Supporting Information

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SI Materials and Methods

Animals. Adult male Long-Evans rats (Harlan) weighing 200 to 250 g served as subjects in all experiments. Animals were maintained on a 12-h/12-h light/dark cycle. All experiments were carried out during the light cycle. All rats were allowed ad libitum access to food and water. All protocols complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Mount Sinai School of Medicine Animal Care Committees.

Morphine Conditioned Place Preference and NTX-Precipitated Withdrawal. The behavioral protocols used were modified from Romieu et al. (1) for mCPP and White et al. (2) for acute NTX-precipitated withdrawal. All experiments took place in a dimly lit room. On day 1, all rats received a single preexposure test in a Plexiglas shuttlebox (Med Associates) composed of two compartments of equal size (20.3 × 15.9 × 21.3 cm) separated by a sliding door. One side consisted of white walls, a grid floor, and a 60 W light that was turned on throughout the experiments. The other side had black walls and a smooth plastic floor and was unlit throughout the experiments. The procedure consisted of three different phases: pretesting (day 1), conditioning (days 2–5), and postconditioning reactivation and testing. During the pretesting phase, each rat was placed in the white compartment with the door open. The animal was allowed to freely explore the apparatus for 10 min. The time spent in each chamber was recorded (unconditioned preference), and then the animals were returned to their home cages. Most animals spent approximately half the time in each chamber. Animals showing a strong unconditioned preference (>540 s) were discarded from the studies (5 of 292 rats were discarded from the entire study). In the subsequent 4 d (days 2–5), place preference conditioning was conducted by using a counterbalanced procedure, such that half the animals in each experimental group were conditioned to the spontaneously preferred side and the other half to the spontaneously nonpreferred side. During conditioning, the animals received an s.c. injection of morphine (10 mg/kg; Sigma) or saline solution (vehicle) and were confined for 30 min to the assigned compartment. On the same day, each animal was also conditioned to vehicle in the opposite chamber. The two daily conditioning sessions were separated by 6 h and the presentation order of morphine and vehicle was alternated between conditioning days. Reactivation of mCPP occurred 1 wk after the last conditioning session and consisted of a single morphine conditioning session. To induce morphine withdrawal, we used the protocol described by White et al. (2). Rats received one morphine conditioning and 4 h later were injected s.c. with the opiate antagonist NTX (0.3 mg/kg; Sigma) and were confined to the original morphine-conditioned (drug-paired) compartment for 30 min. Twenty-four hours later, rats received one vehicle injection and were confined to the vehicle-conditioned (unpaired) compartment for 30 min. The control protocol used for the withdrawal consisted of the same single morphine conditioning and an s.c. injection of the same volume of vehicle solution (saline) 4 h later followed by confinement to the original morphine-conditioned (drug-paired) compartment for 30 min. Testing consisted of placing the animal inside the apparatus and allowing it to freely access both chambers for 10 min. An observer who was blind to treatments and groups recorded the amount of time each animal spent in each chamber. Data were expressed as the difference (in s) between the time spent in the drug-paired compartment on the postconditioning day and the time spent in this compartment in the preconditioning session.

Inhibitor Administration. Systemically, cycloheximide (Sigma) was dissolved in DMSO and diluted to its final concentration in 1% DMSO/saline solution. Cycloheximide (2.2 mg/kg) was injected s.c. first immediately and then 5 h after mCPP reactivation by conditioning (3). Hippocampally, 1 μL of anisomycin (Sigma) was injected bilaterally at 125 μg/μL (3) delivered over 2.5 min. Anisomycin was dissolved as previously described (3). This concentration of anisomycin inhibits approximately 85% of protein synthesis in the hippocampus for at least 6 h (3). Rp-cAMP (Sigma) was dissolved in sterile PBS solution at 36 μg/μL and 1 μL was injected into each hippocampus. This concentration has been successfully used in the amygdala to disrupt the reconsolidation of previously established auditory fear memory (4). All vehicle solutions were prepared accordingly.

Withdrawal Signs Following Disruption of mCPP. Animals underwent mCPP and, 1 wk later, mCPP reactivation using the same protocol described earlier. Immediately afterward, two injections of cycloheximide (2.2 mg/kg) or saline solution were administered s.c., as described earlier. NTX-precipitated withdrawal was induced 24 h later by a single morphine conditioning followed by an s.c. injection of NTX (3.0 mg/kg) 4 h later. The rats were then confined to the morphine-conditioned compartment and videotaped for 15 min within the conditioned compartment. Rats were weighed before NTX injection and then again 60 min later. Physical signs of withdrawal were scored using an adapted protocol of the global rating described by Gellert and Holtzmann (5). This scale consists of graded signs of weight loss, number of "wet dog shakes," instances of abdominal constrictions and checked signs (simply scored as present or absent), including diarrhea, facial fasciculation/teeth chattering, piloerection, ptosis, and penile grooming/erection/ejaculation. According to the Gellert and Holzman scale (5), graded signs, with the exception of weight loss, were given a factor from 1 to 4 based on the frequency of appearance, and checked signs were given a value of 2 to 7, depending on the withdrawal sign but regardless of the frequency of appearance. One point was assigned for each 1% of body weight lost.

Surgical Procedures. Rats were anesthetized and implanted bilaterally with 22-gauge cannulas (Plastics One) positioned 1.5 mm just above the dorsal hippocampi using the following coordinates: anteroposterior, −4.0 mm; mediolateral, ±2.60 mm from bregma; and dorsoventral, −2.0 mm from the skull surface. Rats were allowed 1 wk to recover before undergoing any experimental procedures.

Assay for Nonradioactive Detection of cAMP-Dependent Protein Kinase Activity. Dorsal hippocampi were dissected and frozen on the dry ice. Homogenizations and procedures were performed according to the PepTag kit assay (Promega). Fluorescent-labeled kemptide was used to measure PKA activity. Phosphorylated kemptide was separated from unphosphorylated substrate by agarose gel electrophoresis and visualized with UV light.

Statistical Analyses. One-way or two-way ANOVA followed by Bonferroni post hoc comparisons, as detailed in each experiment. Student *t* test was used for paired comparisons.

1. Romieu P, Phan VL, Martin-Fardon R, Maurice T (2002) Involvement of the sigma(1) receptor in cocaine-induced conditioned place preference: possible dependence on dopamine uptake blockade. *Neuropsychopharmacology* 26:444–455.
2. White DA, Hwang ML, Holtzman SG (2005) Naltrexone-induced conditioned place aversion following a single dose of morphine in the rat. *Pharmacol Biochem Behav* 81: 451–458.
3. Milekic MH, Brown SD, Castellini C, Alberini CM (2006) Persistent disruption of an established morphine conditioned place preference. *J Neurosci* 26:3010–3020.
4. Tronson NC, Wiseman SL, Olausson P, Taylor JR (2006) Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. *Nat Neurosci* 9: 167–169.
5. Gellert VF, Holtzman SG (1978) Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *J Pharmacol Exp Ther* 205:536–546.

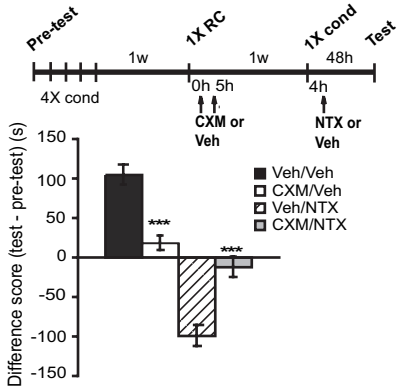

Fig. S1. Disrupting morphine CPP reconsolidation disrupts subsequent NTX-precipitated withdrawal evoked 1 wk later. Experimental timeline is shown above the experiment. The score values are shown in Table S1. Values of preference or avoidance are expressed in seconds as differences (test vs. pretest) and shown as means ± SEM. Cycloheximide significantly disrupts mCPP compared with vehicle (n = 6–7; ***$P < 0.001$). The animals with disrupted CPP also show a significantly disrupted NTX-precipitated withdrawal (n = 7–8; ***$P < 0.001$).

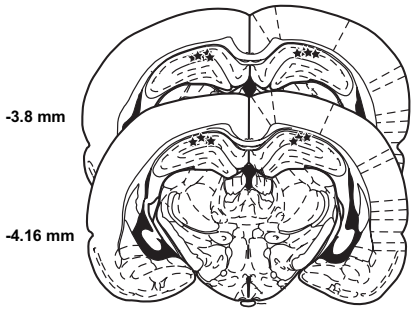

Fig. S2. Hippocampal injections of anisomycin. Representative brain section of dorsal hippocampus cannula placement. Bregma from −3.8 to −4.16 mm, coordinates by Paxinos and Watson (2).

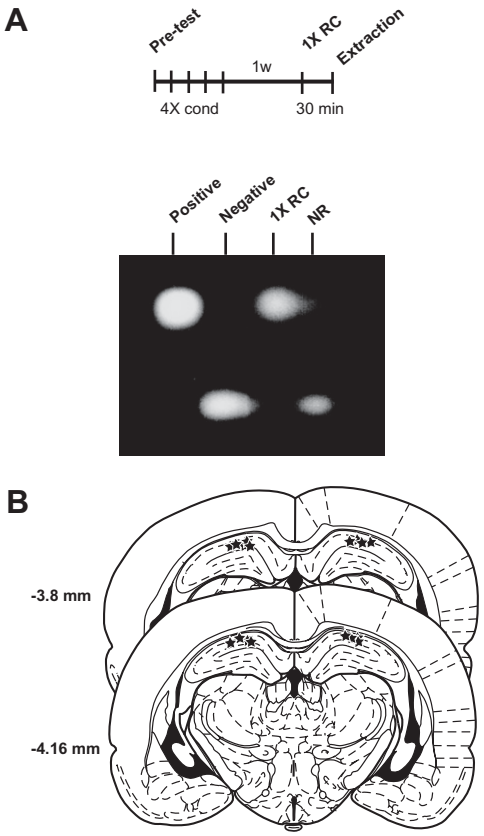

Fig. S3. Hippocampal PKA injection sites and hippocampal PKA activity following mCPP reactivation. (*A*) cAMP-dependent protein kinase assay. Rats were conditioned for 4 d (4× cond) and 1 wk later presented with either 1XRC or remained in the home cage (NR). Thirty minutes later they were decapitated and their dorsal hippocampi processed for PKA activity ($n = 4$ per group). (*B*) Representative brain section of dorsal hippocampus cannula placement. [Bregma −3.8 to −4.16 mm, coordinates by Paxinos and Watson (1).]

1. Paxinos G, Watson C (1998) *The Rat Brain in Stereotactic Coordinates* (Academic, New York).

Table S1. Scores indicating group mean preference or avoidance

Experiment	Groups	Test 1	Test 2	Test 3	Test 4
EXP1 (Fig. 1*A*)	Veh/Veh, *n* = 8	87.4 ± 18.7	107.6 ± 15.4	101.6 ± 8.9	104.5 ± 9.3
	CXM/Veh, *n* = 6	13.2 ± 31.7	20.8 ± 15.3	29.5 ± 20.8	10.3 ± 19.5
	Veh/NTX, *n* = 8	−142.8 ± 24.5	−104.5 ± 14.2	—	—
	CXM/NTX, *n* = 7	−17.1 ± 20.9	−7.6 ± 14.6	—	—
EXP2 (Fig. 1*B*)	Veh/Veh, *n* = 8	112.3 ± 24.5	—	—	—
	CXM/Veh, *n* = 8	122.9 ± 40.9	—	—	—
	Veh/NTX, *n* = 8	−124.0 ± 31.8	—	—	—
	CXM/NTX, *n* = 8	−119.1 ± 29.2	—	—	—
EXP2 (Fig. S4)	Veh/Veh, *n* = 6	104.3 ± 12.7	—	—	—
	CXM/Veh, *n* = 7	17.9 ± 9.1	—	—	—
	Veh/NTX, *n* = 8	−99.2 ± 13.3	—	—	—
	CXM/NTX, *n* = 7	−12.4 ± 13.0	—	—	—
EXP3 (Fig. 2*A*)	Veh/Veh, *n* = 8	55.8 ± 27.8	—	—	—
	CXM/Veh, *n* = 7	−5.6 ± 19.8	—	—	—
	Veh/NTX, *n* = 8	−131.5 ± 22.6	—	—	—
	CXM/NTX, *n* = 8	−113.8 ± 21.5	—	—	—
EXP4 (Fig. 2*B*)	Veh/Veh, *n* = 7	69.4 ± 22.1	—	—	—
	CXM/Veh, *n* = 6	22.0 ± 6.0	—	—	—
	Veh/NTX, *n* = 8	−61.5 ± 19.0	—	—	—
	CXM/NTX, *n* = 6	−107.0 ± 28.8	—	—	—
EXP5 (Fig. 2*C*)	Veh/Veh, *n* = 7	96.6 ± 8.5	—	—	—
	CXM/Veh, *n* = 7	25.9 ± 17.4	—	—	—
	Veh/NTX, *n* = 8	−142.2 ± 15.9	—	—	—
	CXM/NTX, *n* = 7	−182.4 ± 21.5	—	—	—
EXP6 (Fig. 3*A*)	Veh/Veh, *n* = 8	83.0 ± 7.7	73.1 ± 13.3	—	—
	Veh/NTX, *n* = 8	−126.9 ± 18.4	−71.5 ± 19.6	—	—
	ANI/NTX, *n* = 7	−16.3 ± 14.1	−19.8 ± 12.1	—	—
EXP7 (Fig. 3*B*)	Veh/Veh, *n* = 8	115.0 ± 31.5	—	—	—
	Veh/NTX, *n* = 8	−151.9 ± 24.4	—	—	—
	ANI/NTX, *n* = 8	−161.9 ± 24.3	—	—	—
EXP8 (Fig. 4*A*)	Veh/Veh, *n* = 8	116.9 ± 16.5	134.0 ± 28.9	115.5 ± 18.6	103.6 ± 26.5
	Rp-cAMP/Veh, *n* = 8	9.4 ± 14.8	21.3 ± 23.3	29.0 ± 25.6	7.3 ± 19.4
	Veh/NTX, *n* = 8	−174.4 ± 24.9	−151.5 ± 33.4	—	—
	Rp-cAMP/NTX, *n* = 8	−18.3 ± 16.5	−19.6 ± 20.5	—	—
EXP9 (Fig. 4*B*)	Veh/Veh, *n* = 8	125.2 ± 34.0	—	—	—
	Rp-cAMP/Veh, *n* = 8	78.8 ± 52.3	—	—	—
	Veh/NTX, *n* = 8	−124.6 ± 40.7	—	—	—
	Rp-cAMP/NTX, *n* = 8	−80.9 ± 33.3	—	—	—

Preference (positive numbers) or avoidance (negative numbers) ± SEM are shown per experiment described in *Results*. Data are expressed as the difference (in s) between the time spent in the drug-paired compartment on the postconditioning day and the time spent in this compartment in the preconditioning session. ANI, anisomycin; Veh, vehicle.

Table S2. Summary of global rating and individual somatic signs of NTX-precipitated withdrawal following postreactivation disruption of mCPP

Sign	Cycloheximide (n = 6)	Saline (n = 5)
Global rating*	19	17.8
Checked signs†		
Diarrhea	4	5
Teeth chattering/facial fasciculations	4	4
Ptosis	5	4
Piloerection	6	5
Penile grooming/ejaculation	5	3
Graded signs		
Weight loss‡	1.2	1.8
Wet dog shakes§	0.2	0.2
Abdominal constrictions§	4.2	3.2

*Based on weighted scale of Gellert and Holtzman (5). Data are expressed as mean values.
†Data are expressed as fraction of animals exhibiting sign.
‡Data are expressed as mean percent values.
§Data are expressed as mean frequency of sign.