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Brain Research 950 (2002) 1–9

BRAIN RESEARCH

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Research report

# Effects of lesions of various brain areas on drug priming or footshock-induced reactivation of extinguished conditioned place preference

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Accepted 30 April 2002

**Abstract**

We have previously shown with a model of morphine-induced conditioned place preference (CPP) that a brief exposure to footshock stress or a priming dose of morphine could reactivate drug-seeking behavior after a long drug-free period. The present study was designed to examine the possible role of certain brain areas in such a reactivation. After the rats were successfully trained with morphine (4 mg/kg, i.p.) through a CPP paradigm (10 sessions of daily pairing of morphine with one of the two compartments), different parts of nucleus accumbens (NAc), ventral tegmental area (VTA), and central (Ce) or lateral (La) nucleus of amygdala were lesioned with a DC current passing through the respective location. After a 9-day abstinence period, random intermittent footshock (DC square wave, 0.5 mA, 0.5 s width, off time 10–70 s) or drug priming (morphine 0.25 mg/kg, s.c.) reactivated the place preference in sham lesion rats. However, the effect of drug priming could be completely abolished by lesions placed either at VTA, or the majority or shell part, but not the core of NAc. On the other hand, the effect of footshock stressor could be eliminated by a lesion placed at Ce but not La. These results suggest that, while both drug priming and footshock stress are effective in reactivating drug-seeking behavior, they might work through different neurochemical mechanisms and anatomical pathways.


*Theme:* Neural basis of behavior

*Topic:* Opioids: anatomy, physiology, and behavior

*Keywords:* Morphine; Opioid addiction; Psychic dependence; Stress; Drug priming; Relapse

## 1. Introduction

High rate of relapse to drug using behavior after a long period of abstinence characterizes the behavior of experienced addicts [11]. This renders relapse the primary problem for the treatment of addiction [24]. Without thorough understanding of the factors that determine recurred craving and drug-seeking, it would be unlikely for health care professionals to provide effective treatment. Factors that most powerfully trigger the relapse to drug-using behavior in humans are re-exposure to drug [5], exposure to stress [15], or re-exposure to stimuli that have been previously associated with the reinforcing properties of drugs [4]. Drug craving is a subjective description that cannot be directly measured in laboratory animals. However, it is possible to measure relapse directly in a proper operant event, when a laboratory animal reinitiates a particular behavioral response. This relapse to a prior behavioral response, often referred to as reinstatement, is considered capable of reflecting the re-induction of drug-seeking behavior, or craving, following an extinction period from drug-using behavior. Shaham and co-workers have constructed a model for the reinstatement of drug self-administration, using either a small dose of heroin or a brief exposure to footshock [32,34]. The other putative animal model that could mimic human relapse is the reactivation of conditioned place preference (CPP), reported simultaneously by Parker and McDonald [26], Lu et al. [17] and our lab [39]. Although both of these two

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0006-8993/02/$ – see front matter 
PII: S0006-8993(02)02980-3

paradigms could be used to study human relapse behavior, the neurobiological mechanisms might well be different.

The mechanisms involved in the reinstatement of self-administration paradigm by stress and drug priming are still not clear. Some researchers suggested that the mesolimbic dopaminergic system (with the dopaminergic neurons located in the ventral tegmental area and its projection terminated in the shell part of the nucleus accumbens) was involved in both procedures [33,34], while others argued that corticotrophin releasing factor (CRF, densely contained in neurons within the central nucleus of amygdala) or hypothalamo–pituitary–adrenal (HPA) axis played a critical role in relapse to heroin- [35] or cocaine-seeking behavior [6,29], respectively. However, up to date there has been no study addressing the neural basis of the reactivation of place preference.

In the present study, we investigated the role of various brain areas in the reactivation of place preference (as an indication of drug-seeking behavior) induced by drug priming or footshock. To validate the possible involvement of mesolimbic dopaminergic pathway in this reactivation, lesions were placed at nucleus accumbens (NAc, or the shell or core part of it) and ventral tegmental area (VTA). The central (Ce) or lateral nucleus (La) of amygdala was also lesioned to verify whether it is involved in drug priming or footshock-triggered reactivation of CPP.

## 2. Materials and methods

### 2.1. 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 six per 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 Usage of Peking University Health Science Center, and all efforts were made to minimize the pain and discomfort, as well as the number of animals used.

### 2.2. Surgery

Bilateral lesions of selected brain sites were performed under 10% chloral hydrate solution (30 mg/kg) intraperitoneal (i.p.) anesthesia. Lesions were induced by passing a DC current into each site through the anode. The cathode, a crocodile clip, was attached to the skin flap. The brain electrode was made from a stainless steel pin of 0.35 mm in diameter, all insulated except for the tip. Sham lesions were performed by lowering the electrode to the target structure without passing any current. Coordinates are given in Table 1 as millimetres anterior (+) or posterior (−) to bregma, millimetres lateral to bregma, and millimetres ventral to the skull surface, according to the atlas of Paxinos and Watson [27]. All animals were allowed to recover for 9 days before behavioral testing.

Table 1
Parameters of central lesions

| Site | Current (mA) | Duration (s) | Atlas (mm) | | |
|---|---|---|---|---|---|
| | | | Anterior | Lateral | Ventral |
| NAc | | | | | |
| Shell | 1.0 | 15 | +2.2 | 1.2 | 7.9 |
| Core | 1.0 | 20 | +1.6 | 1.8 | 7.7 |
| Majority | 1.0 | 30 | +1.7 | 1.5 | 7.5 |
| VTA | 0.5 | 20 | −5.2 | 0.5 | 7.8 |
| Amygdala | | | | | |
| Central | 0.5 | 20 | −2.4 | 4.5 | 7.5 |
| Lateral | 1.0 | 20 | −2.8 | 5.2 | 8.3 |

### 2.3. Drugs

Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang, China. Chloral hydrate was purchased from the Third Pharmaceutical Factory of Beijing. All the drugs were dissolved in 0.9% saline to their final concentrations.

### 2.4. Footshock

Rats were given randomly delivered intermittent footshock for 15 min (DC, square wave, amplitude 0.5 mA, width 0.5 s, off time randomly distributed between 10 and 70 s with an average of 40 s). The apparatus generating footshock stimulation was a product of Qinghua Electronic Product Company, Beijing.

### 2.5. Procedures

The methods of the CPP paradigm were described in detail elsewhere [40]. Briefly, conditioning took place in one of two distinct environments differing in color and texture, and separated by a removable transparent clapboard. The walls of one compartment were painted with vertical black and white stripes (width: 2 cm), and the floor comprised a layer of fiberboard bedding. In the other compartment, the walls were painted with black dots (diameter: 1.5 cm) sprinkled on white background, and the flooring material was sawdust 1 cm thick. The latter was used as the drug-pairing room since no natural preference was observed for either side (data not shown). 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 (4 mg/kg), and remained there for another 15 min after the injection. This training was performed once a day for 10 days. On the 11th day, rats were given free access to both compartments during a 10-min test session. A rat was

considered to be in a certain compartment when both forepaws were located in that environment. The total time it remained in the drug-paired side was recorded as the place preference score. After that, some rats were retested 1, 3, 5, 7, or 9 days after the last training session for the time course of the abstinence of morphine-induced CPP. Other rats were given appropriate lesion, and then were kept in their home cages for a period of 9 days, for the purpose of full recovery and CPP abstinence. Intermittent footshock (as described above) or priming subcutaneous (s.c.) injection of morphine (0.25 mg/kg) were then given, followed by another CPP testing immediately after the footshock or 15 min after the drug priming.

### 2.6. Histology

Upon completion of behavioral testing, rats were deeply anaesthetized with 10% chloral hydrate (i.p.) 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 for 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 appropriate standardized stereotaxic atlas diagrams [27]. Only animals with bilateral lesions destroying a significant portion of the intended structure, but not extending beyond its boundaries, were included for analysis.

### 2.7. Statistical analysis

Data were processed by commercially available software, GraphPad Prism 3.0. Results were presented as mean±S.E.M. Comparison between means of two groups were calculated with Student's *t*-test, while comparison among three or more related groups were analyzed with two-way analysis of variance (ANOVA) followed by Bonferroni's *t*-test. The accepted level of statistical significance was $P<0.05$.

## 3. Results

### 3.1. Abstinence of morphine-induced place preference

Ninety rats were randomly assigned into 10 groups, with nine in each group. Five of the groups were trained with morphine while the other five with saline as control. They were tested for place preference 1, 3, 5, 7, or 9 days after the last drug-pairing session, respectively. As shown in Fig. 1, after the last drug-pairing session, rats showed similar degree of preference to the drug-pairing room 1 and 3 days after training. However, this place preference became weaker at the 5th day (Bonferroni's post-hoc test, $t(17)=3.731$, $P<0.05$, compared with the CPP scores of the first day); and fell to the same level on the 7th and 9th day as the respective saline control groups. Thus, the morphine-induced CPP would disappear 7 days after the last drug-pairing session in the experimental system we used.

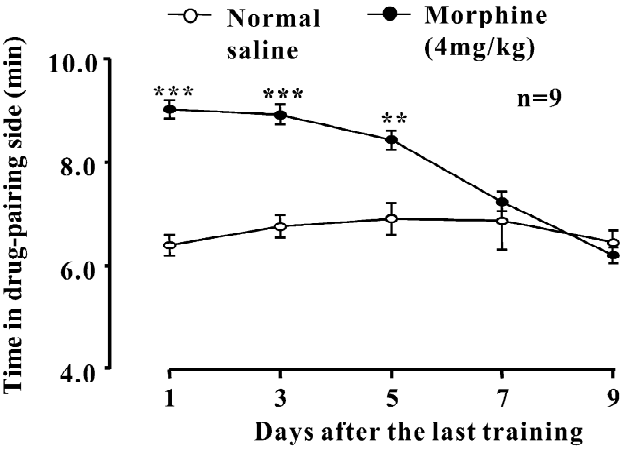


Fig. 1. Spontaneous abstinence of morphine-induced conditioned place preference. **, ***$P<0.01$ and $P<0.001$, respectively, compared with their corresponding saline control groups.

### 3.2. Effect of nucleus accumbens lesions on drug priming-induced reactivation of CPP

Sixty-six rats were randomly distributed into six groups. Four of them were eliminated due to unsuccessful lesion, so finally we had 9–11 rats in each group. After the last CPP training session, these rats obtained an average CPP score (time stayed in the drug-pairing compartment) of 8.7±0.2 min. They were then given lesions at the majority (the *NAc* group), shell (the *shell* group), or core of NAc (the *core* group), or sham lesion to these areas, respectively, before primed with morphine and tested again for place preference. The results are shown in Fig. 2. Rats in the *NAc* and the *shell* group spent 5.5±0.3 min and 6.0±0.5 min in the drug-paired side, respectively, which were significantly lower ($t(19)=4.992$ and 4.131, respectively, $P<0.001$) than corresponding sham lesion groups (8.1±0.3 and 8.6±0.2 min, respectively). However, rats in the *core* group did not show much difference from the sham control (CPP scores, 7.9±0.5 min and 8.5±0.4 min, respectively, $t(19)=1.028$, $P>0.05$). The sites of lesion at different parts of NAc are depicted in Fig. 4A–C. These results suggested that the shell of NAc is an important structure for drug priming-induced reactivation of CPP.

### 3.3. Effect of nucleus accumbens lesions on footshock-induced reactivation of CPP

Sixty rats were randomly distributed into six groups. Two of them were eliminated due to unsuccessful lesion,

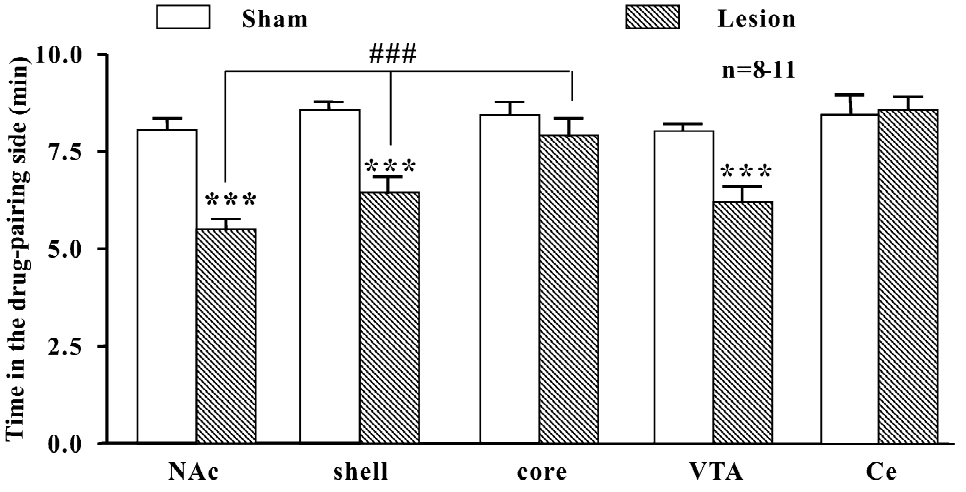


Fig. 2. Effect of lesions placed at different brain areas on drug priming-induced reactivation of conditioned place preference. ***$P<0.001$, compared with corresponding sham control group; $^{###}P<0.001$, compared between lesion groups. *NAc*, group with lesion at majority of nucleus accumbens; *shell*, group with lesion at shell of NAc; *core*, group with lesion at core of NAc; *VTA*, group with lesion at ventral tegmental area; *Ce*, group with lesion at central nucleus of amygdala.

so there were finally 9–10 rats in each group. After the last CPP training session, they obtained an average CPP score of 8.8±0.3 min. They were given the same types of lesion as described above, before footshock was delivered and CPP scores obtained. The results are shown in Fig. 3. Lesions in the *NAc* or *core* group failed to block the footshock-induced CPP reactivation. Rats stayed in the drug-pairing side for 7.8±0.6 min and 8.2±0.4 min, respectively, which were not significantly different ($t(18)=1.493$ and 0.783, respectively, $P>0.05$) from corresponding sham lesion groups (CPP scores, 8.6±0.2 and 8.6±0.3 min, respectively). Rats in the *shell* group showed only a marginal (6.8±0.4 min) but significant ($t(18)=2.736$, $P<0.05$) decrease of place preference, compared with the sham control group (8.3±0.3 min). Reasoning from these results, NAc seems to be less important in the footshock-induced reactivation of CPP.

### *3.4. Effect of VTA lesion on drug priming-induced reactivation of CPP*

Twenty rats were randomly distributed into two groups, with 8–9 in each group after eliminating the three with unsuccessful lesion. After the last CPP training, they obtained an average CPP score of 8.9±0.2 min. They were then given VTA lesion, before primed with morphine and tested for CPP. The result is shown in Fig. 2. The VTA lesion significantly attenuated the CPP reactivated by drug priming, as compared with the sham group (6.3±0.4 min versus 8.2±0.2 min, respectively, $t(19)=7.100$, $P<0.001$).

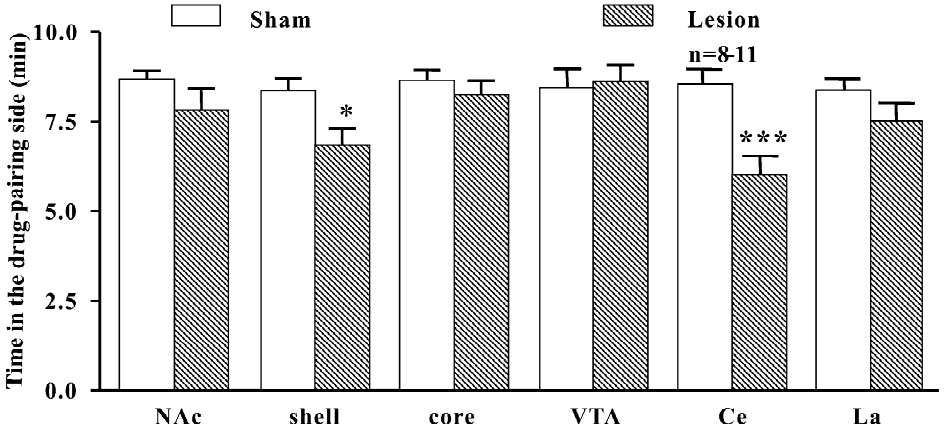


Fig. 3. Effect of lesions placed at different brain areas on footshock-induced reactivation of conditioned place preference. *, ***$P<0.05$ and $P<0.001$, respectively, compared with corresponding sham control group. *NAc*, group with lesion at majority of nucleus accumbens; *shell*, group with lesion at shell of NAc; *core*, group with lesion at core of NAc; *VTA*, group with lesion at ventral tegmental area; *Ce*, group with lesion at central nucleus of amygdala; *La*, group with lesion at lateral nucleus of amygdala.

The site of lesion at VTA is depicted in Fig. 4D. Thus, VTA is another important structure for drug priming-induced reactivation of CPP.

### 3.5. Effect of VTA lesion on footshock-induced reactivation of CPP

Twenty-eight rats were randomly distributed into two groups. Five rats in the lesion group and two in the sham group were dropped due to unsuccessful lesion or other unexpected technical reason. They were trained and lesioned as described above. The average CPP score after training and before lesion is 8.6±0.2 min. Nine days after surgery, they were given the aforementioned footshock before CPP test. The result is shown in Fig. 3. The CPP score of VTA lesion group was 8.5±0.4 min, which was not significantly ($t(16)=0.1678$, $P>0.05$) different from the sham lesion group (8.3±0.5 min). Again, VTA seems not important in the footshock-induced reactivation of CPP.

### 3.6. Effect of Ce and La lesion on footshock-induced reactivation of CPP

So, which brain area might play the major role in footshock-induced CPP reactivation? To address this question, 40 rats were randomly distributed into four groups, with 9–10 in each group after dropping of the rats with a

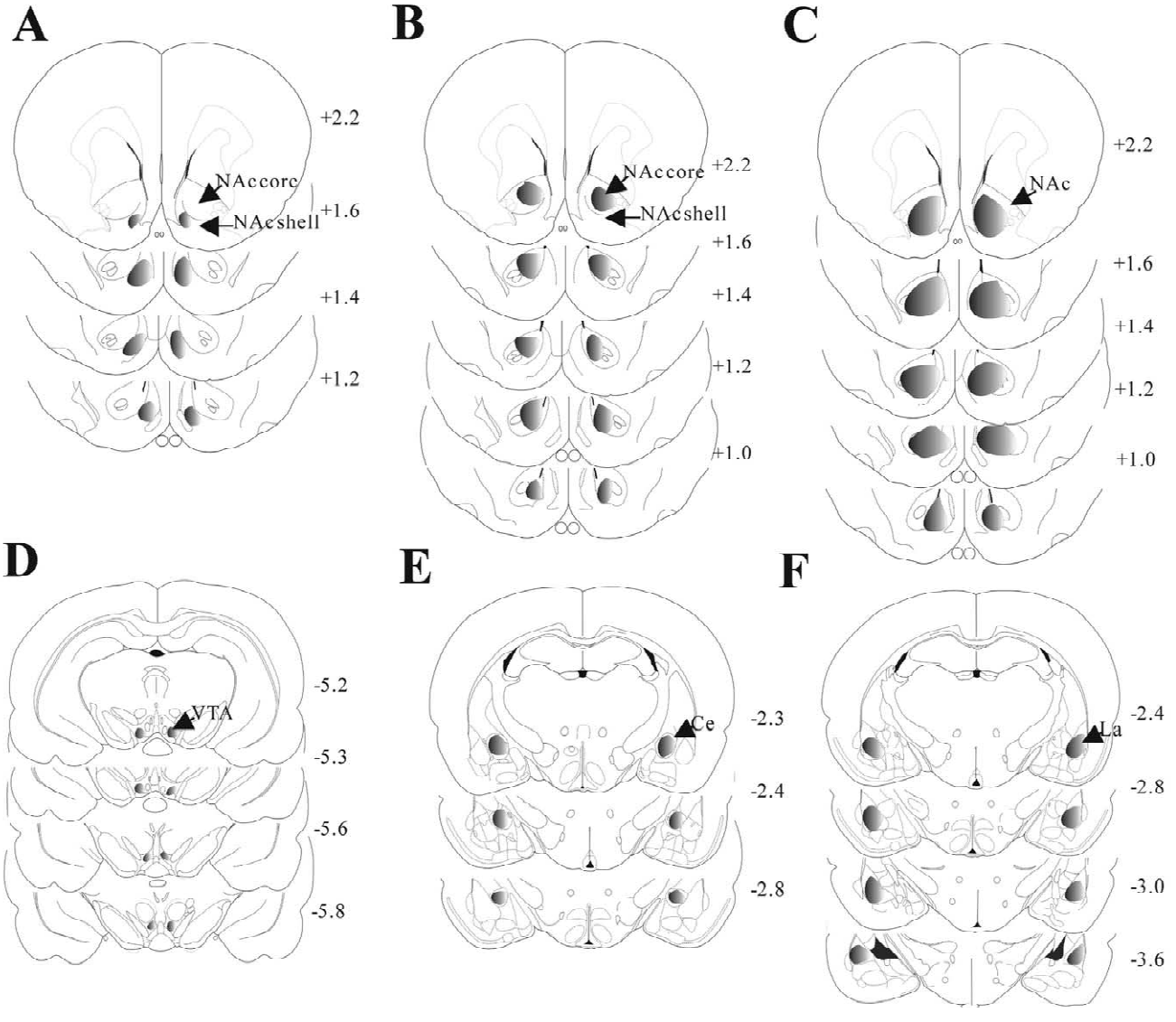


Fig. 4. Schematic representation of coronal sections through the rat brain showing average area (hatched area) destroyed by the lesions in (A) shell of nucleus accumbens (NAc); (B) core of NAc; (C) majority of NAc; (D) ventral tegmental area (VTA); (E) central nucleus of amygdala (Ce), and (F) lateral nucleus of amygdala (La). Drawing is based on illustrations in the atlas of Paxinos and Watson [27]. Numbers beside each section indicate millimetres anterior (+) or posterior (−) from bregma. An animal was included for analysis only with bilateral lesions destroying a significant portion of the intended structure, but not extending beyond its boundaries.

missed lesion. After the last CPP training, their average CPP score is 8.7±0.4 min. Two groups were then given lesions to the central (Ce) or lateral (La) nucleus of amygdala, before given footshock and tested for CPP. The result is shown in Fig. 3. Ce lesion completely abolished the CPP reactivation induced by footshock. Rats stayed in the drug-pairing side for 6.0±0.5 min in the lesion group, which was significantly shorter than its sham lesion control (8.5±0.4 min, $t(18)=4.249$, $P<0.001$). On the other hand, the La lesion has no effect on footshock-induced CPP reactivation (7.6±0.5 min vs. 8.3±0.3 min, $t(18)=1.353$, $P>0.05$). The sites of lesion at Ce and La are depicted in Fig. 4E and F. Thus, Ce seems to be an important area for footshock-induced reactivation of CPP.

### *3.7. Effect of Ce lesion on drug priming-induced reactivation of CPP*

Is Ce also involved in drug priming-induced CPP reactivation? To answer this question, 24 rats were randomly distributed into two groups. There were two rats dropped in the lesion group and one in the sham group for miss-targeted lesion. So finally there were 10–11 rats in each group. After the last training CPP session, they reached an average CPP score of 8.4±0.2 min. Rats in one group were then given Ce lesion, before primed and tested for CPP scores. The result is shown in Fig. 2. Rats in Ce lesion group stayed in the drug-pairing side for 8.5±0.3 min, which was not significantly ($t(20)=0.1827$, $P>0.05$) different from the sham lesion group (8.4±0.5 min). Thus, Ce is apparently not involved in drug priming-induced CPP reactivation.

## 4. Discussion

It is believed that drug addiction is a chronic, recurrent brain disease characterized by relapse [13]. A high rate of relapse after prolonged drug-free periods characterizes the behavior of experienced addicts of heroin and other drugs of abuse [12]. Data from both human and rodent research showed that there are at least three types of factors that could most effectively trigger relapse, i.e. re-exposure to the drug itself [5,38], exposure to stress [1,33], or presentation of drug-associated stimuli or cues [2,21]. Thus, the question arises as to what are the mechanisms underlying the relapses triggered by these factors.

### *4.1. Possible role of the mesolimbic dopaminergic pathway in drug priming-induced CPP reactivation*

Our results showed that lesions of nucleus accumbens, either the majority or the shell but not the core part, could completely abolish the low-dose morphine primed reactivation of an extinguished CPP (Fig. 2). It was reported that the shell and core of NAc have distinct afferent and efferent connections. While the shell represents the character of the true limbic structures, the core portion represents an extension of the sensorimotor striatal complex [28]. Di Chiara and co-workers [7] reported that morphine and psychostimulants preferentially increase extracellular dopamine in the shell of the NAc in rats, where dopamine (DA) is considered as the most important neurotransmitter mediating drug reward [13]. Shaham and Stewart [33] suggested that drug priming might elicit drug-seeking behavior by activating an incentive motivation system, with mesolimbic DA transmission as its most important component [30]. Taken together, we postulated that it is the shell but not the core of the NAc that plays a critical role in drug-induced CPP reactivation.

Ventral tegmental area (VTA) is the source of the dopaminergic projection to the shell of nucleus accumbens. Our results showed that like the shell or majority of NAc lesions, bilateral VTA lesion also abolished the drug priming triggered CPP reactivation. Thus, it is highly possible that the projection from VTA to the shell of NAc, presumably dopaminergic, is involved in mediating the reactivation of CPP for morphine observed in this study. Taken together, these results suggest that the functional integrity of the VTA–NAc projection (presumably mesolimbic dopaminergic) system might be indispensable for the induction of relapse to the morphine-primed drug-seeking behavior.

### *4.2. Mesolimbic dopaminergic pathway may not be involved in footshock-induced CPP reactivation*

It has been well documented that a brief exposure to stressors such as tail pinch or footshock could activate the brain systems involved in the reinforcing effects of drug abuse, including the opioid system [16] and the dopaminergic system in the midbrain [12]. It would thus be interesting to explore whether stress-induced relapse shares the same neural pathway as drug priming-induced relapse, i.e. mesolimbic dopaminergic pathways. To test this hypothesis, we placed lesions at NAc and VTA. As shown in Fig. 3, lesions of both core and majority of NAc had no effect on footshock-induced CPP reactivation. Similarly, lesion of VTA had no effect, either. These results suggested that footshock-induced relapse might be mediated mainly by a mechanism independent of the mesolimbic dopaminergic system. This is in line with the finding that selective dopamine D1- or D2-like receptor antagonists have no effect on footshock-induced reinstatement of heroin self-administration [34].

Interestingly, we found that lesion of the shell part of NAc did produce a marginal effect, though much weaker than its effect on drug priming-induced CPP reactivation. This result is more difficult to interpret. It is possible that a lesion specifically directed to the shell of NAc might be able to damage this area more thoroughly than a lesion directed to the majority of NAc, hence to remove a

possible secondary role of this area in footshock-induced CPP reactivation. Further study will be necessary to verify this hypothesis.

### 4.3. Possible involvement of the central nucleus of amygdala in footshock-induced CPP reactivation

Reports about the relationship between amygdala and conditioned place preference seemed rather conflicting. Hiroi and White [10] first reported that electrolyte or excitotoxic lesion placed at the lateral (La) but not central (Ce) or basolateral (BL) nucleus of amygdala could attenuate the expression of amphetamine-induced CPP. Similar reports revealed that destruction of amygdala blocked cocaine-induced CPP [3], while La lesion blocked the formation of food-related CPP [41], which was possibly through a cholinergic pathway [22]. However, O'Dell et al. [25] stated that intra-Ce but not intra-BL infusion of amphetamine could induce CPP. Thus, different parts of amygdala may be involved differently in the formation and expression of place preference behavior induced by the same or different non-conditioned stimulation.

However, recent evidence has led us to the conclusion that the central nucleus of amygdala (Ce) might play an important role in the footshock-induced reinstatement of drug self-administration. For example, Erb et al. [8] reported that reversible inactivation of Ce with tetrodotoxin attenuated the footshock-induced reinstatement of cocaine self-administration. Similar effect was also observed with footshock-induced reinstatement of heroin self-administration [36]. These results are in line with what we found in the current study that lesion of Ce but not La could block the footshock-induced reactivation of morphine-paired CPP. This is also consistent with the report that inhibition of calcium/calmodulin-dependent protein kinase II in amygdala suppressed the development, maintenance, and reactivation of morphine-induced CPP [18]. So what might be the possible mechanisms that underlie this phenomenon?

Shaham et al. [35] reported that intracerebroventricular (i.c.v.) infusions of CRF could mimic the induction of stress-triggered heroin-seeking behavior, while infusion of CRF receptor antagonist could partially reduce this stress-induced relapse. Koob et al. [14] argued that CRF might contribute to the mechanisms of drug relapse. It would be interesting to note that the amygdaloid CRF plays an important role in the responses to stressful stimuli [9], and a dense population of CRF cell bodies as well as the greatest density of CRF fibers was observed in Ce and adjacent areas [31]. According to the review of Shalev et al. [37], there is a CRF-containing project from Ce to BNST, which had been proved to be involved in footshock-induced reinstatement of cocaine seeking behavior. Thus, Ce lesion might have destroyed the amygdala–BNST CRF transmission that is very important in mediating stress-induced relapse to drug-seeking behavior, i.e. place preference in the current situation, hence to selectively block footshock- but not drug priming-induced reactivation of morphine CPP. This inference was again supported by the works of Lu et al. that pretreatment with $CRF_1$ receptor antagonist CP-154,526 attenuated the stress-induced reactivation of both cocaine- and opiates-seeking behavior [17,19].

### 4.4. Limitation of electrolytic lesion approach

The most important limitation of the electrolytic lesion approach employed in the current study is that it could destroy not only the neuron cell body, but also the pass-by fibers of the targeted area. Due to this limitation, the effect observed here might also be due to a lesion to fibers that happen to pass by the targeted area. It also remains to be elucidated as to what are the specific neurotransmitters involved in the effect we observed in this study, which cannot be answered by the electrolytic approach, either. Thus, further cell-body-specific or neurotransmitter-specific chemical lesion studies would be necessary to answer these questions.

### 4.5. Concerns about the maintenance of the morphine-induced CPP

An earlier study of Mucha and Iversen [23] reported that morphine-induced CPP could maintain at a similar level for at least 1 month after the end of training without any further treatment. However, this result was not always reproducible with different laboratory settings. For example, Lu et al. [20] reported with a similar chamber setting that the morphine-induced CPP would totally disappear 6 days after the end of training without extinction procedures. Thus, the time CPP for morphine could last varies with many experimental conditions, such as details of the training and handling procedures. In the current study, as well as in our previous work [39], we found that the morphine-induced CPP disappeared 7–9 days after the end of training. This rapid disappearance of CPP might be due to several reasons. Firstly, unlike the settings of Mucha and Iversen [23] and later on Lu et al. [19], we were using compartments with different texture of black painting (i.e. vertical strips versus dots) over white background instead of totally black and white compartments. While being an unbiased chamber setting, this might render it more difficult for the rats to keep the memory of the environmental cue related with drug effect. Secondly, the doses of morphine, numbers of training sessions, as well as inter-session intervals of the current study are all different from those employed by the other two groups. Thus, it is possible that the combination of these different settings produced the rapidly disappearing CPP observed in the current study.

In conclusion, the mesolimbic VTA–NAc projection (presumably dopaminergic) is indispensable for morphine

priming-induced relapse to morphine-induced place preference. The central nucleus of amygdala, on the other hand, is very important for the stress-induced reactivation of CPP. The results from the current study will also be valuable for later neural recording studies to ascertain the patterns of activity in these regions that accompany the relapse of place preference.

## Acknowledgements

This study was supported by an NIDA grant DA 03983 to J.-S.H, and by the National Basic Research Program (G 1999 054000) of China.

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