

Original Article

# Blockade of mGluR5 in the nucleus accumbens shell but not core attenuates heroin seeking behavior in rats

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**Aim:** Glutamatergic neurotransmission in the nucleus accumbens (NAc) is crucial for the relapse to heroin seeking. The aim of this study was to determine whether mGluR5 in the NAc core or shell involved in heroin seeking behavior in rats.

**Methods:** Male SD rats were self-administered heroin under a fixed-ratio 1 (FR1) reinforcement schedule for 14 d, and subsequently withdrawn for 2 weeks. The selective mGluR5 antagonist 2-methyl-6-phenylethynyl-pyridine (MPEP, 5, 15 and 50 nmol per side) was then microinjected into the NAc core or shell 10 min before a heroin-seeking test induced by context, cues or heroin priming.

**Results:** Microinjection of MPEP into the NAc shell dose-dependently decreased the heroin seeking induced by context, cues or heroin priming. In contrast, microinjection of MPEP into the NAc core did not alter the heroin seeking induced by cues or heroin priming. In addition, microinjection with MPEP (15 nmol per side) in the NAc shell reversed both the percentage of open arms entries (OE%) and the percentage of time spent in open arms (OT%) after heroin withdrawal. Microinjection of MPEP (50 nmol per side) in the striatum as a control location did not affect the heroin seeking behavior. Microinjection of MPEP in the 3 locations did not change the locomotion activities.

**Conclusion:** Blockade of mGluR5 in NAc shell in rats specifically suppresses the relapse to heroin-seeking and anxiety-like behavior, suggesting that mGluR5 antagonists may be a potential candidate for the therapy of heroin addiction.

**Keywords:** heroin; addiction; relapse; mGluR5; MPEP; the nucleus accumbens; striatum

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## Introduction

Relapse is the most important manifestation of heroin addiction following prolonged periods of abstinence<sup>[1]</sup>. Relapse arises from intense craving that can be triggered by a single drug-taking experience or exposure to an environmental stimulus<sup>[2]</sup>. The pre-clinical literature indicates that long-term drug-induced alterations in glutamatergic transmission within the NAc may underlie relapse to drug-seeking behavior following the re-exposure to drugs and drug-associated stimuli during abstinence of the drug<sup>[3,4]</sup>. Type 5 metabotropic glutamate receptor (mGluR5) as a postsynaptic element is abundantly present throughout in the NAc, where it is positively coupled to NMDA receptor function and mediates various forms of synaptic plasticity<sup>[5,6]</sup>. The deletion of mGluR5 results in marked deficits in responding to the

reinforcing and locomotor stimulating effects of cocaine<sup>[7]</sup>. Furthermore, the administration of the noncompetitive selective mGluR5 antagonist 2-methyl-6 (phenylethynyl) pyridine (MPEP) decreased the self-administration and/or relapse of nicotine<sup>[8,9]</sup>, cocaine<sup>[10,11]</sup>, ethanol<sup>[12–14]</sup>, and heroin<sup>[15,16]</sup>. MPEP, another antagonist of mGluR5, also can attenuate opiate self-administration and opiate-seeking behavior in mice<sup>[17]</sup>. These data indicate mGluR5 plays a critical role in the behavioral responses to multiple substances of abuse.

The NAc consists of two primary functional subdivisions, the shell and core subregions. The NAc receives massive glutamatergic input from various corticolimbic structures, such as the prefrontal cortex (PFC), the amygdala and the hippocampus<sup>[18]</sup>. Inactivation of neurons in the NAc inhibits heroin seeking induced by cues<sup>[19]</sup>. In addition, the reinstatement of heroin-seeking is blocked via the inhibition of prelimbic cortex projections to the NAc or NMDA2b-containing receptors in the NAc<sup>[20,21]</sup>; *N*-acetylcysteine treatment, which decreases the basal level of glutamate, blocks both heroin- and cue-primed reinstatement<sup>[22]</sup>. In contrast, activation of glutamatergic pro-

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jections in the NAc shell promotes heroin relapse<sup>[23]</sup>. However, the effects of mGluR5 blockade in the NAc shell or core on heroin-seeking behavior have not yet been evaluated. In the present study, we observed the effects of MPEP microinjections into the NAc shell or core on the reinstatement of heroin-seeking induced by context, cues or heroin priming.

## Materials and methods

### Animals

Adult male Sprague-Dawley rats obtained from the Experimental Animal Center of Zhejiang Province that weighed 250–300 g at the beginning of experiment were individually housed in a temperature (23±1 °C) and humidity (50%±10%)-controlled vivarium with a reversed 12 h light/dark cycle (7:00 AM lights off–7:00 PM lights on). After arrival, the rats were allowed to acclimate for at least 1 week before the start of the experiment, and all behavioral testing occurred during the dark period. Water was provided *ad libitum*, with the exception of during testing, and rat chow was restricted to 25 g per day following the acquisition of heroin self-administration behavior. All subjects were treated, housed and used in accordance with the National Institutes of Health (NIH) and the Association for the Assessment and Accreditation of Laboratory Animal Care; the experiments were approved by the Animal Care and Use Committee of Ningbo University.

### Drugs

The National Institute of Forensic Science (Beijing, China) generously supplied the diacetylmorphine HCl (heroin), and the heroin was freshly dissolved in 0.9% sterile physiological saline for intravenous (iv) injection. MPEP was purchased from Sigma (Shanghai, China); the doses of MPEP (5, 15, or 50 nmol per side) were selected based on the conventions of the salt in the literature (the minimum dose was 2.5 nmol per side)<sup>[24]</sup>. MPEP was dissolved in sterile saline and microinjected bilaterally into specific brain sites 10 min before testing.

### Intravenous catheterization and intracranial cannula implantation

The rats were anesthetized with sodium pentobarbital (50 mg per kg, ip; Serva, Germany) and then prepared and implanted with intravenous catheters in the right jugular vein as previously described<sup>[25]</sup>. The intracranial guide cannulas were implanted bilaterally to terminate 2 mm above the NAc shell (from bregma: anterior/posterior, +1.7 mm; medial/lateral, ±0.8 mm; dorsal/ventral, -6.5 mm from dura), the NAc core (from bregma: anterior/posterior, +1.7 mm; medial/lateral, ±2.2 mm; dorsal/ventral, -5.5 mm from dura) or the striatum (from bregma: anterior/posterior, +1.7 mm; medial/lateral, ±2.5 mm; dorsal/ventral, -5.0 mm from dura) according to the atlas of Paxinos and Watson using a stereotaxic frame; operations were performed as previously described<sup>[26]</sup>. To prevent postoperative infections and catheter blockage, the rats received an intravenous infusion (0.2 mL) of the antibiotic penicillin (100 mg per kg) and heparin (30 units per mL) daily

after the surgery. The bilateral intracranial guide cannulas were also covered with dust caps.

### Apparatus: testing chambers

Heroin self-administration and reinstatement testing were conducted in operant self-administration chambers (AniLab Software & Instruments Co, Ltd Ningbo, China), which were constructed of metal and plexiglass and housed in sound-attenuated boxes (24 cm×30 cm×28 cm). The chamber had a metal grid floor, and one of the walls had two nose-poke holes (one active and one inactive) with a yellow cue light located inside. The holes were activated by the breaking of a photo-beam 0.5 cm inside the hole with a white stimulus light located near the top of the chambers, which was a Sonalert speaker that provided auditory stimuli during drug delivery or cue-induced reinstatement procedures. A house-light and exhaust fan were located on the opposite wall to mask external noise and odors. Each chamber had a syringe pump outside that was interfaced to a PC to deliver the drug solution to the subjects through Tygon tubing, which was protected by a spring lead.

### Heroin self-administration training and withdrawal

Following recovery from surgery for 1 week, the rats were trained to self-administer heroin in the testing chamber under an FR-1 schedule for 4 h per day for consecutive 14 d. The sessions were initiated with the house-lights off, and the illumination of the yellow light inside the active nose-poke hole signaled the drug availability. Each response in the active hole was immediately reinforced with an infusion of heroin (0.1 mg per kg for d 1–2, 0.05 mg per kg for d 3–6, and 0.025 mg per kg for d 7–14) prepared in 0.9% sterile saline, which was administered via the pump over 4 s as previously described<sup>[25]</sup>. The delivery of each heroin infusion was accompanied by a light cue and was followed by a 20 s time out period, during which responses on the active nose-poke were counted but resulted in no heroin delivery. The session was automatically terminated after 4 h or when the rat received the maximum 80 infusions, whichever occurred first. The rats were returned to their individual home cages shortly after the session. Each rat received one heroin self-administration session per day for a total of 14 d.

During the withdrawal phase, the rats remained undisturbed in their individual home cages for 14 d and did not have access to heroin, the heroin-associated cues, or the heroin self-administration context.

### Intracranial microinfusions

During microinfusions, the injectors were extended 2 mm below the tips of the guide cannulae, and MPEP or saline was infused bilaterally into the NAc shell, core or striatum in a volume of 0.5 µL per hemisphere over 1 min using a microinfusion pump (MD-1001, Bioanalytical Systems, Inc, IN, USA). The injector was left in place for an additional 1 min after the infusion. The rats were then returned to their home cages for 10 min before testing.

### Heroin-seeking induced by context, cues or heroin priming

All rats underwent an identical sequence of behavioral training and withdrawal procedures. The heroin-seeking tests were initiated following withdrawal procedures<sup>[27]</sup>. All rats were tested for heroin-seeking behavior in the original self-administration chambers. For context-induced heroin-seeking, the rats were placed in the chambers without heroin and other conditioned cues. For cue-induced heroin-seeking, an active nose-poke resulted in the presentation of the cues, but no heroin infusions. For heroin-primed heroin-seeking, the rats received a heroin injection (0.25 mg per kg, sc) at 10 min before testing; the rats were then placed in the chambers. For the heroin-seeking induced by heroin-priming with conditioned cues, the rats were given a heroin injection (0.25 mg per kg, sc) at 10 min before testing, and then one active nose-poke resulted in the presentation of the cues, but no heroin infusions during the testing.

### Elevated plus-maze

The elevated plus-maze (EPM) test was evaluated for the rats after heroin abstinence. In brief, the apparatus consisted of a plexiglas maze with two opposite open arms (30 cm×5 cm×0.25 cm) and two opposite enclosed arms (30 cm×5 cm×15 cm) that extended from a central platform (5 cm×5 cm) to form a plus sign. The whole plus-maze apparatus was elevated 50 cm above the floor and placed inside a sound-attenuated room where the rats were habituated for at least 2 h. The trial was initiated by placing the rat on the central platform of the maze facing its head towards an open arm. Then, the proportion of entries into the open arms (OE%) and the proportion of time spent in the open arms (OT%) were recorded during a 5 min test period. An entry into an arm was considered valid only when all four paws of the rat were inside that arm. The animal activities were tracked and recorded via an

overhead video camera linked to a monitor with computer software Smart Version 2.5 (Shanghai Yishu Mdt InfoTech Co, Ltd). The apparatus was thoroughly cleaned with 70% ethanol after each trial.

### Locomotion activity after withdrawal

To further assess the potential nonspecific effects of MPEP or surgery on general motor activity, we examined the effects of MPEP microinjection on locomotion in a novel context. The rats were habituated for 1 h in chambers (AniLab Software & Instruments Co, Ltd, Ningbo, China). After 1 h of habituation in the chamber, the rats ( $n=6$  per group) were microinfused with MPEP in the NAc shell or core. The horizontal locomotion activities were recorded for 1 h. The primary parameter was total distance traveled during the separate 1 h session; data were analyzed as the measure of locomotion using MED Associates SOF-811 Open-field Activity Software<sup>[25]</sup>.

### Histological examination

The rats were deeply anaesthetized with pentobarbital (100 mg/kg, ip) and transcardially perfused with 250 mL of 0.9% saline, followed by 200 mL of 4% paraformaldehyde in 0.01 mol phosphate buffered saline (PBS; pH 7.4). The brains were removed, post-fixed overnight in 4% paraformaldehyde in PBS, and stored in 30% sucrose/PBS. Coronal sections (35  $\mu$ m) were cut through the guide cannula tract using a freezing microtome (Leica, Germany), mounted on gelatin-coated slides and stained with 1% cresyl violet. The cannulae placements in different brain areas were verified. These placements are presented in Figure 1.

### Statistical analysis

All data are presented as the mean±standard errors of the mean (SEM). The dependent measures were total responses

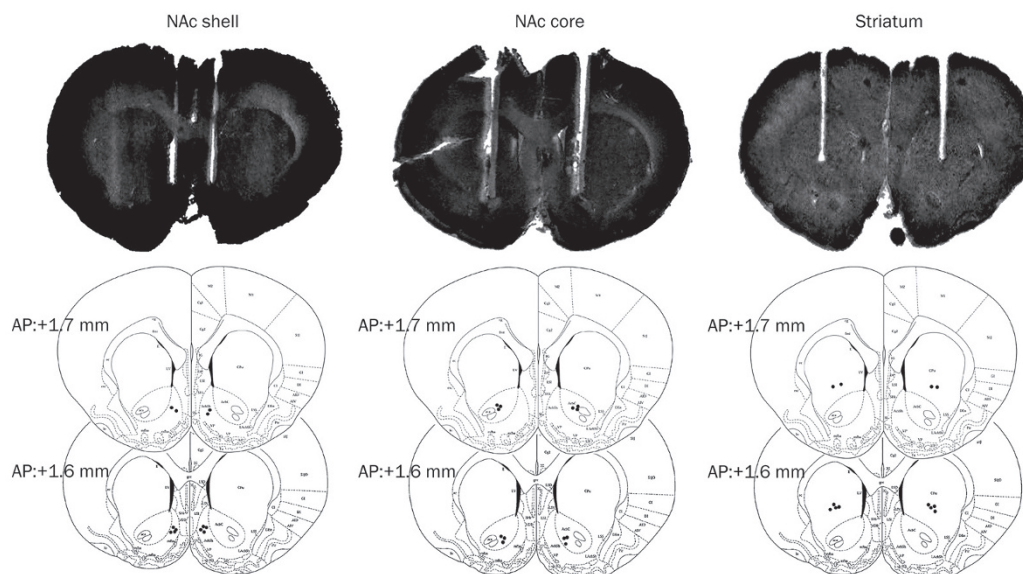


Figure 1. Diagrams of coronal sections of the rat brain showing the location of the microinjector (NAc shell, NAc core and striatum).

on the previously active nose-poke and inactive nose-poke responses. The data were analyzed by one way ANOVA. *Post-hoc* analyses were performed with the Newman-Keuls test, and differences are reported for  $P < 0.05$ .

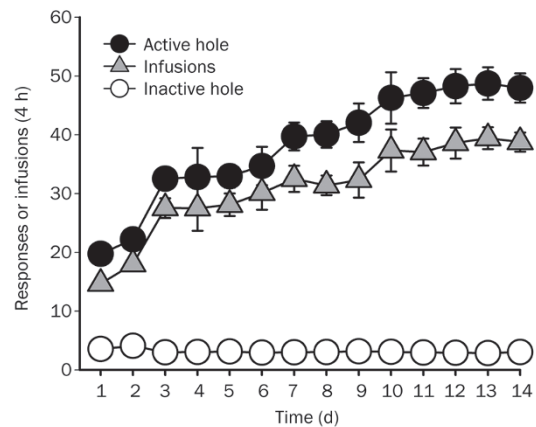
## Results

### Acquisition training of heroin self-administration

Figure 2 shows the number of heroin infusions, active nose pokes and inactive nose-pokes for all rats that were subsequently tested in all experiments. The rats exhibited reliable heroin self-administration, as indicated by the increase in an active nose-poke when the heroin dose was decreased from 0.1 to 0.025 mg per kg bodyweight per infusion ( $P < 0.05$ ).

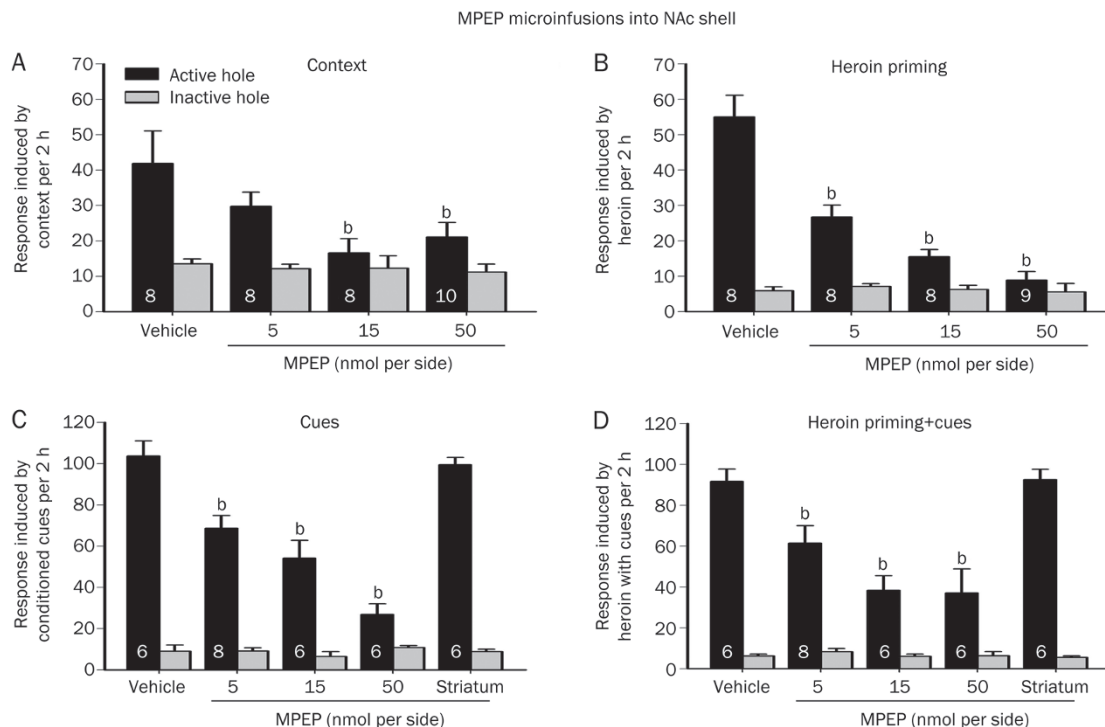
### Effects of MPEP microinfusions into the NAc shell on heroin-seeking induced by context

Bilateral microinfusions were administered into the NAc shell as previously described. The rats ( $n = 8-10$  per group) were placed into the self-administration chamber 10 min after MPEP (vehicle, 5, 15, or 50 nmol per side) was microinfused into the NAc shell. MPEP microinfusions into the NAc shell significantly attenuated the context-induced heroin-seeking behavior. As shown in Figure 3A, one-way ANOVA revealed a significant effect of MPEP per-treatment on active responses ( $F_{(3,30)} = 3.78$ ,  $P = 0.02$ ), and *post hoc* individual group comparisons demonstrated that MPEP at doses of 15 or 50 nmol per



**Figure 2.** Acquisition of heroin self-administration. Number of infusions and active and inactive nose-poke responses during the consecutive 14 d of heroin self-administration training. The rats were trained under an FR1 reinforcement schedule, and the dose of heroin was 0.1 mg per kg for the first 2 d, 0.05 mg per kg for the next 4 d, and 0.025 mg per kg for the last 8 d. Mean  $\pm$  SEM.

side significantly decreased the active responses induced by context compared with the vehicle group (both  $P < 0.05$ ). There was no effect of MPEP microinfusions on the inactive responses at any dose ( $F_{(3,30)} = 0.17$ ,  $P = 0.91$ ).



**Figure 3.** Effects of MPEP microinfusion into the NAc shell on heroin-seeking. The rats were microinjected with vehicle or MPEP (5, 15, or 50 nmol per side) 10 min before test. (A) Microinfusion of MPEP into the NAc shell attenuated heroin-seeking behavior induced by context. (B) Microinfusion of MPEP into the NAc shell attenuated heroin-seeking behavior induced by heroin priming. (C) MPEP microinfusions into the NAc shell attenuated heroin-seeking behavior induced by cues. (D) MPEP microinfusions into the NAc shell attenuated heroin-seeking behavior induced by heroin priming with cues. Mean  $\pm$  SEM of number of active or inactive responses during testing. <sup>b</sup> $P < 0.05$  compared with the vehicle group.

### Effects of MPEP microinfusions into the NAc shell on heroin-seeking induced by heroin priming

The rats ( $n=6-9$  per group) were microinjected with MPEP (vehicle, 5, 15, or 50 nmol per side) into the NAc shell, followed by the injection of heroin at a dose of 0.25 mg per kg (sc). As shown in Figure 3B, MPEP microinfusions attenuated the heroin-seeking behavior induced by heroin priming. One-way ANOVA revealed a significant effect of MPEP pre-treatment in the NAc shell on active responses ( $F_{(3,29)}=29.26$ ,  $P<0.01$ ), and *post hoc* comparisons demonstrated that MPEP microinfusions at all doses significantly decreased the active responses compared with the vehicle group (all  $P<0.05$ ). Pre-treatment with MPEP failed to affect the inactive responses ( $F_{(3,29)}=0.19$ ,  $P=0.91$ ).

### Effects of MPEP microinfusions into the NAc shell on heroin-seeking induced by conditioned cues

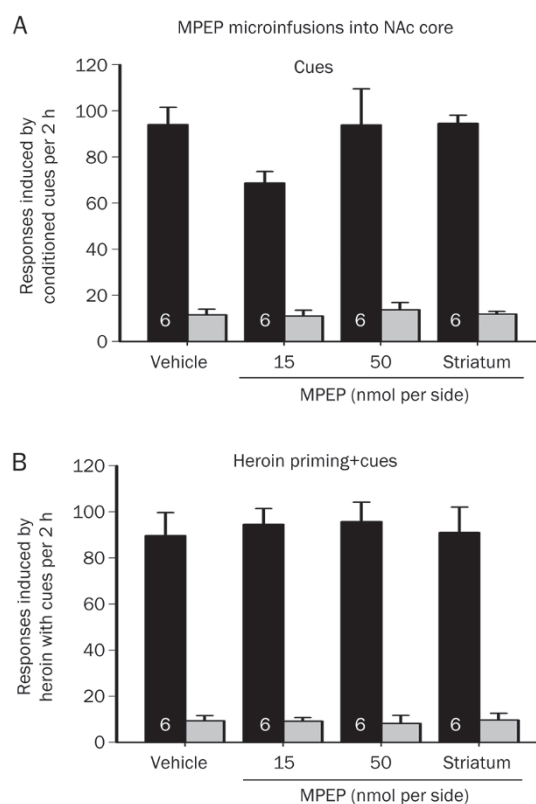
The rats ( $n=6-8$  per group) were placed into the self-administration chamber 10 min after the microinfusion of MPEP (5, 15, or 50 nmol per each side) or vehicle into the NAc shell; MPEP was microinfused into the striatum with the highest dose at 50 nmol per side as an anatomical control. The microinjection of MPEP into the NAc shell dose-dependently reduced cue-induced heroin-seeking behavior. As shown in Figure 3C, one-way ANOVA revealed a significant effect of MPEP pre-treatment on active responses ( $F_{(4,27)}=23.11$ ,  $P<0.01$ ), and *post hoc* comparisons demonstrated that MPEP microinfusions at all doses into the NAc shell significantly decreased the active responses compared with the vehicle group (all  $P<0.01$ ). MPEP microinfusions into the striatum failed to affect the active responses compared with the vehicle group ( $P>0.05$ ). In contrast, MPEP microinfusions did not affect the inactive responses among the groups ( $F_{(4,27)}=0.60$ ,  $P<0.67$ ).

### Effects of MPEP microinfusions into the NAc shell on heroin-seeking induced by heroin priming combined with cues

Bilateral microinfusions of (5, 15, or 50 nmol per side) into the NAc shell ( $n=6-8$  per group) significantly attenuated the heroin-seeking behavior induced by heroin priming combined with conditioned cues. As shown in Figure 3D, one-way ANOVA revealed a significant effect of MPEP pre-treatment on active responses ( $F_{(4,27)}=10.31$ ,  $P<0.01$ ); *post hoc* comparisons demonstrated that MPEP microinfusions at all doses significantly decreased the active responses compared with the vehicle group (all  $P<0.05$ ). There was no significant difference in the active responses between the anatomical control and vehicle groups ( $P=0.94$ ). Thus, MPEP microinfusions failed to affect the inactive responses ( $F_{(4,27)}=1.34$ ,  $P=0.28$ ).

### Effects of MPEP microinfusions into the NAc core on heroin-seeking induced by cues or heroin priming with cues

To assess the role of mGluR5 in the NAc core on heroin-seeking behavior, we also examined the effects of MPEP microinfusion into the NAc core on the heroin-seeking induced by cues or heroin priming with cues. The data, shown in Figure 4, indicate pretreatment with MPEP (15 or 50 nmol per side)

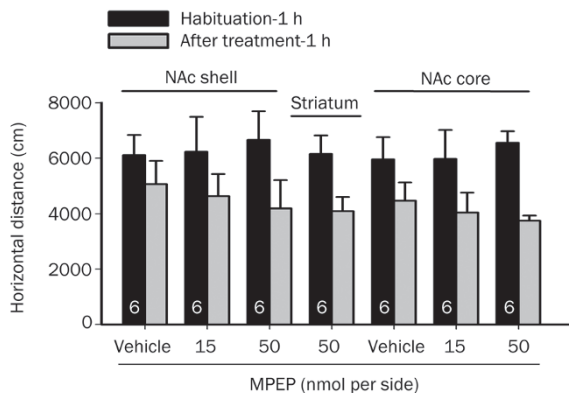


**Figure 4.** Effects of MPEP microinfusions into the NAc core on heroin seeking. The rats were pre-treated with vehicle or MPEP (15 or 50 nmol per side) 10 min before the test. (A) Microinfusion of MPEP into the NAc core had no effect on cue-induced heroin-seeking compared with the vehicle or anatomical control groups. (B) Microinfusion of MPEP into the NAc core had no effect on heroin seeking behavior induced by heroin priming with cues. Mean $\pm$ SEM of number of active or inactive responses during the drug seeking testing.

into the NAc core did not affect the heroin-seeking induced by either conditioned cues or heroin priming with cues. One-way ANOVA revealed no significant effect of MPEP treatment on the active ( $F_{(3,20)}=1.90$ ,  $P=0.16$ ) or inactive ( $F_{(3,20)}=0.22$ ,  $P=0.88$ ) responses induced by cues (Figure 4A). In addition, there was no significant effect of MPEP treatment on the active ( $F_{(3,20)}=0.095$ ,  $P=0.97$ ) or inactive ( $F_{(3,20)}=0.059$ ,  $P=0.98$ ) responses induced by heroin priming (0.25 mg per kg) with conditioned cues (Figure 4B).

### Effects of MPEP microinfusions into the NAc shell or core on locomotion activity

Whether MPEP has a specific effect on heroin seeking remains unknown. Thus, we examined the effects of MPEP injection on locomotion activity. MPEP (15 or 50 nmol per side) was microinfused into the specified brain areas after a 1 h habituation in the chambers, and the total distance was recorded for an additional 1 h. As shown in Figure 5, no significant difference was evident among the groups during the habituation ( $F_{(6,35)}=0.095$ ,  $P=0.99$ ). Pre-treatment with MPEP into the NAc shell, the NAc core or the striatum did not significantly alter



**Figure 5.** Effects of MPEP microinfusions into the NAc shell or core on locomotor activity after withdrawal. The rats were put in chambers for 1 h of habituation and then microinfused with MPEP (vehicle, 15 or 50 nmol per side) into the NAc shell, NAc core or striatum. The total distance (mean±SEM) was recorded for an additional 1 h. There was no difference in the total distance among the groups.

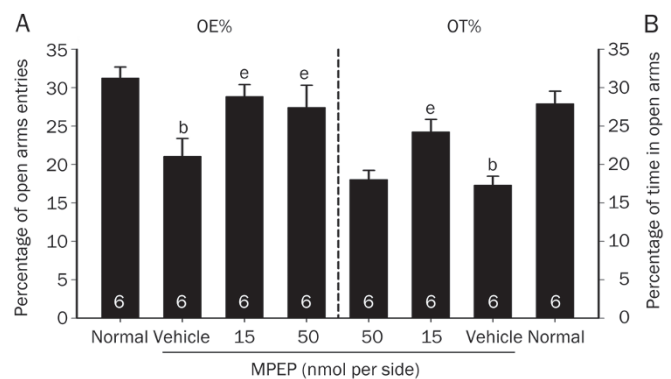
locomotion activity across all tests ( $F_{(6,35)}=0.37$ ,  $P=0.89$ ).

#### Effects of MPEP microinfusions into the NAc shell on anxiety-like behavior

Anxiety disorders are one of the common symptoms during opiate withdrawal, and treatment with MPEP produces an anxiolytic effect. Thus, we also examined the anxiety-like behaviors for the rats with cannulas located in the NAc shell. The rats that had similar experiences and time frames regarding heroin withdrawal and the rats without heroin exposure were tested for EPM as the normal control. As shown in Figure 6, one-way ANOVA revealed a significant effect of pre-treatment with MPEP into the NAc shell on OE% ( $F_{(3,20)}=4.14$ ,  $P=0.019$ ) (panel A) and OT% ( $F_{(3,20)}=12.69$ ,  $P<0.001$ ) (panel B). Compared with the control group, the anxiety-like behavior that was present in the heroin exposed rats indicated reductions of OE% and OT% ( $P<0.05$ ). Pre-treatment with MPEP at the dose of 15 nmol per side significantly reversed the reduction of OE% and OT% in the heroin exposed rats ( $P<0.05$ ). The results suggested that anxiety-like behavior can be induced in heroin addicted rats, and pre-treatment with MPEP can reduce this anxiety-like behavior.

#### Discussion

The main findings of the present study were acute pre-treatment with the mGluR5 antagonist MPEP directly into the NAc shell attenuated the reinstatement of heroin-seeking behavior induced by context, conditioned cues or heroin priming after prolonged withdrawal. In contrast, MPEP microinfused into the NAc core did not affect the heroin-seeking induced by cues or heroin priming. In addition, the effective doses of MPEP failed to produce significant effects on locomotor activity or inactive responding. The results suggested that the inhibitory action of MPEP on heroin-seeking behavior induced by context, cues or heroin priming did not result from its sedative qualities or the impairment of coordinated motor ability.



**Figure 6.** Effects of MPEP microinfusion into the NAc shell on the elevated plus maze test after withdrawal. (A) The percentage of open arms entries. (B) The percentage of time spent in open arms. One group of rats that had similar experiences and a time frame without heroin exposure was used as the normal control. The other heroin withdrawn rats ( $n=6$  per group) were microinfused with MPEP (vehicle, 15 or 50 nmol per side) into the NAc shell and then placed in the elevated plus maze for a 5 min test. Mean±SEM. <sup>b</sup> $P<0.05$  compared with the normal control. <sup>e</sup> $P<0.05$  compared with the vehicle group.

mGluR5s as a postsynaptic element are abundantly expressed in the NAc, where they are positively coupled to NMDA receptor function and enhance glutamatergic transmission. For example, the potentiation of mGluR5 function has been demonstrated to mediate various forms of synaptic plasticity<sup>[5]</sup>. The antagonism of mGluR5 by MPEP attenuated heroin-seeking behavior in the present study, which is consistent with the previous data that systemic administration of MPEP attenuates the reinstatement of various classes of other abused drugs in preclinical models<sup>[5,9,10,14]</sup>. Moreover, another antagonist of mGluR5 also attenuates opiate self-administration and opiate-seeking behavior in mice<sup>[17]</sup>. In the present study, the microinjection of MPEP into the NAc shell but not the core decreased heroin seeking behavior, which indicated that the activation of mGluR5 in the NAc shell may be involved in heroin seeking behavior induced by cues or heroin.

An influential neuroanatomical framework of drug relapse is that the glutamatergic projections from the dorsal mPFC to the NAc core pathway promote cocaine seeking, whereas the projections from ventral mPFC to the NAc shell inhibit cocaine seeking after extinction<sup>[28]</sup>. Interestingly, cocaine seeking induced by context appears to involve subregions of the mPFC and the NAc that are functionally dissociable from the regions involved in heroin seeking<sup>[29]</sup>, and the activation of glutamatergic projections from the ventral mPFC to the NAc shell promotes heroin relapse<sup>[23,30]</sup>. Here, we demonstrated that MPEP treatment in the NAc shell attenuated the heroin seeking behavior induced by context, which indicates the activation of mGluR5 in the NAc shell may be involved in context induced heroin seeking. With respect to opiate drugs, heroin administration leads to long-term alterations in gene expression in the NAc shell<sup>[31]</sup>. The mPFC is a brain area that has a pivotal role in relapse to opiate seeking during periods of drug abstinence.

nence<sup>[32, 33]</sup>. Re-exposure to heroin-conditioned cues results in acute synaptic depression of pyramidal neurons in the ventral mPFC<sup>[34]</sup>. In addition, the basolateral amygdala is a critical component of the neural circuitry that mediates conditioned and heroin-induced reinstatement of heroin-seeking behavior<sup>[35]</sup>, and the hippocampus and stimulation of mGluR in the hippocampus are involved in context-induced cocaine-seeking behavior<sup>[36, 37]</sup>. Thus, the blockade of glutamate transmission in the NAc shell by MPEP in the current study supported the role of the glutamatergic projection from the ventral mPFC and amygdala, as well as the hippocampus to the NAc shell in heroin seeking induced by cues or heroin priming. Additionally, another potential explanation is that pharmacological blockade of mGluR5 may activate the GABAergic projection from the NAc shell to the VTA and inhibit dopamine release from the VTA<sup>[38]</sup>. Thus, the inhibition of the heroin-seeking by MPEP may activate the pathway from the NAc shell to the VTA.

Anxiety-like behavior increased during the period of withdrawal from heroin self-administration. Recent fMRI data have supported the idea that the NAc is involved in not only the reward-based action-contingencies but also the etiology and maintenance of aberrant avoidance behaviors in anxiety disorders<sup>[39]</sup>. The microinjection of corticotropin-releasing factor into the NAc shell induced anxiety-like behaviors in an elevated plus maze and open field, which indicates the role of the NAc shell in anxiety<sup>[40]</sup>. Pretreatment with MPEP in the NAc shell also produced anxiolytic activity, which is consistent with previous studies that found that MPEP attenuated the anxiety-dependent variable in a variety of well-established anxiety test paradigms<sup>[41, 42]</sup>. Given that the magnitude of heroin seeking induced by heroin cues or priming is enhanced by spontaneous withdrawal or naltrexone-precipitated withdrawal<sup>[43, 44]</sup>, it is possible that the inhibitory action of MPEP on anxiety-like behavior may contribute to the blockade of heroin-seeking behavior in the NAc shell.

In conclusion, the present results demonstrated that the activation of mGluR5 in the NAc shell may be involved in heroin-seeking induced by context, cues or heroin priming and suggested that the development of mGluR5 antagonists may provide a novel approach for the treatment of heroin addiction.

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### Author contribution

Wen-hua ZHOU, Lie-min RUAN, and Zhong-ze LOU designed the research. Zhong-ze LOU, Ling-hong CHEN, and Hui-feng LIU performed the experiments and analyzed the data. Zhong-ze LOU, Lie-min RUAN, and Wen-hua ZHOU wrote the manuscript.

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