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Research Report

Peripheral electrical stimulation-induced suppression of morphine-induced CCP in rats: A role for dopamine in the nucleus accumbens

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ABSTRACT

In the previous study we reported that morphine-induced conditioned place preference (CPP) could be suppressed by peripheral electric stimulation (PES), an effect related to the increased gene expression of opioid peptides in the central nervous system. Considering that opioids were known to elevate dopamine (DA) activity in the mesolimbic brain, the present study was designed to further analyze the possible involvement of the mesolimbic dopaminergic system (MLDS) in the suppressive effect of PES on the rewarding effects of morphine in SD male rats. We found that morphine-induced CPP can be successfully suppressed by PES, an effect accompanied by a reversal of the increased tissue contents of DA and its metabolites in the nucleus accumbens (NAc) of morphine-induced CPP rats. Our results suggest that MLDS seems to play important roles in the mechanisms underlying PES's suppression of the rewarding effect of drug-associated environmental cues in the rat.

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1. Introduction

The conditioned place preference (CPP) paradigm involves Pavlovian learning. Pairing of unconditioned stimulus (e.g., reward) with a neutral context to become conditioned stimulus elicits approach behavior known as conditioned response. Drugs of abuse have long-lasting behavioral consequences, one of which is that drug-related behaviors can be elicited and maintained by stimuli associated with the effects of the drug. In the absence of the drug itself, these conditioned stimuli have been demonstrated to maintain drug-craving behavior in rats (Meil and Schechter, 1997; Ma et al., 2007). In human addicts, exposure to drug-associated environmental conditioned stimuli has been demonstrated to increase the urge to drug use (Ludwig and Stark, 1974; O'Brien et al., 1977). Drug-associated conditioned stimuli maintain their effectiveness long after the period of withdrawal from drugs in rats (Ma et al., 2007; Lu et al., 2005) and in humans (O'Brien et al., 1992). Most of the drugs abused in human beings produce CPP in animals. Thus, CPP has become a widely used animal model for the study of the rewarding properties of abused

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Abbreviations: CPP, Conditioned place preference; DOPAC, Dihydroxyphenyl acetic acid; DA, Dopamine; HANS, Han's Acupoint and Nerve Stimulator; HVA, Homovanillic acid; MLDS, Mesolimbic dopamine system; NAc, Nucleus accumbens; PES, Peripheral electric stimulation; PPD, Preprodynorphine; PPE, Preproenkephalin

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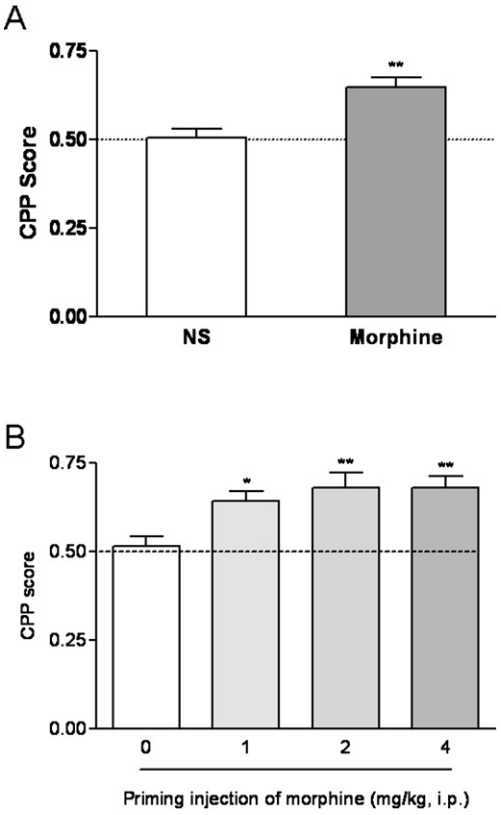

Fig. 1 – Expression and reinstatement of morphine CPP A, Expression of CPP induced by morphine (4 mg/kg, i.p.) The data were analyzed using student *t*-test, n = 11, 9 respectively. B. Reinstatement of extinguished CPP by priming injection of various morphine doses (0, 1, 2, 4 mg/kg, i.p.) The data were analyzed using one-way ANOVA followed by Dunnett's post-test, n = 9- 11 in each group. *P < 0.05, **P < 0.01, compared with the first group.

substances such as opiates and cocaine (Itzhak and Martin, 2002; Shi et al., 2004; Tzschentke, 1998). The CPP paradigm has also been proved a useful tool for testing medications or other approaches for their effects of anti-craving and anti-relapse to drugs of abuse (Bardo and Bevins, 2000).

The mesolimbic dopaminergic system (MLDS) serves a vital role in pathological behavioral changes that occur with repeated exposure to abusive drugs (Koob et al., 1998; Nestler, 2001; Wise, 2002). In the MLDS, dopaminergic neurons originated in the ventral tegmental area project to the nucleus accumbens (NAc), a key neural substrate that is implicated in the rewarding effects of and addiction to morphine and cocaine (Nestler, 2001; Wise, 2002). Opioid and other mu-opioid-receptor agonists increase dopamine (DA) release in terminal regions in the NAc by inhibiting GABAergic neurons in the VTA, which provide tonic inhibition of DA neurons, resulting in increased DA release in terminal regions (Di Chiara and Imperato, 1988; Di Chiara and North, 1992). Thus the overwhelming actions of DA in the NAc lead to neural adaptation that underlies addiction of drugs (Koob et al., 1998; Nestler, 2001).

PES has a great potential of being used for various medical purposes (Ulett et al., 1998). It is developed on the basis of traditional Chinese medicine and has been shown to be able to exert various effects in modulating the central nervous system. The acupoint ST36 (near the knee joint) and SP6 (near the ankle joint) are widely used stimulation points. They have been proved effective in the induction of analgesia (Han et al., 1999), the amelioration of withdrawal syndrome in heroin addicts (Wu et al., 1999), and the attenuation of the drug rewarding effect in morphine- and cocaine-conditioned rats (Shi et al., 2004; Ren et al., 2002). Considering the close interaction between opioid and DA systems in the central nervous system (Di Chiara and North, 1992; Shalev et al., 2002), it would be interesting to examine whether the DA system participates in the inhibitory effect of PES on morphine-induced CPP.

The principal objective of the study was to explore the effect of PES on the maintenance and the reinstatement of morphine CPP and to determine whether MLDS is involved in the effects of PES.

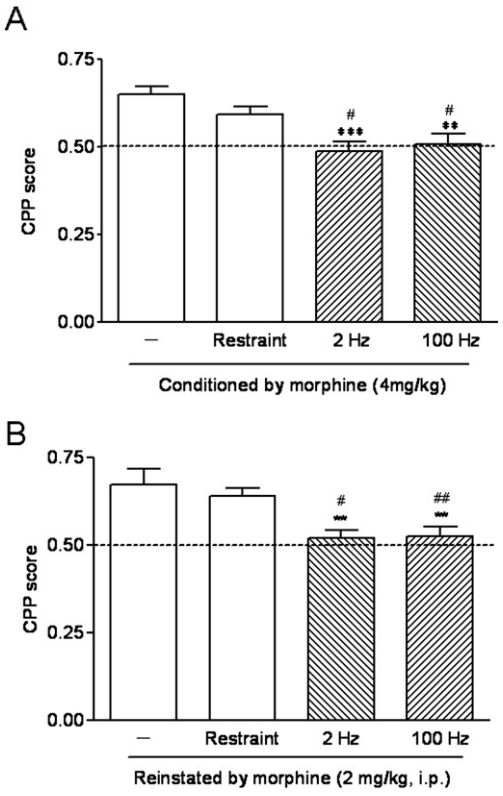

Fig. 2 – Effects of PES on CPP in rats A, Effect of PES on CPP maintenance 24 h after the CPP expression, PES was administrated once a day for 3 days. The effect of PES on the maintenance was tested 24 h after the final PES treatment. *n* = 8–10 in each group. B, Effect of PES on CPP reinstatement induced by priming injection of morphine CPP-extinguished rats received PES once a day for 3 days. The effect of PES on the reinstatement was tested 15-min after the priming injection of morphine in the following day, *n* = 9–11 in each group. The data were analyzed using one-way ANOVA followed by Newman–Keuls post-test, **P < 0.01, ***P < 0.001, compared with control group in the first column. #P < 0.05, ##P < 0.01, compared with restraint group in the second column.

2. Results

2.1. *Expression and reinstatement of morphine CPP*

The preconditioning test showed that animals spent almost equal amount of time in the two end chambers (A: 311±7.19 s, C: 314±6.72 s) and less time in the small center choice chamber (B: 274±10.84 s). There were no significant differences in the time spent in the two end chambers ($P>0.05$). Thus, the CPP apparatus were considered as unbiased in terms of chamber preferences of untreated rats.

Fig. 1A showed the result of CPP expression. Student *t*-test demonstrated a significant difference of CPP score between the rats conditioned by morphine/saline alternative injections *vs.* saline/saline injections [t (18)=3.885, $P=0.0011$], indicating the expression of morphine CPP.

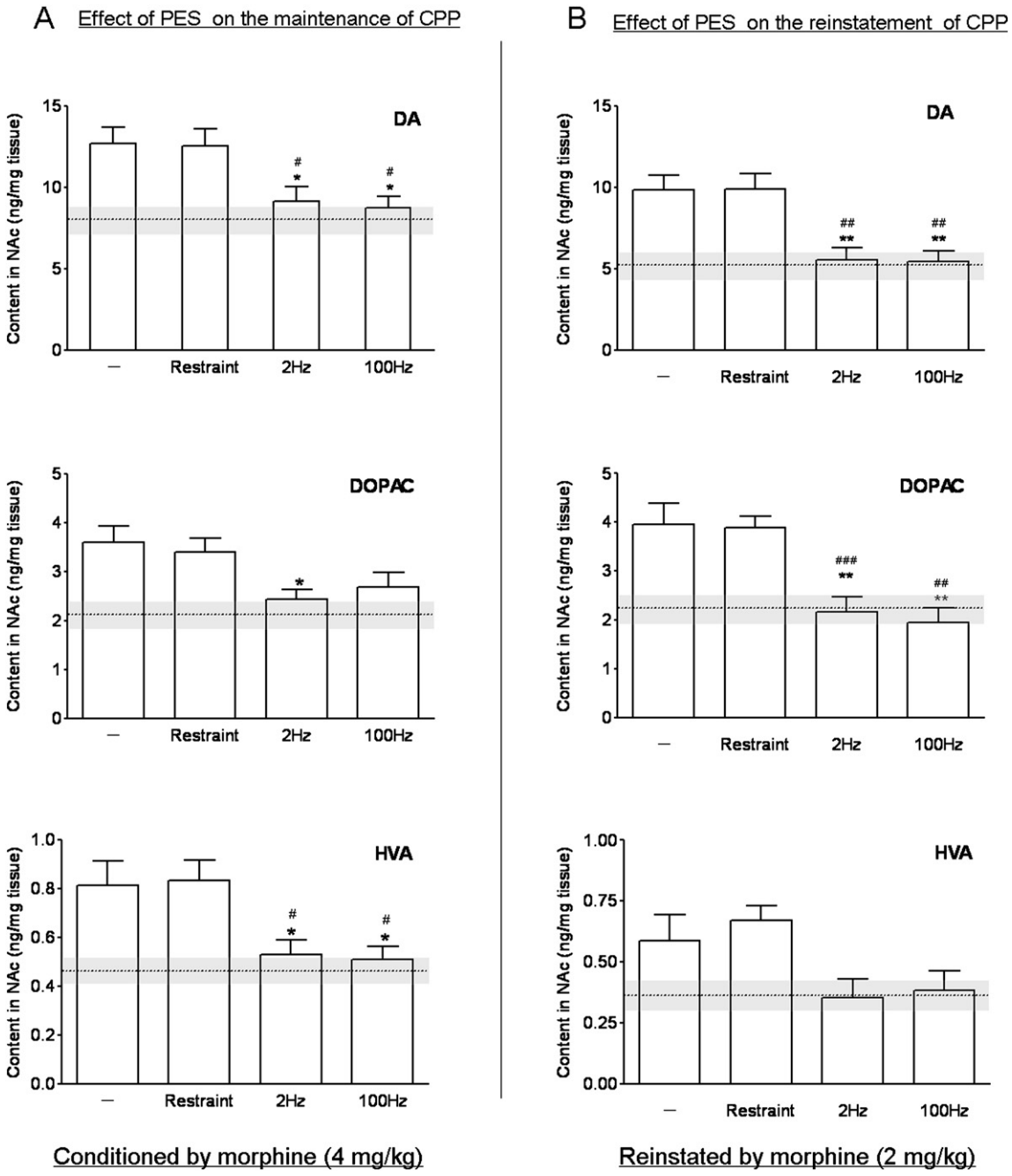

Fig. 3 – Effect of PES on the contents of DA and its metabolites HVA and DOPAC in the NAc of CPP rats. A, Values of rats receiving PES treatments in the maintenance of CPP, $n=5$–6 in each group; B, Values of CPP-distinguished rats receiving PES treatments, $n=7$–8 in each group. The data were analyzed using one-way ANOVA followed by Newman–Keuls post-test. *$P<0.05$, **$P<0.01$ compared with the first control group; #$P<0.05$, ##$P<0.01$, ###$P<0.001$ compared with the Restraint group. The dotted line showed the average of control rats and the shadow presented the range of mean±S.E.M of control rats conditioned by saline (A) or primed by saline (B).

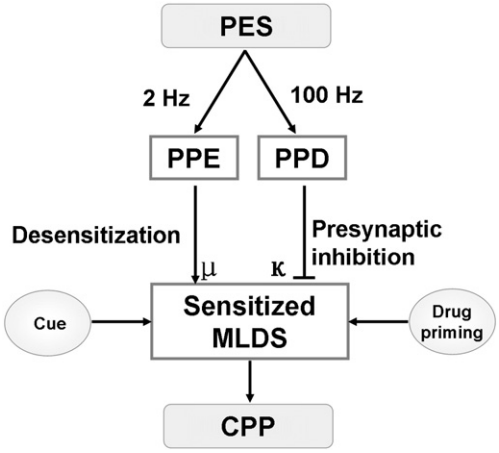

Fig. 4 – Diagram showing the cue-induced CPP or drug priming-induced reinstatement of CPP was suppressed by PES of different frequencies.

Twenty-four hours following the final extinction trial, rats were given morphine priming at doses of 0, 1, 2 or 4 mg/kg (i.p.) and tested for CPP. The results were shown in Fig. 1B. One-way ANOVA showed a significant effect of priming injection of morphine [F (3, 34) = 5.353, P = 0.0040]. Further Dunnett post-test demonstrated that priming injection of morphine at 1, 2, and 4 mg/kg can significantly reinstate the extinguished morphine CPP (*P < 0.05, **P < 0.01, compared with the first group).

2.2. *Effects of PES treatment on the maintenance and reinstatement of morphine CPP*

Twenty-four hours after the CPP expression (induced by 4 mg/kg morphine), 37 rats were randomly distributed into four groups of 8–10 each. Group 1 received no treatment serving as control; group 2 were merely restrained in the holder for 30 min, serving as control for restraint stress; and group 3 and group 4 were given 2 Hz and 100 Hz PES, respectively once a day for three consecutive days. All groups were tested again 24 h after the final PES treatment. One-way ANOVA showed a significant effect of PES treatments on CPP maintenance [F (3, 33) = 8.592, P = 0.0002]. Further Newman–Keuls post-test demonstrated that the morphine-induced CPP was significantly suppressed by PES at 2- or 100-Hz, but not by restraint (Fig. 2A).

In order to investigate the effects of PES treatment on the reinstatement of extinguished CPP, 40 rats were randomly distributed into four groups, with 9–11 in each group. Twenty-four hours following the establishment of the CPP (induced by 4 mg/kg morphine), the rats were given extinction trials for 7 days. As described previously, morphine CPP disappeared by day 7 (Shi et al., 2004). From days 8 to 10, three groups received restraint, 2- or 100-Hz PES treatment, respectively once a day for three consecutive days. The other group received no special treatment serving as a control. On day 11, all the four groups were administered with a priming injection of morphine at 2 mg/kg (i.p.) 15 min before the testing for CPP. One-way ANOVA showed a significant effect of PES treatments [F (3, 34) = 5.353, P = 0.0040]. Further Newman–Keuls post-test demonstrated that the CPP reinstated by priming injection of morphine (2 mg/kg, i.p.) can be suppressed by PES of either 2- or 100-Hz, but not by restraint (Fig. 2B).

2.3. *Effect of PES on the contents of DA and its metabolites in the NAc during the suppression of maintenance and reinstatement of morphine-induced CPP*

The results depicted in Fig. 3A showed a 25–35% decrease of the contents of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the NAc of rats receiving PES of 2-or 100-Hz, compared to those in the control group or restrained rats during the maintenance of morphine-induced CPP. One-way ANOVA showed significant differences of the content of DA [F (3, 20) = 5.106, P = 0.0087], DOPAC, F (3, 20) = 3.902, P = 0.0241] and HVA, F (3, 19) = 5.117, P = 0.0092]. The results depicted in Fig. 3B showed a 40–50% decrease of the contents of DA and its metabolites DOPAC and HVA in the NAc of rats receiving PES of 2-Hz or 100-Hz, compared to those in the control group or restrained rats after reinstatement by morphine (2 mg/kg, i.p.). One-way ANOVA showed significant differences of the contents of DA [F (3, 21) = 9.046, P = 0.0005], DOPAC [F (3, 21) = 11.16, P = 0.0001] and HVA [F (3, 24) = 3.539, P = 0.0298].

3. Discussion

Results of the present study showed that multiple treatments with 2-or 100-Hz PES inhibit the maintenance and the reinstatement of morphine CPP, thus replicating the results published by Shi et al in 2003 and 2004. Inhibition of the maintenance and the reinstatement of morphine CPP suggested blockage of the conditioned response of CPP rats to morphine-associated environmental cues at the early stage of CPP maintenance and to drug priming at CPP reinstatement. The new finding in the present study was that while the content of DA in the NAc was significantly increased in rats showing a profound expression of CPP, it dropped to the baseline level when the CPP was blocked by 2-or 100-Hz PES. Accompanying with the decrease in the content of DA, there was a simultaneous decrease of its metabolites DOPAC and HVA down to the baseline level, suggesting a total quenching of the MLDS activities.

It should be emphasized that the testing of the effects of PES on the maintenance and the reinstatement of CPP in the current experiments was performed 24 h after the final session of PES, and the assessment of brain content of DA was performed 1 h after the CPP testing. So the phenomenon observed in the present study was the manifestation of a delayed or long lasting, rather than an acute or short term effect of PES. Zhu et al. (1997), using microdialysis technique, found that the immediate effect of PES was an acceleration of DA release, which seems difficult to explain the results observed in the present study.

What is the dominant factor to increase the content of DA and its metabolites in the NAc of morphine-induced CPP rats? Two factors became obvious to us. One is the pharmacokinetics of morphine, and the other is the environmental conditioning. Our further experiment was designed to observe the effect of morphine on DA metabolism in the NAc. Rats were given daily alternative injections of morphine and saline for 4 days, in the same way as that in the CPP paradigm. The only

difference was that the injections were made in the animal room with the rats returned to their home cage right after the injection. No significant increase was observed in the content of DA and its metabolites in the NAc (data to be published). These results clearly indicate that the activation of MLDS could only be attributable to the consequence of conditioning (i.e. drug-associated cues) rather than the residual effect of the drug. The present data showed after the PES treatment, the drug-associated cues lost its capability to activate MLDS as well as to maintain CPP (Fig. 2). One could postulate that the inhibitive effect of PES on the maintenance of CPP might result from (1) a blockade of the pathway connecting the cue perception toward the MLDS and/or (2) the direct inhibition of the MLDS. The results shown in Fig. 2B indicate that after the PES treatment, not only the environmental cue, but also the drug priming lost its capability to induce reinstatement, suggesting a powerful reversal of the activation of the MLDS in morphine-induced CPP rats.

Using the reinstatement model of intravenous self-administration of heroin, De Vries et al. (1999) found that in animals with a heroin history, priming with indirect DA agonists (cocaine and amphetamine) resulted in a robust reinstatement of drug seeking for heroin, suggesting that MLDS was involved in the initiation of drug-seeking behavior. In our laboratory, Wang et al. (2000b) also reported that the extinguished morphine CPP could be reinstated by a very small dose (0.25 mg/kg) of morphine or amphetamine. Further study using central lesion technique (electric lesion in the NAc and neurotoxin lesion in the NAc shell by 6-OHDA) revealed that the functional integrity of the MLDS was indispensable for drug priming-induced reinstatement of extinguished morphine CPP. However, to our knowledge, no direct evidence has been reported for the status of activity of the MLDS during reinstatement of drug-seeking behavior. We therefore investigated the contents of DA and its metabolites DOPAC, HVA in the NAc of reinstated rats, and found an obvious up-regulation of the contents of DA and its metabolites in the NAc of rats reinstated by a priming injection of morphine.

In a previous study (Shi et al., 2004) we observed that three consecutive sessions of 2 Hz PES significantly augmented the level of preproenkephalin (PPE) mRNAs and three consecutive sessions of 100 Hz PES significantly augmented the level of preprodynorphine (PPD) mRNAs in the NAc of the reinstated CPP rats. Results obtained in the present study showed that, unlike in the reinstated rats where the MLDS was in an agitated status, in rats treated with PES for 3 days the contents of DA and its metabolites in the NAc remained in the control level. In other words, the suppression of drug-priming-induced CPP reinstatement (behavior expression) was accompanied by an abolishment of the MLDS response (neurochemical indices). Taking together we could summarize that three consecutive sessions of PES produced three consequences: (1) a suppression of the CPP (Fig. 2), which has previously shown to be naloxone reversible (Wang et al., 2000a), (2) an increased expression of PPE and PPD (Shi et al., 2004), and (3) a blockade of MLDS activation (Fig. 3). What then is the internal relationship among these three events?

It is rational to postulate that while both 2 Hz and 100 Hz PES produce similar effects of suppressing the CPP, their mechanisms may not be the same. It has been shown that single session of 100 Hz PES increased the release of dynorphin in the central nervous system (Han, 2003). Repeated 100 Hz PES further increased the PPD gene expression (Shi et al., 2004). An increased release of dynorphin in the NAc as triggered by 100 Hz PES would activate presynaptic kappa-opioid receptor in the NAc, leading to a presynatic inhibition of the release of DA in the NAc. This would mimic the recent finding that exogenously administered kappa-opioid agonist suppressed morphine CPP (Tao et al., 2006).

Concerning the possible mechanisms underlying the blockade of CPP by 2 Hz PES, the following findings should be taken into account. (1) This effect was sensitive to low dose of naloxone (Wang et al., 2000a), suggesting the involvement of mu-opioid receptor; (2) 2 Hz PES stimulation accelerated the release of enkephalin and endorphin rather than dynorphin (Han, 2003); (3) repeated 2 Hz PES stimulations augmented the expression of mRNA encoding PPE (Shi et al., 2004); (4) clinical observation indicated that previous heroin addicts on their first 48 h of drug abstinence were very sensitive to drug (one inhalation is enough to produce euphoria) or endogenously released opioid peptides, as shown by the following finding that one session (30 min) of Han's Acupoint and Nerve Stimulator (HANS) stimulation often leads to a considerable degree of euphoria, although the second session of HANS may not produce such a dramatic effect (Wu LZ, personal communication). Taking together, one could postulate that abstinence of opiate drug leads to the sensitization for opioid receptors, whereas slow (up to 5–10 h), continuous release of enkephalin may cause desensitization, resulting in a lowered response of the MLDS to priming dose of morphine. Indeed, it was speculated that behavioral sensitization played an important role in the development of addiction, especially in the high rate of relapse observed in drug addicts even after a long period of abstinence (Gaiardi et al., 1991). A picture depicting intravenous drug injection is nonsensical for normal persons, yet it produces dramatic cognitive changes (craving) in drug addicts (Zhong et al., 2006), which might be backed by a marked dopaminergic alterations, representing a status of hypersensitivity of MLDS. Three consecutive sessions of 2 Hz PES at low intensity may stimulate the biosynthesis and release of enkephalin (Shi et al., 2004), which interacts with mu-opioid receptor, resulting in the abolishment of the status of sensitization.

It has been suggested that rats receiving any manipulation that leads to memory dysfunction would spend equal time in the morphine vs. saline compared-zone. It has been reported that beta-endorphin reversed pentylenetetrazol-induced amnesia (Baratti et al., 1990), and dynorphin improve amnesia induced by beta-amyloid peptide (Hiramatsu et al., 2000), carbon monoxide (Hiramatsu et al., 1997), cycloheximide (Ukai et al., 1996), galanin (Kameyama et al., 1994) and basal forebrain-lesion (Ukai et al., 1993). The antiamnesic effect of endorphin and dynorphin could be reversed (Baratti et al., 1990; Hiramatsu et al., 1997; Ukai et al., 1996; Itoh et al., 1993) or partially reversed (Hiramatsu et al., 2000) by opioid-receptor antagonists. These studies suggested that the activation of opioid-receptor system could prevent rather than cause amnesia. Since EA of 2 or 100 Hz activated the endogenous mu- and kappa-opioid-receptor pathway, it seems impossible for EA to result in amnesia. The consequences of electrical stimulation in memory tasks may be not universal and can depend on the type of task, conditioned stimulation modality, and type of reflex measured in conditioning. The clinical research on the patients suffered from depression showed that the electroconvulsive shock not only activates the opioid pathway, but also induces retrograde amnesia. In addition, it was

shown that the retrograde amnesia induced by electroconvulsive shock was reversed by opioid antagonists (Prudic et al., 1999). However, Warner-Schmidt et al. (2008) reported that electroconvulsive shock restores neurogenesis and hippocampus-dependent fear memory after disruption by irradiation. Thus the relationship between opioid systems and amnesia induced by electrical stimulation seems not straightforward. In order to exclude the alternative explanation of the inhibitory effects of PES on morphine-induced CPP and its reinstatement by disrupting of association, further study was designed to explore the effect of PES on the CPP induced by natural rewards. Our results showed that confined to the same lateral chamber (familiar side) throughout the 4-day conditioning, rats showed significantly place preference to the other lateral chamber (novel side) during the CPP test. PES treatments had no effect on the novel-environment induced CPP. It was suggested that PES has no impairment on the association in the CPP paradigm. Moreover, experiments from our lab also demonstrated that test of spatial leaning and memory ability using the Morris water maze task revealed that PES of 2 Hz (Chen et al., 2005) and 100 Hz (unpublished data) per se exhibited promoting, rather than a deteriorating effects on the ability of spatial memory. Finally, in our study of reinstatement of cocaine CPP, rats reinstated by drug priming, even pretreated by EA, preferred to the previous cocaine-paired chambers. Consequently, PES is indicated of no impairment on association, making the "memory dysfunction" interpretation unlikely.

In conclusion, our results clearly demonstrated that three consecutive treatments with 2-or 100-Hz PES blocked the dopaminergic response to both environmental cues and drug priming, most probably through the desensitization or deactivation of the MLDS activity (summarized in Fig. 4). The results suggest that PES may serve as a potential therapy in decreasing drug craving and for the prevention of relapse for drug use.

4. Experimental procedures

4.1. *Animal*

All experiments were performed on male Sprague–Dawley rats, obtained from the Peking center of experimental animals, weighing 180–220 g at the beginning of the experiment. Animals were housed 4 per cage in a 12:12 h light/dark cycle (lights on at 07:00) with food and water available at all times. The room temperature was maintained at 22 ± 1 °C and relative humidity at 45–50%. Animals were conditioned and tested during the light phase of the cycle. They were handled daily during the first week after arrival. All experimental procedures were approved by the Animal Use Committee of the Peking University Health Science Center.

4.2. *Conditioned place preference*

4.2.1. *Apparatus*

Conditioning was conducted in black colored rectangular PVC boxes (795 × 230 × 250 mm^3), containing three chambers separated by guillotine doors (Ma et al., 2006, 2007; Shi et al., 2004). The two large black conditioning chambers (A and C, 280 × 220 × 225 mm^3) were separated by a small gray center choice chamber B (135 × 220 × 225 mm^3). Chamber A has 4 light-emitting diodes (LEDs) forming a square on the wall and a stainless-steel mesh floor (22.5 × 22.5 mm^2), chamber C has 4 LEDs forming a triangle on the wall and a stainless-steel rod floor (15 mm apart), whereas chamber B has a gray wooden floor. Fourteen photobeams were placed across chambers with 47.5 mm apart. Through a computer interface, the time spent for the rat in each chamber was recorded by means of infrared beam crossings.

4.3. *Development and expression of CPP*

The methods of CPP have been described in details previously (Shi et al., 2004). Briefly, animals received a single preconditioning test in which they were placed in the center choice chamber with the guillotine doors removed to allow access to the entire apparatus for 15 min. The amount of time spent in each chamber was monitored and used to assess natural preferences. The next day, rats were assigned to receive saline or morphine (4 mg/kg) paired with one of the two conditioning environments in a counterbalanced manner (the 'unbiased' procedure). All animals were allocated to stay for a period of 45-min in the lateral chambers twice daily (09:00 and 15:00) for 4 days, with the saline group receiving 0.9% sodium chloride injections on both sides of the boxes, whereas the morphine group received morphine injection on one side and saline on the other side. Morphine-paired sides were counterbalanced among all groups. The center choice chamber was never used during conditioning and was blocked by guillotine doors. After the 4-day conditionings, rats were placed in the center choice chamber with access to the entire apparatus for 15 min. The time spent in each chamber was recorded.

4.4. *Reinstatement of CPP extinguished by extinction testing*

Frequent place preference testing without rewards would accelerate the spontaneous extinction of already established CPP. Our previous data showed that 7 days was enough for CPP rats to lose the preference to the drug-paired side (Shi et al., 2004). After conditioning and the following initial CPP test, rats were given 15-min tests once a day for 7 days. No injections were given during this extinction period. The day following the last extinction trial, rats received a priming injection of morphine (1, 2 and 4 mg/kg, i.p.) and were placed in the center choice chamber with access to the entire apparatus for 15 min. The time spent in each chamber was recorded.

4.5. *Peripheral electric stimulation*

Rats were kept in specially designed holders, with their hind legs and tails exposed (Han et al., 1991). Two stainless needles of 0.3 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 electrical stimulation produced by a programmed pulse generator (HANS LH-800, produced by Peking University of Astronautics and Aeronautics Aviation) was given via the two needles for a total of 30 min. The frequency of stimulation used was 100 Hz (0.2 ms pulse width) or 2 Hz (0.6 ms pulse width). The intensity of the

stimulation was increased stepwise from 0.5 to 1 mA and 1.5 mA, with each step lasting for 10 min.

Since previous studies have shown that PES was effective to suppress morphine-induced CPP only when it was administered consecutively for three times (Shi et al., 2003), this procedure was followed in the present study.

4.6. *Tissue dissection and preparation*

Rats were decapitated 1 h after the CPP test. The brains were removed and placed on an ice-cooled plate for dissection of the NAc according to stereotaxin atlas of Paxinos and Watson (1986). Immediately afterwards, the tissue samples were weighed and placed in 1.5 ml plastic tubes containing ice-cooled perchloric acid (200 μl, 0.4 M), homogenized for 10-sec using ultrasound (0.5 Hz) and centrifuged for 20 min at 15,000 ×*g* at 4° C. The supernatant was passed through a 0.2 μm filter and kept at 4 °C until HPLC analysis.

4.7. *HPLC analysis for DA and its metabolites*

DA and its metabolites DOPAC and HVA were analyzed using reversed-phase ion-pair chromatography combined with electrochemical detection under isocratic conditions (Teismann and Ferger, 2001). The six-channel detector potentials were set at +50, 100, 200, 300, 400, 500 mV using a glassy carbon electrode and an Ag/AgCl reference electrode. The mobile phase (0.6 mM 1-octanesulfonic acid, 0.27 mM Na_2EDTA, 0.043 M triethylamine and 50 ml acetonitrile/l, adjusted to pH 2.95 with H_3PO_4) was delivered at a flow rate of 0.5 ml/min at 22 °C onto the reversed-phase column (125 mm × 3 mm with pre-column 5 mm × 3 mm, filled with Nucleosil 120–3 C18, Knauer, Berlin, Germany). Ten microliters aliquots were injected by an autoinjector with cooling module set at 4 °C. Data were calculated by an external standard calibration.

4.8. *Statistical analysis*

CPP score represents the index of place preference for each rat, calculated by dividing the time spent in the drug-paired compartment by the time spent in both conditioning compartments (Shi et al., 2003). Data were processed by commercially available software Graph Pad Prism 4.0. Results were presented as mean ± S.E.M. and analyzed with student *t*-test (Fig. 1A), one-way ANOVA followed by Dunnett post-test (Fig. 1B) or by Newman–Keuls (Figs. 2, 3) The accepted level of statistical significance is $P < 0.05$.

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REFERENCES

Baratti, C.M., de Erausquin, G.A., Faiman, C.P., 1990. Brain opioid peptides may participate in the reversal of pentylenetetrazol-induced amnesia. Methods Find Exp. Clin. Pharmacol. 12, 451–456.

Bardo, M.T., Bevins, R.A., 2000. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berl) 153, 31–43.

Chen, J.H., Liang, J., Wang, G.B., Han, J.S., Cui, C.L., 2005. Repeated 2 Hz peripheral electrical stimulations suppress morphine-induced CPP and improve spatial memory ability in rats. Exp. Neurol. 194, 550–556.

De Vries, T.J., Schoffelmeer, A.N., Binnekade, R., Vanderschuren, L.J., 1999. Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal of IV drug self-administration. Psychopharmacology (Berl) 143, 254–260.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl. Acad. Sci. U. S. A. 85, 5274–5278.

Di Chiara, G., North, A.R., 1992. Neurobiology of opiate abuse. Trends Pharmacol Sci 13, 185–193.

Gaiardi, M., Bartoletti, M., Bacchi, A., Gubellini, C., Costa, M., Babbini, M., 1991. Role of repeated exposure to morphine in determining its affective properties: place and taste conditioning studies in rats. Psychopharmacology (Berl) 103, 183–186.

Han, J.S., 2003. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies Trends Neurosci. 26, 17–22.

Han, J.S., Chen, X.H., Sun, S.L., Xu, X.J., Yuan, Y., Yan, S.C., Hao, J.X., Terenius, L., 1991. Effect of low-and high-frequency TENS on Met-enkephalin-Arg-Phe and dynorphin A immunoreactivity in human lumbar CSF. Pain 47, 295–298.

Han, Z., Jiang, Y.H., Wan, Y., Wang, Y., Chang, J.K., Han, J.S., 1999. Endomorphin-1 mediates 2 Hz but not 100 Hz electroacupuncture analgesia in the rat. Neurosci. Lett. 274, 75–78.

Hiramatsu, M., Sasaki, M., Nabeshima, T., Kameyama, T., 1997. Effects of dynorphin A (1–13) on carbon monoxide-induced delayed amnesia in mice. Pharmacol. Biochem. Behav. 56, 73–79..

Hiramatsu, M., Inoue, K., Kameyama, T., 2000. Dynorphin A-(1–13) and (2–13) improve beta-amyloid peptide-induced amnesia in mice. NeuroReport 11, 431–435.

Itoh, J., Ukai, M., Kameyama, T., 1993. U-50,488H, a kappa-opioid receptor agonist, markedly prevents memory dysfunctions induced by transient cerebral ischemia in mice. Brain Res. 619, 223–228.

Itzhak, Y., Martin, J.L., 2002. Cocaine-induced conditioned place preference in mice: induction, extinction and reinstatement by related psychostimulants. Neuropsychopharmacology 26, 130–134.

Kameyama, T., Ukai, M., Miura, M., 1994. Dynorphin A-(1–13) potently improves galanin-induced impairment of memory processes in mice. Neuropharmacology 33, 1167–1169.

Koob, G.F., Sanna, P.P., Bloom, F.E., 1998. Neuroscience of addiction. Neuron 21, 467–476.

Lu, L., Chen, H., Su, W., Ge, X., Yue, W., Su, F., Ma, L., 2005. Role of withdrawal in reinstatement of morphine-conditioned place preference. Psychopharmacology (Berl) 181, 90–100.

Ludwig, A.M., Stark, L.H., 1974. Alcohol craving subjective and situational aspects. Q. J. Stud. Alcohol 35, 899—905.

Ma, Y.Y., Guo, C.Y., Yu, P., Lee, D.Y., Han, J.S., Cui, C.L., 2006. The role of NR2B containing NMDA receptor in place preference conditioned with morphine and natural reinforcers in rats. Exp. Neurol. 200, 343–355.

Ma, Y.Y., Chu, N.N., Guo, C.Y., Han, J.S., Cui, C.L., 2007. NR2B-containing NMDA receptor is required for morphine-but not stress-induced reinstatement. Exp. Neurol. 203, 309–319.

Meil, W.M., Schechter, M.D., 1997. Olanzapine attenuates the reinforcing effects of cocaine. Eur. J. Pharmacol. 340, 17–26.

Nestler, E.J., 2001. Molecular basis of long-term plasticity underlying addiction. Nat. Rev., Neurosci. 2, 119–128.
O'Brien, C.P., Testa, T., O'Brien, T.J., Brady, J.P., Wells, B., 1977. Conditioned narcotic withdrawal in humans. Science 195, 1000–1002.
O'Brien, C.P., Childress, A.R., McLellan, A.T., Ehrman, R., 1992. Classical conditioning in drug-dependent humans. Ann. N. Y. Acad. Sci. 654, 400–415.
Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxin Coordinates, (2nd Ed.). Academic Press, Pub New York.
Prudic, J., Fitzsimons, L., Nobler, M.S., Sackeim, H.A., 1999. Naloxone in the prevention of the adverse cognitive effects of ECT: a within-subject, placebo controlled study Neuropsychopharmacology 21, 285–293.
Ren, Y.H., Wang, B., Luo, F., Cui, C.L., Zheng, J.W., Han, J.S., 2002. Peripheral electric stimulation attenuates the expression of cocaine-induced place preference in rats. Brain Res. 957, 129–135.
Shalev, U., Grimm, J.W., Shaham, Y., 2002. Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev. 54, 1–42.
Shi, X.D., Ren, W., Wang, G.B., Luo, F., Han, J.S., Cui, C.L., 2003. Brain opioid-receptors are involved in mediating peripheral electric stimulation-induced inhibition of morphine conditioned place preference in rats. Brain Res. 981, 23–29.
Shi, X.D., Wang, G.B., Ma, Y.Y., Ren, W., Luo, F., Cui, C.L., Han, J.S., 2004. Repeated peripheral electrical stimulations suppress both morphine-induced CPP and reinstatement of extinguished CPP in rats: accelerated expression of PPE and PPD mRNA in NAc implicated. Brain Res. Mol. Brain Res. 130, 124–133.
Tao, P.L., Liang, K.W., Sung, W.Y., Wu, Y.T., Huang, E.Y., 2006. Nalbuphine is effective in decreasing the rewarding effect induced by morphine in rats. Drug Alcohol Depend. 84, 175–181.
Teismann, P., Ferger, B., 2001. Inhibition of the cyclooxygenase isoenzymes COX-1 and COX-2 provide neuroprotection in the MPTP-mouse model of Parkinson's disease. Synapse 39, 167–174.
Tzschentke, T.M., 1998. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog. Neurobiol. 56, 613–672.
Ukai, M., Kobayashi, T., Kameyama, T., 1993. Dynorphin A-(1–13) attenuates basal forebrain-lesion-induced amnesia in rats. Brain Res. 625, 355–356.
Ukai, M., Shan-Wu, X., Kobayashi, T., Kameyama, T., 1996. Systemic administration of dynorphin A-(1–13) markedly improves cycloheximide-induced amnesia in mice. Eur. J. Pharmacol. 313, 11–15.
Ulett, G.A., Han, S., Han, J.S., 1998. Electroacupuncture: mechanisms and clinical application. Biol. Psychiatry 44, 129–138.
Wang, B., Luo, F., Xia, Y.Q., Han, J.S., 2000a. Peripheral electric stimulation inhibits morphine-induced place preference in rats. NeuroReport 11, 1017–1020.
Wang, B., Luo, F., Zhang, W.T., Han, J.S., 2000b. Stress or drug priming induces reinstatement of extinguished conditioned place preference. NeuroReport 11, 2781–2784.
Warner-Schmidt, J.L., Madsen, T.M., Duman, R.S., 2008. Electroconvulsive seizure restores neurogenesis and hippocampus-dependent fear memory after disruption by irradiation. Eur J Neurosci. 27, 1485–1493.
Wise, R.A., 2002. Brain reward circuitry: insights from unsensed incentives. Neuron 36, 229–240.
Wu, L.Z., Cui, C.L., Tian, J.B., Ji, D., Han, J.S., 1999. Suppression of morphine withdrawal by electroacupuncture in rats: dynorphin and kappa-opioid receptor implicated Brain Res. 851, 290–296.
Zhong, F., Wu, L.Z., Han, J.S., 2006. Suppression of cue-induced heroin craving and cue-reactivity by single-trial transcutaneous electrical nerve stimulation at 2 Hz Addict. Biol. 11, 184–189.
Zhu, C.B., Li, X.Y., Zhu, Y.H., Wu, G.C., Xu, S.F., 1997. Alteration of monoamine contents in microdialysate following droperidol enhanced electroacupuncture. Sheng Li Xue Bao 49, 382–388.