Peripheral electric stimulation inhibits morphine-induced place preference in rats

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Conditioned place preference (CPP) is a commonly used model to detect rewarding effect of drugs. To observe the effect of peripheral electric stimulation (PES) on morphine-induced CPP, we trained the rats with morphine in a CPP paradigm. Twelve hours before the testing phase, rats were given PES via stainless-steel needles with frequencies of 2, 100, or 2/100 Hz, respectively. PES of 2 and 2/100 Hz significantly decreased CPP in morphine-trained animals in a naloxone reversible manner, while PES of 100 Hz, foot shock, needle insertion, or plain restraining, showed no effect. Thus, PES with a low-frequency component (2 Hz) could specifically inhibit the expression of morphine-induced CPP, presumably via activation of opioid receptors. *NeuroReport* 11:1017–1020 © 2000 Lippincott Williams & Wilkins.

Key words: Acupuncture; Conditioned place preference; Frequency dependence; Morphine; Opioid; Opioid addiction; Peripheral electric stimulation; Psychological dependence; Rewarding effect

INTRODUCTION

The rewarding effect of drugs with abuse potential, such as opiates, is the immediate cause of their psychological dependence [1,2]. One of the putative animal models demonstrating the rewarding effect of a drug is conditioned place preference (CPP) paradigm [3], in which animals are trained to prefer the drug-related compartment.

Our previous studies [4–6] showed that transcutaneous electrical nerve stimulation applied on certain acupoints could ameliorate withdrawal syndrome in human heroin addicts, and postpone the relapse of drug use. However, no direct evidence had been shown on whether peripheral electric stimulation (PES) could antagonize the rewarding effect of opiates. The present study was designed to observe the effect of PES with different frequencies on morphine-induced place preference.

MATERIALS AND METHODS

Animals: All experiments were performed on male Sprague–Dawley rats (Institute of Animal Research, Chinese Academy of Science, Beijing), weighing 180–200 g at the beginning of the experiment. They were housed 6/cage, with the room temperature maintained at 24 ± 1°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 the local government.

Drugs: Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang. It was diluted with 0.9% saline to obtain a final concentration of 2 mg/ml. Naloxone HCl was purchased from Sigma Co., Ltd., and dissolved in 0.9% saline to a concentration of 0.5 mg/ml.

Place preference paradigm: The experimental chambers used in our CPP paradigm were rectangular wooden boxes measuring 94 × 44 × 38 cm, divided by a transparent removable clapboard to form two end rooms of 47 × 44 × 38 cm. In one room, the walls 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 white background, and the flooring material was 1 cm thick sawdust. The latter was used as the drug-pairing room.

In the conditioning phase, the chambers were separated by the transparent clapboard. Rats were placed in the drug-pairing room for 5 min before an i.p. injection of morphine and remained there for another 15 min after the injection. This was performed once a day before the end of the conditioning phase. In the testing phase, 24 h after the last training session, the transparent clapboard was removed. At the beginning of the testing phase, rats were put between the two end rooms, with the midline of their body on the the midline of the chamber separating the two end rooms with their head toward one sidewall and tails

to the other. Rats were given free access to both rooms of the apparatus for 10 min. A rat was considered to be in a particular room only when both of its front paws were within that room. Total time spent in the drug-pairing side (min) was recorded.

Peripheral electric stimulation: Rats were kept in special holders, with their hind legs and tails exposed (for details see [7]). Two stainless steel needles of 0.4 mm diameter were inserted into each hind leg, one in the acupoint ST36 (5 mm lateral to the anterior tubercle of the tibia), and the other in SP6 (3 mm proximal to the medial malleolus, at the posterior border of the tibia). Constant current square-wave electric stimulation produced by a HANS LH-800 programmed pulse generator (produced by Beijing University of Astronautics and Aeronautics Aviation) was given via the two needles for a total of 30 min. The frequency of stimulation used was 2, 100, or 2/100 Hz (2 Hz alternating automatically with 100 Hz, each lasting for 3 s; the pulse width was 0.6 ms for 2 Hz and 0.2 ms for 100 Hz). The intensity of the stimulation was increased stepwise from 1 mA to 2 mA and 3 mA, with each step lasting for 10 min.

Foot shock: Twelve hours before the testing session, rats were given intermittent foot shock (amplitude 0.5 mA; width 0.5 s; off time distributed randomly between 10 and 70 s, mean 40 s) for 15 min with SA-II Memory and Behavior Apparatus (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 mean ± s.e.m. Comparisons between means of groups were analyzed with two-way analysis of variance followed by Student–Newman–Keul's test. The accepted level of statistical significance was $p < 0.05$.

RESULTS

Time–effect curve: Ninety-three rats were randomly assigned to one of eight groups, with 11–12 in each group. Four of those groups were trained with 4 mg/kg morphine for 4, 7, 10, or 14 days respectively while the other four groups were trained with saline as control. As shown in Fig. 1, CPP scores of 4- and 7-day training groups were 5.49 ± 0.56 and 7.26 ± 0.51 min, respectively, which showed no significant difference to their corresponding control (5.62 ± 0.39 and 6.76 ± 0.44 min, respectively). However, the CPP score for the 10- and 14-day training groups were 8.31 ± 0.50 and 8.14 ± 0.25 min, respectively, which was significantly higher ($p < 0.001$) than controls (6.71 ± 0.55 and 6.38 ± 0.43 min, respectively). These results showed that with 4 mg/kg dose of morphine, at least 10 days of conditioning was needed to induce a stable place preference.

Dose–effect curve: Fifty-seven rats were randomly distributed into five groups, with 11–12 in each group. Four were trained in CPP paradigm with 1, 2, 4 or 8 mg/kg morphine for 10 days, while the remaining group was trained with saline as control. As shown in Fig. 2, rats trained with 1–8 mg/kg morphine displayed CPP scores of 7.83 ± 0.41, 8.01 ± 0.34, 7.56 ± 0.33, and 7.69 ± 0.28 min, respectively, values which were all significantly higher ($p < 0.001$) than the control group (5.18 ± 0.39 min). However, no significant difference between the four experiment groups were found ($p > 0.05$). This indicated that with a 10-day conditioning phase, morphine in a dose range of 1–8 mg/kg could induce CPP of similar extent, with no apparent dose–effect relationship.

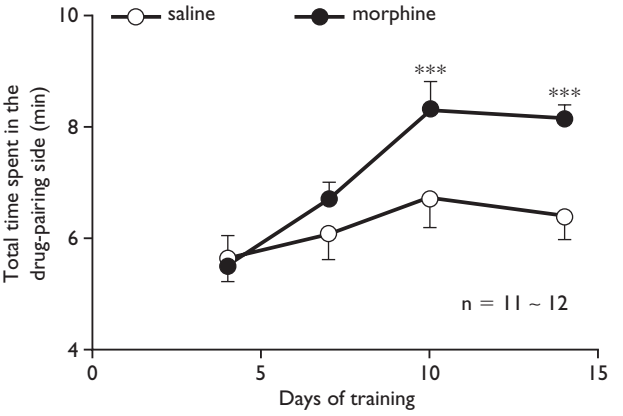

Fig. 1. Time–effect curve for 4 mg/kg morphine-induced CPP. *** $p < 0.001$, compared with experimental groups at 4 or 7 days, as well as corresponding saline control groups.

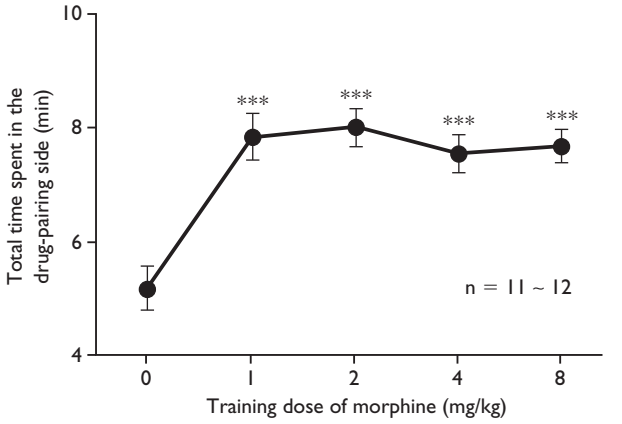

Fig. 2. Dose–effect curve for morphine-induced CPP with 10 days training. *** $p < 0.001$, compared with saline-trained control group.

Effect of peripheral electric stimulation: Eighty-two rats were randomly assigned to one of seven groups, with 11–12 in each group. They received training with 4 mg/kg morphine, i.p., for 10 days. Twelve hours after the last i.p. injection, groups 1, 2 and 3 were given PES of 2, 100, or 2/100 Hz, respectively; group 4 received needle insertion without electric stimulation; group 5 was merely restrained in the holder; group 6 was given foot shock, and group 7 served as CPP control. Each group of rats were given CPP testing 12 h after the treatment. As shown in Fig. 3, CPP score of the restraining, needling, foot shock and 100 Hz-treated groups were 8.35 ± 0.31, 9.02 ± 0.16, 8.76 ± 0.18, and 8.50 ± 0.39 min, respectively, which showed no significant

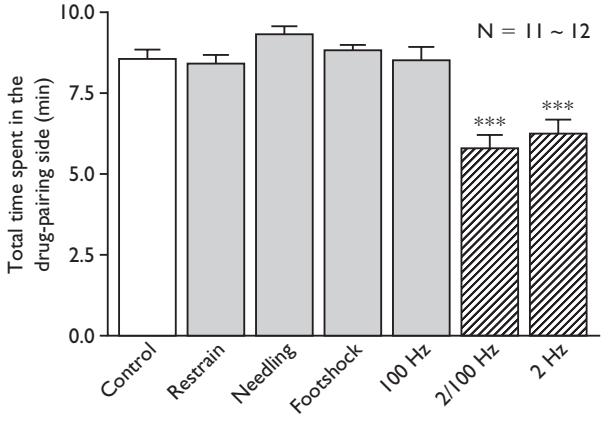

Fig. 3. Effect of peripheral electric stimulation on 4 mg/kg morphine-induced CPP. *** $p < 0.001$, compared with the four control groups as well as the group treated with 100 Hz stimulation.

difference from the CPP control group (8.76 ± 0.22 min). The CPP score of 2 and 2/100 Hz treated groups were only 6.20 ± 0.32 and 5.68 ± 0.31 min, respectively: significantly lower ($p < 0.001$) than the control groups. These results indicated that PES with a component of 2 Hz could specifically inhibit the expression of morphine-induced CPP.

Naloxone blockade: Fifty-seven rats were distributed randomly into six groups, with 9–10 in each group. After training with 4 mg/kg morphine for 10 days and 12 h before the final testing, groups 1 and 2 were given 2 Hz, groups 3 and 4 100 Hz stimulation, and groups 5 and 6 were given only needling as control. Groups 1, 3, and 5 were given naloxone (1 mg/kg, s.c.) and groups 2, 4, and 6 normal saline 15 min before PES or needling. The results are shown in Fig. 4. CPP scores of the 2 Hz + naloxone and 2/100 Hz + naloxone groups were 8.73 ± 0.56 and 8.88 ± 0.24 min, respectively, values which were significantly higher ($p < 0.001$) than their corresponding saline control (5.87 ± 0.32 and 5.68 ± 0.31 min, respectively), but were not significantly different from the saline + needling (8.60 ± 0.34 min) or naloxone + needling group (8.60 ± 0.18 min). These results showed that the effect of low-frequency PES on morphine-induced CPP could be blocked by pretreatment with naloxone.

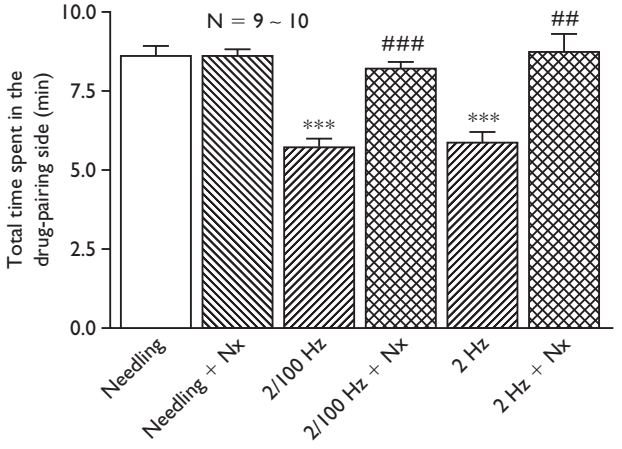

Fig. 4. Naloxone blockade of the inhibitory effect of peripheral electric stimulation on morphine-induced CPP. *** $p < 0.001$, compared with needling control group. ## $p < 0.01$ and ### $p < 0.001$, between the PES-treated groups and their corresponding PES + naloxone-treated groups.

DISCUSSION

The CPP paradigm: Some researchers [9,10] reported that operant action could facilitate CPP building. In the present study, we adopted this idea, to pre-expose the rat in the drug-pairing room for 5 min. This should help to make them realize that it is a prerequisite to enter the specific drug-pairing side in order to obtain the euphoria-inducing injection. Compared with the paradigm without the pre-exposure, the present procedure did facilitate CPP building (data not shown).

The animals' inherent preference for a novel environment is an important factor which cannot be neglected [11]. The most popular method to solve this problem is to expose the rats to both sides of the chamber for several sessions before conditioning. However, there is a latent inhibition in CPP paradigm building. 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 [12]. Martin-Iversion and Reimer [13] have shown that the amount of pre-exposure (habituation) can indeed influence the outcome of a CPP experiment. In their meta-analysis, Bardo *et al.* [14] found that the absence or presence of a pre-conditioning exposure can have a significant influence on the magnitude of the observed conditioning effects, such that in studies without pre-exposure larger effects were generally observed. In the present study, we chose to separate the chamber with a removable transparent clapboard, which permitted the rats to become familiar with the other side when they were confined in the drug-pairing side. We consider this an appropriate compromise between the problem of novelty and habituation.

Some researchers chose a fixed drug-pairing side while others counter-balanced the animal when they assigned the drug-pairing side. Both methods had their advantages and disadvantages (for review see [8]). Since our study showed that drug-naive rats had no preference for any side of our apparatus (data not shown), we decided to choose the former method to avoid unnecessary complexity of experimental design.

The control groups: It is well documented that stress has a strong influence on opiate addiction. Some researchers argued that stressors in the surrounding environment could induce craving in former addicts, which is one of the main causes of relapse (for review see [15]). During our electric stimulation procedure the rat must be kept in a cylindrical holder, with several needles inserted in both hind legs, and received electric stimulation of certain intensity. This procedure might include three stressors: restraining, needling, and electric stimulation. To control these influences, we thought to set three corresponding control groups. Our results showed that none of these

elements had any effect on the place preference induced by morphine. The results convinced us that the effect observed in our study is a specific effect of low frequency electric stimulation rather than stress. The PES protocol was successfully used for the suppression of morphine withdrawal syndrome in rats [16]. It is interesting to note in the present study that the effect of PES in modulating CPP induced by morphine is frequency dependent, rather than a generalized influence.

Possible mechanisms underlining the effect of electric stimulation: As shown above, PES containing a component of low frequency (2 and 2/100 Hz) could diminish the morphine-induced place preference in rats. Furthermore, this effect could be completely prevented by pre-injection of a moderate dose (1 mg/kg) of opioid receptor antagonist naloxone. The latter phenomenon suggests that PES might exert its effect via activation of opioid receptors, especially the μ receptor.

Previous studies in our laboratory have amply shown that PES of identified frequencies could mobilize different kinds of endogenous opioid peptides, acting on their corresponding receptors to induce analgesia [17]. At the spinal level, for example, low frequency (2 Hz) stimulation could increase the release of enkephalin which acts on μ and δ opioid receptors, while high frequency (100 Hz) stimulation could increase the release of dynorphin, which interacts with κ opioid receptors [7,17,18].

The brain areas participating in low- and high-frequency stimulation-induced analgesia are also different. The arcuate nucleus of hypothalamus where β-endorphin neurons aggregate has been shown to be the critical area that mediates the effect of low frequency stimulation, while the parabrachial nucleus of the pons plays a central role in mediating high frequency stimulation-induced analgesia [19–22].

Peripheral electric stimulation could also facilitate the biosynthesis of endogenous opioid peptides. For instance, Guo and his colleagues [23] reported that the production of preproenkephalin mRNA in arcuate nucleus started to increase 4 h after the electric stimulation, peaked at 24–48 h and decreased after 72 h. This can only be logically explained as a long-term activation of the enkephalinergic neurons.

It has been reported that the administration of opioid receptor antagonist, or stopping drug use, can lead to a slight withdrawal reaction after merely several or even one exposure to opiates. This phenomenon, termed acute withdrawal, consists mainly of psychological aversion without observable symptoms or physical signs [24,25]. Some authors argued that acute withdrawal was mainly mediated by the arcuate nucleus of hypothalamus [26]. Since the testing phase of our CPP paradigm occurred 24 h after the last drug-injection, the acute withdrawal might already present. Therefore, it is highly possible that the preference to the drug-pairing environment was due to a motivation to relieve this acute aversion.

Thus, peripheral electric stimulation of low frequency might relieve the acute aversion by activating enkephalinergic and β-endorphinergic neurons in the CNS, especially in the arcuate nucleus [17,19–22], to activate the μ and δ opioid receptors in these areas. This serves to remove the motivation of seeking exogenous opiates, thus inhibit the expression of CPP behavior. Since PES with high frequency (100 Hz) mainly increases the release of dynorphin in the spinal cord [7,22], which has little influence on acute withdrawal, it becomes obvious why 100 Hz stimulation had no effect on morphine-induced CPP.

The influence of CNS activation by PES is not limited to endogenous opioid system. It can also activate other transmitter systems such as cholecystokinin, norepinephrine, dopamine, serotonin, and many others (for review see [27]). The possible roles played by these transmitters systems as related to the effects mentioned above remain to be elucidated.

CONCLUSION

Peripheral electric stimulation with a component of low frequency (2 and 2/100 Hz) could inhibit the expression of morphine-induced conditioned place preference in rats. This effect is naloxone reversible, suggesting a mechanism involving the activation of opioid receptors by endogenous opioid ligands.

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