·论著·

Effect of 6-OHDA lesions of the dopaminergic mesolimbic system on drug priming induced reinstatement of extinguished morphine CPP in rats

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KEY WORDS Oxidopamine; Morphine; Conditioned place preference; Heroin dependence

SUMMARY **Objective:** To evaluate the role played by mesolimbic dopaminergic system in the reinstatement of drug-seeking behavior induced by priming injections of morphine. **Methods:** After the extinguishment of morphine conditioned place preference (CPP), low-dose catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) was bilaterally injected into ventral tegmental area (VTA, 1 g·L^{-1}) and nucleus accumbens (NAc, 5 g·L^{-1}) before being primed with low-dose morphine. **Results:** the effects of drug-priming to induce reinstatement of morphine CPP could be completely abolished by 6-OHDA microinjected into VTA to damage the perikaryon of dopaminergic neurons, or into NAc to lesion the terminal field of the dopaminergic pathyway. **Conclusion:** The functional integrality of the mesolimbic dopaminergic system is indispensable for drug priming-induced reinstatement of conditioned place preference.

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6-羟多巴胺损毁中脑边缘多巴胺能系统抑制药物点燃诱导的大鼠吗啡条件性位置偏爱消退后重建

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[关键词] 羟多巴胺; 吗啡; 条件性位置偏爱; 海洛因依赖

[摘 要] 目的: 考察中脑边缘多巴胺能系统在小剂量吗啡点燃诱导吗啡觅药行为重建中的作用。方法: 将小剂量儿茶酚胺能神经毒素 6-羟多巴胺微量注射到双侧伏核(1 g·L^{-1})或腹侧背盖区(5 g·L^{-1}), 观察其对已消退的吗啡条件性位置偏爱之点燃重建的作用。结果: 6-羟多巴胺微量注射到腹侧背盖区选择性地损毁多巴胺能神经元的胞体, 或注射到伏核以损毁多巴胺能纤维的末梢, 均可完全消除小剂量吗啡(0.25 mg·kg^{-1}, s.c.)的点燃效应。结论: 中脑边缘多巴胺能系统功能的完整性是药物点燃导致条件位置偏爱重建的必要条件。

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Relapse to drug using after long periods of abstinence is a common feature of drug addiction[1]. Along with conditioned drug-related cues and social stressor, another major cause of relapse is the re-exposure to the abused drug itself[2]. Such drug-induced reinstatement of drug-seeking behavior, or relapse, has been reported in animals trained to self-administrate multiple addictive drugs[3-5]. The other putative animal model that could mimic human relapse is the reinstatement of conditioned place preference (CPP), reported simultaneously by Parker and Mcdonald[6], Lu and co-workers[7], and Wang *et al*[8]. While in both models the priming injection of the previously used drug could be considered as renewing the salience of the environmental cues, the animals do not need to do any operation in the training phase in the CPP model, i.e., they do not need to learn to make a response to obtain the drug. So both paradigms could be used to study human relapse behavior, although the underlying neurobiological mechanisms might be different.

Both opiate and psychostimulants could produce rewarding effects by activating the mesolimbic dopamine (DA) system[9,10], which consists of dopaminergic neurons located in the ventral tegmental area (VTA) that projecting to neurons in nucleus accumbens (NAc). According to the incentive-sensitization hypothesis of addiction[11], a drug could prime a response because they could activate the mesolimbic DA system that becomes sensitized upon repeated drug using. This hypothesis has been confirmed by

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considerable evidence obtained from reinstated self-administration models[4,12,13]. Our previous results showed that lesion of VTA and NAc shell could completely abolish drug-priming induced reinstatement of extinguished CPP[14]. Thus, it would be interesting to see whether mesolimbic DA system also play a critical role in drug-priming-induced reinstatement of place preference. In the present study, we microinjected 6-hydroxydopamine (6-OHDA), which selectively damage catecholaminergic neurons[15,16], into VTA or NAc to investigate the role of VTA-NAc dopaminergic pathway in drug-priming-induced reinstatement of morphine-related place preference.

1 Materials and Methods

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 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. Animals were conditioned and tested during the light phase of the cycle. The experimental procedures were approved by the Committee on Animal Care and Use of Peking University Health Science Center, and all efforts were made to minimize the suffering of the animal and the number of animal used.

1.2 Surgery

The rats received an intraplantar (i.p.) injection of desimipramine (DMI: 25 mg·kg^{-1}) and pargyline HCl (50 mg·kg^{-1}) one hour before the surgical operation, to prevent destruction of noradrenergic nerve terminals. 6-OHDA was dissolved in a solution of ice-cold 0.3 mg·ml^{-1} ascorbic acid in 0.9 % saline. Coordinates are given as millimeters anterior (+) or posterior (-) to the bregma, millimeters lateral to bregma, and millimeters ventral to the skull surface, according to the atlas of Paxinos and Watson[17]. For each lesion, control subjects (shams) were used by infusing same volume of the solution of ice-cold 0.3 mg·ml^{-1} ascorbic acid in 0.9 % saline. Under 10 % chloral hydrate (30 mg·kg^{-1}, i.p.) aesthesia, bilateral lesions of NAc were induced by infusing 5 μl of a solution containing 5 μg free base 6-OHDA into NAc (coordinates were +1.7, 1.5, 7.0) over 10 min and the injection needles were left *in situ* for additional 10 min following neurotoxic infusion. To evoke a lesion in VTA, 1 μl of a solution containing 8 μg free base 6-OHDA were injected per side (-5.2, 0.7, 7.8) over 5 min and the injectors were left in the place for additional 5 min following injection. 6-OHDA was kept on ice from light during surgery. All animals were allowed to recover for 14 days before the next behavior testing.

1.3 Drugs

Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang, China. Chloral hydrate was purchased from the Third Pharmaceutical Factory of Beijing. DMI·HCl, 6-OHDA·HCl and pargyline HCl were bought from SIGMA Co., USA.

1.4 Procedure

The methods of CPP paradigm have been described in detail elsewhere[18]. Briefly, conditioning took place in one of the two distinct environments differed from each other in color and texture, and separated by a transparent removable clapboard. The walls of one room were painted with vertical black and white stripes (width: 2 cm), and the floor comprised a layer of fiberboard bedding. In the other room, the walls were painted with black dots (diameter: 1.5 cm) sprinkled on white background, and the flooring material was 1 cm thick sawdust. The later was used as the drug-pairing room. Rats were scored during a 10-min test session as being in a compartment when both forepaws were located in that environment. After that, different kinds of lesions were performed in different groups of rats, and the rats were then kept in their homecages for 14 days for recovery and CPP extinction. Priming injection of morphine (0.25 mg·kg^{-1}, s.c.) was then given, followed by another testing in the CPP chambers 15 min later.

1.5 Histology

On completion of behavior testing, rats were deeply anaesthetized with 10 % chloral hydrate and perfused transcardially with 0.9 % saline, followed by 4 % phosphate-buffered formalin. Brains were removed and stored in formalin until sectioning. All lesion sites were verified using standard Cresyl violet staining methods (40-μm sections every 200 μm). The location and extent of all lesions were identified by determining the area where cells were lost or gliosis was present and silhouettes of lesions were drawn onto appreciate standardized stereotaxic atlas drawings[17]. Only animals with bilateral lesions destroying a significant portion (over 80 %) of the intended structure, but not extending beyond its boundaries, were included for analysis.

1.6 Statistical analysis

Data were processed by commercially available software GraphPad Prism 3.0. Results were presented as $\bar{x} \pm s_{\bar{x}}$. Comparison between means of groups were analyzed with two-way analysis of variance (ANOVA) followed by Bonferroni's post test. The accepted level of statistical significance was $P < 0.05$.

2 Results

2.1 Extinguishing of morphine-induced place preference

One hundred and eight rats were randomly assigned into 12 groups, with 9 in each group. Six of the groups were trained with morphine (4 mg·kg^{-1}) for 10 days in the CPP paradigm, while the other six groups were running the same procedure with saline instead of morphine as the respective control. They

were tested for CPP 1, 3, 5, 7, 9 or 14 days, respectively, after the last drug-pairing session. As shown in table 1, rats showed a similar degree of preference to the drug-pairing room 1 or 3 days after training. However, this place preference became weaker at the fifth day ($P < 0.01$), compared with that of the first day); and fell to the same level on the 7 th, 9 th and 14 th day as the respective saline control group. These results showed that the morphine-induced CPP would disappear seven days after the last drug-pairing session in the experimental system we used.

Table 1 The spontaneous extinction of morphine-induced conditioned place preference

min

Days after training	Time in preferred side	
	Normal saline	Morphine
1	6.40 ±0.20	9.02 ±0.17 **
3	6.76 ±0.22	8.92 ±0.17 **
5	6.91 ±0.31	8.43 ±0.18 *
7	6.88 ±0.57	7.24 ±0.18
9	6.46 ±0.23	6.21 ±0.14
14	5.92 ±0.23	5.64 ±0.48

* $P < 0.05$, ** $P < 0.001$ *vs* normal saline.

2.2 Effect of lesions at nucleus accumbens on drug priming-induced reinstatement of conditioned place preference

Twenty rats were randomly distributed into 2 groups, with 10 in each group. There were two rats dropped in the sham group and one in the lesion group for unexpected technical reasons. The rats were given morphine injection (4 mg·kg^{-1}, i.p.) once a day for 10 days to induce stable CPP. The time spent in the drug-pairing side of the lesion group and the sham lesion group was (7.87 ±0.23) min and (7.91 ± 0.46) min, respectively.

After the surgical lesion at NAc and the priming injection of morphine, the time spent in the drug-pairing side of the NAc lesion group was (5.18 ± 0.40) min, which was significantly lower ($P < 0.001$) than that in the sham lesion group [(8.45 ± 0.20) min]. The results suggested that lesion at NAc completely blocked the effect of drug-priming in the induction of CPP reinstatement.

2.3 Effect of ventral tegmental area lesion on drug priming-induced conditioned place preference

Twenty rats were randomly distributed into two groups. There were one rat dropped in the sham group and two in the lesion group for unexpected technical reasons. The time spent in the drug-pairing side of the lesion and the sham group after CPP training was (7.91 ±0.40) min and (8.52 ±0.24) min, respectively, showing no significant difference.

Ventral tegmental area (VTA) lesion significantly attenuated the reinstated CPP by drug-priming as compared with the sham group [(5.64 ±0.44) min *vs* (8.27 ±0.22) min, respectively, $P < 0.001$]. This result indicates that the integrity of VTA is very important for drug-primed reinstatement of morphine-related place preference.

3 Discussion

The most important finding in the present study is that the functional integrality of the mesolimbic dopaminergic system is indispensable for drug-primed reinstatement of extinguished CPP.

In the model of extinction and reinstatement of self-administration, researchers found that microinjection of morphine into VTA but not NAc could reinstate both heroin- and cocaine-seeking behavior[19]. Wang *et al*[8] suggested that amphetamine priming could reinstate extinguished CPP induced by morphine, and lesion of VTA or NAc shell could abolish CPP reinstatement[14]. Since amphetamine has been known to produce local increase of DA in NAc, while application of morphine directly into the VTA may activate dopamine neurons *via* disinhibition[10] and consequently increase the dopamine release in the NAc[11], the above mentioned data might suggest a critical role of the mesolimbic dopamergic pathway in the reinstatement of drug-seeking behavior. Conflicting results, however, were obtained by using apomorphine, a non-selective DA receptor agonist[20-22]. An alternative approach was to use DA receptor antagonists instead of the agonists[13,23], but the results were often difficult to interpret due to the sedative and aversive effects produced by these ligands. To our knowledge, there is so far no experimental study reported to test whether the DA hypothesis is applicable to account for the drug priming-induced reinstatement of extinguished morphine CPP.

Using 6-hydroxydopamine, the neurotoxin for selective destruction of the dopaminergic axons and cell bodies, we were able to show that lesions placed at either the cell body (VTA) or the terminal field area (NAc) of dopaminergic mesolimbic system could block drug priming-induced CPP reinstatement, suggesting that the VTA-NAc dopaminergic pathway plays a critical role in the priming-induced drug-seeking behavior.

Vries *et al*[20] argued that drug-induced reinstatement of heroin- and cocaine-seeking behavior following long-term extinction is associated with the expression of behavioural sensitization. It has been known that the brain system normally involved in the processing of incentive motivation and reward (e.g., the mesolimbic dopaminergic system) would become hypersensitive ("sensitized") when the individual has been repeatedly exposed to drug and the sensitized state will last for a protracted period even after abstinence[24]. So we postulate that when the sensitized dopaminergic system is activated by priming dose of drugs that increase dopamine release, it will renew the incentive salience of the environment cues of the drug, leading to the reinstatement of the extinguished

CPP. Lesion at one of the important links of the VTA-NAc pathway might block the increase of DA release induced by the priming dose of morphine, thus could block CPP reinstatement.

The drug primed reinstatement of extinguished morphine-induced place preference in the rat could be completely abolished by the selective destruction of the dopaminergic mesolimbic pathway. Thus, a sensitized mesolimbic dopaminergic system seems to be an indispensable substrate mediating drug priming-induced relapse to drug-seeking behavior.

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·消息·

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