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BRIEF REPORT

Cocaine-induced Conditioned Place Preference in Mice: Induction, Extinction and Reinstatement by Related Psychostimulants

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Reinstatement of drug-seeking behavior in animals is relevant to drug relapse in humans. In the present study, we employed the conditioned place preference (CPP) paradigm to investigate the extinction and reinstatement of the place-conditioned response, a model that is consistent with drug-seeking behavior. Cocaine-induced CPP was rendered in Swiss Webster mice and then extinguished after repeated saline injections (8 days) in both the previously cocaine-paired compartment and the saline-paired compartment. Following the extinction phase, the reinstatement of CPP was investigated. Cocaine-experienced mice were challenged with one of the following psychostimulants, cocaine (15 mg/kg), methamphetamine (METH; 0.5 mg/kg), methylphenidate (MPD; 20 mg/kg) and phencyclidine (PCP; 5 mg/kg). The priming injection of cocaine, METH and MPD, unlike PCP, induced a marked preference for the previously cocaine-paired compartment. This finding suggests that all three psychostimulants reinstated the CPP response, and METH and MPD substituted for the reinforcing cue of cocaine. A challenge injection of cocaine administered two and four weeks after the reinstatement of CPP indicated that CPP was maintained up to two weeks. The finding that METH and MPD but not PCP reinstated and supported cocaine-induced CPP suggests that the CPP paradigm may be a useful tool for drug discrimination studies and the reinstatement of drug-seeking behavior.
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KEY WORDS: *Cocaine; Methamphetamine; Methylphenidate; Phencyclidine; Reward; Conditioned place preference*

In animal models, the rewarding properties of various abused substances are determined by drug self-administration studies and the place-conditioning paradigm. The latter is a simple non-invasive procedure which is compatible with the classical Pavlovian conditioning. The rewarding drug serves as an unconditioned stimulus (UCS) that is repeatedly paired with a specific environment that serves as a conditioned stimulus (CS). Subsequently, when animals have the choice of exploring freely the drug-paired (CS) and the non-drug-paired compartments, they prefer the CS environment, indicating the development of conditioned place preference (CPP). This paradigm is used to measure the appetitive value of natural and synthetic substance and particularly to evaluate the rewarding properties of abused substances such as cocaine, amphetamines, opiates and alcohol (for review see Tzschentke 1998; Schechter and Calcagnetti 1998). The conditioned response to drugs of abuse

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is thought to be relevant to human drug-seeking behavior and relapse following exposure to cues previously associated with drug use (Stewart and Vezina 1988; O'Brien et al. 1990).

In drug-self administration studies, the extinction and reinstatement of drug intake serves as another model for drug-seeking behavior and relapse in humans. Overall, the CPP paradigm has not been applied for experiments involving extinction and reinstatement of CPP. It was only recently established that cocaine-induced CPP in rats can be extinguished and then reinstated by a priming injection of cocaine (Mueller and Stewart 2000). The aim of the present study was twofold: a) to investigate whether cocaine-induced CPP in mice can be reinstated not only by cocaine but also by other psychostimulants such as, methamphetamine (METH), methylphenidate (MPD) and phencyclidine (PCP) and b) to determine the duration and maintenance of the conditioned response after its reinstatement.

MATERIALS AND METHODS

Materials

Cocaine-HCl, (+)METH-HCl, and PCP-HCl were purchased from Sigma (St. Louis, MO, USA). (±)MPD-HCl was a gift from the National Institute on Drug Abuse (Washington, DC). All drug solutions were prepared in saline and injected intraperitoneally in a volume of 0.1 ml per 10 g of body weight.

Induction of CPP

Male Swiss Webster mice (32–35 g; Charles River, Wilmington, MA) were maintained on a 12-h light/dark lighting schedule and housed in groups of five with free access to food and water. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. Experiments were carried out essentially as we described previously (Itzhak and Martin 2000; Martin and Itzhak 2000). The conditioned place preference boxes were made of wood except that one side was made of Plexiglas to allow the observation of animals. The dimensions of each compartment were 20 x 15 x 15 cm. One compartment was painted white with a textured floor and the other was painted black with a smooth floor. During the drug- and saline-paired sessions, the compartments were closed by a removable guillotine door. On the first day, each mouse was free to explore the two compartments for a 20-min period. On the second day, the time each mouse spent in the black and white compartments was recorded for a 20-min period with a stopwatch. On the third day, mice (n = 40) received either cocaine (20 mg/kg) or saline (n = 10; control group) and confined for 30 minutes in one compartment of the cage. On the fourth day, animals received a saline injection and paired with the other compartment of the cage for 30 minutes. There was a total of four drug sessions and four saline sessions, and the last day of the treatment was saline. On the test day, day 11, mice received a single saline injection in the test cage, and the time spent in the black and white compartments was recorded for a 20-min period.

In previous experiments we used the 'biased' design, e.g., animals were paired with cocaine in the least-preferred compartment (black) because Swiss Webster mice have shown a slight preference for the white compartment (Itzhak et al. 1998; Itzhak and Martin 2000; Martin and Itzhak 2000). In the present study mice spent 517±13 and 546±18 sec in the black and white compartments, respectively. Since there was no statistical significant difference between the time spent in the two compartments, in the present study we employed the 'unbiased' design. In the first experiment 20 mice (n = 5 per group) were paired with cocaine in the black compartment, and in the second experiment 20 mice (n = 5 per group) were paired with cocaine in the white compartment. Results from the two experiments were not significantly different, thus, in Figures 1 and 2 data are presented as the mean ± SEM time spent in both drug-paired compartments (black and white).

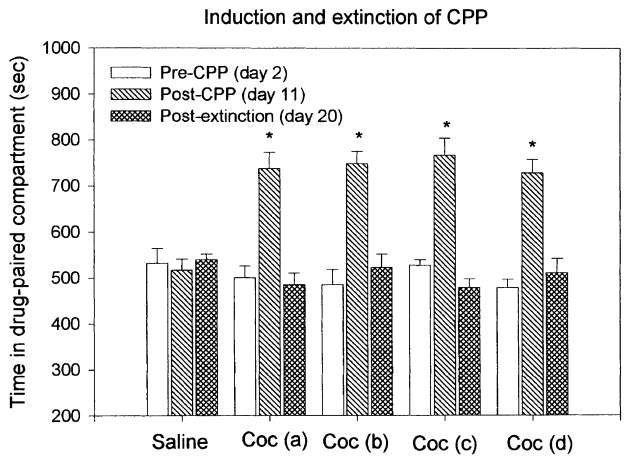

Figure 1. CPP was rendered in Swiss Webster mice by pairing cocaine (20 mg/kg) injection with one compartment of a two-chamber cage and a saline injection on the next day with the other compartment. There was a total of four drug sessions and four saline sessions. Results are presented as the mean ± SEM time spent in the drug-paired compartment. The cocaine injections (groups a, b, c, d; n = 10 each) resulted in a significant preference for the drug paired compartment compared with the time spent in the same compartment before drug administration (pre-CPP; $*p < .01$). The preference of the control group (saline) for the compartment designated as drug-paired did not change significantly. Extinction was rendered by pairing a total of eight saline injection with the previously cocaine- and saline-associated compartments. The latter resulted in complete extinction of the CPP response on day 20.

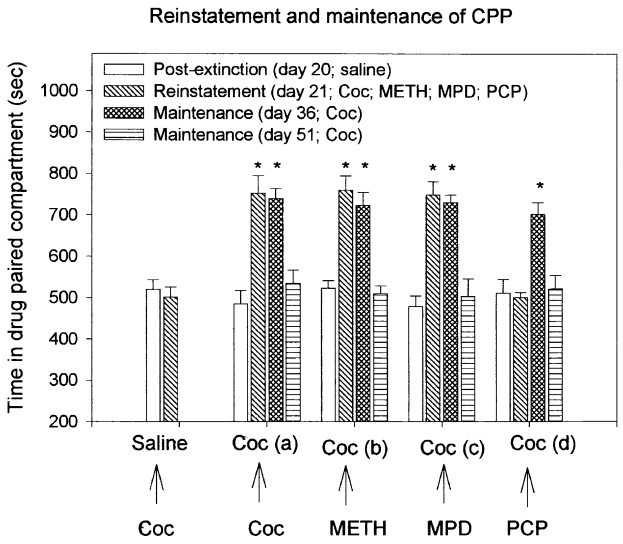

Figure 2. Reinstatement and maintenance of the CPP response in Swiss Webster mice. One day after the extinction of the conditioned response (day 21), the following injections were given: cocaine (15 mg/kg) to control/saline group and cocaine group (a), METH (0.5 mg/kg) to cocaine group (b), MPD (20 mg/kg) to cocaine group (c), and PCP (5 mg/kg) to cocaine group (d). Cocaine, METH and MPD, but not PCP, reinstated the conditioned response in the cocaine experienced mice (*$p < .01$ compared with the time spent in the drug-paired compartment on day 20). Two weeks later, on day 36, animals were re-challenged with cocaine (15 mg/kg). This injection resulted in significant place preference in all cocaine-experienced mice (*$p < .01$ comparison between day 36 and 20). Four weeks later, on day 51, re-challenge with cocaine (15 mg/kg) failed to support the CPP response.

Extinction of Cocaine-induced CPP

Following the establishment of CPP (on day 11), all mice received a saline injection for eight consecutive days (day 12 through 19) in the cocaine- and saline-paired compartments. On the next day (day 20), animals received again a saline injection and the time they spent in the black and white compartments was recorded for 20 minutes. This measurement was determined as post-extinction time spent in the drug-paired compartment (see Figures 1 and 2).

Reinstatement and Maintenance of CPP

One day after the extinction of CPP (day 21), the control group (n = 10) and one of the four cocaine groups (group a; n = 10) were challenged with cocaine (15 mg/kg). The second (group b), third (group c), and fourth (group d) cocaine groups (n = 10 each) were challenged with METH (0.5 mg/kg), MPD (20 mg/kg) and PCP (5 mg/kg), respectively. Immediately after the injection animals were placed in the test cage and the time spent in the black and white compartments was recorded for 20 minutes. Two weeks after the reinstatement of CPP all groups received a cocaine challenge (15 mg/kg) and the time spent in the two compartments of the cage was recorded for 20 minutes. The same experiment was repeated four weeks following the reinstatement of CPP.

Statistical Analysis

Results in Figure 1 are presented as the mean ± SEM time that mice had spent in the drug-paired compartment before and after the induction of CPP and following the extinction of CPP. Results in Figure 2 are presented as mean ± SEM time spent in the drug-paired compartment following extinction, reinstatement and maintenance of CPP. The results were analyzed by a paired 2-tail student's t-test. A p value less than .05 was considered a statistically significant difference.

RESULTS

The administration of cocaine (20 mg/kg) for four alternate days either in the black or the white compartment of the CPP cage resulted in significant ($p < .01$) preference for the cocaine-paired compartment compared with the time spent in the same compartment before drug administration (Figure 1). The increase in time spent in the drug-paired compartment was 215±18 sec, a value that is similar to our previous results from cocaine-induced CPP in Swiss Webster mice (Itzhak et al. 1998; Itzhak and Martin 2000). Saline injections alone did not produce any significant change in compartment preference (Figure 1). Extinction of CPP was rendered following repeated saline injections for eight days in both the drug- and saline-paired compartments. As indicated in Figure 1, this procedure abolished completely the expression of CPP on day 20.

To investigate whether cocaine and other psychostimulants could reinstate the place preference, on day 21 animals were challenged with cocaine (15 mg/kg), METH (0.5 mg/kg), MPD (20 mg/kg) or PCP (5 mg/kg). A challenge cocaine injection given to saline treated mice (control) did not produce any significant change in preference to the black or white compartment (Figure 2). However, the priming injection of cocaine given to cocaine-experienced mice (group a) produced significant preference ($p < .01$) for the previously-cocaine–paired compartment (Figure 2). Similarly, the priming injection of METH and MPD to the cocaine-experienced mice produced a significant ($p < .01$) preference for the previously-cocaine–paired compartment (groups b and c; Figure 2). However, the priming injection of PCP (5 mg/kg) failed to reinstate place preference (group d; Figure 2). In preliminary studies we found that 2 mg/kg PCP also failed to reinstate the conditioned response in co-

caine-experienced mice (data not shown). A high dose of PCP (10 mg/kg) produced marked ataxia which severely impaired animals' movement between the two compartments. Since the dose of 5 mg/kg PCP caused mild hyperactivity which was similar to the intensity of cocaine (15 mg/kg)-induced hyperactivity, the dose of 5 mg/kg PCP was chosen for the subsequent experiments. As indicated in Figure 2, the priming injection of 5 mg/kg PCP (given to group d) failed to reinstate a conditioned preference response. Two weeks after the priming injection was given, all animals were re-challenged with cocaine (15 mg/kg). CPP was maintained in all cocaine-experienced mice, including the mice (group d) challenged previously with PCP that failed to reinstate CPP on day 21. When animals were re-challenged with cocaine (15 mg/kg) four weeks after the reinstatement of CPP, the conditioned response was no longer observed.

DISCUSSION

The major finding of the present study is the potential to use the CPP paradigm as a model for the extinction and the reinstatement of a place-conditioned response by a subset of drugs which elicit reward by the same neurotransmitter system, e.g., dopamine (DA). This model is consistent with the paradigm of drug-seeking behavior previously established in drug self-administration studies.

The expression of cocaine-induced CPP was abolished following repeated pairing of the animals with a saline injection in the previously drug-associated environment. This finding is in agreement with a previous study indicating the extinction of cocaine-induced CPP in rats either by repeated testing of the animals or by pairing saline injections with the two compartments of the cage (Mueller and Stewart 2000). While a challenge cocaine injection did not affect the control group (saline treatment only; Figure 2), the priming injection of cocaine given to the cocaine-experienced mice on day 21 resulted in robust reinstatement of the conditioned response (Figure 2). The finding that both METH and MPD also reinstated the conditioned response on day 21 indicates that these drugs can fully substitute for the cocaine-associated cue. However, the psychotomimetic agent PCP did not substitute for the rewarding effect of cocaine (Figure 2).

Although all four drugs tested are considered psychomotor stimulants, the mechanism of action of PCP is different from the other drugs tested. Both cocaine and MPD block the re-uptake of DA in the ventral tegmental area, nucleus accumbens (NAC), and striatum, causing augmentation in synaptic DA concentration. The increase of DA particularly in the shell of the NAC is believed to be associated with the rewarding properties of these agents (Di Chiara 1999; Leshner and Koob 1999). METH causes the release of newly synthesized DA from presynaptic vesicles and this increase of extracellular DA elicits reward and psychomotor stimulation. PCP, however, is a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist and a psychotomimetic agent (Javitt and Zukin 1991). While some evidence suggests that PCP causes release of DA in rat prefrontal cortex (Hondo et al. 1994), the subjective effects of PCP are different from those caused by the indirect DA agonists (cocaine, METH and MPD).

Attempts to determine whether PCP produces CPP in rats and mice yield equivocal results. In rats, a dose of 2.5 mg/kg PCP resulted in conditioned place aversion, rather than preference (Acquas et al. 1989). Conditioning of rats with a low dose of PCP (0.45 or 0.5 mg/kg) did not produce CPP in one study (Barr et al. 1984), but elicited positive conditioning in another study (Marglin et al. 1989). In mice, results were also quite complex. For instance, while conditioning with 2, 4 and 8 mg/kg PCP produced conditioned place aversion in naive mice, only drug-experienced mice could develop CPP after the conditioning with PCP (Noda et al. 1998).

In drug self-administration studies, the intensity of PCP self-administration in rats varied between different studies. When compared with cocaine, PCP had a lower reinforcing efficacy in rats that self-administered PCP into the NAC (French 1994). However, it has been suggested that the reinforcing effect of NMDA receptor antagonists is not DA-dependent (Carlezon and Wise 1996). In non-human primates the NMDA receptor antagonist, dizocipline (MK-801), did not substitute for cocaine in animals trained to self-administer cocaine (Beardsley et al. 1990). Together, these findings support the present observation indicating the failure of PCP to reinstate cocaine-induced place preference. Thus, it appears that the CPP paradigm may be a useful tool not only for the investigation of drugs with abuse potential, but also for substitution and drug discrimination studies.

Of particular interest is the finding that cocaine reinstated the place-conditioned response two weeks after the extinction phase was completed. The cocaine-experienced mice that did not show the place-conditioned response after PCP administration exhibited CPP two weeks later when they were challenged with cocaine (Figure 2; group d). This finding suggests that the animals remained susceptible to drug-seeking behavior long after the extinction of the drug cue. However, it appears that by the fourth week this sensitivity dissipated since the challenge cocaine injection given four weeks after the extinction failed to produce CPP (Figure 2).

Previous studies have indicated the development of sensitization to the rewarding effect of psychostimulants as determined by the CPP paradigm. For instance, pre-exposure of rats to morphine enhanced the rewarding effect of amphetamine (Lett 1989) and cocaine (Shippenberg et al. 1998) and vice-versa. The design of these

experiments was similar to those used to determine sensitization to the locomotor stimulating effect of psychostimulants, and the results were in agreement with findings pertinent to locomotor sensitization. However, the design of the present study was different and aimed to produce extinction and reinstatement of the conditioned response. Accordingly, the present results cannot be interpreted as sensitization or cross-sensitization between the various drugs tested.

In summary, we have shown that cocaine-induced CPP was abolished following repeated saline injections in the previous cocaine- and saline-paired environments. The place-conditioned response was reinstated by a priming injection of cocaine, methamphetamine and methylphenidate, drugs which produce reward by a dopamine-dependent mechanism. The finding that the NMDA receptor antagonist, phencyclidine, failed to support place-conditioned response suggests that phencyclidine did not substitute for cocaine reward. The present experimental design points to the importance of the CPP paradigm as a tool for drug discrimination studies and the reinstatement of drug-seeking behavior.

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