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ARTICLE

# Modulation of methamphetamine memory reconsolidation by neural projection from basolateral amygdala to nucleus accumbens

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Drug-associated conditioned cues promote subjects to recall drug reward memory, resulting in drug-seeking and reinstatement. A consolidated memory becomes unstable after recall, such that the amnestic agent can disrupt the memory during the reconsolidation stage, which implicates a potential therapeutic strategy for weakening maladaptive memories. The basolateral amygdala (BLA) involves the association of conditioned cues with reward and aversive valences and projects the information to the nucleus accumbens (NAc) that mediates reward-seeking. However, whether the BLA-NAc projection plays a role in drug-associated memory reactivation and reconsolidation is unknown. We used methamphetamine (MeAM) conditioned place preference (CPP) to investigate the role of BLA-NAc neural projection in the memory reconsolidation. Two weeks before CPP training, we infused adeno-associated virus (AAV) carrying the designer receptor exclusively activated by designer drugs (DREADD) or control constructs. We infused clozapine-*N*-oxide (CNO) after the recall test to manipulate the neural activity of BLA-NAc projections in mice. We found that after recall, DREADD-mediated inhibition of BLA neurons projecting to the NAc core blunted consolidated MeAM-associated memory. Inhibition of BLA glutamatergic nerve terminals in the NAc core 1 h after recall disrupted consolidated MeAM-associated memory. However, inhibiting this pathway after the time window of reconsolidation failed to affect memory. Furthermore, under the condition without memory retrieval, DREADD-mediated activation of BLA-NAc core projection was required for amnesic agents to disrupt consolidated MeAM-associated memory. Our findings provide evidence that the BLA-NAc pathway activity is involved in the post-retrieval processing of MeAM-associated memory in CPP.

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## INTRODUCTION

Addictive drugs promote individuals to compulsively seek these drugs regardless of negative consequences. The major problem of addiction is that relapse occurs even when subjects have no longer physical dependence. With repetitive exposure to these drugs, synapses in the reward-related neural circuits have adaptive changes, and neutral contextual cues become motivated and reinforced stimuli [1–3]. When individuals with substance use disorders re-expose to these drug-associated cues, the cues unconsciously retrieve maladaptive reward memory to promote drug relapse [4, 5]. Drug-associated cues are therefore believed to be important triggers of drug relapse [6].

Based on memory theory, a consolidated memory, when retrieved, becomes destabilized and could be reconsolidated to maintain that memory. However, during memory reconsolidation the presence of amnestic agents disrupts the memory. The process is called reconsolidation blockade [7, 8]. Reconsolidation blockade represents a therapeutic strategy for erasing or significantly weakening maladaptive memories, including post-traumatic stress disorder and substance use disorders [9–11]. However, the mechanism of reconsolidation blockade in drug-associated memory remains elusive. Previous studies reported that NR2B-containing NMDA receptors (NMDAR) in the basolateral amygdala (BLA) are required for memory recall-induced destabilization of fear memories [12]. In drug-associated memory, memory recall also induces memory destabilization by the mechanism that NR2B-containing NMDARs in the BLA activate calcineurin and protein phosphatase 1, which in turn results in dephosphorylation of p-GluR1-Ser845 and endocytosis of AMPARs [13]. Thus, the BLA is a potential target area for modulating drug-associated memory reconsolidation.

The BLA is involved in the association of cues with both positive and negative valences [14–17]. It has been shown that the BLA activation is highly correlated with cue-elicited cocaine craving in cocaine abusers [18] and with the retrieval of methamphetamine (MeAM)-associated memories in rats [19]. Neuropharmacological disconnection studies showed that the BLA input drives neuronal responses of the nucleus accumbens (NAc) to reward-predictive cues and facilitates reward-seeking behavior, including sucrose [14, 20, 21] and cocaine [22]. The NAc core is essential for acquiring cue-controlled drug-seeking behavior [23]. The lesions of the NAc core, but not the NAc sell, disrupt the acquisition of cocaine-seeking behavior under the control of drug-associated conditioned reinforcers [22, 24] and reduce cue-induced drug

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craving after abstinence [25]. Moreover, the BLA mediates the associative learning that processes the acquisition of positive reinforcing properties of reward-predictive cues [16]. Indeed, the Cre-dependent chemogenetic study reported that the BLA projecting to the NAc core is required for the acquisition of cocaine-paired cues acting as conditioned reinforcers for cocaine-seeking behavior [26]. While these studies suggest an essential role of the BLA-NAc connection in the acquisition of drug-associated memory in cue-controlled drug-seeking, it is unclear that the BLA-NAc connection mediates the memory retrieval-induced reconsolidation of drug-associated memory.

To this end, the present study used the designer receptor exclusively activated by designer drugs (DREADD) approach to investigate the role of the BLA-NAc core pathway in the reconsolidation of drug-associated memory. We show that, at post-retrieval, the DREADD-mediated inhibition of soma of the BLA neurons projecting to the NAc core (BLA-NAc) disrupted the MeAM-associated memory. Clozapine-*N*-oxide (CNO) infusion into the NAc core to inhibit nerve terminals of the BLA glutamatergic neurons also disrupted the MeAM-associated memory in hM4Di-expressing, but not empty control, mice. Additionally, chemogenetic inhibition of the BLA-NAc pathway after the time window of memory reconsolidation failed to disrupt MeAM-associated memory. These results suggest that activity of the BLA-NAc connection was required for reconsolidation in a time-specific manner. Moreover, anisomycin (ANI)-induced reconsolidation blockade was not observed unless the BLA-NAc pathway was activated. Thus, we found that the BLA-NAc pathway activity is involved in the reactivation and reconsolidation of drug-associated memory.

## MATERIALS AND METHODS

### Animals

Four-week-old male C57BL/6 mice were obtained from the National Laboratory Animal Center, Taiwan. 4–5 mice were housed in a cage in the room with constant temperature (24 ± 1 °C) and humidity levels under a dark/light cycle (12-h-dark, 12-h-light with lights on at 8:00). They were free to access to water and food. These cages were randomly assigned to different experimental groups. All procedures complied with guidelines and were approved by the National Cheng Kung University Medical Center Animal Care and Use Committee.

### Surgery and microinjections

Mice received surgery as detailed in the Supplementary information. Briefly, viruses were purchased from Addgene (#50475-AAVrg-hSyn-hM4Di-mCherry, #1144472-AAVrg-hSyn-mCherry, #50477-AAV5-CaMKIIα-hM4Di-mCherry, #50476-AAV5-CaMKIIα-hM3Dq-mCherry, #50469-AAV5-CaMKIIα-EGFP). The viruses (0.8 µL/side) were bilaterally infused at 0.1 µL/min flow rate. The cannulas (26-gauge) were left in place for 10 min before being withdrawn. These mice recovered for 4 weeks in their home cages before the CPP procedure. For c-Fos fluorescence and histology, the hM4Di mice were sacrificed 90 min after the retest. The hM3Dq mice were sacrificed 90 min after another CNO infusion.

### Drugs

Methamphetamine (MeAM; Sigma) was dissolved in 0.9% saline and was intraperitoneally administrated (ip, 2 mg/kg). Anisomycin (ANI; Sigma) was dissolved in saline-containing 10% DMSO and then the pH value was adjusted to 7.0 (ip, 100 mg/kg; for infusion, 1 µg in 0.8 µL/side). Clozapine-*N*-oxide (CNO, 2 µg in 0.8 µL/side; Enzo Life Sciences) was dissolved in 0.9% saline. For DREADD experiments, ANI and CNO were infused into awakened mice through the implanted cannula at 0.1 µL/min flow rate. For retrograde tracing, red RetroBeads (Red IX RetroBeads, 0.3 µL/side; Lumafluor Inc., USA) were diluted with 0.9% saline.

### Conditioned place preference (CPP) paradigm

As detailed in the Supplementary information, on Day1 (baseline), mice were allowed to explore all three chambers freely for 15 min. During the training phase (Day 2–6), mice were injected with saline and rested in the saline-paired chamber for 30 min. 6 h later, the mice were injected with MeAM and rested in the MeAM-paired chamber for 30 min. On Day 7, the mice were allowed to explore all three chambers freely for 15 min (the CPP test). In the retest, the mice were retested for 15 min. The CPP preference score was calculated as the difference that the amount of time spent in the MeAM-paired chamber was subtracted from the time spent in the saline-paired chamber. The positive value means that mice acquired MeAM-associated memory. The mice that had a positive value in the CPP test, indicating the successful CPP training, were included in the study. The viral injection site was determined as the most ventral point where mCherry fluorescence was brightest. Data from the viral infusion and cannula placement outside the target region were excluded.

### Immunofluorescence

As detailed in the Supplementary information, floating 40-µm coronal brain sections were incubated in primary antibodies (1:200 mouse anti-cFos, Genetex; 1:500 rabbit anti-cFos, Genetex; 1:500 rabbit anti-CaMKIIα, Genetex) in CAS-Block agent (Invitrogen, USA) overnight at 4 °C. Secondary antibodies (1:200, Alexa Fluor-488 Fab goat anti-mouse or Fab goat anti-rabbit; Jackson ImmunoResearch, USA) were in CAS-Block agent. DAPI (1:1000; Sigma) was for nucleus staining. Images were taken by the Leica microscopy (DM2500) coupled to the ANDOR sensor (Oxford Instruments) with MetaMorph software (Molecular Devices). For retrobead quantification, as previous studies described [27–29], we manually counted red retrobead-surrounded DAPI-positive cells within $3 \times 3\,mm^2$ region-of-interest in representative coronal brain sections. We also counted c-fos-positive cells that colocalized with DAPI in representative coronal brain sections. In confocal images, the reporter mCherry signal was enhanced by chicken anti-mCherry (1:1000; ab205402, Abcam) and goat anti-chicken Alexa Fluor-594 (1:500; A-11042, Invitrogen) and was taken by Olympus FV3000.

### Data analysis

All data, using GraphPad Prism6 (GraphPad Software, San Diego, CA), were represented as the mean ± standard error of the mean. For behavioral CPP experiments, mixed two-way ANOVA and repeated one-way ANOVA were followed by Bonferroni's multiple comparisons tests as post hoc tests. For c-Fos expression, the unpaired Student *t*-test was used. The level of significance was $p < 0.05$.

## RESULTS

### Anisomycin administration after memory retrieval disrupts MeAM-associated memory in the CPP

Memory reconsolidation requires *de novo* protein synthesis within 6 h after memory retrieval [8, 30, 31]. First, we confirmed the effect of protein synthesis inhibitor ANI on the reconsolidation of drug-associated memory in the CPP. After CPP MeAM-paired training for 5 days (Day 2–6), on Day 7, these mice were re-exposed to the CPP equipment without giving MeAM to assess MeAM-associated memory. The mice acquired MeAM-associated CPP memory if they spent more time in the MeAM-paired chamber than in the saline-paired chamber. As memory tests reactivated a consolidated memory to become unstable, these mice were injected with vehicle or ANI (100 mg/kg, ip.) 1 h after the CPP test (on Day 7). On Day 8, the mice were retested in the CPP equipment (Fig. 1A). The mixed two-way ANOVA showed a significant treatment × test interaction effect ($F_{(2,24)} = 15.67$, $p < 0.001$). The post-hoc analysis showed that these mice preferred to stay in the MeAM-paired chamber in the CPP test on Day7 (red bars), suggesting that the mice acquired MeAM-associated memory after CPP MeAM-paired training (Fig. 1B, baseline versus CPP test, $p < 0.001$ in vehicle, $p < 0.01$ in ANI). Moreover, ANI-treated mice lost the preference for the MeAM-paired chamber in the retest on Day 8 (green bars) (retest versus CPP test, $p < 0.001$), but vehicle-treated mice still had the preference ($p > 0.05$). In addition, in the retest, vehicle-treated mice had a higher preference score than ANI-treated mice (vehicle versus ANI in the retest, $p < 0.001$). Thus, ANI administration after memory recall disrupted MeAM-associated memory in mice. It confirmed that ANI treatment during memory

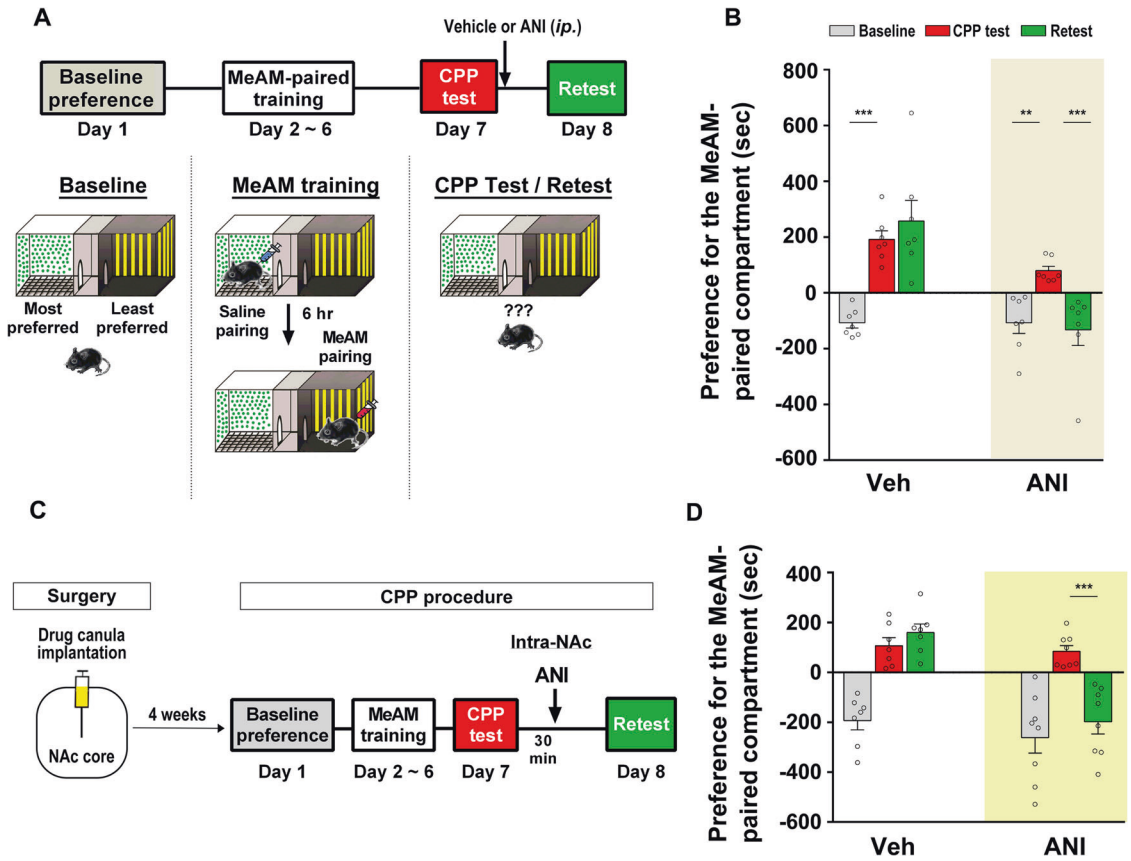



**Fig. 1 Anisomycin administration during reconsolidation disrupts MeAM-associated memory. A** Schematic experimental procedure of conditioned place preference (CPP). On Day 1 (baseline), mice were placed into the central chamber and freely explored all three chambers for 15 min. During the training stage (Day 2–6), saline injection paired with the most preferred chamber and then MeAM (2 mg/kg) injection paired with the least preferred chamber 6 h later. After injection, these mice were placed in the respective chamber for 30 min. On Day 7 (the CPP test) and Day 8 (retest), the mice were placed into the central chamber and freely explored all three chambers for 15 min. 1 h after the test on Day 7, the mice were injected with ANI (100 mg/kg) or vehicle (Veh). **B** The mixed two-way ANOVA showed a significant drug × test interaction effect ($F_{[2,24]} = 15.67$, $p < 0.001$), a significant main effect of drug groups ($F_{[1,12]} = 12.79$, $p < 0.01$), and a significant main effect of repeated tests ($F_{[2,24]} = 24.14$, $p < 0.001$). Bonferroni's multiple comparison tests showed that all mice preferred to stay in the MeAM-paired chamber (red bars) after the CPP MeAM-paired training (baseline versus CPP test, ***$p < 0.001$ in vehicle, **$p < 0.01$ in ANI), indicating that these mice had MeAM-associated memory. Importantly, the retest (green bars) showed that ANI administration after memory retrieval disrupted MeAM-associated memory compared to the vehicle (retest versus CPP test, ***$p < 0.001$ in ANI-treated mice, $p > 0.05$ in vehicle-treated mice). $n = 7$ per group. **C** The experimental timeline of ANI infusion into the NAc core. Another group of mice was infused ANI (1 μg in 0.8 μL/side) or the vehicle into the NAc core 30 min after the CPP test. $n = 7$ and 8 in the vehicle and ANI group, respectively. **D** The mixed two-way ANOVA showed a significant drug × test interaction effect ($F_{[2,26]} = 13.47$, $p < 0.001$), a significant main effect of drug groups ($F_{[1,13]} = 11.32$, $p < 0.01$), and a significant main effect of repeated tests ($F_{[2,26]} = 43.58$, $p < 0.001$). Post-hoc Bonferroni's tests showed that ANI infusion into the NAc core after memory retrieval disrupted MeAM-associated memory compared to the vehicle (retest versus CPP test, ***$p < 0.001$ in ANI-infused mice, $p > 0.05$ in vehicle-infused mice). MeAM methamphetamine, CPP conditioned place preference, Veh vehicle, ANI anisomycin. Data represent mean ± SEM.

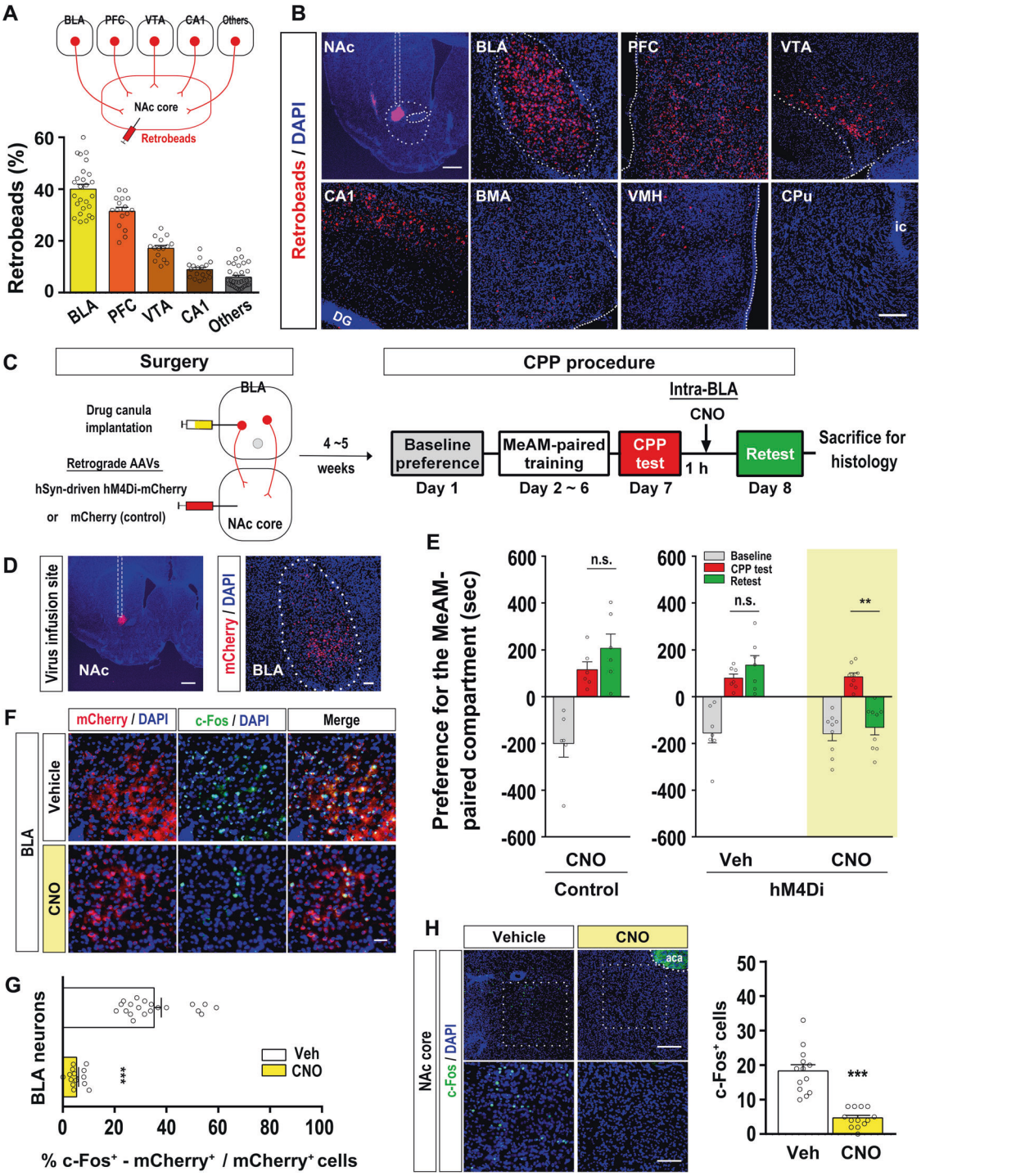
reconsolidation disrupted MeAM-associated memory, which is a phenomenon of reconsolidation blockade.

Next, we examined ANI effects in the NAc core. Another group of mice with CPP MeAM-paired training received ANI (1 μg in 0.8 μL/side) or vehicle infusion into the NAc core 30 min after the CPP test (Fig. 1C). The mixed two-way ANOVA showed a significant treatment × test interaction effect in the CPP preference score (Fig. 1D, $F_{(2,26)} = 13.47$, $p < 0.001$). The intra-NAc core ANI infusion after the CPP test disrupted MeAM-associated memory in the retest (retest versus CPP test, $p < 0.001$), but the vehicle infusion did not affect memory ($p > 0.05$). It indicated that blocking protein synthesis in the NAc core during memory reconsolidation disrupted MeAM-associated memory.

### Basolateral amygdala projecting to the nucleus accumbens is required for reconsolidation of MeAM-associated memory

The NAc core is required for cue-controlled drug-seeking behavior [22, 24, 25] and receives the BLA inputs [17]. We determined the extent of BLA-NAc core connection by infusing the retrograde tracer, red retrobeads, into the NAc core (Fig. 2A). Image quantification showed that the BLA presented the most dominant retrobead signals compared to other brain areas (Fig. 2A, B, $p < 0.001$ in Bonferroni's test). It suggested that the BLA was the major input of the NAc core, which was consistent with previous reports [14, 32].

Next, to examine the role of the BLA-NAc core connection in reconsolidation of MeAM-associated memory, we infused the retrograde adeno-associated virus 2 carrying hM4Di (AAVrg-hSyn-hM4Di-mCherry) or control vectors (AAVrg-hSyn-mCherry) into the NAc core. The cannulas for CNO infusion were implanted into the bilateral BLA. Four weeks later, these mice received the CPP procedure and infused CNO (2 μg/side) or vehicle into the BLA 1 h after the CPP test (Fig. 2C). Figure 2D showed the viral injection site in the NAc core and mCherry expression in the BLA. Supplementary Fig. 1 showed the cannula implantation sites in the BLA. We found that, in the retest, CNO-infused mice did not prefer to stay in the MeAM-paired chamber, but vehicle-infused




mice still preferred it (Fig. 2E, $F_{(2,28)} = 15.65$, $p < 0.001$, the interaction effect in mixed two-way ANOVA; retest versus test, $p < 0.001$ in CNO). The reduced CPP preference score in the retest suggested that CNO infusion 1 h after the CPP test disrupted MeAM-associated memory. Moreover, viral control mice receiving CNO infusion still had the MeAM-associated memory in the retest. These results showed that the hM4Di-mediated inhibition of the BLA-NAc core connection after memory retrieval disrupted MeAM-associated memory.

Next, we investigated the BLA-NAc core activity in response to memory reconsolidation. The retest induced c-Fos expression, the neuronal activation marker, in the BLA (Fig. 2F) and NAc core (Fig. 2H) in vehicle-infused mice. However, CNO infusion significantly reduced c-Fos expression in the BLA (Fig. 2G) and NAc core (Fig. 2H; vehicle versus CNO, $p < 0.001$, unpaired $t$-test). It suggested that the CNO infusion after the CPP test disrupted reactivated MeAM-associated memory and thus no MeAM-associated memory in the retest induced c-Fos expression in the CNO group. These results showed that CNO infusion inhibited the BLA-NAc core activity and disrupted MeAM-associated memory. Thus, the BLA-NAc connection was required for reconsolidation of MeAM-associated memory.

**Fig. 2 Post-retrieval chemogenetic inhibition of the BLA neurons projecting to the NAc disrupts the consolidated MeAM-associated memory. A** (Top) schematic representation showed that the retrograde tracer, red retrobeads, infused into the NAc core. (Bottom) Quantification of images showed that retrobead signals were present in the BLA (40 ± 1.83%), the prefrontal cortex (PFC, 31 ± 1.53%), and the ventral tegmental area-substantia nigra (VTA-SN, 17.1 ± 1.11%). Minor signals were also observed in CA1 of the ventral hippocampus (8.88 ± 0.8%), the basomedial amygdala (BMA), and the ventromedial hypothalamus (VMH). The one-way ANOVA indicated that the BLA was the major input of the NAc core ($F_{(4,\ 103)} = 146.9$, $p < 0.001$; BLA versus PFC, $p < 0.001$ in Bonferroni's multiple comparisons test). Brain slices from three mice. Data represent mean ± SEM. **B** Representative images showed the injection site of retrobeads in the NAc core. Scale bar represents 550 μm. The distribution of retrobeads included the BLA, the PFC, the VTA, the CA1, the BMA, and the VMH. The CPu was a negative control brain area with no retrobead signals. Scale bar represents 200 μm. NAc, the nucleus accumbens; BLA, the basolateral amygdala; PFC, the prefrontal cortex; VTA, the ventral tegmental area; CA1, the ventral hippocampal CA1; others include the basomedial amygdala (BMA) and the ventromedial hypothalamus (VMH). CPu, the caudate putamen. **C** Schematic representation of the virus infusion and cannula implantation sites and the experimental procedure. CNO (2 μg/side) or vehicle was infused 1 h after the test on Day 7. The mice were sacrificed for histology analysis 90 min after the retest. **D** Representative images showed the retrograde AAV infusion site in the NAc core (left) and the reporter mCherry in the BLA (right). Scale bars represent 550 μm and 100 μm in the NAc core and in the BLA, respectively. **E** In the CPP procedure, all mice after CPP training preferred the MeAM-paired chamber (baseline versus CPP test, $p < 0.05$ and $p < 0.001$ in control and hM4Di-expressing mice, respectively, Bonferroni's multiple comparisons test). In the retest on Day 8 (green bars), intra-BLA CNO infusion reduced the preference for the MeAM-paired chamber compared to the vehicle infusion in hM4Di-expressing mice (retest versus CPP test, $p < 0.001$ in CNO infusion, Bonferroni's multiple comparisons test). CNO infusion into the BLA had no effect in transduced control viral mice (retest versus CPP test, $p > 0.05$, Bonferroni's multiple comparisons test). The mixed two-way ANOVA in hM4Di-expressing mice, the drug × test interaction, $F_{[2,28]} = 15.65$, $p < 0.001$, $n = 7$ and 9 in vehicle and CNO, respectively; the repeated one-way ANOVA in control mice, $F_{(1.8,\ 9.2)} = 18.62$, $p < 0.001$, $n = 6$. **F** Representative images showed c-Fos expression on hM4Di-expressing BLA neurons in the vehicle- and CNO-infused mice. The scale bar represents 40 μm. Green, c-Fos; red, mCherry; blue, DAPI. **G** CNO infusion decreased retest-induced c-Fos expression on hM4Di-expressing neurons in the BLA compared to the vehicle. Vehicle versus CNO, $p < 0.001$, unpaired $t$ test. $n = 7$ and 9 mice in vehicle and CNO, respectively. **H** CNO infusion also decreased retest-induced c-Fos-positive cells in the NAc core compared to the vehicle. The bottom images show the magnification of the white dotted box in the top images. Vehicle versus CNO, $p < 0.001$, unpaired $t$-test. $n = 7$ and 9 mice in vehicle and CNO, respectively. The scale bars represent 200 and 100 μm in the top and bottom images, respectively. Green, c-Fos; blue, DAPI. Data represent mean ± SEM.

### BLA glutamatergic neurons projecting to the NAc core are necessary for reconsolidation of MeAM-associated memory

Previous studies reported that the NAc receives BLA glutamatergic inputs in reward-seeking behavior [14, 21, 30, 31]. Our data showed that in the mice with retrograde AAVs infusion into the NAc core (Fig. 3A), 76.48% of mCherry-positive BLA neurons were CaMKIIα-positive (Fig. 3B, C), supporting that the principal BLA neurons projecting to the NAc core are glutamatergic neurons.

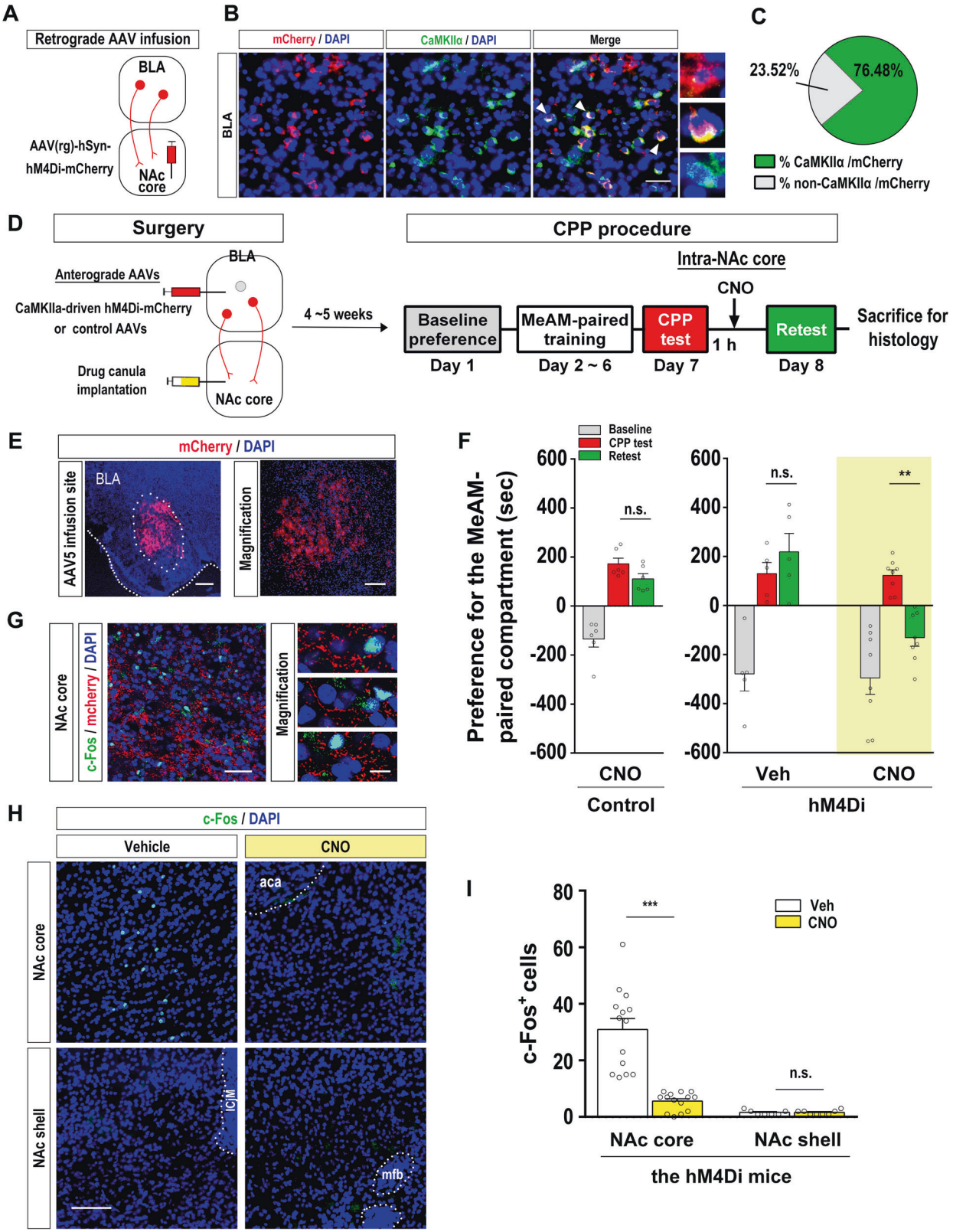
Next, we examined whether MeAM-associated memory reconsolidation required glutamatergic BLA neurons projecting to the NAc core. AAV5-CaMKIIα-hM4Di-mCherry or control AAVs were infused into the BLA, and cannulas were implanted into the NAc core to affect nerve terminals of BLA glutamatergic neurons. These mice with the MeAM-paired CPP training were infused CNO into the NAc core 1 h after the CPP test (Fig. 3D). We found that intra-NAc core CNO infusion blunted the preference to stay in the MeAM-paired chamber compared to the vehicle infusion in the hM4Di-expressing mice (Fig. 3F, $F_{(2,22)} = 6.88$, $p < 0.01$, the interaction effect; retest versus CPP test, $p < 0.01$ in CNO, $p > 0.05$ in vehicle). In viral control mice, CNO infusion did not affect the MeAM-associated memory in the retest (retest versus CPP test, $p > 0.05$). The result indicated that the activity of glutamatergic BLA-NAc core neurons was necessary for MeAM-associated memory reconsolidation.

In histology analysis, Fig. 3E showed the viral injection site in the BLA, and Supplementary Fig. 2 showed the cannula implantation sites in the NAc core. Figure 3G showed the mCherry-labeled BLA axon terminals in the NAc core. Additionally, CNO infusion reduced c-Fos expression in the NAc core but not in the shell (Fig. 3H, I, $p < 0.001$ in the core, $p > 0.05$ in the sell) in the hM4Di mice, implying that CNO infusion was restricted in the core part.

We next examined whether the effect of chemogenetic inhibition was restricted within the time window of memory reconsolidation. AAV5-CaMKIIα-hM4Di-mCherry was infused into the BLA of another group of mice (Fig. 4A). Delaying CNO infusion was administrated into the NAc core 18 h after memory retrieval. Figure 4B showed that both the vehicle- and CNO-infused hM4Di mice had MeAM-associated memory in the retest ($F_{(2,18)} = 0.05$, $p > 0.05$, the interaction effect; retest versus CPP test, $p > 0.05$ both in CNO and vehicle). It indicated that inhibiting the glutamatergic BLA-NAc core pathway after the time window of reconsolidation failed to affect MeAM-associated memory. Supplementary Fig. 3 showed the cannula implantations in the NAc core. Taken together, the effect of inhibiting glutamatergic BLA neuronal nerve terminals in the NAc core was restricted within the time window of memory reconsolidation.

### Anisomycin treatment after chemogenetic activation of BLA-NAc pathway disrupts MeAM-associated memory

Next, we examined whether the BLA-NAc core pathway activity is sufficient to trigger MeAM-associated memory reconsolidation. Another group of mice was transduced AAV5-CaMKIIα-hM3Dq-mCherry into the BLA. Since memory reconsolidation is sensitive to amnesic agents [8], ANI was used to check reconsolidation status. On Day 8, CNO was infused, and then ANI (1 μg/side) was infused into the NAc core 1 h later, but the memory test was not performed (Fig. 5A). The retest was performed on the next day (Day 9). Figure 5B showed the viral injection site in the BLA, and Supplementary Fig. 4 showed the cannula implantations in the NAc core. Figure 5C showed a significant interaction effect of drug infusion × test in MeAM-associated memory ($F_{(4,36)} = 12.57$, $p < 0.001$, mixed two-way ANOVA). ANI-only infusion failed to affect the MeAM-associated memory in the retest (Fig. 5C left, retest versus CPP test, $p > 0.05$ in vehicle+ANI infusion), supporting that the amnesic agent without memory recall had no effects on MeAM-associated memory. Since ANI treatment disrupted MeAM-associated memory only during reconsolidation, we used ANI-disrupted memory as the indicator for memory reconsolidation. If activation of the BLA-NAc core pathway reactivates the consolidated MeAM-associated memory, ANI treatment would disrupt MeAM-associated memory even in the condition without memory retrieval. The result showed that CNO-only infusion failed to affect the MeAM-associated memory in the retest (Fig. 5C middle, $p > 0.05$). However, ANI infusion reduced MeAM-associated memory in CNO-infused mice (Fig. 5C right, retest versus CPP test, $p < 0.001$). It indicates that ANI infusion disrupted MeAM-associated memory only when activating the glutamatergic BLA-NAc core pathway. Thus, the combination of




protein synthesis inhibition (by ANI treatment) with the BLA-NAc core pathway activation impaired MeAM-associated memory.

Next, we checked whether CNO infusion activated hM3Dq-expressing neurons. The hM3Dq mice were sacrificed 90 min after receiving intra-NAc core CNO infusion (Fig. 5D). The quantification showed that CNO infusion induced higher levels of c-Fos expression in the BLA (Fig. 5D, E, $p < 0.05$) and the NAc core (Fig. 5F, $p < 0.001$) compared to the vehicle infusion. It confirmed that CNO infusion activated the glutamatergic BLA-NAc core pathway. Together, activating the glutamatergic BLA-

**Fig. 3 Chemogenetic inhibition of nerve terminals of glutamatergic BLA neurons in the NAc core after retrieval disrupts the consolidated MeAM-associated memory. A** The retrograde virus was infused in the NAc core, and the mCherry reporter expression was analyzed in the BLA. **B** Representative images showed that mCherry signals (red) colocalized with CaMKIIα signals (green), the glutamatergic neuron marker, in the BLA. Triangles pointed to the colocalized cells. Scale bar represents 40 μm. The insets on the right: amplification images for the mCherry-only positive cell (top), the colocalized cell (middle), and the CaMKII-only positive cell (bottom). **C** Image quantification revealed that 76.48% of mCherry-positive BLA neurons were CaMKIIα-positive. $n = 8$ brain slices from three mice. **D** Schematic representation of the virus infusion and cannula implantation sites and the experimental procedure. CNO (2 μg/side) or vehicle was infused into the NAc core 1 h after the CPP test. **E** Representative images showed the anterograde AAV infusion site in the BLA (left) and its amplification image (right). Scale bar represents 550 μm (left) and 200 μm (right). **F** In hM4Di-expressing mice, CNO infusion reduced the preference for the MeAM-paired chamber in the retest compared to vehicle infusion, suggesting that inhibiting the nerve terminals of glutamatergic BLA neurons in the NAc core during reconsolidation disrupted MeAM-associated memory. Mixed two-way ANOVA, the drug × test interaction, $F_{[2,22]} = 6.88$, $p < 0.01$; $n = 5$ and 8 in vehicle and CNO, respectively; retest versus test, $p < 0.01$ in CNO, Bonferroni's multiple comparisons test. By contrast, in control EGFP-expressing mice, CNO infusion had no effect on the retest. Repeated one-way ANOVA, $F_{(1.8,\ 7.2)} = 21.59$, $p < 0.01$; $n = 5$; retest versus test, $p > 0.05$, Bonferroni's multiple comparisons test. **G** Confocal images showed fiber-like mCherry-labeled BLA axon terminals in contact with c-Fos-positive cells in the NAc core. The scale bar represents 50 μm in the left and 10 μm in the right magnification photos. **H** Representative images showed c-Fos-positive cells (green) in the NAc core (top) and in the shell (bottom) of the vehicle and CNO-infused mice. The scale bar represents 100 μm. **I** Image quantification revealed that CNO infusion reduced c-Fos expression in the NAc core compared to vehicle infusion. CNO infusion had no effects on the NAc shell. CNO versus vehicle in the NAc core, $p < 0.001$, unpaired *t*-test, n = 14 brain slices from four mice; the NAc shell, $p > 0.05$, $n = 7$ and 8 brain slices from four mice. Data represent mean ± SEM.

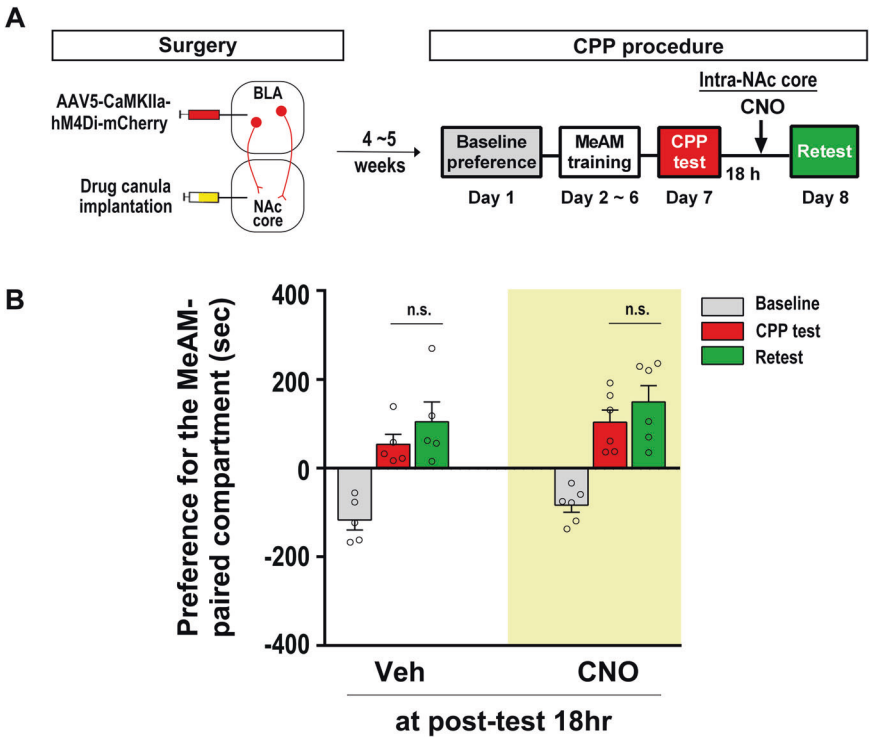


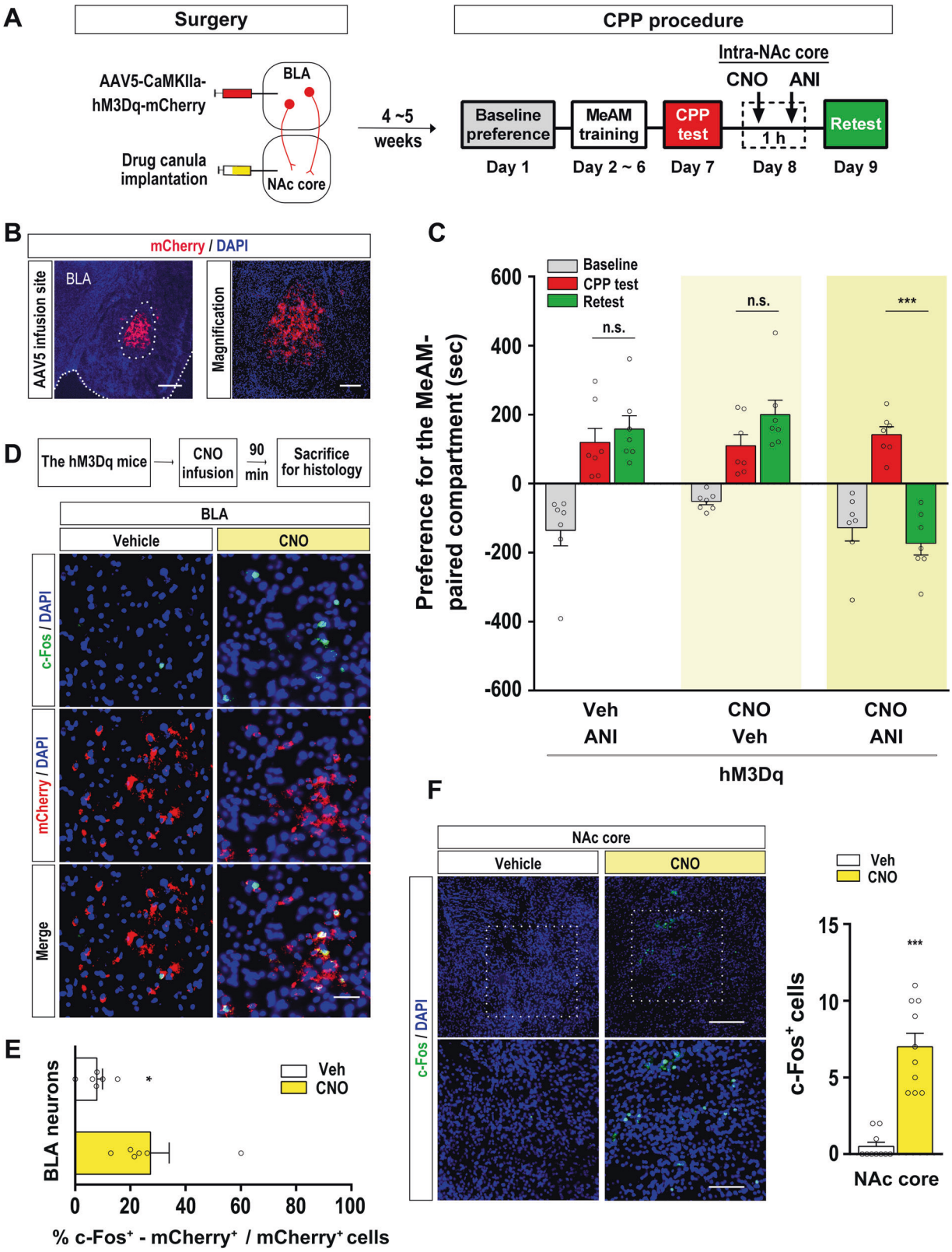

**Fig. 4 Delayed inhibition of BLA neuronal nerve terminals in the NAc core after the time window of memory reconsolidation fails to affect the consolidated MeAM-associated memory. A** Diagram of the virus infusion and cannulas implantation sites and the experimental procedure. CNO (2 μg/side) or vehicle was infused into the NAc core 18 h after the CPP test. **B** Delayed CNO infusion failed to affect MeAM-associated memory in hM4Di-expressing mice. Mixed two-way ANOVA, the drug × test interaction, $F_{[2,18]} = 0.05$, $p = 0.95$; $n = 5$ and 6 in vehicle and CNO, respectively; retest versus test, $p > 0.05$, Bonferroni's multiple comparisons test. Data represent mean ± SEM.

NAc core pathway is sufficient for ANI to disrupt MeAM-associated memory.

Next, we examined whether activating the BLA-NAc core pathway was able to rescue ANI-mediated reconsolidation blockade. To this end, we changed the time course of CNO and ANI treatment. Another group of hM3Dq-mice was intraperitoneally injected ANI 30 min after the CPP test, and then was infused intra-NAc core CNO 30 min later (Supplementary Fig. 5A). In the retest, the post-CNO infusion failed to rescue ANI-disrupting MeAM-associated memory (Supplementary Fig. 5B, retest versus CPP test, $p > 0.05$ in vehicle+CNO infusion, $p < 0.001$ in ANI + CNO infusion). The data suggested that ANI-induced reconsolidation blockade could not be reversed by activation of the BLA-NAc core pathway.

## DISCUSSION

Current understanding of the mechanism underlying drug relapse and how to prevent relapse remains incomplete. The present study demonstrated that the BLA-NAc core pathway is involved in the reconsolidation of MeAM-associated memory. We used chemogenetic approaches to inhibit the BLA-NAc core neuronal activity after memory retrieval, resulting in the disruption of consolidated MeAM-associated memory. Inhibiting BLA nerve terminals in the NAc core after retrieval disrupted the consolidated MeAM-associated memory, which ruled out the effects of other BLA-projecting brain areas. We also found that the requirement of the BLA-NAc core activity was restricted within the time window of memory reconsolidation. Furthermore, the




BLA-NAc core pathway activation primed mice to be sensitive to amnestic agents, disrupting MeAM-associated memory.

The BLA-NAc core pathway has been demonstrated to mediate neutral cues to acquire the property with second-order reinforcement, which increases and maintains cocaine-seeking behavior [26]. It suggests that NAc core-projecting BLA neurons are likely part of memory engrams of conditioned reward-predictive cues. Memory theory indicates that a consolidated memory becomes unstable after memory recall and could be disrupted by amnesic agents [31]. Given that NAc core-projecting BLA neurons are drug-

**Fig. 5 Activating the glutamatergic BLA-NAc core pathway is sufficient for anisomycin to disrupt MeAM-associated memory. A** Schematic representation of the virus infusion and cannula implantation sites and the experimental procedure. Twenty-four hours after the CPP test, the mice received CNO infusion into the NAc core (Day 8) to activate nerve terminals of glutamatergic BLA neurons. 1 h later, ANI was infused into the NAc core, and the mice did not experience a memory test. On the next day (Day 9), the mice received the memory retest. **B** Representative images showed the anterograde AAV infusion site in the BLA (left) and its amplification image (right). Scale bar represents 550 μm (left) and 200 μm (right). **C** ANI-only or CNO-only infusion on Day 8 failed to affect MeAM-associated memory in the retest. The combination of CNO infusion with ANI infusion disrupted the consolidated MeAM-associated memory of hM3Dq-expressing mice in the retest, suggesting that ANI disrupted the MeAM-associated memory only when activating the BLA-NAc core pathway. Mixed two-way ANOVA, the drug × test interaction, $F_{[4,36]} = 12.57$, $p < 0.001$; $n = 7$ per group; retest versus CPP test, $p > 0.05$ in Veh+ANI and CNO + veh, $p < 0.001$ in CNO + ANI, Bonferroni's multiple comparisons test. **D**, **E** Several days after the CPP experiments, these hM3Dq mice received CNO infusion into the NAc core and then were sacrificed for histology 90 min later. CNO infusion induced c-Fos expression on mCherry-positive neurons in the BLA of hM3Dq-expressing mice compared to vehicle infusion. CNO versus vehicle, $p < 0.05$, unpaired *t*-test, $n = 6$ brain slices from three mice per group. **F** Representative images showed c-Fos-positive cells (green) in the NAc core. CNO infusion induced c-Fos expression in the NAc core compared to the vehicle. The bottom images show the magnification of the white dotted box in the top images. Vehicle versus CNO, $p < 0.001$, unpaired *t*-test. $n = 10$ brain slices from three mice per group. The scale bars represent 200 and 100 μm in the top and bottom images, respectively. Green, c-Fos; blue, DAPI. Data represent mean ± SEM.

related memory engrams, it is possible that inhibiting the BLA-NAc core pathway after memory recall could disrupt drug-associated memory. Indeed, our data showed that mice lost MeAM-associated memory by suppressing the BLA-NAc core pathway after memory recall. In addition, memory retrieval induced c-Fos expression in BLA neurons and their downstream target NAc core neurons. These results support that NAc core-projecting BLA neurons are MeAM-associated memory engrams that mediate memory reconsolidation. Moreover, the extracellular signal-regulated kinase (ERK) activation triggers protein synthesis in memory formation and reconsolidation [33]. ERK activation in the BLA [34] and the NAc core [35] is necessary for drug-paired contextual cue memory reconsolidation. As hM4Di couples to Gi protein, hM4D-mediated inhibition is likely to suppress ERK activation and thus blunt memory reconsolidation. In addition, intra-NAc core infusion of ERK inhibitors blocks cocaine CPP reconsolidation [35], which is consistent with our finding that protein synthesis in the NAc core is required for memory reconsolidation. Moreover, by chemogenetic approaches, here we found that the consolidated CPP memory was susceptible to ANI-induced reconsolidation blockade in the BLA-NAc core pathway.

CPP is a drug-associated memory model that links drug reward with neutral environment cues. In the CPP training phase, the contiguous drug-paired context is associated with rewarding responses and acquires secondary rewarding effects to elicit approach responses [36]. Context-evoked MeAM memories are measured by the preference for the MeAM-paired chamber. The CPP is used to study the mechanism of cocaine memory recall and extinction [37–40]. Thus, CPP provides critical information on the role of drug-associated environmental cues in the modulation of drug memory. Interestingly, the NAc core is critical to the incubation of MeAM craving after voluntary abstinence [25]. In the hM4Di experiments of this study, CNO infusion was after the CPP test, and the retest was on the next day. Thus, short-term suppressing craving feelings by CNO is unlikely to reduce the preference for the MeAM-paired chamber on the next day. Furthermore, the experiment of delayed CNO infusion 18 h after memory recall showed no effect on the preference for the MeAM-associated chamber. Hence, it is reasonable that the effect of inhibiting the BLA-NAc core pathway on the preference for the MeAM-associated chamber comes from disrupting a consolidated MeAM-associated memory rather than suppressing the craving feeling.

The NAc is a hub that receives inputs from several brain areas. By retrograde tracers, we confirmed that the NAc core receives inputs from the BLA, the PFC, and the VTA. Moreover, we found that the most robust retrobead signals were located on the BLA. The BLA-NAc projection has been reported to mediate reward-related and motivated behaviors [14, 21, 41] and the reinforcing effect of cocaine-associated cues to increase cue-induced cocaine-seeking behavior [26]. In this study, recalling MeAM-associated memory induced c-Fos expression in the BLA and NAc core, and inhibiting the BLA-NAc core activity after retrieval impaired the consolidated MeAM-associated memory. By contrast, under the condition without memory retrieval, activating the BLA-NAc core pathway was required for ANI to disrupt the consolidated MeAM-associated memory. Based on these results, we propose a schematic model: cue-induced memory retrieval activates the BLA-NAc core pathway to induce MeAM-associated memory from a stable to a destabilized status, which stage is sensitive to ANI treatment to disrupt memory.

This study proved that ANI treatment disrupted MeAM-associated memory only during the BLA-NAc core pathway activation. Because new memory formation requires protein synthesis, it raises the possibility that ANI also blocks new memory formation that accelerates CPP extinction. However, our findings showed that CNO-only infusion failed to affect MeAM-associated memory (Fig. 5C, middle). Thus, the disappeared MeAM-associated memory in the CNO + ANI group is unlikely to the hM3Dq-mediated new memory formation that accelerated CPP extinction.

It is reported that contextual cues are not necessary to induce the destabilization and reconsolidation of a consolidated memory, which depends on the boundary condition. The degree of differences between the initial learning contexts and reactivation conditions decides the cue-induced degree of memory strength [42]. In this study, memory tests were measured in the original CPP equipment of MeAM-paired training, but MeAM was not given. Thus, the prediction error in boundary conditions might allow memory destabilization and reconsolidation. When memory tests reactivate the BLA-NAc core pathway in the condition without MeAM, it might create the prediction error to induce memory reactivation that is sensitive to amnesic agents.

Conditioned reinforcing effects of drug-associated cues are the major factor for drug relapse after prolonged abstinence. Enhancement of memory destabilization and facilitation of reconsolidation blockade to impair conditioned drug-associated cue memory is a potential strategy to prevent relapse [7, 43]. In conclusion, our findings provide evidence that the BLA-NAc core pathway is involved in the post-retrieval process of MeAM-associated cue memory in CPP. Our data support the hypothesis that MeAM-paired cues reactivate and reconsolidate conditioned MeAM-associated memory through the BLA-NAc core pathway. These findings imply that targeting the BLA-NAc core pathway is a potential strategy for the effective intervention of MeAM relapse.

## REFERENCES

1. Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci. 2005;8:1481–9. https://doi.org/10.1038/nn1579

2. Gipson CD, Kupchik YM, Shen H, Reissner KJ, Thomas CA, Kalivas PW. Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. Neuron. 2013;77:867–72. https://doi.org/10.1016/j.neuron.2013.01.005
3. Sampedro-Piquero P, Ladrón de Guevara-Miranda D, Pavón FJ, Serrano A, Suárez J, Rodríguez de Fonseca F, et al. Neuroplastic and cognitive impairment in substance use disorders: a therapeutic potential of cognitive stimulation. Neurosci Biobehav Rev. 2019;106:23–48. https://doi.org/10.1016/j.neubiorev.2018.11.015
4. Bossert JM, Liu SY, Lu L, Shaham Y. A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. J Neurosci. 2004;24:10726–30. https://doi.org/10.1523/jneurosci.3207-04.2004
5. Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. J Neurosci. 2007;27:12655–63. https://doi.org/10.1523/jneurosci.3926-07.2007
6. LeBlanc KH, Ostlund SB, Maidment NT. Pavlovian-to-instrumental transfer in cocaine seeking rats. Behav Neurosci. 2012;126:681–9. https://doi.org/10.1037/a0029534
7. Nader K. Memory traces unbound. Trends Neurosci. 2003;26:65–72. https://doi.org/10.1016/s0166-2236(02)00042-5
8. Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature. 2000;406:722–6. https://doi.org/10.1038/35021052
9. Exton-McGuinness MTJ, Milton AL. Reconsolidation blockade for the treatment of addiction: challenges, new targets, and opportunities. Learn Mem. 2018;25:492–500. https://doi.org/10.1101/lm.046771.117
10. Lee JLC, Nader K, Schiller D. An update on memory reconsolidation updating. Trends Cogn Sci. 2017;21:531–45. https://doi.org/10.1016/j.tics.2017.04.006
11. Milton AL, Everitt BJ. The persistence of maladaptive memory: addiction, drug memories and anti-relapse treatments. Neurosci Biobehav Rev. 2012;36:1119–39. https://doi.org/10.1016/j.neubiorev.2012.01.002
12. Ben Mamou C, Gamache K, Nader K. NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci. 2006;9:1237–9. https://doi.org/10.1038/nn1778
13. Yu YJ, Huang CH, Chang CH, Gean PW. Involvement of protein phosphatases in the destabilization of methamphetamine-associated contextual memory. Learn Mem. 2016;23:486–93. https://doi.org/10.1101/lm.039941.115
14. Ambroggi F, Ishikawa A, Fields HL, Nicola SM. Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron. 2008;59:648–61. https://doi.org/10.1016/j.neuron.2008.07.004
15. Janak PH, Tye KM. From circuits to behaviour in the amygdala. Nature. 2015;517:284–92. https://doi.org/10.1038/nature14188
16. Keefer SE, Gyawali U, Calu DJ. Choose your path: divergent basolateral amygdala efferents differentially mediate incentive motivation, flexibility and decision-making. Behav Brain Res. 2021;409:113306 https://doi.org/10.1016/j.bbr.2021.113306
17. Koob GF. A basolateral amygdala microcircuit for drug craving: is there a craving engram. Biol Psychiatry. 2021;89:323–5. https://doi.org/10.1016/j.biopsych.2020.11.008
18. Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, et al. Neural systems and cue-induced cocaine craving. Neuropsychopharmacology. 2002;26:376–86. https://doi.org/10.1016/s0893-133x(01)00371-2
19. Chen YY, Zhang LB, Li Y, Meng SQ, Gong YM, Lu L, et al. Post-retrieval extinction prevents reconsolidation of methamphetamine memory traces and subsequent reinstatement of methamphetamine seeking. Front Mol Neurosci. 2019;12:157 https://doi.org/10.3389/fnmol.2019.00157
20. Shiflett MW, Balleine BW. At the limbic-motor interface: disconnection of basolateral amygdala from nucleus accumbens core and shell reveals dissociable components of incentive motivation. Eur J Neurosci. 2010;32:1735–43. https://doi.org/10.1111/j.1460-9568.2010.07439.x
21. Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, et al. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature. 2011;475:377–80. https://doi.org/10.1038/nature10194
22. Di Ciano P, Everitt BJ. Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. J Neurosci. 2004;24:7167–73. https://doi.org/10.1523/JNEUROSCI.1581-04.2004
23. Scofield MD, Heinsbroek JA, Gipson CD, Kupchik YM, Spencer S, Smith AC, et al. The nucleus accumbens: mechanisms of addiction across drug classes reflect the importance of glutamate homeostasis. Pharm Rev. 2016;68:816–71. https://doi.org/10.1124/pr.116.012484
24. Ito R, Robbins TW, Everitt BJ. Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. Nat Neurosci. 2004;7:389–97. https://doi.org/10.1038/nn1217
25. Rossi LM, Reverte I, Ragozzino D, Badiani A, Venniro M, Caprioli D. Role of nucleus accumbens core but not shell in incubation of methamphetamine craving after voluntary abstinence. Neuropsychopharmacology. 2020;45:256–65. https://doi.org/10.1038/s41386-019-0479-4
26. Puaud M, Higuera-Matas A, Brunault P, Everitt BJ, Belin D. The basolateral amygdala to nucleus accumbens core circuit mediates the conditioned reinforcing effects of cocaine-paired cues on cocaine seeking. Biol Psychiatry. 2021;89:356–65. https://doi.org/10.1016/j.biopsych.2020.07.022
27. Kim JH, Jung AH, Jeong D, Choi I, Kim K, Shin S, et al. Selectivity of neuromodulatory projections from the basal forebrain and locus ceruleus to primary sensory cortices. J Neurosci. 2016;36:5314–27. https://doi.org/10.1523/JNEUROSCI.4333-15.2016
28. Li SJ, Vaughan A, Sturgill JF, Kepecs A. A viral receptor complementation strategy to overcome CAV-2 tropism for efficient retrograde targeting of neurons. Neuron. 2018;98:905–17.e5. https://doi.org/10.1016/j.neuron.2018.05.028
29. Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, et al. A circuit mechanism for differentiating positive and negative associations. Nature. 2015;520:675–8. https://doi.org/10.1038/nature14366
30. Morris RG, Inglis J, Ainge JA, Olverman HJ, Tulloch J, Dudai Y, et al. Memory reconsolidation: sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. Neuron. 2006;50:479–89. https://doi.org/10.1016/j.neuron.2006.04.012
31. Nader K, Hardt O. A single standard for memory: the case for reconsolidation. Nat Rev Neurosci. 2009;10:224–34. https://doi.org/10.1038/nrn2590
32. Deroche MA, Lassalle O, Castell L, Valjent E, Manzoni OJ. Cell-type- and endocannabinoid-specific synapse connectivity in the adult nucleus accumbens core. J Neurosci. 2020;40:1028–41. https://doi.org/10.1523/JNEUROSCI.1100-19.2019
33. Medina JH, Viola H. ERK1/2: a key cellular component for the formation, retrieval, reconsolidation and persistence of memory. Front Mol Neurosci. 2018;11:361 https://doi.org/10.3389/fnmol.2018.00361
34. Wells AM, Arguello AA, Xie X, Blanton MA, Lasseter HC, Reittinger AM, et al. Extracellular signal-regulated kinase in the basolateral amygdala, but not the nucleus accumbens core, is critical for context-response-cocaine memory reconsolidation in rats. Neuropsychopharmacology. 2013;38:753–62. https://doi.org/10.1038/npp.2012.238
35. Miller CA, Marshall JF. Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron. 2005;47:873–84. https://doi.org/10.1016/j.neuron.2005.08.006
36. Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward. Psychopharmacology. 2000;153:31–43. https://doi.org/10.1007/s002130000569
37. Visser E, Matos MR, Mitrić MM, Kramvis I, van der Loo RJ, Mansvelder HD, et al. Extinction of cocaine memory depends on a feed-forward inhibition circuit within the medial prefrontal cortex. Biol Psychiatry. 2021. https://doi.org/10.1016/j.biopsych.2021.08.008
38. Zhou Y, Zhu H, Liu Z, Chen X, Su X, Ma C, et al. A ventral CA1 to nucleus accumbens core engram circuit mediates conditioned place preference for cocaine. Nat Neurosci. 2019;22:1986–99. https://doi.org/10.1038/s41593-019-0524-y
39. Augur IF, Wyckoff AR, Aston-Jones G, Kalivas PW, Peters J. Chemogenetic activation of an extinction neural circuit reduces cue-induced reinstatement of cocaine seeking. J Neurosci. 2016;36:10174–80. https://doi.org/10.1523/jneurosci.0773-16.2016
40. Stefanik MT, Kupchik YM, Kalivas PW. Optogenetic inhibition of cortical afferents in the nucleus accumbens simultaneously prevents cue-induced transient synaptic potentiation and cocaine-seeking behavior. Brain Struct Funct. 2016;221:1681–9. https://doi.org/10.1007/s00429-015-0997-8
41. Stefanik MT, Kalivas PW. Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. Front Behav Neurosci. 2013;7:213 https://doi.org/10.3389/fnbeh.2013.00213
42. Schroyens N, Beckers T, Kindt M. In search for boundary conditions of reconsolidation: a failure of fear memory interference. Front Behav Neurosci. 2017;11:65 https://doi.org/10.3389/fnbeh.2017.00065
43. Ma YY, Lee BR, Wang X, Guo C, Liu L, Cui R, et al. Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections. Neuron. 2014;83:1453–67. https://doi.org/10.1016/j.neuron.2014.08.023

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## AUTHOR CONTRIBUTIONS

JYL and CHC performed experiments and analyzed data. CLS designed some experiments and wrote the manuscript. YJY designed some experiments and analyzed some data. YQS performed some fluorescence experiments. CHC and PWG supervised JYL's works, and PWG supervised YJY. CHC and PWG designed experiments and wrote the manuscript.

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## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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