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Supplementary Material for

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A memory retrieval-extinction procedure to prevent drug craving and relapse

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Method

Subjects

Male Sprague-Dawley rats (weighing 240-260 g upon arrival; total n = 231) were housed in groups of five under controlled temperature (23 ± 2ºC) and humidity (50 ± 5%) and maintained on a 12 h/12 h light/dark cycle with free access to food and water. All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experiments were approved by the University Animal Use Committee.

Participants in the human study were 66 formerly heroin-dependent male inpatients at the treatment centers of Beijing Ankang and Tian-Tang-He Drug Rehabilitation Center. During the study, all participants remained hospitalized and received their usual drug-free rehabilitation treatment. Inclusion criteria were: (1) male; (2)18-50 years old; (2) DSM- IV criteria for heroin dependence, but opiate-free for at least one month; (3) no history of other drug use during the past 6 months before admission to the hospital. Exclusion criteria were: (1) current or past cardiovascular disease; (2) systolic pressure below 90 mmHg or above 140 mmHg or diastolic pressure below 50 mmHg or above 90 mmHg; (3) history of allergy (food, medicine); (4) current or past psychiatric illness; (5) neurological signs and/or history of neurological disease; (6) current medical

illness; and (7) participation in other clinical trials of medications within the past 3 months. All participants gave written informed consent. The study was approved by Human Investigation Committee of the Peking University Health Center. In addition, the study participants were clearly informed that participation or dropping out would have no consequences for their usual treatment in the rehabilitation center.

Experimental procedures

Rat experiments

Training for cocaine or morphine CPP and initial CPP test (Test 1) (Exp. 1-3).

The CPP procedure was conducted in accordance with methods we used in previous studies (*47*). The apparatus for CPP conditioning and testing consisted of 23 polyvinyl chloride boxes, identical except for their floors. The boxes had two large side chambers (27.9 cm long × 21.0 cm wide × 20.9 cm high) with different floors (bar or grid, respectively), separated by a smaller chamber (12.1 cm long × 21.0 cm wide × 20.9 cm high with a smooth polyvinyl chloride floor). In each box, the three chambers were separated by manual guillotine doors.

To determine baseline preference, rats were initially placed in the middle chamber with the doors removed and were allowed free access to all compartments for 15 min (baseline test). A computer measured the time spent in each of the chambers during the 15 min session through interruptions of infrared beams. Most rats spent approximately one-third of the time in each chamber (data not shown). Twenty-one of the 164 rats were excluded because of a strong unconditioned preference toward one chamber (>540 s). Thus, conditioning was performed using an unbiased, balanced protocol.

Each rat was conditioned for 8 consecutive days with alternating injections of drug (cocaine, 10 mg/kg, i.p. or morphine, 10 mg/kg, s.c.) and saline (1 ml/kg, ip.or s.c.) (*15, 47, 48*). After each injection, the rats were confined to the corresponding conditioning chambers for 45 min before being returned to their home cages. The day after the last conditioning session, the rats were tested for the expression of drug-induced

CPP (Test 1) under conditions identical to those described in the baseline test. The CPP score was defined as the time spent in the drug-paired chamber minus the time spent in the saline-paired chamber (*49*).

Retrieval of drug CPP memories (Exp. 1-3)

The retrieval manipulation was based on previous studies with some modifications (*15, 50*). The retrieval test was the same as the initial CPP test, except that the duration was 10 min.

Extinction of drug CPP (Exp. 1-3)

Extinction training was identical to the initial CPP training except that no injections were given. The day after the 8 consecutive days of extinction training, the rats were tested again for CPP expression (Test 2).

Drug-priming-induced drug CPP (Exp. 1 and 2)

The manipulation of drug-induced reinstatement of drug CPP was based on previous studies (*15, 47, 51*). Five min before the CPP reinstatement test, the rats received a drug injection (cocaine, 5 mg/kg, i.p. or morphine, 5 mg/kg, s.c.). These doses were based on previous findings (*15, 47, 51*). Conditions during the reinstatement test were the same as those for the baseline preference test and Tests 1-2.

Intravenous drug self-administration (Exp. 4-7)

Rats (weighing 300-320 g when surgery began) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). Catheters were inserted into the right jugular vein with the tip terminating at the opening of the right atrium as previously described (*52*). All rats were allowed to recover for 5-7 days after surgery. The operant chambers used (AniLab Software and Instruments) were equipped with two nosepoke operandi (ENV-114M; Med Associates) located 5 cm above the chamber floor. Nosepokes in the active operandum led to cocaine or heroin infusions that were accompanied by a 5-s tone-light cue. Nosepokes in the inactive operandum were also recorded but had no programmed consequences. The modified cannula on the rat's skull was connected to a liquid swivel with polyethylene-50 tubing protected by a metal spring and leading to a 10-ml syringe infusion pump. Rats were trained to self-administer cocaine hydrochloride (0.75

mg/kg/infusion) or heroin (0.05 mg/kg/infusion) during three 1-h daily sessions separated by 5 min over 10 days. The sessions began at the onset of the dark cycle. A fixed-ratio one reinforcement schedule was used, with a 40 s timeout period after each infusion. Each session began with the illumination of a houselight that remained on for the entire session. To facilitate the acquisition of drug self-administration, food was removed from the chambers during the 3-h sessions on the first 5 days. The number of drug infusions was limited to 20 per h. At the end of the training phase, the groups in the different experimental conditions were matched for their drug intake during training. These training procedures were based on procedures we had used in our previous studies (*52, 53*).

Retrieval of drug cues' memories (Exp. 4-7)

The retrieval manipulation was the same as the extinction procedure below, except that the duration was 15 min.

Extinction of drug-reinforced responding (Exp. 4-7)

During extinction, the conditions were the same as during training, except that drug was no longer available; that is, nosepoke responses led to a 5-sec tone-light cue under an FR1 40-sec timeout reinforcement schedule. The rats were given extinction training until responding on the active nosepoke operandum decreased to less than 20% of mean responding during the last 3 days of cocaine or heroin self-administration for at least 2 consecutive days.

Test for drug seeking (Exp. 4-7)

Once responding on the active nosepoke operandum was successfully extinguished according to the criterion described above, testing commenced. The testing conditions were the same as during training, with the exception that active nosepokes were not reinforced by drug. Each session began with illumination of the houselight, which remained on for the entire session. Nosepoke responding during the test sessions resulted in contingent presentations of the tone-light cue that had previously been paired with drug infusions

under the FR1 40-sec timeout reinforcement schedule.

Tissue sample preparation and Western blot assay (Exp. 8)

The assay procedures were based on those used in our previous studies (*15, 32, 54*). Twenty-four h after the end of the last extinction session, the rats were decapitated. Their brains were quickly extracted, frozen in -60ºC *N*-hexane, and transferred to a -80ºC freezer. Using a freezing cryostat (-20ºC; Reichert-Jung 2800 Frigocut E), bilateral tissue punches (12 gauge) of the infralimbic cortex, prelimbic cortex, basolateral amygdala, and central amygdala were taken from 1 mm thick coronal sections approximately 2.9 mm from bregma. Tissue punches were homogenized (10-15 s × 3, 5 s interval) with an electrical disperser (Wiggenhauser, Sdn Bhd) after 30 min, with RIPA lysis buffer (Beyotime Biotechnology, Jiangsu, China; 20 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM EDTA, 1% Na3VO4, 0.5 μg/ml leupeptin, 1 mM phenylmethanesulfonyl fluoride). The homogenate was then subjected to 10,000 × *g* centrifugation for 10 min at 4°C. All of the above procedures were performed at 0-4ºC. Protein concentrations in all samples were determined using the bicinchoninic acid assay (Beyotime Biotechnology, Jingasu, China). Samples were further diluted in RIPA lysis buffer to equalize the protein concentrations.

Four × loading buffer (16% glycerol, 20% -mercaptoethanol, 2% sodium dodecyl sulfate [SDS], 0.05% bromophenol blue) was added to each sample (3:1, sample:loading buffer) before boiling for 3 min. Samples were cooled and subjected to SDS-polyacrylamide gel electrophoresis (10% acrylamide/0.27% N,N'-methylenebisacrylamide resolving gel) for about 40 min at 80 V in stacking gel and about 1 h at 130 V in resolving gel. Proteins were transferred electrophoretically to Immobilon-P transfer membranes (Millipore, Bedford, MA) at 0.25 A for 2.5 h. Membranes were washed with TBST (Tris-Buffered Saline plus 0.05% Tween-20, pH 7.4) and then dipped in blocking buffer (5% bovine serum albumin [BSA] in TBST) overnight at 4ºC. The next day, the membranes were incubated for 1 h at room temperature on an orbital shaker with

anti-PKMζ antibody (1:1000; Upstate USA, 07-264) or β-actin (1:1000; Santa Cruz) in TBST plus 5% BSA and 0.05% sodium azide. After three 5-min washes (3 times) in TBST buffer, the blots were incubated for 45 min at room temperature on a shaker with horseradish peroxidase-conjugated secondary antibody (PKMζ was goat anti-rabbit IgG and β-actin was goat anti-mice; Santa Cruz; PI-1000; Vector Labs) diluted 1:5000 in blocking buffer. The blots were then washed three times for 5 min each in TBST and incubated with a layer of Super Signal Enhanced chemiluminescence substrate (Detection Reagents 1 and 2, 1:1 ratio, Pierce Biotechnology (Rockford, IL) for 1 min at room temperature. Excess mixture was dripped off before the blots were wrapped with a clean piece of plastic wrap (no bubbles between blot and wrap), and then the blots were exposed to X-ray film (Eastman Kodak Company) for 5-60 s. Band intensities for PKMζ were quantified by two observers who were blind to the experimental groups using Quantity One software (version 4.4.0, Biorad, Hercules, CA, USA). Band intensities from each test sample were compared to the band intensities from the standard curves. The amount of the protein of interest in each sample was interpolated from the standard curve. The standard curve runs in all Western blots in our study showed that the band intensities for each of our test samples were within the linear range of detection.

Exp. 1: Effect of memory retrieval-extinction on reinstatement of cocaine CPP

This experiment was designed to determine how extinction at different intervals after cued retrieval of the cocaine memory would affect subsequent cocaine-priming-induced reinstatement of drug CPP. The rats underwent sessions for cocaine-induced CPP for 8 d and were tested one day later to confirm that CPP had been established (Test 1). They were then divided into four groups with equivalent cocaine CPP scores and treated as follows: (1) Group 1 rats received 8 consecutive days of extinction without memory-retrieval trials (No memory retrieval + Extinction); (2) Group 2 rats were given 10-min memory-retrieval trials 10 min before every extinction session (Memory retrieval + 10 min delay + Extinction); (3) Group 3 rats were given a retrieval trial 1 h before every extinction session (Memory retrieval + 1 h delay + Extinction); (4) Group 4 rats

were given a memory-retrieval trial 6 h before every extinction session (Memory retrieval + 6 h delay + Extinction). All rats were retested for cocaine CPP expression (Test 2) the day after the last extinction session. Then the rats underwent a reinstatement session, during which they were given a cocaine injection (5 mg/kg, i.p.) five min before testing (Fig. S1).

Exp. 2: Effect of memory retrieval-extinction on reinstatement of morphine CPP

In Exp. 2 we assessed whether the findings obtained with cocaine in Exp. 1 would generalize to the opiate drug morphine. After 8 consecutive days of morphine CPP sessions, the rats were tested to confirm the establishment of morphine CPP and divided into four groups with equivalent morphine CPP scores after test 1. The four groups were treated and tested as described in Exp. 1. Then the rats underwent a reinstatement session, during which they were given a priming morphine injection (5 mg/kg, s.c.) five min before the reinstatement test (Fig. 1).

Exp. 3: Effect of memory retrieval-extinction on spontaneous recovery of cocaine CPP

To further test how extinction at different intervals after memory retrieval would affect cocaine CPP, we assessed spontaneous recovery of CPP 14 d after the retrieval-extinction manipulation. The rats underwent cocaine CPP sessions and were divided into 4 groups as described in Exp. 1. The rats were tested for cocaine CPP expression (Test 2) the day after the last extinction session. The rats were then housed in their homecages for 14 days, after which they were tested for spontaneous recovery of the extinguished CPP (Fig. S2).

Exp. 4-5: Effect of memory retrieval-extinction on cocaine- or heroin-priming-induced operant drug seeking

To further test how extinction at different intervals after memory retrieval would affect drug seeking, we tested reinstatement in an intravenous self-administration model (*21*). In Exp. 4, the rats were trained to self-administer cocaine for 10 days, and one day later they were divided into three groups with equivalent cocaine intake: (1) Group 1 rats underwent 3.25 h extinction without memory retrieval (No memory retrieval

+ Extinction); (2) Group 2 rats were given memory retrieval 10 min before every extinction session (Memory retrieval + 10 min delay + Extinction); (3) Group 3 rats were given memory retrieval 6 h before every extinction session (Memory retrieval + 6 h delay + Extinction). Twenty-four h after the rats met the extinction criterion, they underwent a priming test initiated by a non-contingent cocaine injection (5 mg/kg, i.p.) 5 min prior to the test sessions; during these sessions, nosepokes led to contingent delivery of the tone-light cue previously paired with cocaine infusions but not cocaine (Fig. S3).

In Exp. 5, the rats were trained to self-administer heroin for 10 d, and one day later they were divided into two groups with equivalent heroin intake: (1) Group 1 rats underwent 3.25 h extinction without memory retrieval (No memory retrieval + Extinction); (2) Group 2 rats were given memory retrieval trials 10 min before the extinction session (Memory retrieval + 10 min delay + Extinction). Twenty-four h after the rats met the extinction criterion, they underwent a priming test initiated by a non-contingent heroin injection (0.25 mg/kg, s.c.) 5 min prior to the test sessions (Fig. 2). The cocaine and heroin priming doses were based on findings from previous extinction-reinstatement studies (*55, 56*).

Exp. 6: Effect of retrieval-extinction on spontaneous recovery of cocaine seeking

To test how extinction at different intervals after retrieval would affect subsequent cocaine-seeking behavior, we assessed spontaneous recovery. Rats were trained to self-administer cocaine for 10 d and then divided into two groups that were matched for their cocaine intake and treated as follows: (1) Group 1 rats underwent 3.25 h extinction training without memory retrieval (No memory retrieval + Extinction); (2) Group 2 rats underwent a memory retrieval trial 10 min before every extinction session (Memory retrieval + 10 min delay + Extinction). After the rats met the extinction criterion, they were returned to the homecages for 4 weeks. Then they were tested for spontaneous recovery of extinguished drug-seeking behavior in an extinction session in which nosepokes led to contingent delivery of the tone-light cue previously paired with cocaine infusions but not cocaine (Fig. S4).

Exp. 7: Effect of retrieval-extinction on renewal of cocaine seeking

We used a modification of the ABA renewal procedure (*57*) that has been used over the last decade to study the role of context in relapse to drug seeking (*23, 28*). Rats were first trained to self-administer cocaine in Context A. They were then divided into two groups that were matched for their cocaine intake and given extinction training in Context B: Group 1 rats underwent 3.25 h extinction training without memory retrieval (No memory retrieval + Extinction), and Group 2 rats underwent memory retrieval 10 min before every extinction session (Memory retrieval + 10 min delay + Extinction). The retrieval manipulation was a short 15-min exposure to the training context during which nosepokes resulted in presentation of the discrete cues but not cocaine. When the rats met the extinction criterion, they underwent a renewal test in Context A; nosepokes led to contingent delivery of the tone-light cue previously paired with cocaine infusions but not cocaine (Fig. S5).

Exp. 8: Effect of retrieval-extinction on PKMζ levels in medial prefrontal cortex and amygdala

Rats were divided into 4 groups that were matched for their cocaine intake during training (10 d), and treated as follows: (1) Group 1 rats received cocaine self-administration training without extinction sessions (No retrieval + No extinction); (2) Group 2 rats underwent 3.25 h extinction training without memory retrieval (No memory retrieval + Extinction); (3) Group 3 rats were given memory retrieval 10 min before every extinction session (Memory retrieval + 10 min delay + Extinction); (4) Group 4 rats were given memory retrieval 6 h before every extinction session (Memory retrieval + 6 h delay + Extinction). Twenty-four h after the experimental conditions, the rats were euthanized and their brains were removed for Western blots to measure levels of PKMζ in mPFC and amygdala (Fig. S6).

Human study: Effect of retrieval-extinction on drug craving in formerly heroin addicts

Each participant received a baseline assessment of demographic and heroin-abuse characteristics, as well as the Hamilton Anxiety Scale (HAMA) and the Beck Depression Inventory (BDI). Heroin craving was

assessed using a visual analog scale (VAS), i.e., an undivided line marked at the left and right ends with 0 (“not at all”) and 10 (“extremely high”), on which the participants had to rate their current craving for heroin, before and immediately after exposure to a neutral cue and a heroin cue. Neutral and heroin cues were both 5-min videotapes. The neutral videotape showed natural scenery; the heroin videotape (available upon request) showed scenes of heroin smoking and injection. Heart rate (HR) and blood pressure were monitored pre- and post-cue exposure. There was a 10-min interval between the neutral and the heroin cue exposure session. To avoid sending participants away in a vulnerable or distressed state, the session did not end until the participant’s physiological measures had returned to baseline levels.

Sixty-six participants provided informed consent and completed the study. After baseline assessments on Day 1, the participants were randomly assigned to 3 groups: 1) Neutral cue exposure + Extinction; 2) Heroin cue exposure + 10 min delay + Extinction; 3) Heroin cue exposure+ 6 h delay + Extinction. The 3 groups received different exposure + extinction manipulations on Days 2 and 3. Cue-induced changes in heroin craving were assessed for all participants 1 day (Day 4), 30 days (Day 34), and 180 days (Day 184) after the baseline session.

Twenty-four h after the baseline session described above, the participants underwent different retrieval-extinction manipulations for 2 consecutive days: 5 min neutral-cue exposure (no retrieval) + 60 min extinction training (Group 1), or 5 min heroin-cue exposure (memory retrieval) + 60 min extinction training, with an interval of either 10 min (Group 2) or 6 h (Group 3) between retrieval and extinction training. During the extinction sessions, the participants were given 4 consecutive sessions of repeated exposures to 3 different kinds of heroin-related cues (5-min slides, 5-min video and 5-min inspection and handling of drug-use material, including simulated heroin). Subjective craving, HR, and blood pressure were assessed before extinction started and after the extinction session. Ratings of cue-induced heroin craving were taken again on day 4, day 34, and day 184 using procedures identical to those described for Day 1 (Fig. 4). The

experimental conditions were based on those used in previous studies (*3, 4, 20, 58-61*). The effectiveness of heroin-related cues during extinction training had been established in a preliminary study in our laboratory (data not shown); the 3 types of heroin-related cues had each increased heroin craving and blood pressure, measures of sympathetic activation.

Statistical analyses

Data were expressed as mean±SEM and were analyzed by ANOVAs with the appropriate between- and within-subjects factors for each experiment (see Main Text). Significant main effects and interactions ($p<0.05$, two-tailed) from the factorial ANOVAs were followed by one-way ANOVAs and Turkey's post-hoc tests.

Table S1. Demographic and clinical characteristics of subjects with heroin dependence

Group	No-Retrieval -Extinction (n=22)	Retrieval-10min -Extinction (n=22)	Retrieval-6h -Extinction (n=22)	P value
Age (years)	36.3±1.4	39.1±1.8	36.9±1.5	0.4
Education (years)	9.4±0.6	8.6±0.6	9.2±0.6	0.6
Heroin use (years)	9.7±1.1	11.4±1.2	11.1±1.0	0.5
Daily dose (g)	0.8±0.1	1.2±0.4	1.5±0.7	0.6
BDI score	10.2±1.2	12.2±1.4	11.4±1.5	0.6
HAMA score	11.3±0.9	10. 5±1.0	11.2±1.1	0.8

Table S2. Summary of statistical results. SA: self-administration

Experiments	Main effect 1	Main effect 2 (and 3)	Interaction
Exp. 1: cocaine CPP reinstatement test	Group [F (3,34) = 40.5, p < 0.001]	Test [F(1,34) = 62.2, p < 0.001]	Group × Test interaction [F(3,34) = 37.9, p < 0.001]
Exp. 2: morphine CPP reinstatement test	Group [F(3,25) = 3.6, p = 0.027]	Test [F(1,25) = 22.2, p < 0.001]	Group × Test interaction [F(3,25) = 9.5, p < 0.001]
Exp. 3: cocaine CPP spontaneous recovery test	Group [F (3,28)=16.0, p<0.001]	Test [F (1,28)=47.5, p<0.001]	Group × Test interaction [F(3,28)=21.5, p<0.001]
Exp. 4: cocaine SA extinction training	Group [F(2,21) = 9.6, p = 0.001]	Extinction session [F (13,273) = 19.8, p < 0.001]	Group × Extinction session [F(26,273) = 1.7, P = 0.021]
Exp. 4: cocaine SA reinstatement test	Group [F(2,21) = 8.8, p = 0.002]	Reinstatement Condition [F(1,21) = 130.9, p < 0.001]	Group × Reinstatement Condition interaction [F(2, 21) = 6.1, p = 0.008]
Exp. 5: heroin SA extinction training	Group [F(1,10) = 2.2, p = 0.172]	Extinction session [F (11,110) = 10.5, p < 0.001]	Group × Reinstatement Condition interaction [F (11, 110) = 0.7, p = 0.775]
Exp. 5: heroin SA reinstatement test	Group [F(1,10) = 9.8, p = 0.011]	Reinstatement Condition [F (1,10) = 19.9, p = 0.001]	Group × Reinstatement Condition interaction [F(1, 10) = 6.9, p = 0.025].
Exp. 6: cocaine SA extinction training	Group [F(1,16) = 0.1, p = 0.716]	Extinction session [F (11,176) = 18.9, p < 0.001]	Group × Extinction session interaction [F(11,176) = 3.2, p < 0.001]
Exp. 6: cocaine SA spontaneous recovery test	Group [F(1,16)=7.5, p=0.015]	Test Condition [F (1,16)=186.9, p<0.001]	Group × Test Condition interaction [F(1, 16)=14.8, p=0.001]
Exp. 7: cocaine SA extinction training	Group [F(1,12) = 1.3, p = 0.276]	Extinction session [F (11,132) = 9.9, p < 0.001]	Group × Extinction Session [F(11,132) = 1.4, p = 0.171]
Exp. 7: cocaine SA renewal test	Group [F(1,12) = 6.8, p = 0.023]	Test Condition [F(1,12) = 44.4, p < 0.001]	Group × Test Condition interaction [F(1,12) = 6.7, p = 0.024]
Exp. 8: PKMζ levels in infralimbic cortex	Not applicable	Group [F(3,24)=3.4, p=0.037]	Not applicable
Exp. 8: PKMζ levels in basolateral amygdala	Not applicable	Group [F(3,24) = 5.9, p = 0.004]	Not applicable

Human experiments: cue-induced craving	Group [F(2,38.5) = 14.6, p < 0.001]	Test Day [F(3,22.3) = 5.7, p < 0.001] Cue type [F(1,650.6) = 493.6, p < 0.001]	Group × Test Day × Cue type [F(6,8.85) = 1.1, p = 0.35 Group × Test Day [F(6,8.31) = 1.1, p = 0.39] Test Day × Cue type [F(3,144) = 19.9, p < 0.001] Group ×Cue type [F(2,23.75) = 4.9, p = 0.011]
Human experiments: Systolic blood pressure	Group [F(2,1987.26) = 13.77, p < 0.001]	Test Day [F(3,97.91) = 0.45, p = 0.716] Cue type [F(1,13114.51) = 181.8, p < 0.001] Test Day × Cue type [F(3,256.94) = 1.19, p = 0.314]	Group × Test Day× Cue type [F(6,770.07) = 1.78, p = 0.102] Group × Test Day [F(6,539.55) = 1.25, p = 0.281] Group ×Cue type [F(2,343.25) = 2.38, p = 0.094]
Human experiment: Diastolic blood pressure	Group [F(2,649.33) = 4.20, p = 0.0157]	Test Day [F(3,83.32) = 0.35, p = 0.786] Cue type [F(1,6692.09) = 86.5, p < 0.001] Test Day × Cue type [F(3,457.46) = 1.97, p = 0.118]	Group × Test Day× Cue type [F(6,170.46) = 0.37, p = 0.90] Group × Test Day [F(6,483.12) = 1.04, p = 0.40] Group ×Cue type [F(2,374.46) = 2.42, p = 0.09]
Human experiment: Heart rate	Group [F(2,8.64) = 0.13, p = 0.88]	Test Day [F(3,18.27) = 0.18, p = 0.91] Cue type [F(1, 4301.70) = 130.4, p < 0.0001] Test Day × Cue type [F(3,321.97) = 3.2, p = 0.022]	Group × Test Day× Cue type [F(6,112.04) = 0.57, p = 0.76] Group × Test Day [F(6,163.26) = 0.82, p = 0.55] Group ×Cue type [F(2,7.45) = 0.11, p = 0.89]

Figure legends

Fig S1. *Memory retrieval 10 min or 1 h before extinction sessions prevented drug-priming-induced reinstatement of cocaine CPP.* (**A**) Timeline of the experimental procedure. Training and retrieval-extinction manipulations were identical to those shown in Fig. 1A except that the drug administered was cocaine (10 mg/kg, i.p., during training; 5 mg/kg, i.p., in the reinstatement test). (**B**) Effect of the experimental manipulations on the CPP score. Data are mean ± SEM of preference score in sec (time spent in the cocaine-paired chamber minus time spent in the saline-paired chamber) during the CPP tests. * Different from the "No Memory Retrieval" condition, $p < 0.05$; n = 9 -11 per experimental condition.

Fig S2. *Memory retrieval 10 min or 1 h before the extinction sessions prevented spontaneous recovery of cocaine CPP.* (**A**) Timeline of the experimental procedure. Training and retrieval-extinction manipulations were identical to those shown in Fig.1A except that spontaneous recovery of cocaine CPP was tested 14 d later. The conditions during the spontaneous-recovery test were identical to those of CPP tests 1 and 2. (**B**) Effect of the experimental manipulations on the CPP scores. Data are mean ± SEM of preference score in sec during the CPP tests. * Different from the "No Memory Retrieval" condition, $p < 0.05$; n = 9-10 per experimental condition.

Fig S3. *Memory retrieval 10 min before the extinction sessions accelerated extinction responding and attenuated cocaine-priming-induced reinstatement of drug seeking.* (**A**) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into three groups and given different retrieval-extinction manipulations: 195 min extinction training (in one group) or 15 min memory retrieval + 180 min extinction training (in the other two groups—with 10 min or 6 h delay between memory retrieval and extinction training). The rats were then tested for reinstatement of nosepoke responding after non-contingent priming injections of cocaine (5 mg/kg, i.p.). (**B-C**) Number of responses (mean ± SEM) on the active and inactive nosepoke

devices during the extinction phase and the reinstatement test. * Different from the “No Memory Retrieval” condition, $p < 0.05$; n = 9-10 per experimental condition.

Fig. S4. *Memory retrieval 10 min before the extinction sessions attenuated spontaneous recovery of cocaine seeking.* (**A**) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into two groups and given different retrieval-extinction manipulations: 195 min extinction training or 15 min memory retrieval + 180 min extinction training, with a 10 min delay between memory retrieval and extinction training. Twenty-eight days later, the rats were tested for spontaneous recovery of cocaine seeking in a single extinction test. (**B**) Number of responses (mean ± SEM) on the active and inactive nosepoke devices during the last extinction session and spontaneous recovery test. * Different from the “No Memory Retrieval” condition, $p < 0.05$; *n* = 6-8 per experimental condition.

Fig. S5. *Memory retrieval 10 min before the extinction sessions attenuated renewal of cocaine seeking when the rats returned to the cocaine self-administration environment after extinction of drug-reinforced responding in a non-drug context.* (**A**) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into two groups and given different retrieval-extinction manipulations in a different non-drug context (green): 195 min extinction training or 15 min memory retrieval + 180 min extinction training, with 10 min delay between memory retrieval and extinction training. The rats were then tested for renewal of cocaine seeking in a single extinction test conducted in the original cocaine self-administration context. (**B**) Number of responses (mean ± SEM) on the active and inactive nosepoke devices during the last extinction session and the renewal test. * Different from the “No Memory Retrieval” condition, $p < 0.05$; n = 8-9 per experimental condition.

Fig S6. *Different retrieval-extinction manipulations differentially affected PKMζ levels in medial*

prefrontal cortex and amygdala. (A) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into four groups and given different memory retrieval-extinction manipulations: No retrieval—No extinction, 195 min extinction training (in one group) or 15 min memory retrieval + 180 min extinction training (in the other two groups—with 10 min or 6 h delay between memory retrieval and extinction training). Twenty-four h after the last extinction session (session XX), the rats were euthanized and their brains were removed for subsequent Western blots to measure levels of PKMζ. (B-E) Effect of the different retrieval-extinction manipulations on PKMζ levels in the infralimbic cortex, prelimbic cortex, basolateral amygdala, and central amygdala. Data are percent difference values (mean ± SEM) from a group of naïve rats that did not participate in the behavioral experiment. The brains of the rats in the No retrieval—No extinction condition were taken one day after the last self-administration training session. * Different from the self-administration group, $p < 0.05$; # Different from each of the other three groups, $p < 0.05$. *n* = 6-10 per experimental condition.

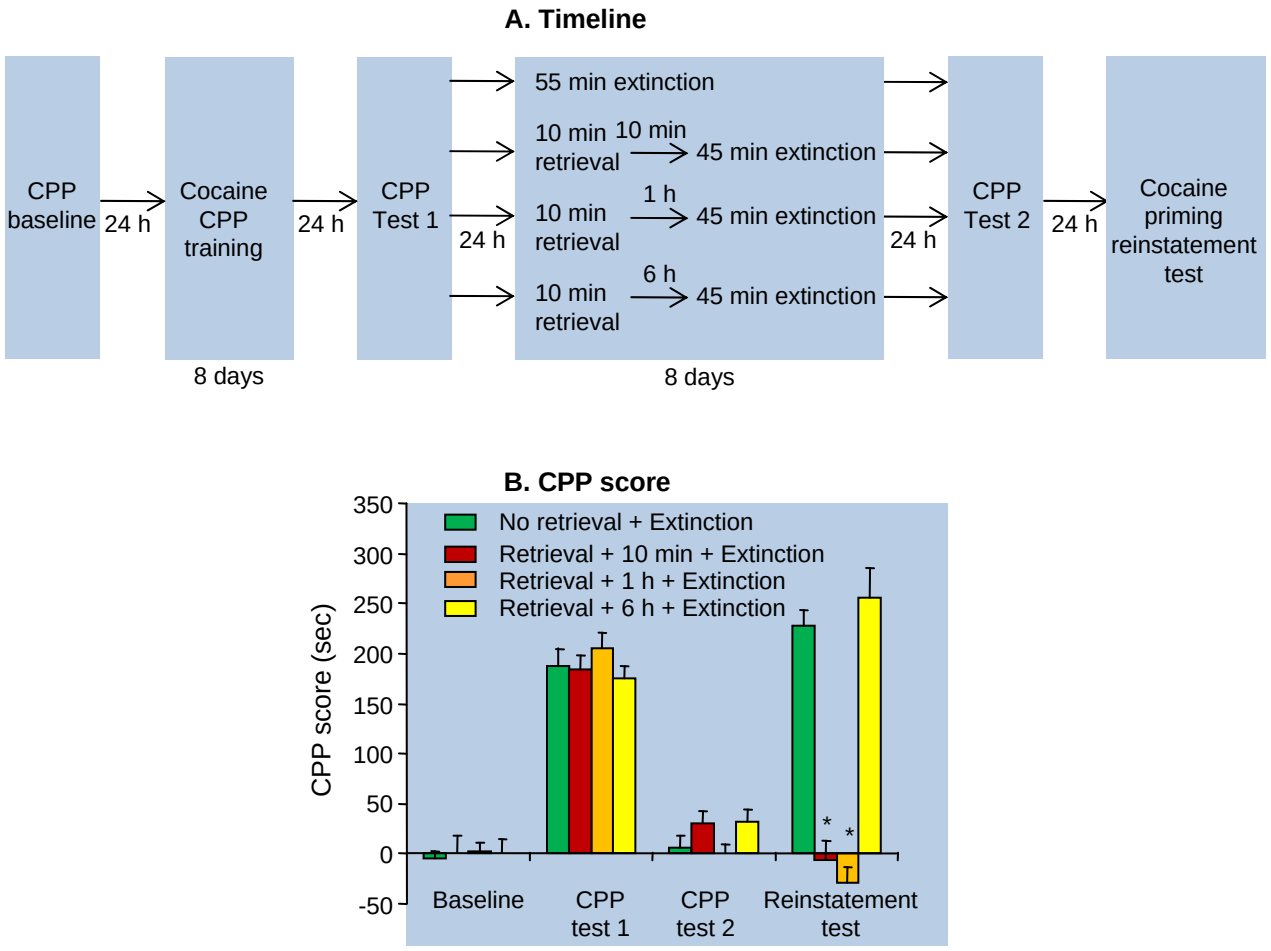

Fig S1. *Memory retrieval 10 min or 1 h before extinction sessions prevented drug-priming-induced reinstatement of cocaine CPP.* (**A**) Timeline of the experimental procedure. Training and retrieval-extinction manipulations were identical to those shown in Fig. 1A except that the drug administered was cocaine (10 mg/kg, i.p., during training; 5 mg/kg, i.p., in the reinstatement test). (**B**) Effect of the experimental manipulations on the CPP score. Data are mean ± SEM of preference score in sec (time spent in the cocaine-paired chamber minus time spent in the saline-paired chamber) during the CPP tests. * Different from the "No Memory Retrieval" condition, $p < 0.05$; *n* = 9 -11 per experimental condition.

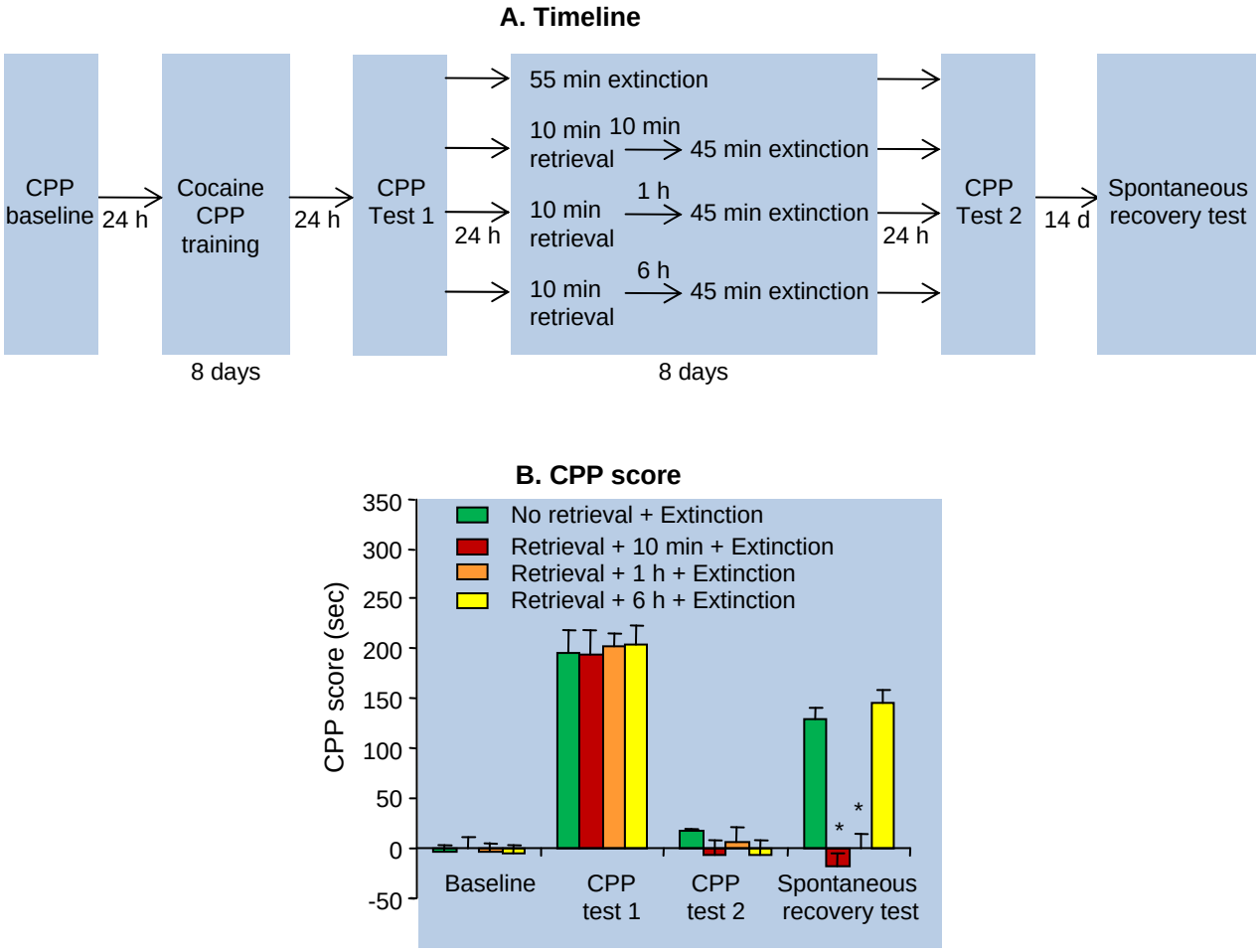

Fig S2. *Memory retrieval 10 min or 1 h before the extinction sessions prevented spontaneous recovery of cocaine CPP.* (**A**) Timeline of the experimental procedure. Training and retrieval-extinction manipulations were identical to those shown in Fig.1A except that spontaneous recovery of cocaine CPP was tested 14 d later. The conditions during the spontaneous-recovery test were identical to those of CPP tests 1 and 2. (**B**) Effect of the experimental manipulations on the CPP scores. Data are mean ± SEM of preference score in sec during the CPP tests. * Different from the "No Memory Retrieval" condition, $p < 0.05$; *n* = 9-10 per experimental condition.

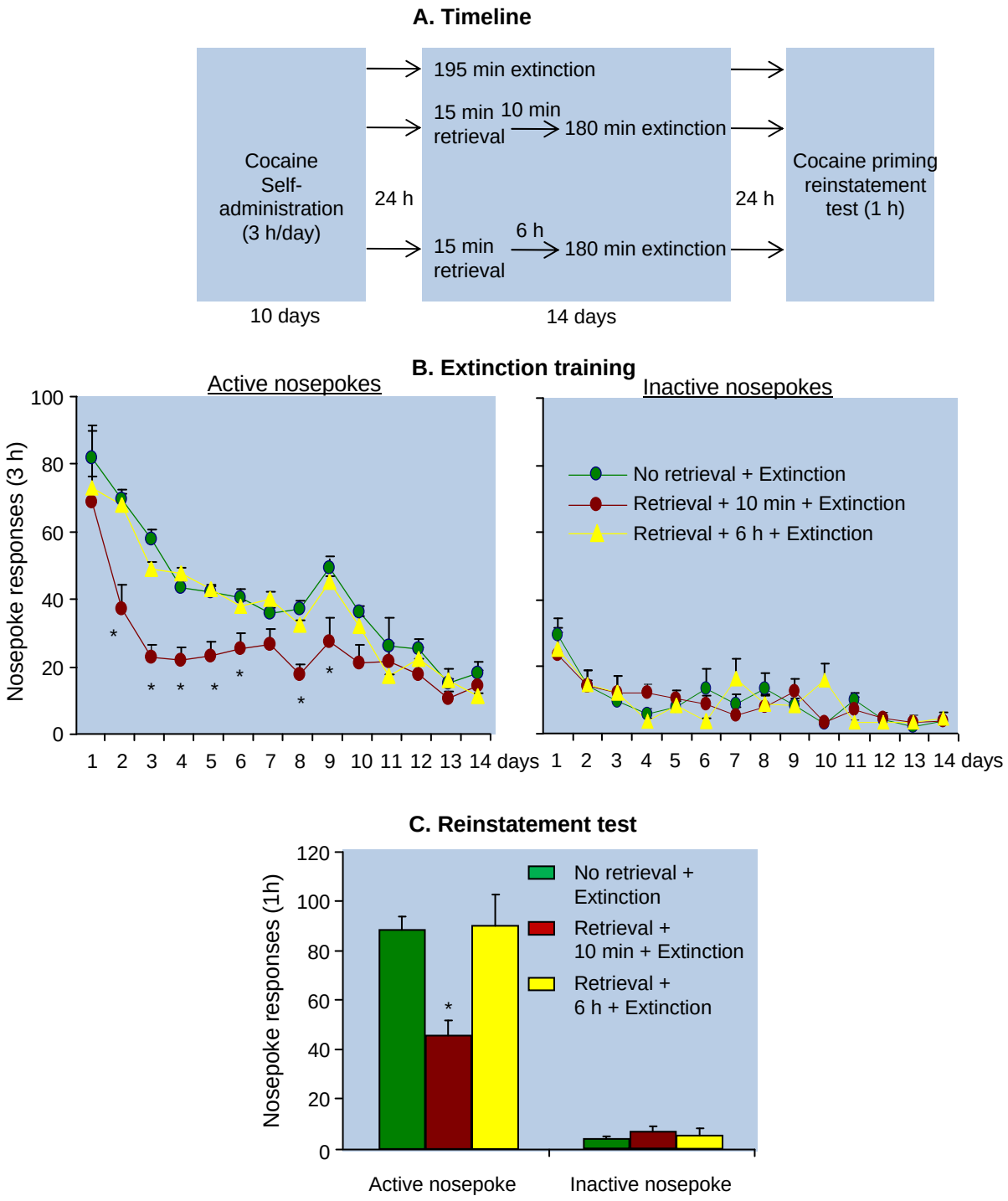

Fig S3. *Memory retrieval 10 min before the extinction sessions accelerated extinction responding and attenuated cocaine-priming-induced reinstatement of drug seeking.* (**A**) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into three groups and given different retrieval-extinction manipulations: 195 min extinction training (in one group) or 15 min memory retrieval + 180 min extinction training (in the other two groups—with 10 min or 6 h delay between memory retrieval and extinction training). The rats were then tested for reinstatement of nosepoke responding after non-contingent priming injections of cocaine (5 mg/kg, i.p.). (**B-C**) Number of responses (mean ± SEM) on the active and inactive nosepoke devices during the extinction phase and the reinstatement test. * Different from the "No Memory Retrieval" condition, $p < 0.05$; n = 9-10 per experimental condition.

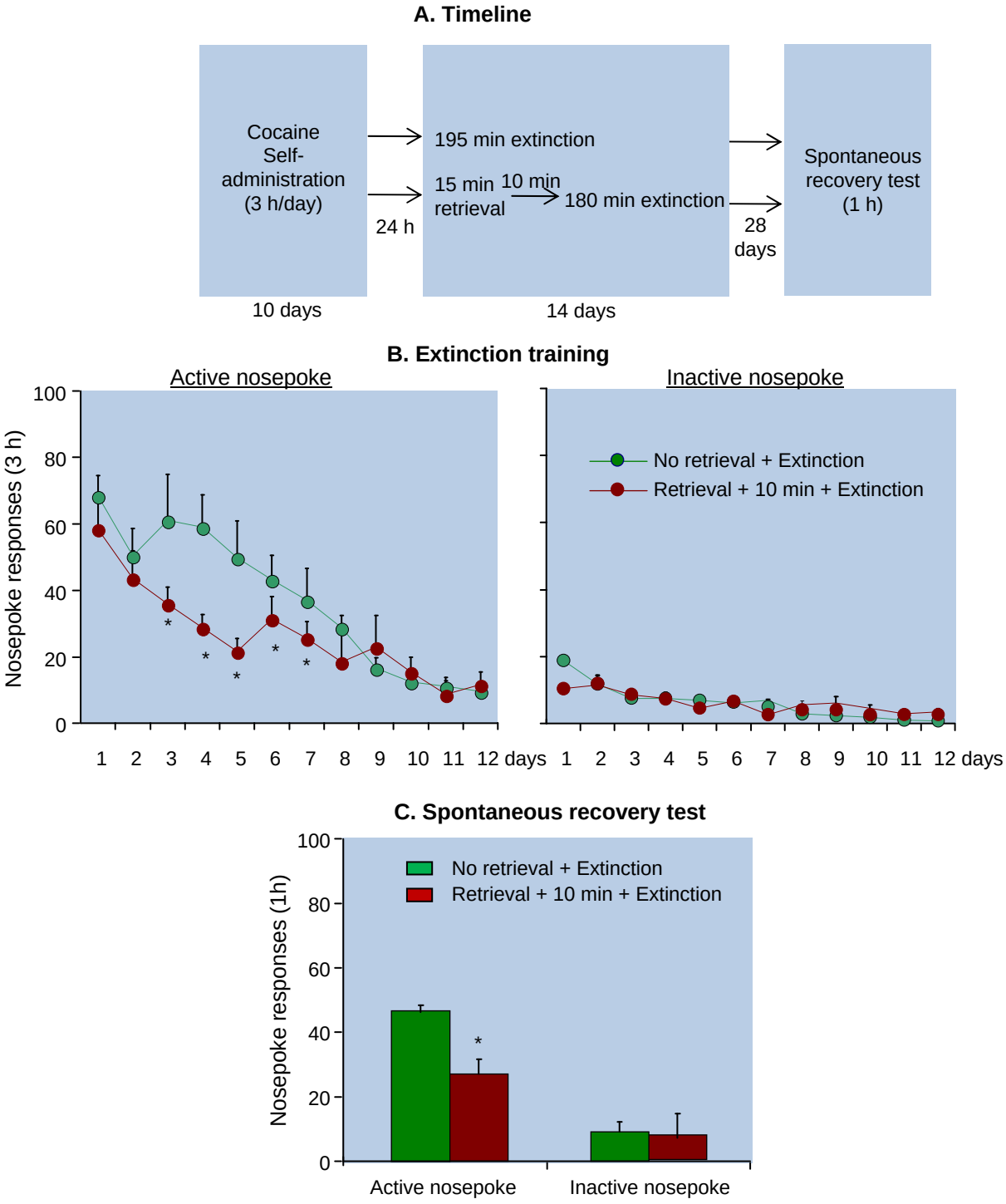

Fig. S4. *Memory retrieval 10 min before the extinction sessions attenuated spontaneous recovery of cocaine seeking.* (**A**) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into two groups and given different retrieval-extinction manipulations: 195 min extinction training or 15 min memory retrieval + 180 min extinction training, with a 10 min delay between memory retrieval and extinction training. Twenty-eight days later, the rats were tested for spontaneous recovery of cocaine seeking in a single extinction test. (**B**) Number of responses (mean ± SEM) on the active and inactive nosepoke devices during the last extinction session and spontaneous recovery test. * Different from the "No Memory Retrieval" condition, $p < 0.05$; *n* = 6-8 per experimental condition.

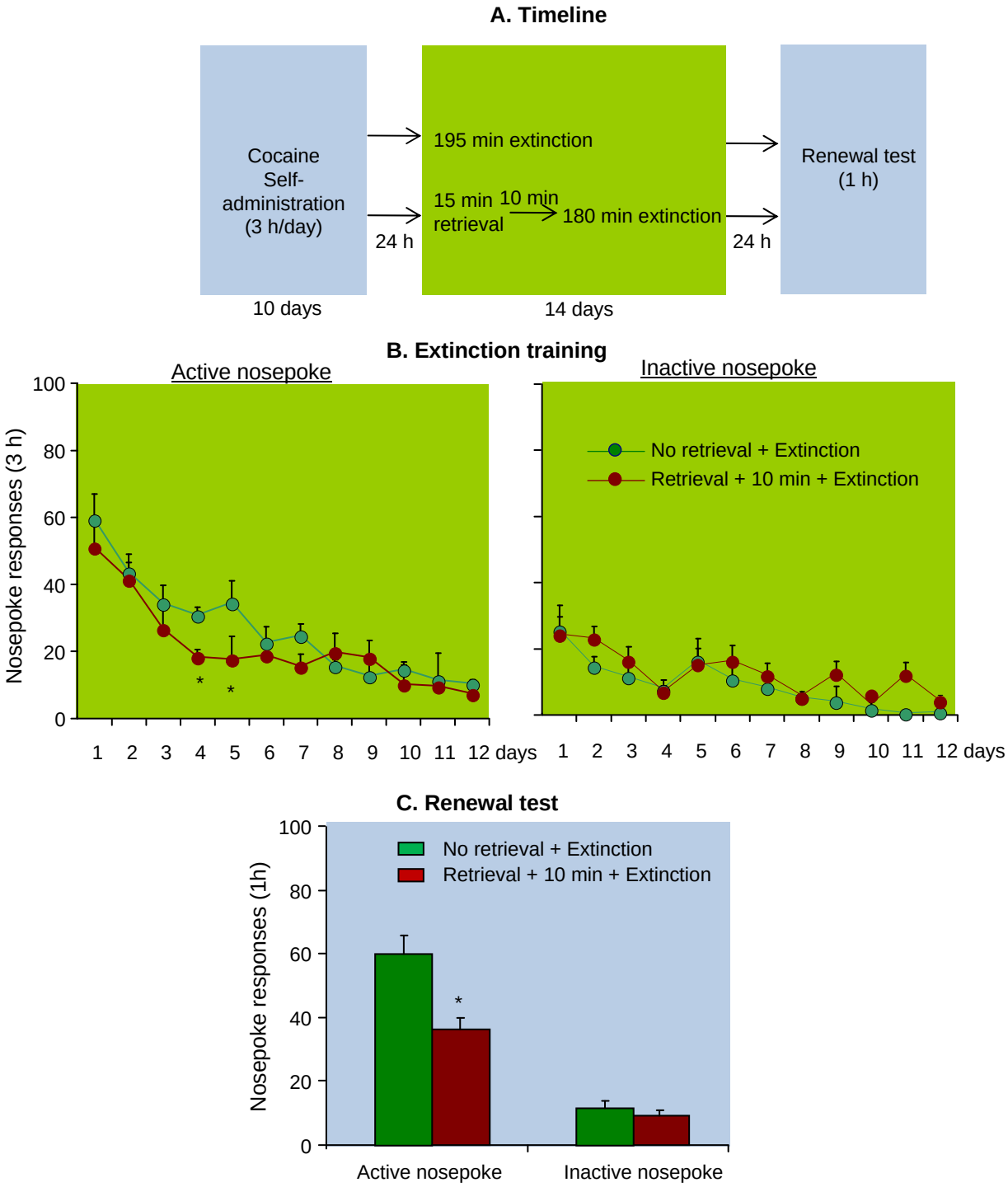

Fig. S5. *Memory retrieval 10 min before the extinction sessions attenuated renewal of cocaine seeking when the rats returned to the cocaine self-administration environment after extinction of drug-reinforced responding in a non-drug context.* (**A**) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into two groups and given different retrieval-extinction manipulations in a different non-drug context (green): 195 min extinction training or 15 min memory retrieval + 180 min extinction training, with 10 min delay between memory retrieval and extinction training. The rats were then tested for renewal of cocaine seeking in a single extinction test conducted in the original cocaine self-administration context. (**B**) Number of responses (mean ± SEM) on the active and inactive nosepoke devices during the last extinction session and the renewal test. * Different from the "No Memory Retrieval" condition, $p < 0.05$; n = 8-9 per experimental condition.

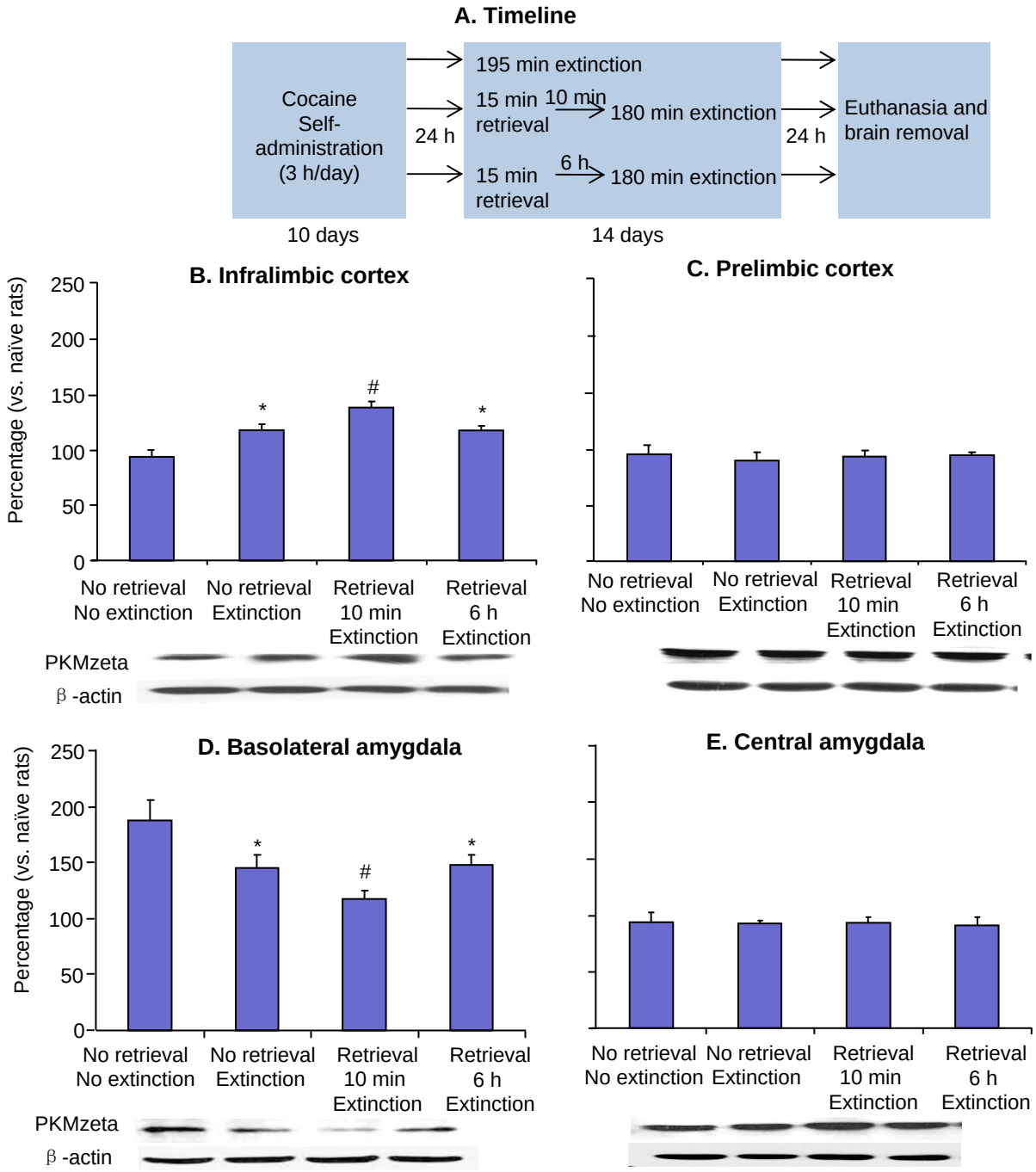

Fig S6. *Different retrieval-extinction manipulations differentially affected PKM ζ levels in medial prefrontal cortex and amygdala.* (**A**) Timeline of the experimental procedure. Rats were trained to self-administer intravenous cocaine during three 1-h daily sessions over 10 days. Twenty-four h later, the rats were divided into four groups and given different memory retrieval-extinction manipulations: No retrieval—No extinction, 195 min extinction training (in one group) or 15 min memory retrieval + 180 min extinction training (in the other two groups—with 10 min or 6 h delay between memory retrieval and extinction training). Twenty-four h after the last extinction session (session 14), the rats were euthanized and their brains were removed for subsequent Western blots to measure levels of PKM ζ . (**B-E**) Effect of the different retrieval-extinction manipulations on PKM ζ levels in the infralimbic cortex, prelimbic cortex, basolateral amygdala, and central amygdala. Data are percent difference values (mean ± SEM) from a group of naïve rats that did not participate in the behavioral experiment. The brains of the rats in the No retrieval—No extinction condition were taken one day after the last self-administration training session. * Different from the self-administration group, $p < 0.05$; # Different from each of the other three groups, $p < 0.05$. *n* = 6-10 per experimental condition.

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