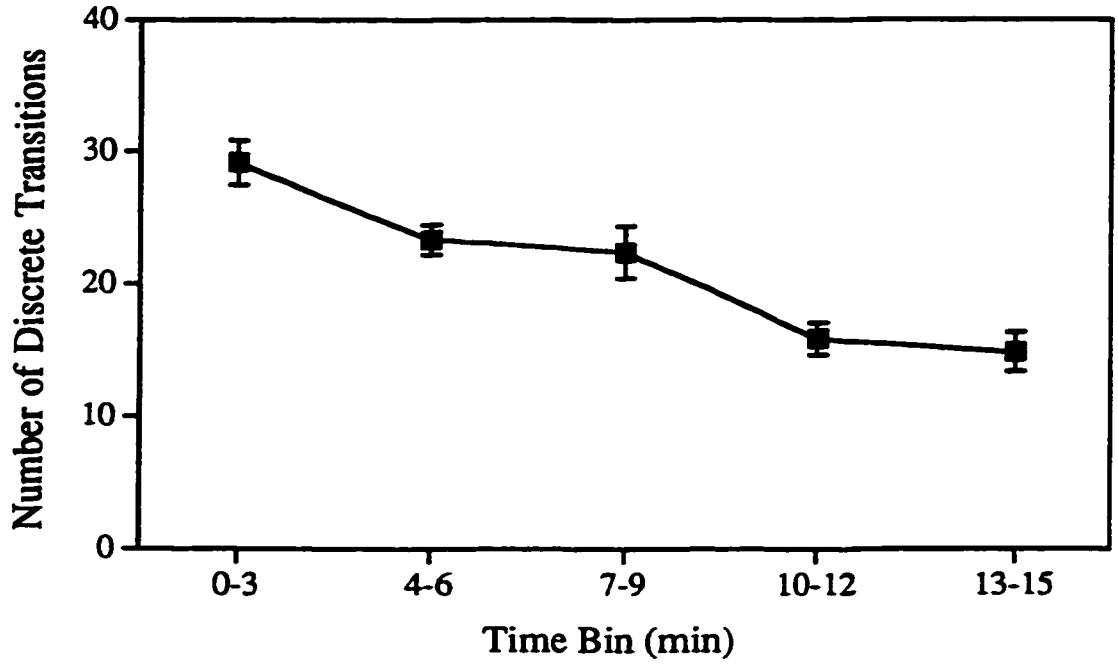
Discrete Chamber Transitions

As seen in Figure 4, the number of transitions from one chamber to another made per unit time decreased over the course of the test. This was confirmed by a repeated measures ANOVA for the number of transitions, revealing a decrease in transitions over time ($F(4,92) = 17.817$, $p<.0001$). 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 ($p<.05$).

Discussion

As in previous studies, a robust conditioned place preference for the cocaine-paired chamber was found. Further, the temporal analysis of the conditioned place preference revealed that the cocaine-paired side was preferred throughout the entire test session. As well, a decrease in the number of discrete chamber transitions was observed over the duration of the test.

Figure 4. **Discrete Chamber Transitions: Mean (±SEM) number of discrete chamber transitions in 3-minute bins over the 15-minute test for conditioned place preference.**


40
30
20
10
0
Number of Discrete Transitions
0-3
4-6
7-9
10-12
13-15
Time Bin (min)

Inasmuch as cocaine has been shown to reliably induce a conditioned place preference (see Schechter & Calcagnetti, 1993, 1998), the results from this experiment are not in themselves surprising. Cocaine is a powerful reinforcer which is widely abused in the human population. Place conditioning measures reward as operationally defined as approach and maintenance of contact with drug-related cues. Thus, the environment (the CS) previously associated with the effects of cocaine (the UCS) can elicit an approach response.

The fact that rats in the present study were shown to approach and maintain contact with the environment previously paired with cocaine throughout the course of the test implies that they are sensitive to the drug-related cues in that environment. In fact, a conditioned place preference was evident even within the first three minutes of testing. This suggests that evidence for a clear preference can be established using a relatively short test duration. However, one point of consideration is the role of novelty in the test for a conditioned place preference. In the present experiment, rats received a single exposure to the entire apparatus prior to conditioning. As Carr et al. (1988) demonstrated, a single exposure to a chamber results in a preference for that chamber relative to a chamber to which the animal was exposed six times. Thus, the relative novelty of the middle chamber could mask a conditioned place preference in a short test. It can be seen, however, from Figure 3B that the rats no longer showed an equal preference for the cocaine-paired and middle chambers by six minutes, suggesting that a test of six or more minutes is sufficient to demonstrate a clear preference following conditioning with cocaine. It would appear as well that, because there were no clear changes in preference over the remainder of the test, longer tests could be used. It should be pointed out that long tests might induce extinction of the preference. Because the rats are exposed to the environment (the CS) in the absence of the drug (the UCS), long tests may reduce the effectiveness of the CS.

The decrease in discrete chamber transitions suggests that the rats initially engage in exploratory behaviour. This behaviour, however, did not interfere with the tendency for rats to spend more time in the previously drug-paired chamber. Previous work has demonstrated that locomotor activity does not play a significant role in conditioned place preference (Carr et al., 1988; Kosten & Miserendino, 1998, Martin-Iversen et al., 1997). The decline in activity is likely influenced by the novelty of the test situation. Novelty induces increases in locomotor activity on its own, but the novelty lessens over time. Thus, although the novel nature of the test appears to affect locomotor activity, the effect gradually diminishes within a test session.

At this point, a dose characterization for this particular apparatus was deemed necessary. Thus, in Experiment 2, the role of the dose used for conditioning in establishing a conditioned place preference was examined. Further, the effects of time on the maintenance of an established conditioned place preference were explored.

Experiment 2

This second experiment was designed to examine the effect of the dose of cocaine on the development of cocaine-induced conditioned place preference. Although few researchers have studied dose effects, a review of these studies by Bardo et al. (1995) revealed little if any effect of dose of cocaine on the development of a conditioned place preference. It was felt, however, that dose effects should be determined in this new apparatus, and, as a result, the doses of 5, 10, and 20 mg/kg IP administered cocaine were tested. More importantly, Experiment 2 was designed to study the maintenance of the conditioned place preference over time. It would be expected that if a conditioned place preference involves associative learning processes, it would be maintained for a considerable time in the absence of the opportunity for extinction. In the present experiment, therefore, following an initial test for conditioned place preference, animals

were tested following either a delay of 2, 4, or 6 weeks. Those animals receiving their second test at 2 and 4 weeks were subsequently tested at 2-week intervals up to the sixth week.

Method

Subjects

Thirty-six Long-Evans male rats (Charles River Canada) weighing from 360 to 490 g at the beginning of the experiment were used as subjects. The animals were maintained as outlined in the General Methods.

Procedure

All animals underwent pre-exposure, conditioning, and tests for conditioned place preference as described in the General Methods. Prior to conditioning, the rats were divided into three groups (n=12 per group) corresponding to the three doses of cocaine (5, 10, and 20 mg/kg, IP) used for conditioning.

Following the first test for a conditioned place preference, four animals from each dose group were assigned to one of three delay periods of 2, 4, or 6 weeks. Further, these new groupings were matched according to the amount of time spent on the cocaine-paired side of the apparatus during the first conditioned place preference test. A one-way ANOVA for time spent on the cocaine-paired side revealed no differences among the three groups ($F(2,33) = .003$, p=ns). Group 1 (n=12) was tested 2, 4, and 6 weeks after the first conditioned place preference test. Group 2 (n=12) was tested 4 and 6 weeks after and group 3 (n=12) was tested only at 6 weeks.

Results

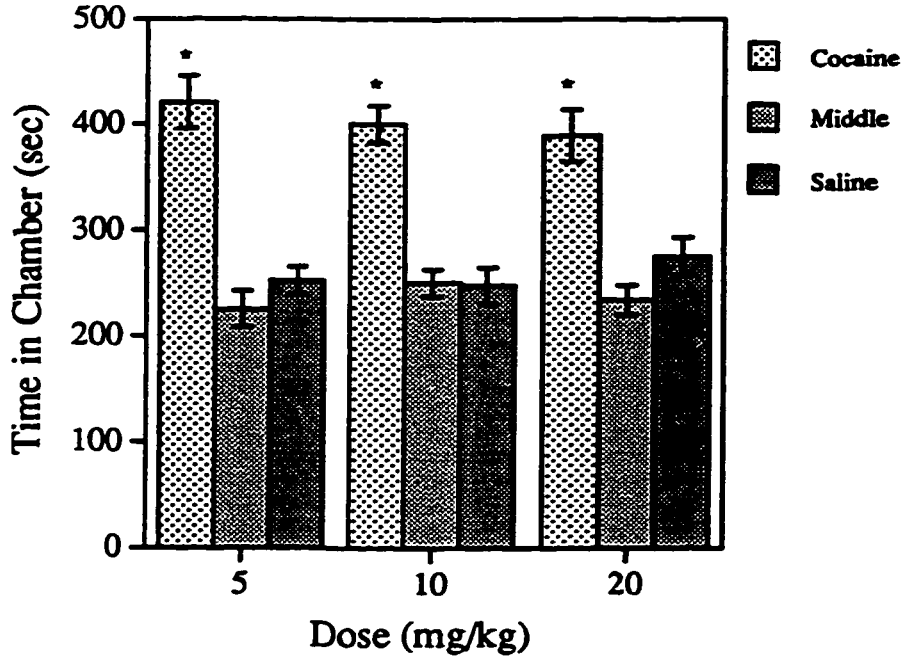
Pre-exposure Test

The pre-exposure test, as expected from the results of Experiment 1, demonstrated a lack of prior preference for either side chamber. The repeated measures ANOVA on time spent in each of the three chambers (Wire Screen, Middle, Steel Mesh) revealed, however, a significant effect of Chamber ($F(2,70) = 25.849$, $p<.0001$). Post hoc comparisons revealed that the effect was attributable to the relatively small amount of time spent in the centre choice area ($p<.05$). There was no difference in the time spent in the two outer chambers (p=ns); the mean time (±SEM) spent in the wire screen and the steel mesh chambers respectively was 328.8 (±13.7) and 351.6 (±11.8) sec.

Conditioned Place Preference Test

The results of the initial conditioned place preference test are shown in Figure 5. It can be seen that the animals spent a greater amount of time on the cocaine-paired side than the saline-paired side at all doses used for conditioning. This was confirmed by a mixed ANOVA for Chamber (Cocaine-Paired, Middle, Saline-Paired) and Dose (5, 10, 20 mg/kg), which revealed only an effect of Chamber ($F(2,66) = 50.395$, $p<.0001$). Simple effects analyses for Chamber were performed demonstrating a significant effect of Chamber for 5 ($F(2,22) = 20.816$, $p<.0001$), 10 ($F(2,22) = 20.247$, $p<.0001$), and 20 mg/kg ($F(2,22) = 11.698$, $p<.001$) IP administered cocaine as the conditioning dose. Post hoc pair-wise comparisons for the three chambers revealed that animals spent more time in the cocaine-paired side compared to either the saline-paired or the centre choice chamber (ps<.05).

Figure 5. Conditioned place preference test: Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in the 15-minute test for conditioned place preference following conditioning with 5, 10, or 20 mg/kg IP cocaine. * Different from the Saline side, $p<.05$.

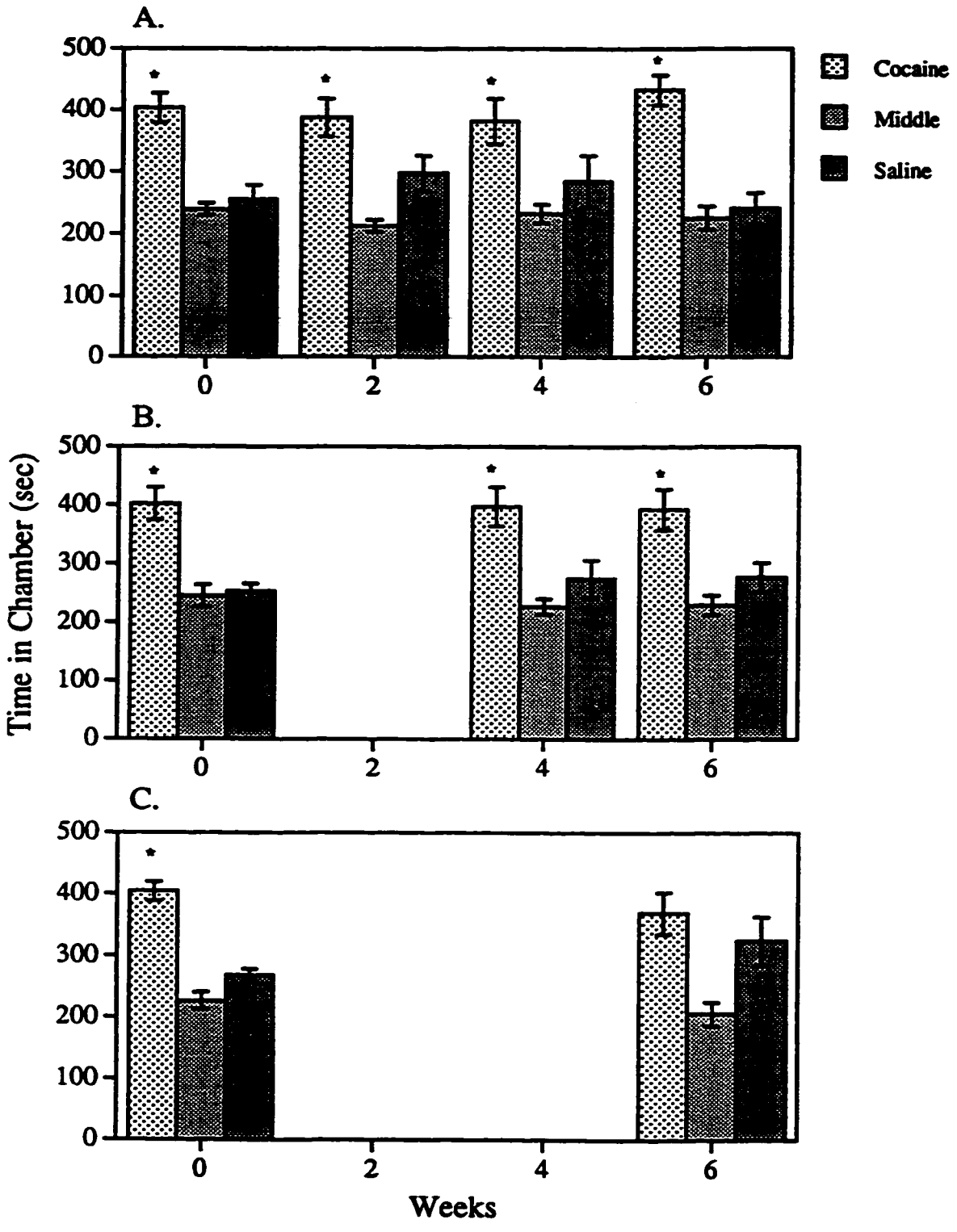

500
400
300
200
100
0
Time in Chamber (sec)
5
10
20
Dose (mg/kg)
Cocaine
Middle
Saline

Time Course of Maintenance of Conditioned Place Preference

Figure 6 shows the results of the conditioned place preference test carried out at either 2, 4, or 6 weeks after the initial test. Animals in groups 1 and 2, tested after a delay of 2 and 4 weeks respectively, continued to spend significantly more time in the cocaine-paired side, whereas those in group 3, tested for the second time after 6 weeks had elapsed, did not (see Figure 6A,B,C). For this second test, a mixed ANOVA for Chamber and Group revealed an overall Chamber effect ($F(2,66) = 18.169$, $p<.0001$). Animals tested after 2 and 4 weeks always spent more time in the cocaine-paired side ($p<.05$), whereas animals tested for the second time after 6 weeks no longer spent significantly more time in the cocaine- than the saline-paired side.

Figure 6A shows the mean (±SEM) time spent in each chamber at tests given at 0, 2, 4, and 6 weeks for group 1. It can be seen that rats spent more time in the cocaine-paired chamber at all time points. A repeated measures ANOVA for Chamber (Cocaine-Paired, Middle, Saline-Paired) by Week (0,2,4,6) revealed only a significant effect of Chamber ($F(2,22) = 16.885$, $p<.0001$). Simple effects analyses for Chamber were performed, reaching statistical significance at 0 ($F(2,22) = 13.640$, $p<.0001$), 2 ($F(2,22) = 8.287$, $p<.01$), 4 ($F(2,22) = 3.492$, $p<.05$), and 6 ($F(2,22) = 16.931$, $p<.0001$) weeks. In all cases, post hoc comparisons revealed that animals spent more time on the cocaine-paired side than on the saline-paired side ($p<.05$). Similar analyses were conducted for group 2 (see Figure 6B). Again, only an effect of Chamber was significant ($F(2,22) = 23.759$, $p<.0001$). Subsequent simple effects analyses revealed a Chamber effect at 0 ($F(2,22) = 12.336$, $p<.001$), 4 ($F(2,22) = 6.895$, $p<.01$) and 6 weeks ($F(2,22) = 6.771$, $p<.01$). Post hoc comparisons revealed that animals spent more time in the cocaine-paired chamber than in either the middle or saline-paired chamber ($ps<.05$). Figure 6C shows the mean (±SEM) time spent in each chamber at tests given at 0 and 6 weeks for group 3. It can be seen that, after 6 weeks had elapsed, the animals no longer spent more

Figure 6. Endurance of conditioned place preference: A: Group 1. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in 15-minute tests for conditioned place preference at 0, 2, 4, and 6 weeks post-conditioning. B: Group 2. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in 15-minute tests for conditioned place preference at 0, 4, and 6 weeks post-conditioning. C: Group 3. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in 15-minute tests for conditioned place preference at 0 and 6 weeks post-conditioning. * Different from the Saline side, $p<.05$.


A.
B.
C.
Cocaine
Middle
Saline
Time in Chamber (sec)
500
400
300
200
100
0
0
2
4
6
Weeks

time in the cocaine-paired chamber than in the saline-paired chamber. Again, the repeated measures ANOVA for Chamber by Week revealed only an effect of Chamber ($F(2,22) = 18.643$, $p<.0001$). Separate repeated measures ANOVAs for Chamber at 0 and 6 weeks demonstrated a statistically significant effect of Chamber at both tests ($F(2,22) = 32.425$, $p<.0001$ and $F(2,22) = 4.699$, $p<.05$, respectively). At week 0, the Chamber effect was the result of animals spending more time in the cocaine-paired side ($p<.05$). However, at 6 weeks, the Chamber effect was due to a tendency to spend less time in the centre chamber only ($p<.05$), with no difference in time spent between the cocaine and the saline-paired side evident.

Discussion

In the present experiment it was found that, within the dose range studied (5, 10, and 20 mg/kg IP), four pairings of cocaine with either of the end chambers once every other day produced a conditioned place preference for the cocaine-paired chamber at test. More importantly, the conditioned place preference, once established, was shown to endure up to 4 weeks following the initial conditioned place preference test, and longer with repeated testing at 2-week intervals.

Using this 'unbiased' procedure with a balanced apparatus, in which animals showed no unconditional preferences for the two conditioning chambers, a strong cocaine-induced conditioned place preference was established at all doses studied (5, 10, and 20 mg/kg IP). The lack of effect of dose observed for the cocaine-induced conditioned place preference is similar to the findings of several researchers who were unable to demonstrate an effect of dose when cocaine was administered IP (Bell, Stewart, Thompson, & Meisch, 1997; O'Dell et al., 1996; Spyraki et al., 1982) and is consistent with the results of a recent meta-analysis carried out by Bardo et al. (1995). It should be noted, however, that even in these previous studies there was some suggestion that dose mattered; very low

doses produced little effect while increasingly greater doses led to somewhat larger cocaine conditioned place preferences. In all cases, however, these small dose-related increases in time spent on the cocaine-paired side did not reach statistical significance. Thus, the place conditioning method may not be sensitive enough to the effects of dose with IP administration.

The general lack of effects of dose found with IP administration of cocaine may arise from several factors. First, the half-life of IP cocaine is about 0.3 h (Nayak et al., 1976), a relatively short period of time. With conditioning sessions of 20 minutes, as was the case in the present study, peak effects of the drug are experienced during the conditioning trial and animals are removed from the apparatus before the drug effects fully dissipate. Longer durations for conditioning trials, however, have been shown to produce similar or weakened conditioned place preferences (Bell et al., 1997; Parker et al., 1994). Second, the IP route of administration results in slower onset of the actions of cocaine than the IV route of administration. It may not be surprising therefore that dose effects have been found when cocaine is administered intravenously (IV). Nomikos & Spyraki (1988), for example, showed that a relatively narrow range of doses (0.5-2.5 mg/kg IV) produced a conditioned place preference. This finding, however, appears to be the result of the aversive effects of cocaine at high doses. Rats were found to convulse at doses higher than 2.5 mg/kg IV, an effect that became sensitized with repeated administration (Nomikos & Spyraki, 1988). Drugs which produce convulsant effects, such as picrotoxin (Spyraki et al., 1985), produce conditioned place aversions. Thus, the rewarding properties of cocaine at these higher doses are likely masked. This inverted U-shaped effect of dose may not be seen with IP administration due to the restricted range of doses used. Very high doses of IP cocaine are required to increase plasma drug levels to concentrations similar to that produced by IV cocaine.

In the place conditioning literature, the most common IP dose used is 5 mg/kg

(e.g., Bardo et al., 1986; Morency & Benninger, 1986; Nomikos & Spyraki, 1988). There is little consistency from study to study, however, in the dose of cocaine required for successful place conditioning. Durazzo et al. (1994) demonstrated, using IP administration, that doses of 10 mg/kg or greater were required to induce a cocaine conditioned place preference. In that study a range of doses from 0.32 to 32 mg/kg was tested and 6 conditioning trials were given. Shippenberg and Heidbreder (1995) found that a minimum dose of 7.5 mg/kg IP cocaine was required to induce a conditioned place preference using 4 conditioning trials and a range of doses (1-10 mg/kg). Mayer and Parker (1993) demonstrated cocaine conditioned place preferences using a range of doses (5-20 mg/kg IP), but the effect was greatest with a dose of 15 mg/kg IP. In order to establish maximal conditioned place preference with minimal risk, 10 mg/kg IP cocaine was chosen as the conditioning dose for the remaining experiments. This is a common dose chosen by many researchers (e.g., Martin-Iverson et al., 1997; Nomikos & Spyraki, 1988), and the one which we originally used in Experiment 1.

The second finding, that a conditioned place preference once established endures over time, is an important demonstration. Mucha and Iversen (1984) reported a conditioned place preference one month following the last conditioning trial using either morphine or naloxone administered subcutaneously (SC). Similar results have been reported for cocaine with repeated testing up to 4 weeks (Nomikos & Spyraki, 1988). However, the authors found that the preference for the cocaine-associated side was attenuated by the seventh day following the last conditioning trial, and after tests given on days 1 and 4. They interpret this to be due to an extinction effect that may have built up over successive short interval tests during which the animals were presented with the CS (environment) in the absence of the UCS (drug). In the present study, the cocaine conditioned place preference was maintained up to 4 weeks in the absence of any intervening tests. If the development and maintenance of a conditioned place preference

is the result of associative learning, endurance over time would be expected. The conditioned response (CR) of approaching and maintaining contact with the drug-paired stimulus environment (the CS) should persist in the absence of any opportunity for extinction. However, the passage of time may eventually lead animals to forget or confuse the cues in the conditioning environments. Thus, after 6 weeks without exposure, animals no longer demonstrated a significant place preference (see Figure 6C).

In this experiment, repeated testing with long delays maintained, and appeared to enhance, a cocaine conditioned place preference following the initial attenuation seen at the time of the second test (see Figure 6A). The fact that repeated delayed testing results in an apparent recovery of the initially attenuated preference has considerable significance for drug-free cocaine users. Perhaps repeated tests at sufficiently long intervals serve as reminders, maintaining the approach response. If so, occasional exposures to a previously drug-paired environment may come to induce drug-seeking behaviour. Without frequent and explicit extinction training, the CS (environment) may maintain or become even more potent in eliciting the CR (approach and contact) with delayed exposure. Parallel findings have been reported with avoidance conditioning, leading Eysenck (1968) to propose a theory of incubation, defined as an increment in CR strength occurring during a period of time when only unreinforced presentations of the CS are made, i.e., when traditionally extinction would be expected to occur. This theory was based on the finding that a single pairing of a painful stimulus (UCS) with a neutral stimulus (CS) results in an enhanced CR in the presence of the CS alone (Dykman, Mack, & Ackerman, 1965; Napalkov, 1963). Similarly, Spear and colleagues (Spear & Parsons, 1976; Smith & Spear, 1984) have demonstrated that, following avoidance conditioning, escape behaviour could be reinstated by "prior-cuing" or "reminder" treatment. That is, the presentation of a CS associated with the original fear-eliciting UCS (e.g., footshock) results in escape behaviour following long retention intervals (Spear & Parsons, 1976),

proactive interference (Gordon & Spear, 1973), retroactive interference (Smith & Spear, 1979), and other performance deficit-inducing procedures. The present finding, that 2-week intervals maintain and possibly enhance conditioned place preference, suggests that, for cocaine users, occasional encounters with a previously drug-associated environment without formal extinction training could result in an episode of relapse.

The present study demonstrated that a cocaine-induced conditioned place preference was not easily diminished by the passage of time alone. In the next experiment, the explicit extinction of approach and maintenance of contact (the putative CR) with the previously drug-paired side was explored.

Experiment 3

Classical conditioning requires that a neutral stimulus (CS) be predictive of an unconditioned stimulus (UCS). The effectiveness of the learned association can then be tested by exposing the subject to the CS alone. Thus, using the place conditioning method, a previously neutral environment (CS) paired with the rewarding effects of a drug can serve to elicit an approach response (the CR) in the absence of the drug itself (the UCS). However, the CS-UCS relation is expected to weaken with repeated exposures to the CS alone. As such, a conditioned place preference should be extinguishable by explicit and frequent drug-free tests.

Previous work has demonstrated an attenuation in the magnitude of a conditioned place preference following repeated tests (Bardo et al., 1986; Mithani et al., 1986). However, there is little evidence for complete abolishment of a conditioned place preference. The acquisition and maintenance of a cocaine-induced conditioned place preference has been shown to be attenuated by testing between each conditioning session (Bardo et al., 1986). Further, using a 'biased' procedure, Calcagnetti and Schechter (1993) demonstrated that repeated saline-paired exposures to the previously drug-paired

chamber results in the abolishment of a cocaine conditioned place preference. This study is confounded by the fact that the side of drug pairings was the previously nonpreferred side. These studies suggest that a conditioned place preference is amenable to extinction training, but neither are conclusive.

Following extinction training, using the self-administration method, it has been demonstrated repeatedly that a priming injection of the drug that was self-administered induces reinstatement of responding on the previously drug-paired lever (de Wit & Stewart, 1981; Erb et al., 1996). If the drug alone is sufficient to induce drug-seeking behaviour following extinction, a noncontingent priming injection of the drug should reinstate a conditioned place preference. The drug injection recreates the interoceptive events that were previously experienced in the drug-paired chamber. Thus, the animal is likely to attend to stimuli previously associated with the drug-state and the approach response will be reinitiated. In essence, the drug itself should serve as a potent reminder of the importance of environmental stimuli previously paired with the drug.

In this experiment, rats were conditioned and subsequently tested for a cocaine conditioned place preference using the apparatus described, an 'unbiased' procedure, and equally preferred end chambers. The rats were then subjected to daily repeated tests to determine whether a conditioned place preference could be extinguished. Following the extinction procedure, rats were given a priming injection of cocaine to determine whether the former conditioned place preference could be reinstated.

Method

Subjects

Twenty-four male Long-Evans rats (Charles River Canada) weighing between 370 and 490 g served as subjects. All rats were treated in accordance with the General Methods.

Procedure

The place conditioning procedure was the same as previously described. The dose used for conditioning was 10 mg/kg cocaine, administered IP. Following the conditioned place preference test, the rats were divided into two groups. The two groups were matched on time spent in the cocaine-paired side as revealed by a one-way ANOVA ($F(1,22) = .21$, p=ns). Group 1 (n=12) was subjected to repeated 15 minute daily testing in the same manner as the conditioned place preference test. That is, each rat was placed in the centre choice area with the guillotine doors removed allowing access to the entire apparatus in a drug-free state. This first group continued to be given daily tests, with no injection, until the initial conditioned place preference was abolished. Animals in group 2 (n=12) served as controls and were left in their home cages during the time in which group 1 was receiving repeated tests. When the first group no longer showed a conditioned place preference, both groups received a 15 minute test for preference. The day following this test, all rats received a priming injection of cocaine (5 mg/kg, IP) and were placed in the centre choice area with access to the entire apparatus for 15 minutes.

Results

Pre-exposure Test

As expected from the results of the previous experiments, the pre-exposure test showed that animals spent an equal amount of time (X±SEM sec) in the two outer chambers (wire: 359.5±15.0; steel: 340.5±15.1) and less time in the smaller centre choice chamber (198.3±10.3). The repeated measures ANOVA for Chamber revealed a significant effect ($F(2,46) = 27.772$, $p<.0001$). Post hoc comparisons confirmed that animals spent more time in the end chambers than in the centre ($p<.05$), with no differences found in time spent in either end chamber.

Conditioned Place Preference Test

The results of the initial conditioned place preference test are shown in Figure 7. It can be seen that the animals spent more time in the cocaine-paired chamber than in either the saline-paired or centre chamber. The repeated measures ANOVA on time spent in each of the three chambers at test revealed a significant effect of Chamber ($F(2,46) = 56.242$, $p<.0001$). Post hoc tests revealed that the Chamber effect was attributable to a greater portion of time spent in the cocaine-paired chamber ($p<.05$).

Extinction Training

Figure 8A shows the results of the the repeated tests carried out daily for the extinction group. 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 Block (1, 2, 3, 4) by Chamber (Cocaine-Paired, Middle, Saline-Paired) revealed a significant effect of Chamber ($F(2,22) = 37.732$, $p<.0001$), as well as a Chamber by Block interaction ($F(6,66) = 4.50$, $p<.001$). Simple effects analyses carried out for the effect of Chamber revealed significant differences for days 1 through 12 (all $Fs(2,22) > 7.7$, $p<.01$). Post hoc comparisons revealed that the animals spent more time on the cocaine-paired side than the saline-paired side on days 1 through 7, and on day 10 (all $ps<.05$). On days 8, 9, 11, and 12, the Chamber effect was the result of a decreased amount of time spent in the middle chamber than in either end chamber (all $ps<.05$). Figure 8B shows the results of the conditioned place preference test given to the delay group on day 12. It can be seen that these animals spent more time on the cocaine-paired side than on the saline-paired side. The repeated measures ANOVA for Chamber for the delay group revealed a significant effect of Chamber ($F(2,22) = 8.131$, $p<.01$). Post hoc comparisons revealed that animals in the delay group spent more time in the

Figure 7. Conditioned place preference test: Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in the 15-minute test for conditioned place preference. * Different from the Saline side, $p<.05$.

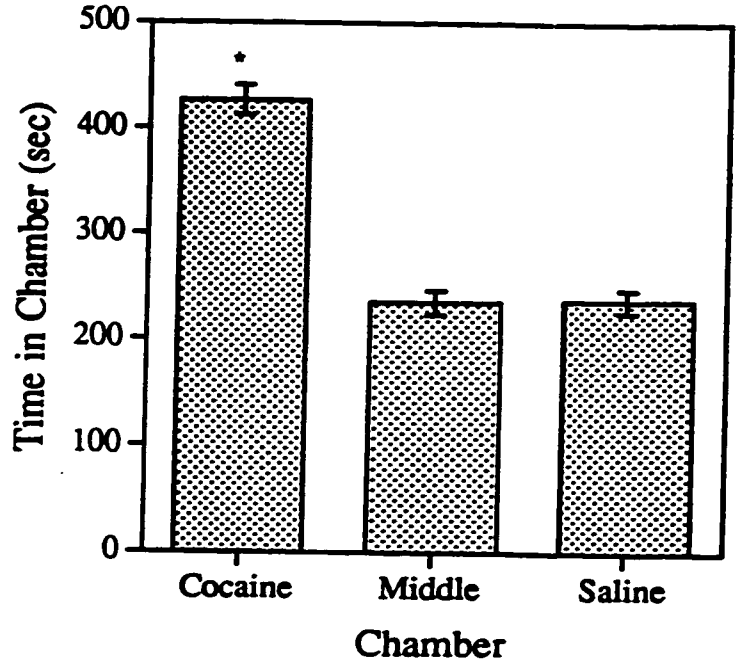
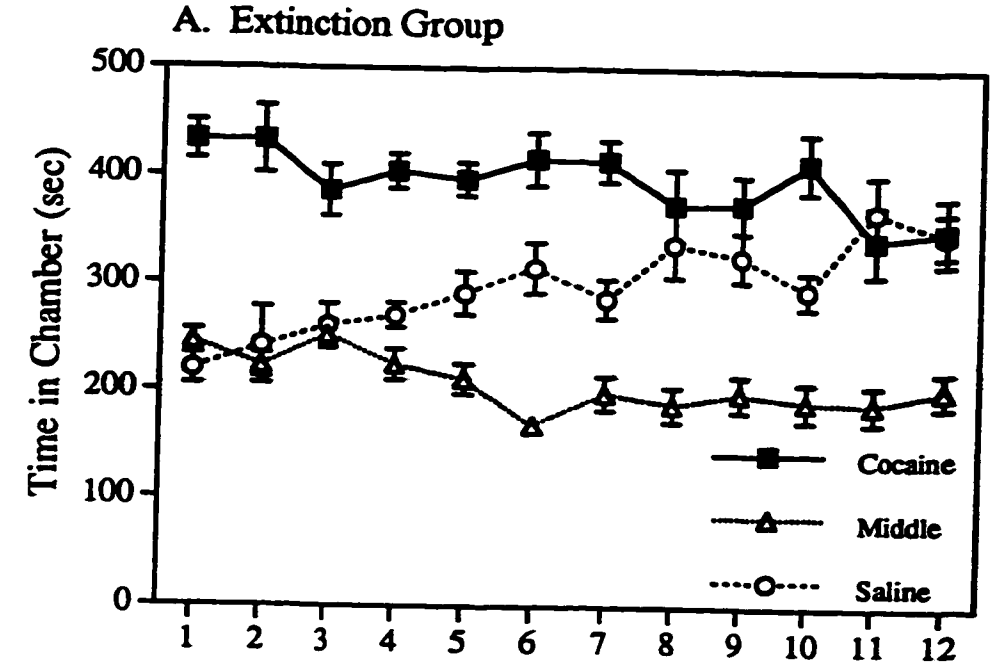
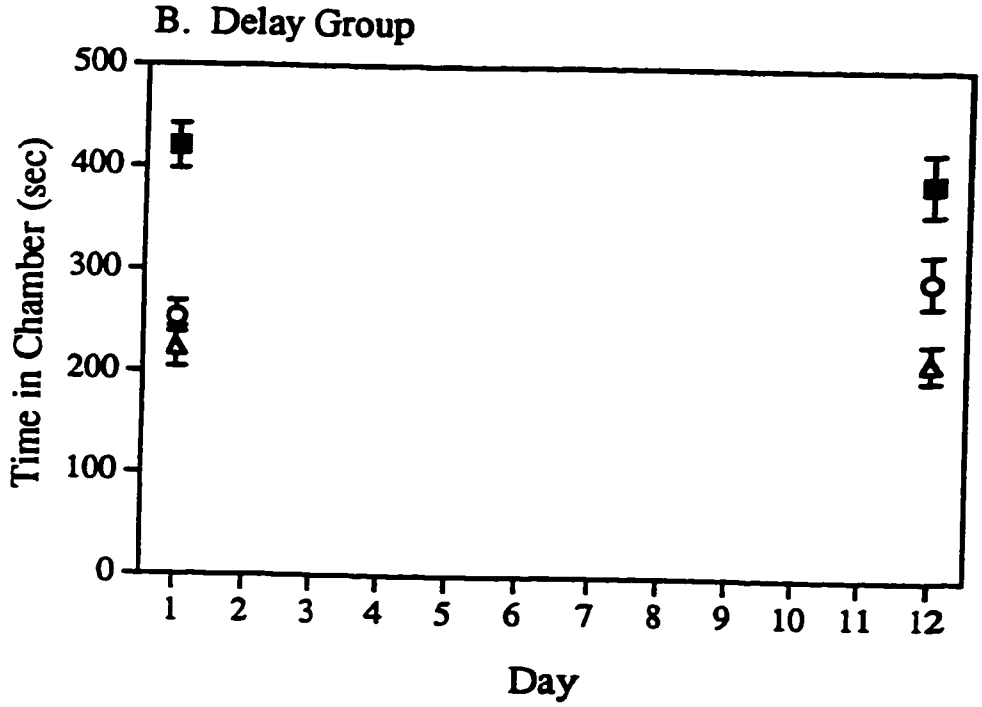

500
400
300
200
100
0
Time in Chamber (sec)
*
Cocaine
Middle
Saline
Chamber

Figure 8. Extinction training: **A**: Extinction group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in 12 daily 15-minute tests for conditioned place preference. **B**: Delay group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chamber in 15-minute tests for conditioned place preference 1 and 12 days post-conditioning.


A. Extinction Group
Time in Chamber (sec)
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
Cocaine
Middle
Saline


B. Delay Group
Time in Chamber (sec)
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
Day

cocaine-paired chamber than in either the saline-paired or centre chamber ($p<.05$).

Priming and Reinstatement Test

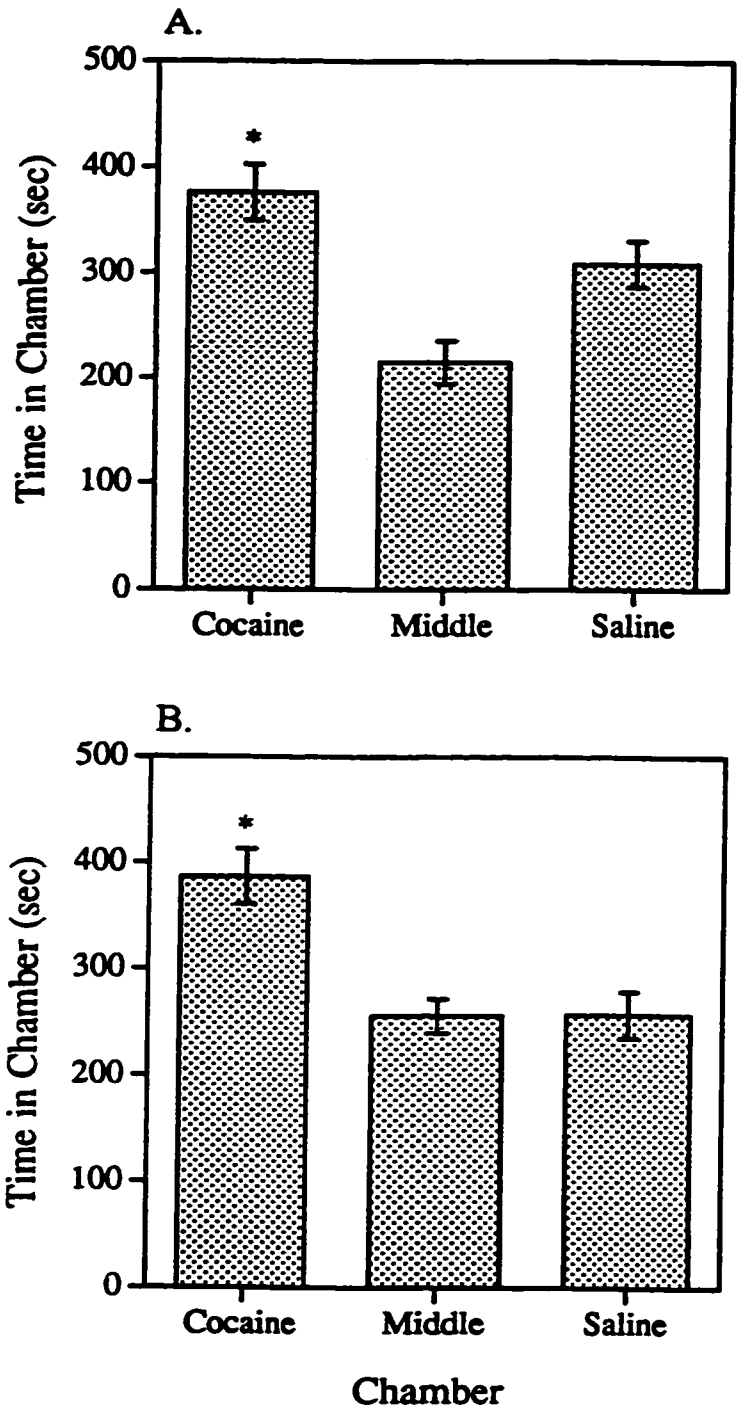
Figure 9A shows the effects of the priming injection of cocaine given to the extinction group. It can be seen that the rats spent more time in the chamber previously associated with cocaine. The repeated measures ANOVA for Chamber revealed a significant effect ($F(2,22) = 8.312$, $p<.01$). Post hoc pair-wise comparisons revealed that animals in the extinction group spent more time in the cocaine-paired chamber than in either the saline-paired or centre chamber ($p<.05$). Figure 9B shows the results of the priming injection of cocaine given to the delay group. The repeated measures ANOVA for Chamber revealed a significant effect ($F(2,22) = 8.102$, $p<.01$), also due to animals spending a greater portion of time on the cocaine-paired side ($p<.05$).

Discussion

The results of the present experiment indicate that repeated testing, as a form of extinction training, leads to the abolishment of a cocaine-induced conditioned place preference. Over daily tests, the animals became indifferent to the previously drug-paired environment. Eventually, the lack of preference was reminiscent of the baseline lack of side preference. This suggests that repeated exposure to the previously drug-paired environment (CS) in the absence of the drug (UCS) leads to a gradual decline and eventually an absence of the approach response (CR). This observation provides further evidence that place conditioning follows associative learning principles.

Previous attempts have been made to explicitly extinguish a conditioned place preference by repeated testing. Bardo, Miller, and Neisewander (1984) gave a total of six daily tests, reporting little decline in duration of time spent on the morphine-paired side. Although total time spent in the chambers remained similar to the initial conditioned place

Figure 9. Priming and reinstatement test. **A**: Extinction group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers following a priming injection of cocaine (5 mg/kg). **B**: Delay group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers following a priming injection of cocaine (5 mg/kg). * Different from the Saline side, $p<.05$.


A.
500
400
300
200
100
0
*
Time in Chamber (sec)
Cocaine
Middle
Saline
B.
500
400
300
200
100
0
*
Time in Chamber (sec)
Cocaine
Middle
Saline
Chamber

preference test, the animals made fewer transitions from chamber to chamber following extinction training. In a study using cocaine, Bardo et al. (1986) demonstrated that the acquisition of a cocaine place preference was disrupted by giving intermittent tests between conditioning trials. In the present study, when extinction trials were given following acquisition of the cocaine place preference, there was a gradual decline and subsequent abolition of this established preference (see Figure 8A).

Uncertainty remains concerning the nature of the CR established and extinguished in a place preference. Most researchers agree that approach behaviour is the CR, but approaching an environment involves a complex set of behaviours. Locomotion is an obvious part of approach behaviour, but does not correlate well with time spent in the paired chambers (Hemby, Jones, Justice, & Neill, 1992; Jones, Hooks, Juncos & Justice, 1994; Spyraki et al., 1982). Maintaining contact with the environmental cues which serve as the CS is the end result of approach behaviour. The place conditioning method usually involves a drug-free test for preference in which the animal is not experiencing the internal state induced by a drug once associated with a particular set of cues. Yet the animal will continue to approach the previously drug-paired environment. The association of the drug state with the environmental cues may result in the environment alone becoming sufficient to provoke physiological and behavioural responses similar to those produced by the drug (see Eikelboom & Stewart, 1982; Stewart & Eikelboom, 1987).

In the present experiment, rats that remained in their home cage for 12 days continued to show a conditioned place preference at test, although attenuated compared to the initial test, for the previously drug-paired environment. This finding was expected from the results of experiment 2, in which rats continued to show a preference for the drug-paired chamber even after 4 weeks without exposure. Following this delayed test, a subsequent test in which animals were given a priming injection of cocaine resulted in a conditioned place preference similar in magnitude to the initial one. Thus, the drugged

state may have served as a reminder of the previously drug-associated cues. This issue will be addressed in more detail in the next experiment.

Animals that had received extinction training showed reinstatement of conditioned place preference after receiving a priming injection of cocaine. Similar results have been reported using the self-administration method. For example, Erb et al. (1996) reported a reinstatement of responding on a lever once associated with IV cocaine infusions following prolonged extinction when animals were given a priming injection of cocaine. Thus, both the self-administration and the place conditioning methods are amenable to studies of drug-induced reinstatement. Such findings appear to be comparable to those in which IV cocaine administration in humans has been demonstrated to elicit cocaine craving in experienced users (Jaffe, Cascella, Kumor, & Sherer, 1989), an affective state thought to result in drug seeking. Note, however, that the resulting reinstatement of a conditioned place preference in the present experiment was not robust and was in fact attenuated compared to the initial conditioned place preference test. The cocaine-induced internal state likely served as a reminder to approach the previously drug-paired environment, but the repeated testing procedure used to extinguish the approach response may have served to attenuate cocaine-induced reinstatement. In particular, the initial conditioned place preference test is novel compared to the preceeding conditioning phase and has been suggested to reduce the time spent in contact with the drug-associated cues (Vezina & Stewart, 1987). Although repeated testing may result in the extinction of the CR, such a procedure may also lead to the attenuated preference seen following a priming injection of the drug itself. As the test phase becomes familiar, it is possible that the exploratory behaviour of the animals will decrease. Therefore, it was concluded that an extinction procedure similar to the conditioning procedure might allow for a more robust reinstatement of a conditioned place preference as the animals would have less experience with the test phase. This possibility was addressed in Experiment 4.

Experiment 4

The results of experiment 3 indicate that daily repeated testing eventually leads to the abolishment of a conditioned place preference which can then be reinstated by a priming injection. In experiment 4, a different extinction procedure was used to determine whether it would lead to similar findings. Calcagnetti and Schechter (1993), using the 'biased' technique, showed that a conditioned place preference could be extinguished by pairing saline with the previously drug-paired chamber on four occasions. As already discussed, however, there are problems inherent in the 'biased' design, making a replication using an 'unbiased' procedure appear necessary. An additional demonstration that time spent in the drug-paired context is subject to extinction would provide further support for the idea that place conditioning follows the principles of associative learning. Therefore, one objective of the present experiment was to determine whether, in cocaine-conditioned subjects, subsequent pairings of that environment with saline injections would attenuate the expression of conditioned place approach as measured by the time spent in presence of the originally drug-paired cues. A second objective of this experiment was to demonstrate that an injection of the conditioning drug given before testing would reinstate the extinguished conditioned place preference. Thus, after the establishment of a conditioned place preference, rats were given 4 exposures to each end chamber after receiving saline injections. This procedure reproduces the conditioning procedure, but without any drug pairings. A preference test was used to assess the effectiveness of this form of extinction training. As in experiment 3, a test for reinstatement involving a priming injection of cocaine followed.

Method

Subjects

The subjects were 24 male Long-Evans rats (Charles River Canada), weighing between 370 and 490 g on the pre-exposure test day. All animals were treated as outlined in the General Methods.

Procedure

The place conditioning procedure was identical to that described in the General Methods, using a dose of 10 mg/kg IP cocaine for conditioning. Following the initial conditioned place preference test, the 24 animals were divided into two groups (n=12 per group). The groups were matched on time spent in the cocaine-paired chamber at test, as confirmed by a one-way ANOVA ($F(1,22) = .009$, p=ns). The extinction group was subsequently subjected to saline pairings over 8 days, in which an IP injection of saline was given prior to confinement to both end chambers. The animals did not receive any cocaine during this period. Following the termination of the extinction phase, the extinction group was given a preference test. For the duration of the extinction phase, animals in the delay group remained in their home cages. They were given a second test for conditioned place preference on the same day as the extinction group. The following day, both groups received a priming injection of cocaine (5 mg/kg IP) immediately prior to a test for conditioned place preference.

Results

Pre-exposure Test

In contrast to previous experiments, the rats tended to spend a somewhat longer time (mean±SEM sec) on the wire mesh side (389.2±19.4 sec) than on the steel grid side (333.1±15.8 sec) during the pre-exposure test, although they did spend less time in the

centre choice chamber (176.3±10.3 sec). The repeated measures ANOVA for time spent in each Chamber (Wire, Middle, Steel) revealed a significant effect ($F(2,46) = 33.189$, $p<.0001$). Post hoc comparisons confirmed that animals spent more time on the wire mesh side than on the steel grid side ($p<.05$), although more time was spent in both end chambers than in the centre chamber ($ps<.05$). As this was the only discrepant result of the pre-exposure test, it was decided to proceed with conditioning. As previously described in the General Methods, the side of conditioning was counterbalanced across animals.

Conditioned Place Preference Test

Figure 10 shows the mean (±SEM) time spent in the three chambers of the apparatus during the test for conditioned place preference. For all 24 animals, the repeated measures ANOVA for Chamber at time of test revealed a significant effect ($F(2,46) = 49.784$, $p<.0001$). Post hoc comparisons showed that the effect of Chamber was the result of animals spending an increased amount of time in the cocaine-paired chamber than in either the saline-paired or centre choice chamber ($ps<.05$).

Extinction Training

Figure 11A shows the results for the extinction group pre- and post-extinction. It can be seen that, following extinction training, animals no longer showed a side preference. The repeated measures ANOVA for time spent in each Chamber by Test (pre-extinction, post-extinction) revealed a significant effect of Chamber ($F(2,22) = 6.636$, $p<.01$) and a Chamber by Test interaction ($F(2,22) = 5.641$, $p<.05$). During the initial test for conditioned place preference, animals in the extinction group spent more time in the chamber previously associated with cocaine injections ($p<.05$). However, after extinction training, no side preferences were found, with mean (±SEM) time spent in the cocaine-

Figure 10. Conditioned place preference test. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in the 15-minute test for conditioned place preference. * Different from the Saline side, $p<.05$.

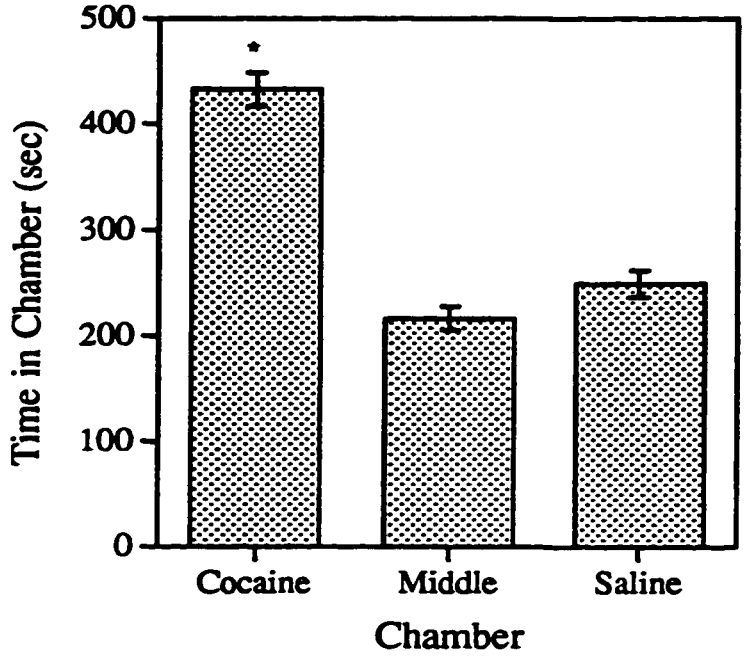
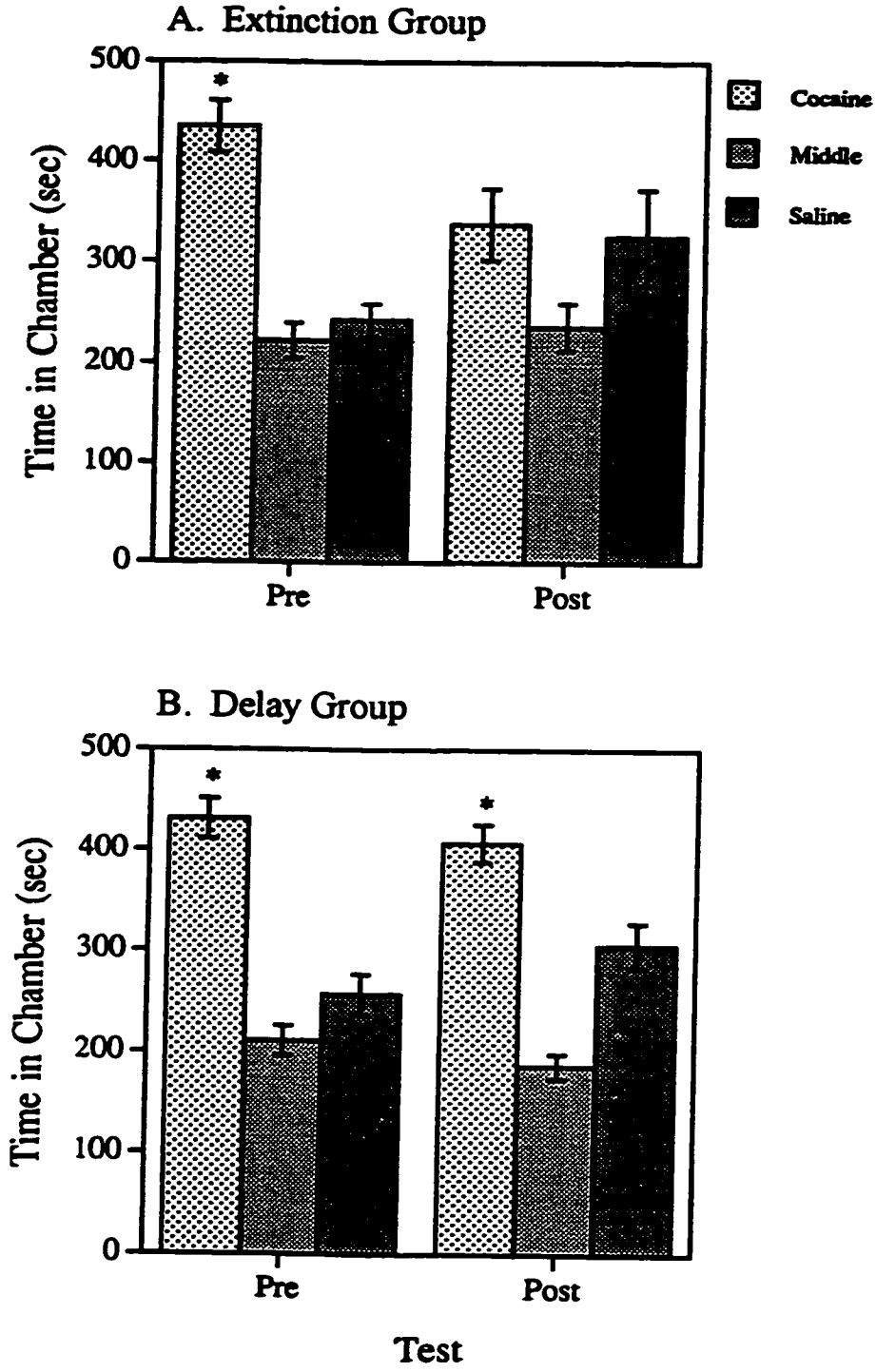

500
400
300
200
100
0
Time in Chamber (sec)
*
Cocaine
Middle
Saline
Chamber

Figure 11. Extinction training: **A**: Extinction group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers in pre- and post-extinction 15-minute tests for conditioned place preference. **B**: Delay group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chamber in 15-minute tests for conditioned place preference 1 and 12 days post-conditioning. * Different from the Saline side, $p<.05$.

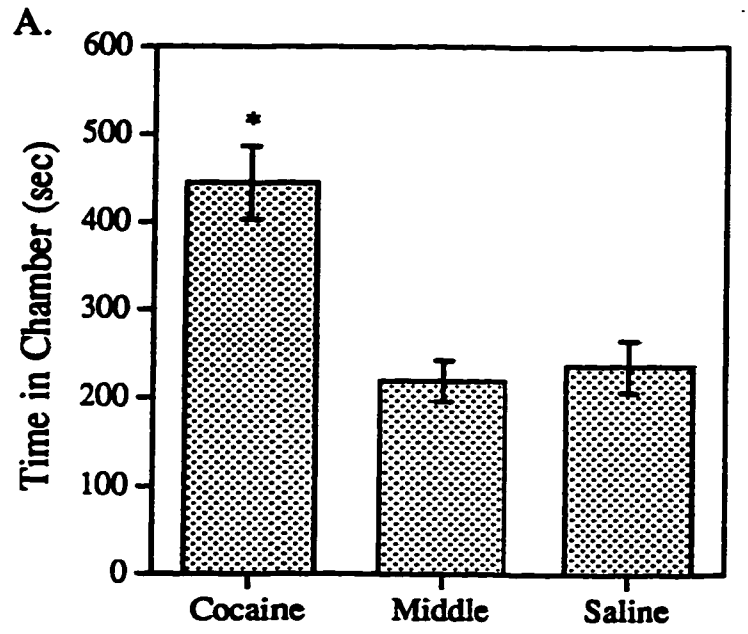

A. Extinction Group
500
400
300
200
100
0
Time in Chamber (sec)
*
Pre
Post
Cocaine
Middle
Saline
B. Delay Group
500
400
300
200
100
0
Time in Chamber (sec)
*
*
Pre
Post
Test

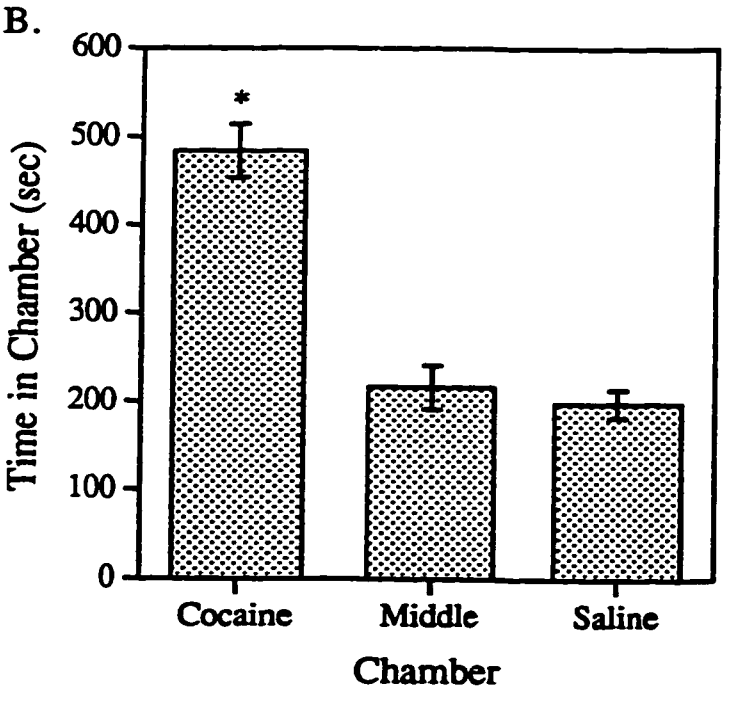
and the saline-paired chambers being 337.3±35.4 and 325.1±47.1 sec, respectively. Figure 11B shows the results of the conditioned place preference tests administered to the delay group on days 1 and 12 post-conditioning. A parallel analysis of time spent in the three chambers for the delay group across both tests revealed a significant effect of Chamber ($F(2,22) = 32.428$, $p<.0001$) and a Chamber by Test interaction ($F(2,22) = 3.99$, $p<.05$). Post hoc comparisons revealed that animals spent a greater portion of time on the cocaine-paired side than on the saline-paired side during the initial test ($p<.05$). Following the delay, the animals continued to spend more time on the cocaine-paired side than the saline-paired side ($p<.05$), and less time in the centre choice chamber than in the end chambers ($ps<.05$).

Priming and Reinstatement Test

As seen in Figure 12A, a priming injection of cocaine administered to animals in the extinction group resulted in a reinstatement of a side preference for the previously cocaine-paired side. The repeated measures ANOVA for Chamber revealed a significant effect of Chamber ($F(2,22) = 10.153$, $p<.001$). Post hoc pair-wise comparisons revealed that the animals spent a greater amount of time in the cocaine-paired chamber than in the saline-paired one ($p<.05$). Figure 12B shows the effects of a priming injection of cocaine on time spent in the three chambers for the delay group. The repeated measures ANOVA revealed an effect of Chamber ($F(2,22) = 28.418$, $p<.0001$). Post hoc comparisons revealed that animals spent more time on the cocaine-paired side than on the saline-paired side ($p<.05$).

Figure 12. Priming and reinstatement test. **A**: Extinction group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers following a priming injection of cocaine (5 mg/kg). **B**: Delay group. Mean (±SEM) time spent in the cocaine-paired, middle, and saline-paired chambers following a priming injection of cocaine (5 mg/kg). * Different from the Saline side, $p<.05$.


A.
600
500
400
300
200
100
0
Time in Chamber (sec)
*
Cocaine
Middle
Saline


B.
600
500
400
300
200
100
0
Time in Chamber (sec)
*
Cocaine
Middle
Saline
Chamber

Discussion

One objective of the present study was to establish that cocaine-induced place preference followed the principles of associative learning. The present findings support the hypothesis that the effects of a previously learned association between a chamber and cocaine could be reduced by extinction training. Four saline-paired (extinction) trials following cocaine-paired trials reversed the expression of the chamber preference observed after the end of the conditioning procedure and returned the time spent in the chambers to baseline levels. This outcome contrasts with that found for the delay group not subjected to extinction sessions. The delay group continued to show a conditioned place preference even after 12 days in the home cages. Furthermore, it was found that a priming injection of cocaine produced a robust reinstatement of the extinguished conditioned place preference.

The delay group, following a priming injection of cocaine, also showed an enhanced conditioned place preference for the previously drug-paired side. A similar increase in the magnitude of a cocaine conditioned place preference following a priming injection of cocaine has recently been reported 24 hours following the initial conditioned place preference test (Cramer et al.,1998; Cramer, Hubbell, & Reid, 1998). These results combined suggest that the drug-induced state can facilitate the approach response towards cues previously paired with drug. Such an enhanced response is reminiscent of the view long familiar to scientists engaged in the study of animal or human memory, that retention is most probable when the circumstances of testing best approximate those of learning (Spear, 1978). Thus, the drug-induced state may serve as the most potent reminder of the CS-UCS association, resulting in an enhanced approach response.

General Discussion

The present experiments clearly demonstrate that a place preference developed on the basis of pairing an environment with cocaine administration follows the rules of classical conditioning. The stimulus environment (CS) paired with the effects of cocaine (UCS) did elicit approach and maintenance of contact (CR) with the environmental cues when presented alone. Thus, a greater amount of time was spent in the drug-paired chamber than in both the saline-paired and centre choice chambers. Evidence for the preference was obtained in a relatively short-duration test, and was accompanied by fewer transitions between chambers as the test progressed. Furthermore, approach towards the previously drug-paired chamber was maintained following one month without exposure to the CS, and longer with repeated infrequent exposures. An extinction regimen of repeated daily tests in the apparatus without cocaine attenuated and eventually abolished the conditioned place preference. Similar results were found when the previously drug-paired and saline-paired environments were subsequently paired with saline for an equal number of trials as used during the conditioning phase. Thus, following training, extinction conditions led to the diminution of a conditioned place preference. Collectively, these results suggest that the development, maintenance, and extinction of a conditioned place preference follow the principles of classical conditioning.

In the present study, both of the extinction procedures used resulted in comparable findings, i.e., the diminution and eventual abolishment of a conditioned place preference. The explanation for extinction initially posited by Pavlov (1927) was that an inhibitory CS-UCS association countered the excitatory association that was formed during acquisition; extinction was thus a form of learning rather than an eradication of earlier learning. If extinction training involves new learning, then the possible differences between what was learned under the two extinction procedures should be considered.

Repeated daily tests resulted in the gradual diminishment of the conditioned place preference. Apart from reducing the association between the previously drug-paired environment and the effects of cocaine, this form of extinction training allowed the animals to become familiar with the testing context. Over days, the exact whereabouts of the previously drug-paired cues became known such that the animals could remain in the presence of these cues even when not in the cocaine-paired chamber. In the second extinction procedure, explicit non-pairings of the chambers with the drug were given. Thus, under these conditions, the animal had more time to learn that the previously drug-paired chamber was no longer paired with the effects of the drug.

In animals given extinction training, a priming injection of cocaine reinstated the conditioned place preference. As Smith and Spear (1984) point out, reactivation of memories best occurs when, at test, the stimuli best approximate those during training. The drug-induced interoceptive state was clearly similar to the training experience in which an injection of cocaine was paired with a stimulus environment. Thus, the previous association of the environmental cues with the drug may have been recalled, overcoming any new learning that had taken place. The saline-pairing procedure can be compared to a proactive interference procedure, which is overcome by the drug-induced state produced by a priming injection. In contrast, the repeated testing procedure may have resulted in a less robust reinstatement of a conditioned place preference due to familiarity with the test context. That is, the animals were aware of the location of the drug-related cues but perhaps only maintained contact with them initially. This explanation could be examined by observing the time course for the reinstatement of a conditioned place preference.

Reinstatement of a Conditioned Place Preference

The reinstatement of the cocaine conditioned place preference observed in the present series of studies has similarities to that seen in studies of self-administration using cocaine (de Wit & Stewart, 1981, Erb et al., 1996), heroin (de Wit & Stewart, 1983; Shaham & Stewart, 1995), and amphetamine (Gerber & Stretch, 1973; see also De Vries, Schoffelmeer, Binnekade, Mulder, & Vanderschuren, 1998). An additional feature of these self-administration studies was that drugs with similar pharmacological properties as the training drug could induce reinstatement of drug-seeking as typically assessed by lever-pressing. For example, amphetamine reinstates cocaine seeking (de Wit & Stewart, 1981). Similar studies carried out using the place conditioning technique could assess further the similarities between the reinstatement induced in place conditioning and self-administration methods, as well as the equivalence of these two methods for measuring drug motivation and reward.

Previous work has repeatedly demonstrated the ability of the UCS to reinstate a conditioned response. For example, Smith and Spear (1984) demonstrated that the most efficient "prior-cuing" (or reinstatement) treatments for classical conditioning included the UCS. In that study, the latency to cross into a 'safe' environment was recorded following the presentation of a light associated with footshock. If the animals had experienced footshock (with or without the CS) in a novel environment prior to testing with the CS alone, they performed better than animals which had not. How the UCS reminder comes to enhance subsequent responding has been considered. Rescorla and Heth (1975) demonstrated that a conditioned avoidance response to an extinguished CS could be reinstated by the presentation of the UCS (footshock) or by an unconditional loud noise that induces a similar affective state. This finding led these authors to conclude that the affective aspects of the UCS are important for reinstatement, i.e., the internal state induced by the fear-eliciting UCS. Thus, stimuli that come to induce a similar affective

state can serve to reinstate responding.

In studies of conditioned place preference, it has been argued that the affective state induced by a drug becomes associated with an environment. If, following extinction, a priming injection of the drug is given to the animal, it is argued that the animal would reexperience the affective state. When given free access to the apparatus, it was observed that the animal responds by approaching those cues associated with the drug. Not only did the priming injection of the drug reinstate an extinguished approach response, but it also resulted in an enhanced response in animals that had previously shown an attenuated preference. If the affective state induced by the drug is responsible for the reinstatement of responding, then drugs with similar pharmacological properties should also reinstate responding.

Several studies have shown that stress can reinstate drug-seeking behaviour. In particular, using the self-administration method, footshock has been demonstrated to produce a robust reinstatement of cocaine (Erb et al., 1996), heroin (Shaham & Stewart, 1995, 1996; Shaham, Rajabi, & Stewart, 1996), and ethanol seeking (Lê, Quan, Juzytch, Fletcher, Joharchi, & Shaham, 1998). It seems possible, therefore, that stress may also reinstate drug seeking as measured by place approach. An obvious problem that arises, however, when trying to compare stress-induced reinstatement in the place conditioning method to that in the self-administration method is that the configuration of the apparatus used is very different. Whereas self-administration studies generally use a single chamber with steel rod flooring (e.g., Erb et al., 1996), place conditioning studies require multiple chambers, often with diverse forms of floors. Thus, a rat cannot receive footshock in the apparatus unless confined to a single area. If the area chosen is the central choice area, as logic would assume, the result could be that rats actively avoid that area at test. Since the rat may associate the central choice area with footshock it may, at test, initially avoid and escape that area when given free access to the entire apparatus. This could result in

animals staying on the side they initially escape to, regardless of past pairings with drug or saline. On the other hand, giving footshock in the drug-paired environment, which is what occurs in self-administration studies, would likely lead to an active avoidance of that chamber, at least for some period of time. This could disrupt any approach behaviour of the animals resulting in a lack of reinstatement. If the initial response of the animal is a fear-elicited one, a considerable amount of time may be necessary to see any stress-induced reinstatement. Normally, a short test duration of 15 to 20 minutes is used in place conditioning studies. Thus, the typical apparatus used to study conditioned place preference, as well as the short duration of test, may not be suitable for the study of stress-induced reinstatement. With these considerations in mind, the use of a longer test may lead to the observation of stress-induced reinstatement with footshock.

What is measured by place conditioning?

Little consideration has been given to what is learned in studies using the conditioned place preference procedure and to the basis of the expression of the preference. Young (1959) proposed that the affective state of an organism at any given time can be conceptualized as a point on a hedonic continuum ranging from negative to indifferent to positive. Stimuli that induce a positive affective state are rewarding, i.e., they activate the neurochemical brain mechanisms of motivation and set the context for learning. Thus, one operational measure of rewarding stimuli is their ability to elicit approach responses. This idea is similar to that of Schneirla (1959), who posited that approach and withdrawal are the only empirical terms that are applicable to all motivated behaviours. More recently, a definition of reward in relation to learning has been expanded and made even more explicit. Beninger, Hoffman, and Mazurski (1989) define reward as a "a biologically important stimulus that elicits approach" and the "consequences of reward would be to enhance the incentive properties of stimuli

associated with reward". Approach as an operational definition of reward seems appropriate to characterize what is measured in conditioned place preference testing.

Place conditioning studies utilize a classical conditioning procedure in which the chamber cues (CS) via pairings with the drug effects (UCS) acquire conditioned rewarding properties and are thus able to elicit approach (CR). Thus, the CS elicits approach in the absence of the drug. One problem that often arises when using drugs as unconditioned stimuli is that the UCR that a drug may be producing is an unspecified moiety (Wise, 1989) which, in itself, does not elicit a behavioural response. Thus, the idea that the CR is really an approach response is difficult to explain in the absence of the UCR counterpart. Furthermore, Hoffman (1989) postulated that there cannot be a UCR corresponding to an approach response because a drug enters the central nervous system without activating any sensory systems. Thus, the approach response elicited by the drug-associated environment must result from the predictive relation or association between the drug and the environment. This view corresponds with that of Tolman (1932) in that, as a result of CS-UCS pairings, the CS becomes predictive of the UCS. Thus, according to Tolman, what is learned in place conditioning is a relation between the affective state induced by the drug and external cues specified in the environment. These environmental cues, when animals are tested in a drug-free state, come to signal a positive affective state. Thus, the cues are approached and more contact is maintained with these cues.

According to Konorski (1967), CRs are diffuse expressions of a general emotional state, such as approach when the UCS is appetitive. CRs thus reflect the general affective value of the UCS. The implication is that conditioning depends on an association between the CS and the motivational attributes of the UCS. The CS, therefore, comes to elicit the emotional state rather than simply predict it. In place conditioning, as previously discussed, the CR involves approach and maintenance of

contact with the environmental cues (CS). It is the nature of the CS that is determinant of the precise response elicited by its presentation, i.e., the characterization of the response elicited is subject to the sensory modality of the CS. For example, in a study of autoshaping in young chicks, when the CS was a key-light signalling a brief increase in temperature (the UCS), the CS alone elicited approach, pecking and snuggling (Wasserman, Hunter, Gutowski, & Bader, 1975). In contrast, a rise in temperature alone resulted in the subjects becoming immobile. In the present studies, the environment paired with the effects of cocaine had both tactile and visual cues. Thus, the CR elicited by the presentation of the CS alone involves approach towards the environmental cues and maintenance of contact, either directly or at a distance.

In conclusion, the present discussion points to some inadequacies in our theoretical understanding of what is measured in place conditioning. It does appear, however, that perhaps what is being measured is conditioned reward as defined operationally by approach. In the present experiments, approach was instituted as a result of pairing cocaine, a drug assumed to induce a positive affective state, with a set of environmental cues. Furthermore, the results from the studies on extinction support the hypothesis that the stimulus cues of a cocaine-paired environment will be approached less following the repeated experience of no drug in association with those cues.

What are the implications of the present study for cocaine users?

For drug users, an attempt to abstain from drug use without any intervention will likely result in an episode of relapse. The results presented in the present study suggest that the occasional encounter with an environment previously associated with a drug may provoke drug-seeking behaviour. This conclusion arises from the fact that in the present studies, infrequent tests given without drug apparently helped to maintain the preference for the drug-related environment. In fact, studies with detoxified former cocaine users

have shown that they report cocaine craving when they encounter drug-buying locations, or virtually any stimuli that have been repeatedly associated with getting and using cocaine (O'Brien, Childress, McLellan, & Ehrman, 1992). Furthermore, the present studies suggest that drug seeking would be further enhanced following a drug infusion; a drug injection may lead the user to seek out environments previously associated with drugs. That environment may then further enhance drug seeking because of the presence of stimuli which have been paired with the physiological and behavioural effects of the drug. The likelihood that drug is available in an environment associated with drug taking is high, such that the abstinent user is faced with the opportunity to obtain it.

The present findings appear to have important implications for the abstinent drug user who wants to avoid an episode of relapse. It was found in these experiments that approach towards a previously drug-paired environment can be extinguished. With frequent and explicit extinction training, stimuli associated with drug taking appear to gain a relatively neutral status. In the case of a long-term drug user, it is clear that a program of extinction training would need to be extensive and broad-based. After long term cocaine use, there are usually numerous stimuli within the person's normal environment that have strong links to cocaine. One program of treatment that has been studied involves giving abstinent users repeated exposure to cocaine "reminders" while they are in a safe environment in an attempt to reduce the craving and arousal often triggered by these stimuli (O'Brien, Childress, McLellan, & Ehrman, 1990). Using this treatment method, it should be possible to reduce or extinguish the power of such cues to trigger the conditioned responses that could lead to drug use and relapse. In practice, the effectiveness of such a treatment program has been somewhat successful; patients who underwent extinction treatment had a higher proportion of clean urines than patients who were only detoxified (Childress, Hole, Ehrman, Robbins, McLellan, & O'Brien, 1993). This result is promising for the future development of treatment programs and suggests

that extinction training should play an integral part.

In developing treatment programs, a consideration of the limitations of extinction training is necessary. In particular, extinction is context specific. Bouton and his colleagues have shown that when training occurs in one context and extinction occurs in another, CRs are still obtained if testing occurs in the first context (Bouton & Bolles, 1979; Bouton & King, 1983). If testing occurs in a third context, then CRs are also observed. These results suggest that, rather than responding being specific to the acquisition context, nonresponding is specific to the extinction context. These results were interpreted in terms of the context serving as a salient retrieval cue, with the context used for acquisition training having a broader stimulus generalization gradient than the context used for extinction training (Bouton, 1993; Brooks & Bouton, 1994). Bouton has supported this account by showing that even when acquisition and extinction occur in the same context, so that there are no CRs in that context at the end of extinction, CRs are again observed when testing occurs in another context (Bouton & Ricker, 1994). In fact, testing in the same context following extinction training and the passage of time often leads to the phenomenon of spontaneous recovery (Pavlov, 1927), in which an extinguished response is recovered. These findings suggest that comprehensive extinction training would be more effective in relapse prevention if it was carried out in the natural environment of the drug user over an extended period of time.

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