·论著·

Inhibition by peripheral electric stimulation of the reinstatement of morphine-induced place preference in rats and drug-craving in heroin addicts

WANG Bin, ZHANG Benguo, GE Xuecai, LUO Fei, HAN Jisheng△
(Neuroscience Research Institute and Department of Neurobiology, Peking University, Beijing 100083, China)

KEY WORDS Morphine; Heroin dependence; Conditioned place preference; Drug craving; Transcutaneous electric nerve stimulation

SUMMARY **Objective:** To test the hypothesis that peripheral electric stimulation (PES) may suppress the reinstatement of morphine-induced conditioned place preference (CPP) in rats as well as the drug craving of detoxified heroin addicts in a frequency-dependent manner. **Methods:** CPP model of the rat was constructed with two compartment automatic CPP apparatus, and the craving of the heroin addicts was assessed with a visual analogue scale (VAS). **Results:** (1) PES of low frequency could prevent the drug priming- or foot shock-induced reinstatement of morphine CPP; (2) this effect was naloxone-reversible, suggesting a possible involvement of endogenous opioid mechanisms; and (3) PES of low frequency could also accelerate the rate of natural decay of drug craving in heroin addicts after successful abstinence. **Conclusion:** PES might serve as a therapeutic measure for the treatment of heroin addiction. *(J Peking Univ [Health Sci], 2003, 35: 241-247)*

外周电刺激抑制大鼠吗啡条件性位置偏爱的复发及海洛因成瘾者的心瘾

王 玢,张本国,葛学采,罗 非,韩济生△
(北京大学神经科学研究所,北京大学基础医学院神经生物学系,北京 100083)

[关键词] 吗啡;海洛因依赖;条件性位置偏爱;心瘾;经皮神经电刺激

[摘 要] 目的:探讨外周电刺激(PES)是否能频率依赖性地抑制吗啡条件性位置偏爱(CPP)的复发和抑制海洛因成瘾者脱毒后的心瘾。方法:用二室自动 CPP 箱记录大鼠条件性 CPP,用视觉模拟尺测量海洛因成瘾者的"心瘾(渴求)"。结果:(1)低频 PES 能抑制大鼠小剂量吗啡点燃、或脚底电刺激诱发的吗啡 CPP;(2)上述效应可被小剂量吗啡受体拮抗剂纳洛酮(1 mg·kg^{-1})翻转,提示有内源性阿片机制参与;(3)低频 PES 还能使海洛因成瘾者脱毒后对毒品"心瘾"的自然消退过程加速。结论:外周神经电刺激可能是治疗海洛因成瘾的一种有效方法。

[中图分类号] R338 [文献标识码] A [文章编号] 1671-167X(2003)03-0241-07

Drug addiction involves not only the acquisition and maintenance of drug-using behavior, but also the reinstatement of this behavior after successful abstinence. High rate of relapse after long period of abstinence characterizes the behavior of experienced users of heroin and other drugs of abuse (Jaffe, 1990). Over the past several decades, most of the studies were focusing on the neurobiological mechanisms of drug reward, which was viewed as a central factor in drug abuse[1,2]. Only recently had scientists begun to focus on the neurobiology of relapse, a better understanding of which could lead to more effective treatment strategies for addictive disorders.

Drug craving is a subjective description that can only be measured in human subjects. However, reinstatement of an operant event can be directly measured when a laboratory animal reinitiates a particular behavioral response. This reinstatement is thought to be able to mimic the induction of drug seeking following extinction from drug using. A model of the reinstatement of self-administration had been built by Shaham[3,4] and co-workers using either a small (priming) dose of morphine or a brief exposure to foot shock stress. Another putative animal model for this purpose is the reinstatement of conditioned place preference (CPP), reported simultaneously by Parker

基金项目:国家重点基础研究规划项目"脑功能和重大疾病的基础研究"(G1999054000),基础研究重大项目前期研究专项"阿片成瘾与复吸的神经机制及非药物抗复吸的研究"及美国国立卫生研究院基金资助 Supported by the National Basic Research Program of China (G1999054000), Department of Science and Technology of China (Grant 2001CCA01200) and NIDA, NIH, USA (DA 03983)

△Corresponding author email, hanjisheng@bjmu.edu.cn

© 1995-2004 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

and McDonald[5], Lu and co-workers[6] and a team in our lab[7]. All these models can be employed for the study of human relapse behavior. But until now, few studies were reported aiming at the possible prevention of relapse on these paradigms.

Our previous works had shown that peripheral electric stimulation (PES), especially those with a component of low frequency (2 Hz), could block the expression of CPP induced by morphine[8] in a naloxone-reversible manner. Other studies in our lab have amply shown that PES of high frequency (100 Hz) can suppress morphine withdrawal syndrome both in rats[9,10] and heroin addicts[11,12]. Hence it becomes interesting to test the hypothesis whether PES could also prevent the reinstatement of morphine-induced CPP in laboratory animals, or more directly, to diminish drug craving in heroine addicts.

1 Animal experiment

1.1 Materials and Methods

1.1.1 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 per cage, with the room temperature maintained at (24 ±1) ℃, relative humidity at 50 %, under a 12 ∶12 h light-dark cycle. The experimental procedures were approved by the Committee of Animal Care and Use of Peking University.

1.1.2 Drugs Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang, China. Naloxone HCl was purchased from SIGMA Co., Ltd. All drugs were dissolved in 0.9 % saline to their final concentrations.

1.1.3 Conditioned place preference The methods of CPP paradigm have been described in detail elsewhere[8]. Briefly, conditioning took place in one of the two distinct environments, which differed in color and texture and separated by a removable transparent 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 white background, and the flooring material was 1 cm thick of sawdust. The later was used as the drug-paring room. In the acquisition phase, rats were trained once a day for 10 days by giving intraperitoneal (i.p.) injection of morphine (4 mg·kg^{-1}) 5 min after the animals were put into the drug-pairing area, where they were kept for another 15 minutes. On the 11th day, the transparent clapboard was removed, and the rats were scored during a 10-min test session with their time spared in drug-paring compartment. Rats were only considered as being in a compartment when both forepaws were located in that environment. Rats that did not show sufficient place preference were discarded. Thus numbers of rats in different groups might not be equal.

1.1.4 Extinction and reinstatement of CPP Our previous work has shown that morphine (4 mg·kg^{-1}, once a day for 10 days)- induced CPP in rats extinguished 7 days after the last drug-pairing session[7]. In the present study, observation was made 9 days after the last drug-pairing session. Rats were either injected with a priming dose (0.25 mg·kg^{-1}, s.c.) of morphine 15 min before testing, or exposed to intermittent foot shock (with square waves of amplitude of 0.5 mA, width of 0.5 s, the off time randomly distributed among 10 - 70 s with an average of 40 s) for 15 min immediately before testing. The test procedure was the same as that mentioned above. Apparatus generating the foot shock stimulation was bought from Qinghua Electronic Product Company, Beijing, China.

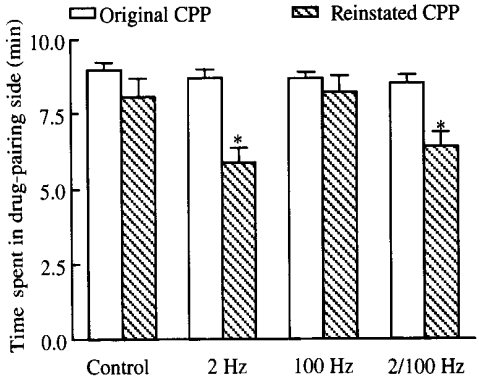
1.1.5 Peripheral Electric stimulation for the rat Rats were kept in special holders, with their hind legs and tails protruding[13]. Two stainless steel needles of 0.3 mm diameter were inserted into each hind leg, one in the acupoint ST36 (5 mm lateral to the anterior tubercle of the tibia), and the other in SP6 (3 mm proximal to the medial malleolus, at the posterior border of the tibia). Constant current square-wave electric stimulation produced by a programmed pulse generator (HANS LH-800, produced by Beijing University of Aeronautics and Astronautics) was delivered 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 in 2 Hz and 0.2 ms in 100 Hz. The intensity of the stimulation was increased stepwise from 1 mA to 2 and 3 mA with each step lasting for 10 min. In this study, PES was administered to rats 18 hours before CPP reinstatement. The control group received mock PES, with the needles being inserted *in situ* without administering electric stimulation. The motor behavior of the rats was un-distinguishable between the PES group and control group before the CPP testing.

1.2 Results

1.2.1 Effect of PES on low dose morphine- or foot shock- induced reinstatement of CPP Seventy-six rats were randomly distributed into 8 groups, with 9 - 10 in each group. All groups were trained with i.p. injection of morphine (4 mg·kg^{-1}), once per day for 10 days. After that they were kept in home cages for an 8-day extinction. Each two groups of rats were then given normal saline (s.c.) 15 min prior to PES of 2-, 100-, or 2/100- Hz, or needling without electrical stimulation as control, respectively. Eighteen hours after the PES, one of the two groups received a priming injection of morphine (0.25 mg·kg^{-1}, s.c.). Fifteen minutes later, they were tested in the training apparatus again. The other four groups were tested immediately after an intermittent foot shock stress of 15 min. The results are shown in Fig. 1 and Fig. 2. Training with 4 mg·kg^{-1} mor-

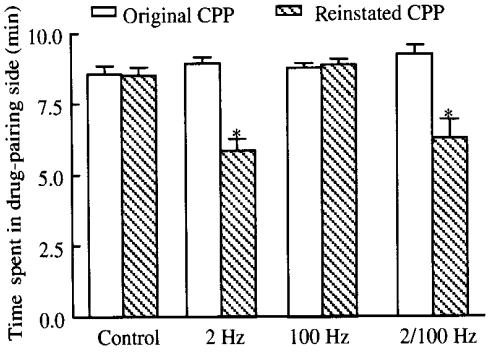
© 1995-2004 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

phine for 10 days established a stable CPP (original CPP). The extinguished CPP was successfully reinstated by our drug-priming paradigm and foot shock stress (reinstated CPP). Treatment with 2 Hz or 2/100 Hz PES blocked the reinstatement of the faded CPP ($P < 0.001$), while 100 Hz PES or needling had no such effect ($P > 0.05$).



* $P < 0.001$, compared with control.

Figure 1 Effect of PES on drug-priming-induced CPP reinstatement



* $P < 0.001$, compared with control.

Figure 2 Effect of PES on foot shock-induced CPP reinstatement

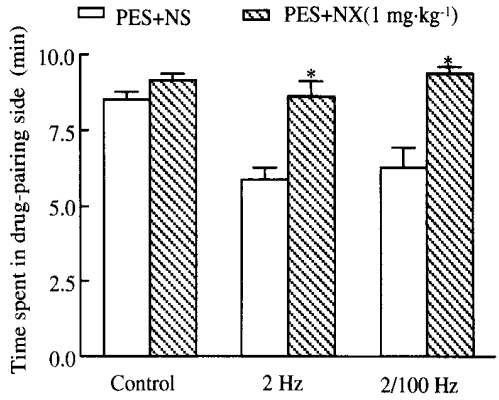
1.2.2 Naloxone blocks the effects of PES on drug priming- and foot shock- induced CPP reinstatement

Sixty-four rats were randomly distributed into 6 groups, with 10 - 11 in each group. The training, testing, and extinction procedures were exactly the same as mentioned above. Rats were injected with naloxone (1 mg·kg^{-1}, s. c.) 10 min prior to needling or PES of 2 Hz or 2/100 Hz, respectively. Eighteen hours later, they were either injected with 0.25 mg·kg^{-1} (s. c.) morphine 15 min before CPP test, or given intermittent foot shock immediately before the testing for CPP. Naloxone of 1 mg·kg^{-1} completely reversed the effect of either 2 Hz- or 2/100 Hz- PES on drug priming- (Fig. 3) and foot shock- (Fig. 4) induced reinstatement of the extinguished CPP.

2 Human observations

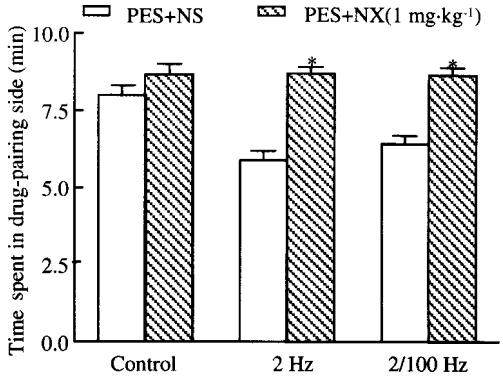
2.1 Materials and Methods

2.1.1 Human subjects One hundred and seventeen heroin abusers, aged 18 - 41 years old, were involved in this study. All but four of the subjects were male. They have been diagnosed as heroin dependence according to the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (SCID). Their average time period of heroin abuse was 3 years [(2.95 ± 1.05) years], and the dose of heroin used per day was estimated to be in the range of 0.5 - 2.0 g (purity not identified, but estimated to be in the range of 30 % - 70 %). The i. v. route of administration comprised about 30 % - 35 % of the cases, with the rest being taken *via* inhalation. The research protocol has been approved by the Department of Scientific Research, Peking University Health Science Center, and the Department of Public Security of the Zhan-Jiang County, Guangdong Province. All the participants had provided written informed consent to participate in the study.



* $P < 0.001$, compared with corresponding only saline + PES group; NS, nornal saline; NX, naloxone.

Figure 3 Naloxone blocks the effects of PES on drug-priming-induced CPP reinstatement



* $P < 0.001$, compared with corresponding saline + PES group.

Figure 4 Naloxone reversed the effects of PES on foot shock-induced CPPreinstatement

2.1.2 Transcutaneous electrical nerve stimulation Constant current square-wave electric stimulations were delivered from a HANS LH-800 stimulator through two pairs of skin electrodes. One pair was put on the acupoint of Hegu (LI-4, on the back of the hand, between the first and the second metacarpus, near the central point of the second metacarpus) and Laogong (P-8, on the palmer side of the hand, opposite to the Hegu point), and the other pair on Neiguan (P-6, located at the palmer side of the forearm, 2 inches above the palmer groove, in between the two tendens) and Waiguan (TE-5, at the opposite side of the P-6 point). The frequency of electrical

© 1995-2004 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

stimulation used was 2-, 100-, or 2/100- Hz. The pulse width was 0.6 ms in 2 Hz and 0.2 ms in 100 Hz. The intensity of the stimulation was adjusted to the maximal value that was tolerable by the subject without causing any painful feeling. The threshold value (T) was 5 - 7 mA. The average intensity that was tolerable for the subject in day 1 was 8 - 10 mA (1.5 ×T), followed by 10 - 15 mA (2 ×T) in day 2, 15 - 20 mA (3 ×T) or more in day 3 and thereafter. The treatment was administered once a day (30 minutes per session) for 10 days.

In the control group, the skin pads were placed on the same points and connected to the HANS device. The frequency was set to 2/100 Hz AM (amplitude modulation) mode and intensity adjusted to threshold level (5 - 7 mA), remaining there for less than 2 minutes, and switched to 1 mA thereafter. This intensity has been shown to produce no significant changes in pain threshold in humans[14].

2.1.3 Rating of craving scores Self-rating of heroin craving scores were obtained with the use of a visural analog scale (VAS). The craving rate ranged from 0 (not at all, marked on the left extreme of the scale) to 10 (the strongest one could imaging, marked on the right extreme of the scale). The assessment was performed in the morning by a trained research assistant, unaware of the grouping of the population.

2.1.4 Treatment procedures The detoxification procedure was conducted by the application of Han's acupoint nerve stimulator (HANS), supplemented with a small dose of methadone for the first 2 - 5 days as described previously[15]. The subjects were enrolled in the current research at least one month after the successful detoxification protocol. A total of 117 heroin addicts were randomly assigned into 4 groups, i.e., control (n = 29), 2 Hz-(n = 30), 100 Hz-(n = 29) and 2/100 Hz-(n = 29) HANS group. The whole study consisted of 3 phases: (1) Pre-HANS Phase: from day 1 to day 10, the craving scores were accessed once a day, without HANS treatment; (2) HANS treatment phase: from day 11 to day 20, the subjects received HANS of different frequencies or sham-treatment (control group) once a day, preceded by the assessment of craving score; (3) Post-HANS phase: from day 21 to day 30, the craving score was assessed once a day, without HANS treatment.

2.1.5 Statistical analysis Data were processed by commercially available software GraphPad Prism 3.0. Results were presented as $\bar{x} \pm s_{\bar{x}}$. In the animal study, comparisons between means of groups were made with two-way analysis of variance (ANOVA) followed by Students Newman-Keul's test. In the human study, for each group of patients and in each treatment phases, linear regression analysis was performed, with the average craving score as the dependent variable, and days after successful extinction as independent variable. The slope of the regression line was calculated as an indication of the rate of decay of the craving score. The accepted level of statistical significance was $P < 0.05$.

2.2 Results

The effect of PES on subjective craving score in the ex-heroin abusers was shown in Fig. 5. During the pre-treatment phase, the craving scores of patients in these 4 groups decreased gradually in a similar rate. The rate of decay of the craving scores for the control, 100 Hz, 2 Hz, and 2/100 Hz group were - 0.084 ±0.013, - 0.081 ±0.005, - 0.093 ± 0.008, and - 0.082 ±0.013, respectively. The rate of decay remained constant in the control group during the treatment phase (- 0.083 ±0.004) and the post treatment phase (- 0.087 ±0.004). In the 100 Hz group, the rate of decay showed a moderate acceleration during the treatment phase (- 0.102 ± 0.006) and the post-treatment phase (- 0.102 ± 0.005), but the difference as compared to the sham group was statistically not significant. In contrast, this rate was accelerated significantly in 2 Hz and 2/100 Hz groups (- 0.174 ±0.005 and - 0.220 ± 0.007, respectively; $P < 0.05$ and $P < 0.01$, compared with the control group, respectively) during the treatment phase, resulting in a net decrease of 1.0 - 1.5 scores as compared to the other two (control and 100 Hz) groups. In the post-treatment phase, the slope of decay remained at a level (2 Hz group, - 0.131 ± 0.008; 2/100 Hz group, - 0.109 ±0.002) midway between the pre-treatment and treatment phase. These results suggested that PES containing a low frequency component could accelerate the natural decay of heroin craving in human subjects.

3 Discussion

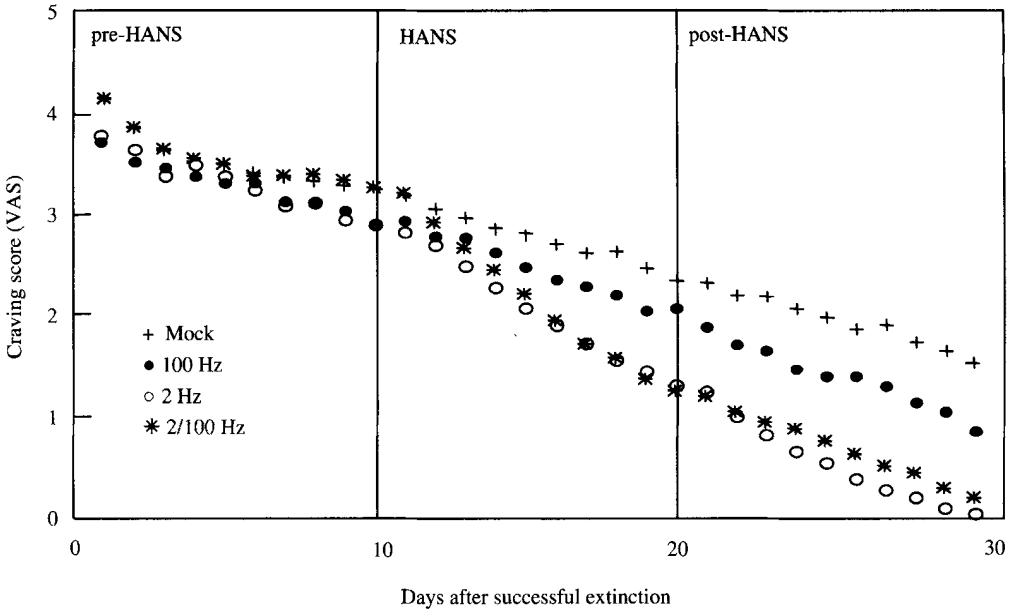
The most important finding in the present study is that PES containing a low-frequency component (either pure 2 Hz or 2/100 Hz) could effectively antagonize the reinstatement of drug-seeking behavior induced by drug-priming or by foot shock stress in experimental animals. This effect could be completely prevented by pretreatment with a small dose (1 mg · kg^{-1}) of naloxone, the opioid receptor antagonist. In the meantime, it could also accelerate the decay of the craving score in the heroin abusers, with an identical frequency dependency compared with that of the rat.

3.1 The possible neural mechanism underlying the inhibitory effect of low-frequency PES on CPP reinstatement induced by drug-priming or foot shock

Although both stress and re-exposure to drugs are known to be important factors for inducing relapse in humans[16,17] as well as in rodents[3,4], the mechanisms underlying the two relapse inducers seemed to be different. While dopamine release in the nucleus accumbens (NAc) is essential for opiate priming-induced relapse, this was less important in stress-induced CPP reinstatement[3,4], despite the fact that there is a greater induction of heroin-seeking behavior by stressors[3,4]. This discrepancy suggests that stress-induced relapse may involve certain dopamine-independent mechanisms, including the corticotropin

© 1995-2004 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

releasing factor (CRF) neurotransmission system, or the non-HPA axis stress system[18].



PES of 2 Hz or 2/100 Hz accelerated the decay of craving scores during the treatment period. $P < 0.01$, compared between the 2 Hz (or 2/100 Hz) and the mock group. $P > 0.05$, compared between the 100 Hz and the mock stimulation group.

Figure 5 Effect of PES on heroin craving of humans

The present study showed that low-frequency PES is effective in preventing both drug priming- and stress- induced relapse, suggesting that there are some common mechanisms shared by these two relapse pathways. Since these effects of PES could be blocked by naloxone, it is suggested that the aforementioned common relapse pathway might involve endogenous opioid receptors, presumably the μ receptor, which is sensitive to small dose of naloxone.

Previous studies in our lab have amply shown that PES of identified frequencies could mobilize different kinds of endogenous opioid peptides, interacting with relevant receptors to induce analgesia[19]. At the spinal level, for example, low frequency (2 Hz) stimulation increases the release of enkephalin that interacts with μ and δ opioid receptors, while high frequency (100 Hz) stimulation increases the release of dynorphin that interact with κ-opioid receptor[13, 19, 20]. At higher level of the central nervous system, the arcuate nucleus of hypothalamus (ARC) where β-endorphin containing 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 (PBN) plays central role in mediating high frequency stimulation-induced analgesia[21-24].

Moreover, PES could also facilitate the biosynthesis of endogenous opioid peptides. For instance, Guo and his colleagues[25] reported that the expression of preproenkephalin mRNA in ARC started to increase at 4 h after the electric stimulation, peaking at 24 - 48 h, and returned to normal level only after 72 h. This can only be logically explained as a long-term increased activity of the enkephalinergic neurons. Thus, PES might generate its effect of anti-CPP reinstatement, at least in part, by such a long-term activation of enkephalinergic neurons in certain central areas like ARC.

Kreek[26, 27] and Zhou[28] revealed that the stress responsive system (both HPA axis and non-HPA axis) in humans and animal models is partly under a negative feedback modulation by the endogenous opioids. In the present study, we tested the effect of PES on stress-induced relapse 18 h after the administration of PES. In that period the enkephalinergic and β-endorphinergic neurons in central nervous system, especially in the arcuate nucleus[19, 21-24, 29], should be fully prepared to release enkephahlin and endorphin to suppress the stress responsive system, thus removing the motivation for drug-seeking behavior. As we mentioned above, 100 Hz PES stimulate dynorphin release in spinal cord level rather than in brain, that may explain why PES of pure high frequency can not suppress drug-seeking behavior induced by foot shock stress[4].

3.2 The possible mechanisms underlying the effect of low-frequency TENS in suppressing subjective craving in heroin addict

Two types of PES are applicable clinically, namely, (1) the electroacupuncture (EA) that requires the inserting of needles into the body sites, and (2) TENS that only requires the attachment of the self sticking electrodes onto the skin. A series of studies have shown that both EA and TENS could induce similar degree of analgesic effect[30] with very similar, if not identical, mechanisms of action. Thus we employed TENS as the type of PES in the present observation. To distinguish with the conventional TENS device, which produces only high-frequency (100 - 200 Hz) narrow-pulse (0.1 - 0.5 ms) output

© 1995-2004 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

based on the gate control theory, we used a device entitled HANS, which produces both high-frequency narrow-pulse output as well as low-frequency wide-pulse output, plus a dense-and-disperse (DD) mode of stimulation[14]. This method of stimulation provides more flexibility in treatment and more opportunity for the exploration of the possible mechanisms of action.

It has been generally accepted that a high concentration of exogenous opiate in the body would induced a negative feedback control on the expression and release of endogenous opioid peptides[31, 32]. When the exogenous opiates were abstinent, it would naturally generate a motivation to seek exogenous opiates to maintain a pathological homeostasis. Since low frequency peripheral stimulation can activate the endogenous opioidergic (enkephalinergic and β-endorphinergic) system[14], it would be helpful to prevent the relapse of drug-seeking behavior.

Most of the studies on the acupuncture treatment of heroin addiction focused on its efficacy for detoxification, i. e., for the treatment of physical dependence[33, 34]. In contrast, the present study focused on the efficacy of PES on the amelioration of craving. Moreover, the present study stressed the importance of the frequency- rather than the site- specificity of the PES. This is based on the previous findings that the specificity of the site of stimulation (so called the "acupoint") is only relative, rather than absolute. At the very beginning of our study on acupuncture-induced analgesia (1965), we compared the efficacy of 10 commonly used acupoints in producing a hypoalgesic effect. Potasium iontophoresis method was applied for the detection of the pain threshold of the skin at 8 different sites of the body. Stimulation of any one of the 10 points produced an increase of the pain threshold with a very similar slow onset and slow decay curve. The difference was only quantitative rather than qualitative, with LI-4 showing the highest efficacy[35]. In addition, the sites at the hand and the aim are easy to reach if one would like to put on the skin pads by oneself.

It has been reported that either 100 Hz or 2 Hz EA could ameliorate morphine withdrawal syndrome in the rats[9, 10, 36] and in humans[12], with 100 Hz EA significantly more effective than 2 Hz EA. This is in line with the finding that spinal κ-opioid system plays an important role in suppressing morphine withdrawal syndrome in the rat[37, 38]. Why then the high frequency stimulation did not work for the relief of craving? Since high frequency PES accelerated both the release[20, 36] and biosynthesis[29] of dynorphin, and dynorphin *per se* induces aversive rather than euphoric effect[39], it would not be surprised that 100 Hz PES was not helpful to reduce craving.

It has been shown that PES could also activate neurotransmissions other than the endogenous opioid system, such as cholecystokinin, norepinephrine, dopamine, serotonin, and many others[40]. The possible roles played by these transmitters systems in the effects mentioned above should also be elucidated in future studies.

In conclusion, PES with a component of low frequency (pure 2 Hz, or 2 Hz alternating with 100 Hz) is very powerful in preventing the drug priming- or foot shock- induced reinstatement of morphine-seeking behavior in rats, which is naloxone reversible. It could also accelerate the natural decay of subjective craving in heroin addicts. PES of pure 100 Hz produced little, if any effect. Taken together, low frequency PES may serve as a putative therapeutic measure for preventing the relapse of drug abuse in heroin addicts.

References

1 Koob GF, Moal LM. Drug abuse: hedonic mechanisms of drug dependence[J]. Science, 1997, 278: 52-58
2 Self DW, Nestler EJ. Relapse to drug-seeking: neural and molecular mechanisms[J]. Drug Alcohol Depend, 1995, 51: 49-50
3 Shaham Y, Stewart J. Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal [J]. Psychopharmacology (Berl), 1995, 119: 334-341
4 Shaham Y, Stewart J. Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats[J]. Psychopharmacology (Berl), 1996, 125: 385-391
5 Parker LA, Mcdonald RV. Reinstatement of both a conditioned place preference and a conditioned place aversion with drug primes [J]. Pharmacol Biochem Behav, 2000, 66: 559-561
6 Lu L, Ceng X, Huang M. Corticotropin-releasing factor receptor type Ⅰ mediates stress-induced relapse to opiate dependence in rats [J]. Neuroreport, 2000, 11: 2373-2378
7 Wang B, Luo F, Zhang WT, *et al*. Stress or drug priming induces reinstatement of extinguished conditioned place preference[J]. Neuroreport, 2002, 11: 2781-2784
8 Wang B, Luo F, Xia YQ, *et al*. Peripheral electric stimulation inhibits morphine-induced place preference in rats[J]. Neuroreport, 2000, 11: 1017-1020
9 Han JS, Zhang RL. Suppression of morphine abstinence syndrome by body electroacupuncture of different frequencies in rats[J]. Drug Alcoh Depend, 1993, 31: 169-175
10 Wu LZ, Cui CL, Han JS. Agonists of κ receptor inhibit morphine withdrawal syndrome [J]. Chinese J Neuroscience, 1995, 2: 168-172
11 Han JS, Wu LZ, Cui CL. Heroin addicts treated with transcutaneous electrical nerve stimulation of identified frequencies[J]. Regul pept, 1994, 54: 115-116
12 Wu LZ, Cui CL, Han JS. Clinical research on Han's acupoint nerve stimulator for the treatment of opiate withdrawal syndrome [J]. Chin J Pain Med, 1995, 1: 30-38
13 Chen XH, Han, JS. Analgesia induced by electroacupuncture of different frequencies is mediated by different types of opioid receptors: another cross-tolerance study[J]. Behav Brain Res, 1992, 47: 143-149
14 Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies [J]. Trends Neurosci, 2003, 26: 17-22
15 Wu LZ, Cui CL, Han JS. Reduction of methadone dosage and relief of depression and anxiety by 2/100 Hz TENS for heroin detoxification[J]. Chin J Drug Depend, 2001, 10: 124-126
16 Stewart J, de Wit H. Reinstatement of drug-taking behavior is a method of assessing incentive motivational properties of drug. In: Methods of Accessing the Reinforcing Properties of Abused Drugs [M]. New York: Springer, 1987. 211-227
17 Kosten TR, Rounsaville BJ, Kleber HD. A 2.5-year follow up of depression, life crises, and treatment effects on abstinence among opioid addicts[J]. Arch Gen Psychiatry, 1986, 43: 733-739
18 Self DW, Nestler EJ. Relapse to drug-seeking: neural and molecular mechanisms[J]. Drug Alcohol Depend, 1998, 51: 49-50
19 Han JS, Wang Q. Mobilization of specific neuropeptides by peripheral stimulation of identified frequencies[J]. News Physiol Sci, 1992, 7: 176-180
20 Han JS, Chen XH, Sun SL, *et al*. Effect of low-and-high-frequen-

© 1995-2004 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

cy TENS on met-enkephalin-Arg-Phe and dynorphin A immunoreactivity in human lumbar CSF[J]. Pain, 1991, 47:295-298
21 Wang Q, Mao LM, Han JS. The arcuate nucleus of hypothalamus mediates low but not high frequency electroacupuncture analgesia in rats[J]. Brain Res, 1990, 513:60-66
22 Wang Q, Mao LM, Han JS. Analgesic electric stimulation of hypothalamic arcuate nucleus: tolerance and its cross-tolerance to 2 Hz or 100 Hz electroacupuncture[J]. Brain Res, 1990, 518:40-46
23 Wang Q, Mao LM, Han JS. Naloxone-reversible analgesia produced by microstimulation of arcuate nucleus of hypothalamus in pentobarbital anesthetized rats[J]. Exp Brain Res, 1990, 37:201-204
24 Wang Q, Mao LM, Han JS. The role of parabrachial nucleus in high frequency electroacupuncture analgesia in rats[J]. Chin J Physiol Sci, 1991, 7:363-367
25 Guo HF, Cui X, Hou YP, *et al*. c-Fos proteins are not involved in the activation of preproenkephelin gene expression in rat brain by peripheral electric stimulation (electroacupuncture)[J]. Neurosci Lett, 1996, 207:163-166
26 Kreek MJ. Multiple drug abuse patterns and medical consequence. In: Meltzer HY. ed. Psychopharmacolgy: The Third Generation of Progress[M]. New York: Raven Press, 1987. 1957-1604
27 Kreek MJ. Rationale for maintenance pharmacotherapy of opiate dependence. In: O'Brien CP, Jaffe JH, ed. Addictive States[M]. New York: Raven Press, 1992. 205-230
28 Zhou Y, Spangler R, Maggos CE, *et al*. Modulation of CRF-R1 mRNA in rat anterior pituitary by dexamethasone: correlation with POMC mRNA[J]. Peptides, 1996, 17:435-441
29 Guo HF, Tian JH, Wang XM, *et al*. Brain substrates activated by electroacupuncture of different frequencies I: comparative study on the expression of oncogene c-fos and genes coding for three opiid peptides[J]. Mol Brain Res, 1996, 43:157-166
30 Wang JQ, Mao L, Han JS. Comparison of the antinociceptive effects induced by electroacupuncture and transcutaneous electrical nerve stimulation in the rat[J]. Int J Neurosci, 1992, 65:117-129
31 Gudehithlu KP, Tejwani GA, Bhargava HN. Beta-endorphin and methionine-enkephalin levels in discrete brain regions, spinal cord, pituitary gland and plasma of morphine tolerant-dependent and abstinent rats[J]. Brain Res, 1991, 553: 284-290
32 Bhargava HN, Gulati A. Down-regulation of brain and spinal cord mu-opiate receptors in morphine tolerant-dependent rats[J]. Eur J Pharmacol, 1990, 90: 305-311
33 Wen HL. Fast detoxification of heroin addicts by acupuncture and electrical stimulation (AES) in combination with naloxone[J]. Comp Med Est West, 1977, 5:257-263
34 Washburn AM, Fullilove RE, Fullilove MT, *et al*. Acupuncture heroin detoxification: a single-blinded clinical trial[J]. J Subst Abuse Treat, 1993, 10: 345-351
35 Research Group of Acupuncture, Beijing Medical College. The effects of acupuncture on the skin pain threshold in humans[J]. Chin J Med, 1973, 3: 151-157
36 Wu LZ, Cui CL, Tian JB, *et al*. Suppression of morphine withdrawal by electroacupuncture in rats: dynorphin and kappa-opioid receptor implicated[J]. Brain Res, 1999, 851: 290-296
37 Takemori AE, Loh HH, Lee NM. Suppression by dynorphin A-(1-13) of the expression of opiate withdrawal and tolerance in mice[J]. Eur J Pharmacol, 1992, 221: 223-226
38 Cui CL, Wu LZ, Han JS. Spinal kappa-opioid system plays an important role in suppressing morphine withdrawal syndrome in the rat[J]. Neurosci Lett, 2000, 295:45-48
39 Funada M, Suzuki T, Narita M, *et al*. Blockade of morphine reward through the activation of kappa-opioid receptors in mice[J]. Neuropharmacology, 1993, 32: 1315-1323
40 Han JS. Acupuncture and stimulation-produced analgesia. In: Herz A, ed. Opioids II (Handbook of Experimental Pharmacology)[M]. Berlin: Springer, 1993. 105-125

(Received 2003-03-17)
(Edited by ZHAO BO)

短篇报道 ·

中国人群出生缺陷核心家庭永生细胞株的建立

裴丽君△,李 竹,任爱国,赵文睿,曲 梅,朱江辉
(北京大学生育健康研究所,北京 100083)

EB 病毒(Epstein-Barr virus)转化人外周血 B 淋巴细胞,使其成为能连续分裂永久生存的类淋巴母细胞(lymphoblastoid),利用这一特点建立各种疾病人群永生细胞株(immortalized cell lines),可永远传代,并且保存了每个个体的完整基因组,其生化和分子生物学特性不发生变化,可作为建立生物标本库的首选方法。

目的:利用 EB 病毒转化人类外周血 B-淋巴细胞方法建立各种出生缺陷家庭或家系永生细胞株,为出生缺陷遗传流行病、分子生物学等研究提供永久性实验材料。

方法:标本来源于中美预防出生缺陷与残疾合作项目现场,对象为既往监测到的、仍存活的出生缺陷(神经管畸形、先心病、唇腭裂、染色体异常、先天肢体畸形、多发畸形等)儿童及其亲生父母;研究方案通过了北京大学医学伦理学委员会批准,并在签署知情同意书后方可采集静脉血;利用 EB 病毒和免疫抑制剂环孢菌素 A 转化人外周血 B 淋巴细胞,同时比较了 CO_2 和非 CO_2 两种培养方法。EB 病毒制备方法为使 B95-8 细胞经过培养、饥饿、病毒释放过程,最终收集、冻存于 -70 ℃或液氮备用。

结果:成功建立了 40 个出生缺陷核心家庭 117 个个体永生细胞株;细胞冻存 2 个月后复苏 45 株,复苏成功率为 100%;CO_2 培养法的转化成功率(100%)高于非 CO_2 法(66.7%);前者比后者所需时间缩短约 20～30 d,两种方法在转化成功率、转化时间和建株时间上,经 χ^2 检验差异均有显著性($P<0.01$)。

结论:EB 病毒转化人外周血 B-淋巴细胞建立出生缺陷永生细胞库是可行的,采用 CO_2 培养可提高转化成功率,EB 病毒的质量是 B-淋巴细胞转化成败的关键。永生细胞株可帮助解决许多罕见病、遗传病研究对象来源不足的困难,对出生缺陷遗传流行病、分子生物学等研究具有重要价值。

(2002-11-27 收稿)
(本文编辑:赵 波)

基金项目:国家重点基础研究专项(G1999055905)资助
△Corresponding author email, Peilj@ncmih.bjmu.edu.cn

© 1995-2004 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.