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This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods

Rat study

Subjects: We housed male Sprague-Dawley rats (weighing 260-280 g, Vitalriver Company) in individual cages in a temperature- (23±2°C) and humidity- (50±5%) controlled animal facility with free access to food and water. We kept the rats on a reverse 12 h/12 h light/dark cycle. We performed the experimental procedures in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the procedures were approved by the Biomedical Ethics Committee for animal use and protection of Peking University. We used 156 rats, of which 4 rats were excluded because of loss of the dental cement and catheter patency during the SA training, and 10 rats were excluded because they exhibited strong unconditioned side preference (> 540 s) during the nicotine preconditioning phase.

Training for nicotine conditioned place preferences (CPP) and initial CPP test

The CPP procedure is based on our previous studies. The apparatus for CPP conditioning and testing consisted of 20 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, we initially placed rats in the middle chamber with the doors removed and allowed them 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). The conditioning was performed using an unbiased, balanced protocol.

We performed CPP training over 8 consecutive days with alternating injections of nicotine (0.5 mg/kg, s.c.) and saline (1 ml/kg, s.c.). After each injection, we confined the rats to the corresponding conditioning chambers for 45 min before being returned to their home cages. The day after the last conditioning session, we tested the rats for the expression of nicotine-induced CPP (Test 1) under conditions identical to those described for the baseline test. We calculated the CPP score as the time spent in the nicotine-paired chamber minus the time spent in the saline-paired chamber.

**Extinction of nicotine CPP**

Extinction training was identical to the initial CPP training, except that no injections were given. The day after the 4 consecutive days of extinction training, which included 2 sessions in the nicotine-paired side and 2 sessions in the saline-paired side, we tested again the rats for CPP expression (Test 2).

**Nicotine-priming-induced reinstatement of CPP**

The nicotine priming-induced reinstatement of CPP procedure is based on our previous studies. Five min before the CPP reinstatement test, we injected the rats with nicotine (0.25 mg/kg, s.c.). The experimental procedure during the reinstatement test was the same as those for the baseline preference test and CPP tests 1-2. The reinstatement tests were performed by an investigator blinded to condition.

**Intravenous nicotine self-administration (SA)**

We anesthetized the rats (weighing 300-320 g when surgery began) with sodium pentobarbital (60 mg/kg, i.p.) and inserted the catheters into the right jugular vein with
the tip terminating at the opening of the right atrium as previously described. We allowed the rats to recover for 5-7 days after surgery. The operant chambers (AniLab Software and Instruments) were equipped with two nosepoke operandi (AniLab Software and Instruments) located 5 cm above the chamber floor. Nosepokes in the active operandum led to nicotine 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. We trained the rats to self-administer nicotine bitartrate (0.03 mg/kg/infusion) during two 1-h daily sessions separated by 5 min over 14 days. The sessions began at the onset of the dark cycle. We used a fixed-ratio 1 (FR1) reinforcement schedule, 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. At the end of the training phase, the groups in the different experimental conditions were matched for their nicotine intake during training. These training conditions and nicotine unit dose are based on previous studies.

**Sucrose self-administration**

The experimental conditions were identical to those described above for nicotine self-administration, with the exception that active nosepoke responses led to 0.1 ml of sucrose (10%) delivered into a liquid receptacle.

**Extinction of nicotine-reinforced responding**

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

**Nicotine priming-induced reinstatement of operant responding**

Once responding on the active nosepoke operandum was successfully extinguished per the criterion described above, testing commenced. The extinction tests were performed by an investigator blinded to condition. The test conditions were the same as during training, with the exception that active nosepokes were not reinforced by nicotine. 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 nicotine infusions but not nicotine. During the nicotine priming reinstatement tests, we injected nicotine (0.3 mg/kg, s.c.) 5 min before the start of the sessions. The nicotine priming dose is based on previous studies.5,6

**Drugs and injections**

We dissolved nicotine tartrate (Xinxiang Crude Medicinal Nicotines Co.), and propranolol HCl (#A9789, St. Louis, Sigma–Aldrich) in 0.9% physiological saline and intraperitoneally injected at a dose of 10 mg/kg; this dose is based on previous studies.8-10

**Exp. 1: Effect of systemic injections of propranolol on reconsolidation of nicotine CPP memories and SA memories in rats** (eFigure 4A and 5A)

In the CPP experiment, we first assessed baseline preferences of rats (8 groups, n=8-10 per group) for the two chambers of the CPP apparatus (test 1, 15 min session).
Next, we trained the rats to associate one context with the effect of nicotine injections (0.5 mg/kg, s.c., 4 pairings) and to associate another context with the effect of saline injections (1 ml/kg, 4 pairings); we then assessed the expression of CPP (test 2, 15 min session). Twenty-four h later, we divided the rats in the first run into 4 groups (n=8-10 per group). We exposed two groups of rats to the nicotine-paired side of CPP apparatus for 5 min [CPP-conditioned stimulus (CPP-CS) retrieval] and injected two other groups of rats with a low dose of nicotine (0.15 mg/kg, s.c.; unconditioned stimulus (UCS) retrieval). Immediately after CPP-CS or UCS retrieval, we injected the rats with propranolol (10 mg/kg, i.p.) or its vehicle (saline). CPP expression was assessed 1 day (test 3, 15 min session) and 14 days (test 4, 15 min session) later. Next, we gave rats 4 days of extinction training and tested for extinction of CPP (test 5, 15 min session). Twenty-four h later, we determined reinstatement of nicotine CPP after an acute injection of nicotine priming (0.25 mg/kg, s.c., test 6, 15 min session).

In the 2nd run of the CPP experiment, we used 4 other groups of rats to determine whether the effect of propranolol is temporally specific and dependent on UCS retrieval. We performed the baseline preference (test 1), CPP training, and CPP expression (test 2) as described above. Twenty-four h later, we exposed two groups of rats (n=8 per group) to nicotine UCS retrieval in homecage (0.15 mg/kg, s.c.) and injected propranolol (10 mg/kg, i.p.) or vehicle 9 h later. We also injected two other groups of rats (n=8 per group) with saline in homecage and immediately after that injected them propranolol or vehicle. We then determined CPP expression 1 day later (test 3).

In the self-administration experiment, we used two groups of rats (n=9-12 per group) that we trained to self-administer intravenous nicotine (0.03 mg/kg/infusion, infusion
volume 50 µl) during two 1-h daily sessions over 14 days under an FR1 40-s timeout reinforcement schedule; nicotine infusions were paired with a 5-s compound tone-light cue. Twenty-four h later, we exposed the rats to a low dose of nicotine (0.15 mg/kg, s.c.; UCS retrieval) in the homecage and immediately after that injected them with propranolol (10 mg/kg, i.p.) or saline. We then determined cue-induced nicotine seeking under extinction conditions 1 day (extinction test 1) and 30 days (extinction test 2) later. Next, we exposed the rats to extinction training for 5 daily two 1-h sessions. Twenty-four h after last extinction session, we tested the rats for reinstatement of nicotine seeking induced by a priming injection of nicotine (0.3 mg/kg, s.c., test 3, 1-h session). The nicotine priming dose is based on previous studies 5,6,11.

**Exp. 2: Effect of systemic injections of propranolol on reconsolidation of multiple nicotine reward memories in rats (eFigure 1 & 2)**

We first trained all rats in the nicotine SA procedure (14 d), as described in Exp. 1. Next, we determined baseline preference (CPP test 1), and then trained the rats in the CPP procedure (8 d), as described in Exp. 1, and subsequently assessed the expression of learned CPP (CPP test 2). Twenty-four h later, we divided the rats into 4 groups. We injected two groups of rats (n=7-9 per group) with a low dose of nicotine (0.15 mg/kg, s.c.; UCS retrieval) and exposed two other groups of rats to a 15 min-extinction session in the SA chamber (operant-CS retrieval); immediately after UCS retrieval or operant-CS retrieval, we injected the rats with propranolol (10 mg/kg, i.p.) or vehicle (saline). We then determined the CPP expression 1 d (CPP test 3) and 15 days (CPP test 4) after the retrieval manipulations, and cue-induced nicotine seeking under extinction conditions 2 days (extinction test 1) and 16 days (extinction test 2) after these manipulations.
We then used four groups of rats (n=8-10 per group) to examine whether systemic nicotine UCS retrieval plus propranolol affect sucrose seeking (eFigure 6A). We trained the rats to nosepoke for oral sucrose (a potent non-nicotine reward in rodents) solution (10%) over 10 days and nicotine CPP over 8 days. Twenty-four h later, we exposed the rats to a low dose of nicotine (0.15mg/kg, s.c.; nicotine UCS retrieval), and immediately injected them with propranolol (10 mg/kg, i.p.) or vehicle (saline). We then determined subsequent nicotine CPP expression (CPP test 3) and cue-induced sucrose seeking under extinction conditions (extinction test).

**Human study**

The experimental procedure included six sessions in the human study.

1. **baseline measures (day 1):** The participants completed questionnaires assessing their basic demographics, nicotine/alcohol/illicit nicotine use, and medical and psychiatric history, and were then shown a series of choices of GIF images to be used later as CS, consisting of shapes (square, circle and triangle) in different colors (black, white, red, green, and blue). We measured subjective ratings of “liking” and “craving” visual analogue scales (range 5-5 for “liking” rating, and range 0-10 for “craving” rating) after exposure to the future CS and after exposure to established smoking-associated cues, including a lighter, ashtray, and a new pack of cigarettes (termed herein ‘preexisting CS’). The effectiveness of these preexisting CS was established in a preliminary study (data not shown).

2. **Conditioning training (days 2-4):** This phase included two days of CS+ trials and one day of a CS- trial in a counterbalanced order. During the CS+ trials, we instructed the
participants to pay attention to the CS1 or CS2 (one of two CS on each day) on the computer screen, and to smoke two cigarettes of their preferred brand during a 10-min period. During the CS- trial, we instructed the participants to pay attention to the CS-image for 10 min without smoking. We counterbalanced the images used as CS+ and CS- across participants, and we randomized the sequence of exposure to the CS1, CS2, unpaired CS- within each subject. We exposed each subject to one pairing trial with each CS.

3. Conditioning test (day 5): During this test, we measured subjective ratings of ‘liking’ and ‘craving’ on the VAS after exposure to CS1 or CS2 (termed herein newly learned CS) or the unpaired CS-. We presented the CS1, CS2, and CS- in a random order for each subject. We then exposed the participants to the pre-existing nicotine CS 1 h later.

4. UCS memory retrieval (day 6): During the UCS memory reactivation session, the participants smoked two puffs of a cigarette (1/6 of a cigarette), and either 1 h before or 6 h after UCS retrieval we gave them oral propranolol (40 mg) or placebo.

5-6. Post-retrieval and priming tests (days 7-8): We measured subjective ratings of ‘liking’ and ‘craving’ on the VAS after exposure to CS1 or CS2 (the newly learned nicotine CS) or the unpaired CS-and exposure to the pre-existing nicotine CS (day 7), which was identical to conditioning test. We also measured subjective ratings of ‘liking’ and ‘craving’ after the participants smoked two puffs from their preferred cigarette brand (a nicotine priming manipulation) (day 8).
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<td>Group (propranolol, vehicle), Test (Test 1, 2, 3, 4)</td>
<td>Group ($F_{1,18} = 8.24, P = 0.01$)</td>
<td>Test ($F_{3,54} = 16.76, P &lt; 0.01$)</td>
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<td>Test ($F_{2,28} = 16.75, P &lt; 0.01$)</td>
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<td>Group ($F_{1,19} = 29.32, P &lt; 0.01$)</td>
<td>Test ($F_{1,19} = 5.83, P = 0.03$)</td>
<td>Group $\times$ Test ($F_{1,19} = 0.57, P = 0.46$)</td>
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<td>Exp. 2: UCS-retrieval, nicotine CPP test (Fig 1B)</td>
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<td>Group $(F_{1,15}=6.59, P=0.02)$</td>
<td>Test $(F_{3,45}=15.85, P&lt;0.01)$</td>
<td>Group × Test $(F_{3,45}=5.73, P&lt;0.01)$</td>
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<td>Exp. 2: UCS-retrieval, nicotine-seeking test (Fig 1C)</td>
<td>Group (propranolol, vehicle), Test (Extinction Test 1, 2)</td>
<td>Group $(F_{1,13}=0.02, P=0.88)$</td>
<td>Test $(F_{3,39}=12.72, P&lt;0.01)$</td>
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<td>Group $(F_{1,16}=7.79, P=0.01)$</td>
<td>Test $(F_{2,32}=18.47, P&lt;0.01)$</td>
<td>Group × Test $(F_{2,32}=4.50, P=0.02)$</td>
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<td>Exp. 2: nicotine UCS-retrieval, nicotine CPP test (eFig 6B)</td>
<td>Group (propranolol, vehicle), Test (CPP Test 1, 2, 3)</td>
<td>Group $(F_{1,16}=0.49, P=0.49)$</td>
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<td>Exp. 2: nicotine UCS-retrieval, sucrose-seeking test (eFig 6C)</td>
<td>Group (propranolol, vehicle)</td>
<td>Group $(F_{1,198}=3.24, P=0.04)$; Cue type $(F_{2,198}=14.96, P&lt;0.01)$</td>
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<td>Group × Test $(F_{2,198}=7.75, P&lt;0.01)$; Cue type × Test $(F_{2,198}=4.69, P=0.01)$</td>
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**Human study**

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<td>Group $(F_{2,198}=3.24, P=0.04)$; Cue type $(F_{2,198}=14.96, P&lt;0.01)$</td>
<td>Test $(F_{1,198}=2.73, P=0.1)$</td>
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<td>Exp. 3: liking rating for learned cues after priming (Fig 3B)</td>
<td>Group (propranolol, vehicle), Cue type (CS1, CS2, CS-)</td>
<td>Group ( (F_{2,198}=10.19, P&lt;0.01); ) Cue type ( (F_{2,198}=6.75, P&lt;0.01) )</td>
<td>Group × Cue type ( (F_{4,198}=0.21, P=0.93); ) Group × Cue type × Test ( (F_{4,198}=1.47, P=0.21) )</td>
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<td>Exp. 3: liking rating for pre-existing cues in real- life 1 day after treatment (Fig 3C)</td>
<td>Group (propranolol, vehicle), Test (day 5, 7)</td>
<td>Group ( (F_{2,66}=1.95, P=0.15) )</td>
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<td>Exp. 3: craving rating for learned cues 1 day after treatment (Fig 4A)</td>
<td>Group (propranolol, vehicle), Cue type (CS1, CS2, CS-), Test (day 5, 7)</td>
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<td>Test ( (F_{1,198}=5.33, P=0.02) ) Group × Test ( (F_{2,198}=4.17, P=0.02); ) Cue type × Test ( (F_{2,198}=0.16, P=0.85); ) Group × Cue type ( (F_{4,198}=0.81, P=0.52); ) Group × Cue type × Test ( (F_{4,198}=0.75, P=0.56) )</td>
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<td>Exp. 3: craving rating for learned cues after priming (Fig 4A)</td>
<td>Group (propranolol, vehicle), Cue type (CS1, CS2, CS-)</td>
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<td>Exp. 3: craving rating for pre-existing cues in real- life 1 day after treatment (Fig 4B)</td>
<td>Group (propranolol, vehicle), Test (day 5, 7)</td>
<td>Group ( (F_{2,66}=1.02, P=0.37) )</td>
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<td>Exp. 3: craving rating for pre-existing cues in real- life after priming (Fig 4B)</td>
<td>Group (propranolol, vehicle)</td>
<td>Group ( (F_{2,66}=7.91, P&lt;0.01) )</td>
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**eFigure 1.** Experimental Timeline for the Effect of Propranolol on Reconsolidation of Specific Operant-Related Nicotine Reward Memories After Operant-CS Memory Retrieval.

We trained rats in the nicotine self-administration and CPP procedures. Twenty-four h later, we exposed the rats to a short 15-min extinction session in the self-administration training chamber (operant-CS retrieval), and immediately after that injected them with propranolol or vehicle. We then tested them for CPP expression (CPP tests 3 and 4) and operant nicotine seeking (extinction tests 1s and 2).
eFigure 2. Experimental Timeline for the Effect of Propranolol on Reconsolidation of Multiple Nicotine Reward Memories After UCS Memory Retrieval.

We trained rats in the nicotine self-administration and CPP procedures. Twenty-four h later, we exposed the rats to the nicotine UCS retrieval (0.15 mg/kg, in homecage) and immediately after this injected them with propranolol (10 mg/kg, i.p.) or vehicle. We then tested the rats for CPP expression (CPP tests 3 and 4) and operant nicotine seeking (extinction tests 1 and 2).
**eFigure 3.** Experimental Timeline for the Effect of Propranolol Administration Before UCS Memory Retrieval Decreased Subsequent Preference for Nicotine-Associated CS and Cue-Induced Craving in Humans.

The experiment included six phases: (1) baseline test of preference (liking) and craving for pre-existing nicotine CS (e.g., ashtray, cigarette package) and for three different CS to be paired (CS1, CS2, ‘newly learned’ nicotine CS) or not paired (CS-) with smoking (day 1); (2) conditioning training during which CS1 and CS2 were paired with smoking and CS- was not (days 2-4; see extended Methods); (3) post-training test of preference and craving for the new learned CS (day 5); (4) UCS memory retrieval (smoking two puffs of smoking a cigarette) that preceded (1 h) by propranolol (40 mg, p.o) or placebo administration, or UCS memory retrieval that was followed 6 h later by propranolol administration (day 6); (5) post-UCS retrieval tests of preference and craving after exposure to the newly learned and pre-existing CS (day 7); and (6) nicotine priming (smoking two puffs of a cigarette, day 7).
**eFigure 4.** Propranolol Disrupts Reconsolidation of Nicotine Reward Memory After Nicotine CPP-CS and Nicotine UCS Memory Retrieval.

(A) **Experimental timeline:** CPP study. We trained rats in the CPP procedure and tested them for CPP expression (CPP test 2). Twenty-four h later, we exposed the rats to the nicotine-paired side of CPP apparatus (CPP-CS retrieval), or a low dose of nicotine (0.15 mg/kg subcutaneously).
mg/kg, UCS retrieval) or saline in the homecage; we then injected the rats with propranolol (10 mg/kg) immediately after the retrieval manipulations or 9 h later. We determined nicotine CPP expression 1 day (test 3) and 14 days (test 4) later. Next, we gave the rats extinction trainings and verified that nicotine CPP was extinguished (test 5). Twenty-four h later, we tested the rats for reinstatement of CPP after priming injections of nicotine (0.25 mg/kg) (Test 6). (B-E) Systemic injections of propranolol immediately after exposure to CPP-CS (Vehicle, n=10; Propranolol, n=10) or UCS (Vehicle, n=10; Propranolol, n=9) decreased subsequent nicotine CPP (repeated-measures ANOVA, Group × Test interaction, $F_{3,54}=6.33, P=0.001$ for CPP-CS exposure; $F_{3,51}=5.31, P=0.003$ for UCS exposure) and nicotine priming-induced reinstatement of CPP after extinction (repeated-measures ANOVA, Group × Test interaction, $F_{1,18}=13.05, P=0.002$ for CPP-CS exposure; main effect of Group, $F_{1,17}=7.45, P=0.014$ for UCS exposure [Test 3: Cohen’s $d$ estimate, 1.72, 95%CI, 0.63-2.77; Test 4: Cohen’s $d$ estimate, 1.46, 95%CI, 0.42-2.47; Test 6: Cohen’s $d$ estimate, 1.28, 95%CI, 0.27-2.26.]); propranolol had no effect on nicotine CPP when injected 9 h after UCS retrieval (Vehicle, n=8; Propranolol, n=8) or after saline (nicotine vehicle, Vehicle, n=8; Propranolol, n=8) injections (All $P>0.1$). Data are mean±SEM of preference score in sec (time spent in the nicotine-paired chamber minus time spent in the saline-paired chamber) during the CPP tests. * Different from the vehicle group, † Different from test 5 of vehicle group.
eFigure 5. Propranolol Disrupts Reconsolidation of Nicotine Reward Memory After Nicotine Operant-CS and Nicotine UCS Memory Retrieval.

(A) Experimental timeline: SA study. We trained rats to self-administer intravenous nicotine (0.03 mg/kg/infusion). Twenty-four h later, we injected the rats with a low dose of nicotine (0.15 mg/kg) in their homecage (UCS retrieval), and immediately after that with propranolol or vehicle. We then tested the rats for cue-induced nicotine seeking under extinction conditions 1 day (extinction test 1) and 30 days (extinction test 2) later.
Next, we gave the rats extinction training until the operant response was extinguished (last extinction session). Twenty-four h later after last extinction session, we tested the rats for nicotine priming-induced nicotine-seeking (Test 3). (B-C) Propranolol injections immediately after UCS retrieval decreased cue-induced nicotine seeking in extinction tests 1-2 (repeated-measures ANOVA, main effect of Group, $F_{1,19}=29.32, P<0.001$) and nicotine priming-induced reinstatement after extinction (repeated-measures ANOVA, main effect of Group, $F_{1,19}=8.82, P=0.008$). Vehicle, $n=9$; Propranolol, $n=12$. **Extinction test 1:** Cohen’s $d$ estimate, 1.61, 95%CI, 0.59-2.60; **Extinction test 2:** Cohen’s $d$ estimate, 1.69, 95%CI, 0.66-2.69; **Priming test:** Cohen’s $d$ estimate, 1.61, 95%CI, 0.59-2.60. Data are mean ± SEM of number of responses on the active and inactive nosepoke devices during testing. * Different from the vehicle group, # Different from the last extinction session of vehicle group, Repeated-measures ANOVA, $P<0.05$. 

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eFigure 6. Propranolol Injections After Nicotine UCS Retrieval Has No Effect on Sucrose Seeking.

(A) Experimental timeline. We trained rats in the sucrose self-administration and nicotine CPP procedures. Twenty-four h later, we exposed the rats to nicotine UCS retrieval (0.15 mg/kg, in homecage) and immediately after this injected them with propranolol (10 mg/kg) or vehicle. We then tested them for CPP expression (CPP tests 3) and cue-induced operant sucrose seeking (extinction test 1). (B-C) Systemic injections of propranolol immediately after nicotine UCS exposure decreased subsequent expression of nicotine CPP (repeated-measures ANOVA, main effect of Group, $F_{1,16}=7.79, P=0.013$) but had no effect on sucrose seeking (one-way ANOVA, $F_{1,16}=0.49, P=0.494$) in the extinction tests. Vehicle, $n=8$; Propranolol, $n=10$. Data are mean±SEM of number of
responses on the active and inactive nosepoke devices during the test. * Different from the vehicle group, mixed ANOVA, $P<0.05$. 


