

Stress or drug priming induces reinstatement of extinguished conditioned place preference

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Received 23 May 2000; accepted 14 June 2000

To construct a model for the relapse of drug use, we investigated the reinstatement of morphine-induced conditioned place preference (CPP) in rats. After the morphine CPP paradigm was established, rats were left extinguishing for 9 days, then exposed to 15 min of random foot shock or s.c. drug priming with different doses of morphine or amphetamine, respectively. Foot shock or a higher dose (0.25 mg/kg) of

both drugs could reinstate the CPP induced by 4 mg/kg of morphine after a 9-day extinction, while a lower dose (0.125 mg/kg) of both drugs had no effect. It is concluded that the CPP extinction–reinstatement paradigm might be used as a model to investigate the mechanism of relapse in addicts. *NeuroReport* 11:2781–2784 © 2000 Lippincott Williams & Wilkins.

Key words: Amphetamine; Conditioned place preference; Extinction; Morphine; Opioid addiction; Psychological dependence; Reinstatement; Relapse; Stress

INTRODUCTION

Relapse to drug-using behavior is the key obstacle in successful abstinence of drug addiction. The main difficulty is that the mechanism of relapse remains unclear. Since the rewarding effect of drugs with abuse potential is thought to be the immediate cause of their psychological dependence [1,2], it is also presumed to account for much of the relapse phenomenon. To date, the only animal model addressing the relapse phenomenon is the extinction–reinstatement of i.v. self-administration [3,4]. The conditioned place preference (CPP) paradigm [5] is widely used in drug reinforcement research [6]. However, very few studies have addressed the question of whether the extinguished CPP induced by morphine could be reinstated.

The most important environmental event that may lead to relapse in humans is thought to be exposure to a stressful condition [7]. Shaham and his colleagues [8] argued that stress was even more effective in reinstating drug-seeking behavior in a self-administration paradigm than re-exposure to drugs. It was also found that opioids could reinstate self-administration of psychostimulants, and vice versa [9]. In the present study, we tested the possibility of reinstating morphine-induced place preference with foot shock stress, low-dose morphine or amphetamine priming in rats, to establish a model for the future study of relapse of drug use.

MATERIALS AND METHODS

Animals: All experiments were performed on male Sprague–Dawley rats (provided by the Institute of Animal

Research, Chinese Academy of Science), weighing 180–200 g at the beginning of the experiment. They were housed 6/cage, with the room temperature maintained at $24 \pm 1^\circ\text{C}$, relative humidity at 50%, under a 12:12 h light–dark cycle. The experimental procedures were approved by the Committee on Animal Care and Use of Beijing Medical University.

Drugs: Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang. Amphetamine sulfate was purchased from Sigma. All drugs were dissolved in 0.9% saline to their final concentrations.

Place preference paradigm: The methods of CPP have been described in detail previously [10]. Briefly, conditioning took place in one of two distinct environments differing in color and texture and separated by a transparent removable clapboard. The walls of one room were painted with vertical black and white stripes (width 2 cm), and the floor comprised a layer of fiberboard bedding. In the other room, the walls were painted with black dots (diameter 1.5 cm) sprinkled on a white background, and the flooring material was 1 cm thick sawdust. The latter was used as the drug-pairing room. Rats were scored during a 10 min test session as being in a compartment when both forepaws were located in that environment.

Foot shock: Rats were given randomly delivered intermittent foot shock for 15 min, with an amplitude of 0.5 mA, a width of 0.5 s, the off time distributed randomly between

10 and 70 s with an average of 40 s. The apparatus generating the foot shock stimulation was SA-II Memory and Behavior Apparatus, kindly provided by the Institute of Psychology, Chinese Academy of Science.

Statistical analysis: Data were processed by commercially available software GraphPad Prism 3.0. Results are presented as means \pm s.e.m. Comparisons between means of groups were analyzed with two-way analysis of variance (ANOVA) followed by Student–Newman–Keul's test. The accepted level of statistical significance was $p < 0.05$.

RESULTS

Dose-effect of morphine-induced place preference: Seventy-seven rats were evenly and randomly distributed into seven groups. One rat was dropped from each of two of the seven groups for unexpected technical reasons. Six of the groups were trained in CPP paradigm with morphine (0.25, 0.5, 1, 2, 4 or 8 mg/kg) for 10 days, one group was given saline as a control. As shown in Fig. 1, rats trained with morphine of moderate to high dose (1–8 mg/kg) all displayed significant CPP ($p < 0.001$ vs control group). However, rats trained with a lower dose (0.25 and 0.5 mg/kg) of morphine showed no significant difference compared with the control group. These results indicated that with a 10-day conditioning phase, morphine in a dose range of 1–8 mg/kg could induce a place preference of similar degree, while lower doses failed to induce any place preference.

Extinguishing of morphine-induced place preference: Seventy-two rats were randomly assigned into eight groups, with nine in each group. Four of the groups were trained with morphine (4 mg/kg) for 10 days in the CPP paradigm, while the other four groups were given the same procedure with saline instead of morphine as the respective control. They were tested for CPP 1, 3, 5, or 7 days, respectively, after the last drug-pairing session. As shown in Fig. 2, rats showed a similar degree of preference to the

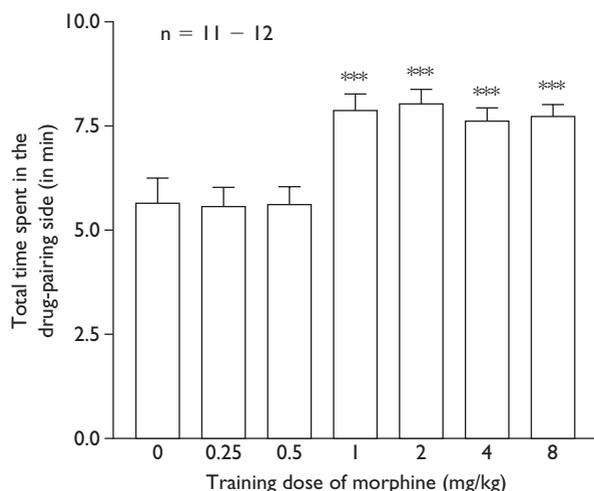


Fig. 1. Dose-effect curve of morphine-induced CPP with 10-day training. *** $p < 0.001$ vs saline-trained control group.

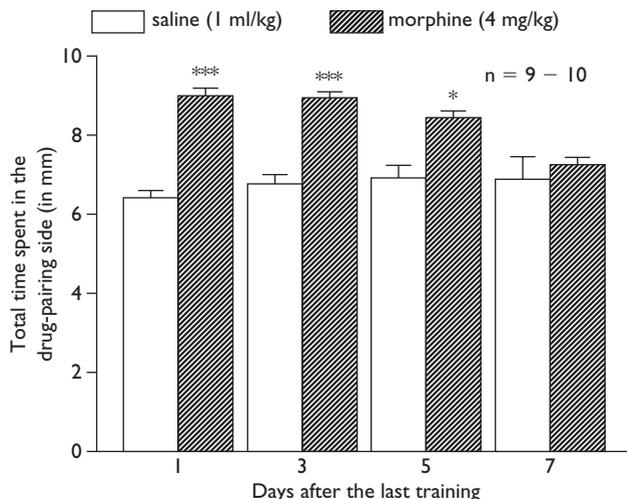


Fig. 2. The spontaneous extinction of morphine-induced conditioned place preference. * $p < 0.05$, *** $p < 0.001$ vs corresponding saline control groups.

drug-pairing room 1 or 3 days after training. However, this place preference became weaker at the fifth day ($p < 0.05$ vs first day); and fell to the same level on the seventh day as the respective saline control group. These results showed that the morphine-induced CPP would disappear after a 7-day extinguishing period.

Reinstatement of the extinguished place preference with a priming injection of morphine or amphetamine: Seventy-five rats were randomly allocated into eight groups, with 9–10 in each group. Three groups (1–3) were trained with saline whereas five groups (4–8) were trained with 4 mg/kg of morphine for 10 days. As shown in Fig. 4, a 10-day training with 4 mg/kg morphine (groups 4–8) but not saline (group 1), induced a stable place preference. After 9 days of extinction, the CPP disappeared (group 4). The rats receiving a priming injection of 0.25 mg/kg of morphine (group 4) or amphetamine (group 6) showed a clear preference for its former drug-pairing side ($p < 0.001$ vs control groups). Rats receiving 0.125 mg/kg of these two drugs (groups 5 and 7), however, showed no preference for the morphine-pairing side. The results indicate that the extinguished CPP could be reinstated by priming injection with a moderate dose of either morphine or amphetamine.

Reinstatement of the extinguished place preference with foot shock: Twenty-four rats were randomly distributed into 2 groups, with 12 in each group. One group was trained with morphine (4 mg/kg) for 10 days, the control groups was administrated with saline. Nine days after the last drug-pairing session when the CPP was expected to be extinguished as shown in Fig. 3, rats were given intermittent foot shock (as described in Materials and Methods section) and then given another testing immediately in the CPP chambers. The results are shown in Fig. 4. The morphine-trained group displayed a stable place preference ($p < 0.001$ vs saline control group) one day after the training. Nine days after the training, an intermittent foot

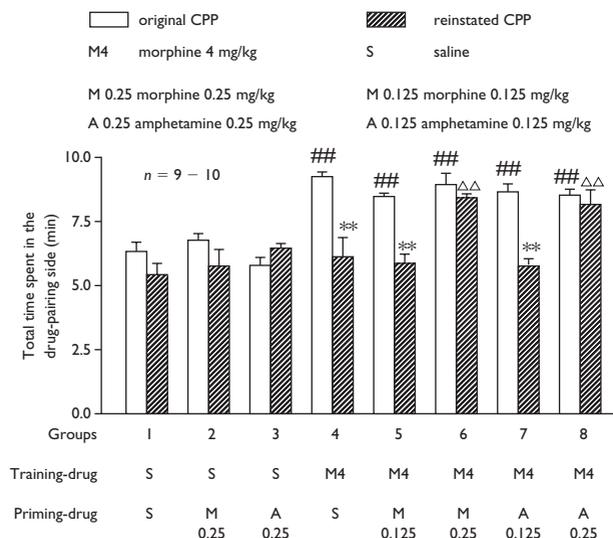


Fig. 3. The effect of low doses morphine or amphetamine on the extinguished place preference to morphine-paired compartment. $###p < 0.001$ vs saline-trained groups; $**p < 0.001$ vs relevant original CPP; $\delta\delta p < 0.001$ vs relevant 0.125 mg/kg priming group, or the saline priming group (negative control).

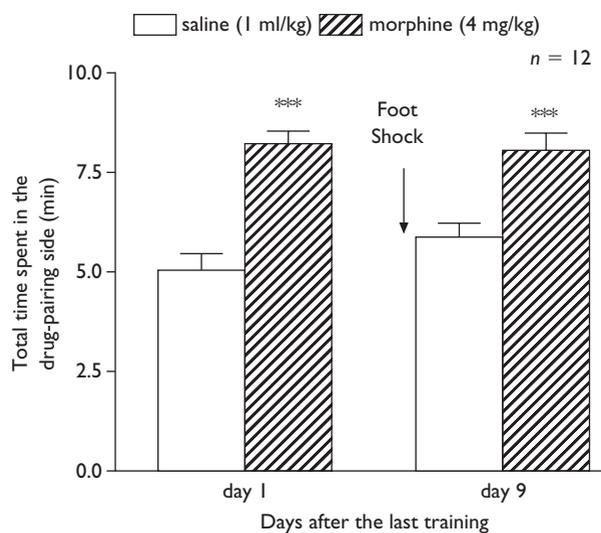


Fig. 4. The effect of intermittent foot shock on the extinguished place preference to morphine-paired compartment. $***p < 0.001$ vs corresponding saline control groups.

shock stress was given for 15 min, which produced a recurrence of place preference ($p < 0.001$ vs foot shock treated saline control group), to much the same extent as the original CPP. These results indicated that the extinguished CPP could be reinstated by foot shock stress.

DISCUSSION

It is believed that drug addiction is a chronic and recurrent condition characterized by relapse [1]. A high rate of relapse after prolonged drug-free periods characterizes the

behavior of experienced users of heroin and other drugs of abuse [11]. Data from research on both humans and rodents showed that factors which can most effectively trigger relapse were re-exposure to the drug itself [9,12], exposure to stress [3,13] or presentation of drug-associated stimuli or cues [14,15]. To date, most, if not all research on relapse in animals adopted the extinction-reinstatement model of intravenous self-administration, which needs a relatively long training period that always leads to tolerance and physical dependence. To some extent, this model could hardly distinguish the motive of drug-seeking and drug-using behavior driven by avoidance of withdrawal syndrome from that of seeking euphoria or psychological reward without any physical dependence developed. Hence, it may not mimic the relapse that occurs in addicts who have never developed to a drug-dependent and withdrawal state [16]. Our early work showed that the rats in our CPP model had never developed physical dependence to morphine, since naloxone could not precipitate a withdrawal syndrome in them (data not shown). So we consider that our model might be more close to the relapse in non-dependent individuals.

Our results showed that a brief presentation of a stressor of intermittent foot shock could induce a clear reinstatement of drug-seeking behavior in the former place-prefering rats induced by morphine of moderate dosage. We also found that this stressor could effectively induce drug-seeking behavior in as long as 2 weeks from the last drug-pairing session (data not shown). Shaham and his co-workers [3,4] obtained similar results in their self-administration model. However, what is the basis for stress-induced reinstatement of drug-seeking behavior? Some researchers hold that stressors mimic the facilitating or priming effects that brief exposures to the incentive objects themselves have on these behavior [8]. On the other hand, since withdrawal from early opioid using might induce a slight psychic aversive effect, a phenomenon called acute withdrawal [14,17], we wonder whether the place preference reflects an avoidance of the aversive effect occurring after the period of euphoria. Our previous work showed that CPP behavior could be eliminated by mild low-frequency (2 Hz) peripheral electric stimulation, an effect that can be blocked by systemic application of naloxone [10]. The low-frequency peripheral electric stimulation had been proved to be able to activate endogenous opioidergic neurons in the arcuate nucleus of hypothalamus, spinal cord, and many other central sites [18]. Hence we implied that the suppressing effect of peripheral electric stimulation on CPP might be due to the activation of endogenous opioidergic neurons that in turn relieve the aversive effect of withdrawal from early drug-using. With these considerations in mind we tend to believe that foot shock as a stressor might present to the rats a similar aversive feeling as the early withdrawal did, leading the animals to recall the motive to approach the former drug-pairing chamber even after the extinguishing of CPP behavior. However, this hypothesis needs to be verified by further studies.

We also found in our present study that a low dose (0.25 mg/kg) of morphine could recall the extinguished CPP, and a similar dose of amphetamine could have cross-priming effect. Considerable evidence suggested that opiates and psychostimulants could all activate the mesolimbic

bic dopamine system to produce their rewarding effects [19,20], while dopamine receptor antagonists could block the priming effect of heroin, amphetamine and cocaine in the extinguished self-administration model [21,22]. Taken together, we presume that dopamine release can be a common mechanism underlying the relapse to drug-seeking behavior for opiates and psychostimulants, hence one drug might cross-prime the other via this common path. Since a lower dose (0.125 mg/kg) of morphine or amphetamine failed to induce the relapse of drug-seeking behavior, we imply that the dopamine release must reach a certain threshold level in order to trigger the relapse of drug-seeking behavior. Again, this speculation needs further verification.

CONCLUSION

That the extinguished morphine CPP could be reinstated by foot shock stress or by a moderate dose of morphine or amphetamine seems to mimic the relapse in opiates addicts after exposure to stressors or opiates, and after priming by psychostimulants. It might serve as a useful model in addition to the self-administration model, for the investigation of the mechanism of drug-abuse, or more specifically, to the relapse of drug use, in human beings.

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Acknowledgements: The authors want to thank Professor Nan Sui and Dr Jing Chen at the Institute of Psychology, Chinese Academy of Science, for their constructive advice on the establishment of the animal model, and generous supply of the apparatus for electric foot shock. We also feel grateful to Dr Yan-Hua Ren at the Chinese National Institute of Drug Dependence for her kind help in many aspects. This study is supported by a grant from NIDA, USA, DA03983, and the Ministry of Science and Technology, P. R. China.