Supporting Information

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SI Materials and Methods

Animals. Male C57BL/6J mice (8 wk old; Jackson Laboratory) had access to food and water ad libitum. All procedures were performed during the light portion of the 12-h light/dark cycle and conducted in accordance with National Institutes of Health guidelines for animal care and use and. The study was approved by the University of California Irvine's Institutional Animal Care and Use Committee.

Drugs. All drugs were administered at a volume of 10.0 mL/kg. RGFP966 (Repligen) was dissolved in DMSO and diluted in a vehicle of 30% (wt/vol) hydroxypropyl-β-cyclodextrin and 100 mM sodium acetate (pH 5.4). The final DMSO concentration was 10% (vol/vol) for drug and vehicle. RGFP966 was administered s.c. Cocaine-HCl (Sigma-Aldrich; 20 mg/kg) was dissolved in saline and administered i.p. Distribution of RGFP966 to the CNS was quantified by LC/MS/MS at 15, 30, 60, and 120 min after administration.

Distribution of RGFP966 in Brain and Plasma. Animals dosed with RGFP966 (10 mg/kg s.c.) were killed at 15, 30, 60, and 120 min after administration (n = 4–5 per time point). Brains were harvested, blotted, and weighed. After tissue homogenization, samples were diluted sixfold (wt:vol) with acetonitrile containing an internal standard, homogenized, and filtered. Concentrations of RGFP966 in filtrates were determined by LC/MS/MS (API-4000 triple quadrupole; ABSciex), equipped with a CTC Pal autosampler (Leap Technologies) and an Agilent 1100 high-performance liquid chromatograph. Samples were analyzed using the MRM quantitation method, in which signals resulting from a specific parent–daughter ion transition, specifically 364.2–236.8 atomic mass unit for RGFP966, were measured after separation of the sample on an HPLC column (50 × 4.6 mm, 5-μm Gemini C18 column; Phenomenex) using a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile from 95:5 to 5:95 vol:vol. Peak areas of RGFP966, normalized to an internal standard, were quantified from the regression equation (quadratic regression with $1/x^2$ weighting) obtained by quantification of standards prepared from stock solution of drug diluted in blank mouse brain (Bioreclamation) and treated as described above for unknown samples. All analyses and calculations were performed using Analyst software (ABSciex). Plasma samples obtained from the same animals were quantified following a similar protocol. The brain:plasma ratio was calculated as the ratio of maximum concentrations in brain and plasma.

Object Recognition. Subthreshold training and a 24-h retention test for location-dependent object recognition memory (OLM) and novel object recognition memory (ORM) were performed as described previously (1–4). Mice received an injection of RGFP966 (3, 10, or 30 mg/kg s.c) or vehicle alone either 1 h before or immediately after a 3-min training seeion.

Cocaine-Conditioned Place Preference: Extinction and Reinstatement. Place conditioning and extinction were performed as described previously (5). Conditioned place preference (CPP) tests (CPP score: difference in time spent in the cocaine compartment versus the saline compartment) were assessed in a drug-free state (15 min), after which mice were conditioned for 4 d with alternating injections of cocaine and saline. Immediately after extinction, mice received RGFP966 (3 or 10 mg/kg s.c) or vehicle. After both treatment groups achieved our a priori extinction criterion (5), all mice underwent a reinstatement test on the same day immediately after a priming injection of cocaine (10 mg/kg i.p.). A stronger CPP protocol with an additional 4 d of conditioning (8 d total conditioning) was used to assess the effectiveness of HDAC3 inhibition on the extinction of cocaine-CPP. The time spent in each chamber was tracked automatically (6).

Extinction of Cocaine-CPP Followed by OLM. Mice were conditioned with cocaine and tested as described above. Each day for 5 consecutive d, at 1 h after pretest and conditioning, mice were habituated to the OLM apparatus. CPP was assessed with the mice in a drug-free state (posttest 1), and 1 h later the mice were trained on OLM for 3 min. The mice received an injection of RGFP966 (10 mg/kg) or vehicle either immediately after posttest 1 or (as a negative control) 8 h after OLM training. The next day, CPP was assessed (posttest 2), and 1 h later an OLM retention test was performed.

Tissue Preparation. At 1 h after the first extinction session, mice were euthanized by cervical dislocation and brains were flash-frozen. Coronal sections and tissue punches from the infralimbic cortex (IFC), nucleus accumbens (NAc), and dorsal hippocampus (HIPP) were collected for immunofluorescence and ChIP analyses as described previously (5, 7–8).

Immunofluorescence. Slides were fixed in 4% paraformaldehyde for 10 min at room temperature, blocked in 8% normal goat serum (Jackson ImmunoResearch Laboratories) with 0.3% Triton X-100 in PBS, and incubated overnight at 4 °C with primary antibody in block solution, followed by a 2-h incubation at room temperature with secondary antibody. Primary antibodies used were acetyl-histone-H4K8 (1:2,000 dilution; Cell Signaling), acetyl-histone-H3K14 (1:1,000; Millipore), and acetyl-histone-H2BK12 (1:1,000; Abcam). The secondary antibody was goat anti-rabbit IgG-FITC (1:1,000; Chemicon). Immunolabeling was quantified by measuring the optical density in the area corresponding to the IFC [anterior–posterior (AP), +1.8 mm to +1.4 mm], dorsal HIPP (area CA1; AP, −1.5 mm to –2.0 mm), or NAc (including core and shell; AP, +1.3 mm to +1.0 mm) from comparable 20× images using ImageJ software.

Quantitative RT-PCR. Quantitative RT-PCR was performed to examine *c-fos* and *Nr4a2* expression as reported previously (3–4, 9). Tissue was collected from 1-mm^2 punches of IFC, NAc (core and shell), and dorsal HIPP (including CA1, CA2, CA3, and DG). Total RNA (75 ng) was reverse-transcribed and analyzed using the Roche proprietary algorithms and REST 2009 software based on the Pfaffl method (10–11). All values were normalized to GAPDH expression levels. Primers were derived from the Roche Universal Probe Library: c-Fos left primer, 5′-ggggcaaagtagagcagcta-3′; c-Fos right primer, 5′-agctccctcctccgattc-3′; probe, atggctgc; Nr4a2 left primer, 5′-ttgcagaatatgaacatcgaca-3′; Nr4a2 right primer, 5′-gttccttgagcccgtgtct-3′; probe, ttctcctg (c-Fos and Nr4a2 probes are conjugated to the dye FAM); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) leftprimer, 5′-atggtgaaggtcggtgtga-3′; right primer, 5′-aatctccactttgccactgc-3′; probe, tggcggtattgg (GAPDH probe is conjugated to Lightcycler Yellow 555).

ChiP. Tissue was collected from mice at 1 h after the post-extinction injection of vehicle or RGFP966 (10 mg/kg). For each sample, bilateral IFC tissue was isolated from two 500-μm sections. ChIP was performed based on the protocol from Millipore

ChIP kit. In brief, tissue was cross-linked using 1% formaldehyde (Sigma-Aldrich), lysed, and sonicated, and chromatin was immunoprecipitated overnight with anti-HDAC3 (Millipore), anti-H4K8Ac (Millipore), or nonimmune rabbit IgG (negative control; Millipore). The immunoprecipitation was collected using magnetic Protein A beads (Millipore). After washing, chromatin was eluted from the beads and reverse cross-linked in the presence of proteinase K. DNA was purified and then quantified by quantitative PCR. *c-fos* promoter enrichment in ChIP samples was measured by quantitative PCR using a Roche Lightcycler 480 and SYBR Green. Primer sequences used to amplify the promoter region were designed by the Primer 3 program (c-Fos: 5′-tacgacccctttcaggcatac-3′ and 5′-gtttttaaaggacggcagcac-3′). A total of 5 μL of input, ChIP, or IgG sample was used in each reaction in duplicate, with eight biological samples for each condition. Percent input was calculated for both the ChIP and IgG samples, and fold enrichment was calculated as a ratio of the ChIP to IgG. An in-plate standard curve was used to determine amplification efficiency (AE) and the 100-fold dilution factor for the input was included. The equation used was $AE^{[\text{input cycle threshold (Ct)-ChIP Ct}]} \times 100/AE^{(\text{input Ct-IgG Ct})} \times 100$. Samples were then normalized to the vehicle condition.

Statistics. Datasets were analyzed using the independent-samples *t* test, repeated-measures ANOVA, two-way ANOVA, or one-way ANOVA. Bonferroni post hoc tests were performed when appropriate. Simple planned comparisons were done using the Student *t* test, with α levels held at 0.05.

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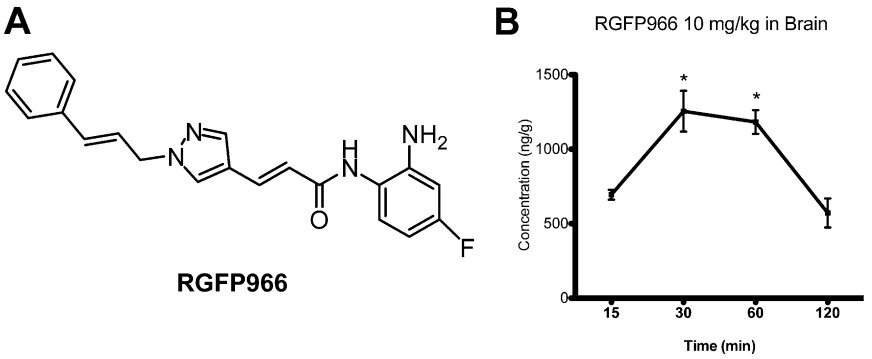

Fig. S1. Structure of RGFP966 and concentration in brain. (*A*) Chemical structure of the HDAC3 inhibitor, RGFP966. International Union of Pure and Applied Chemistry name: (*E*)-N-(2-amino-4-fluorophenyl)-3-(1-cinnamyl-1*H*-pyrazol-4-yl)acrylamide. (*B*) Systemic administration of RGFP966 (10 mg/kg) enters the brain within 15 min. Peak concentration is reached at 30 min, is maintained through 60 min, and begins to decline by 120 min.

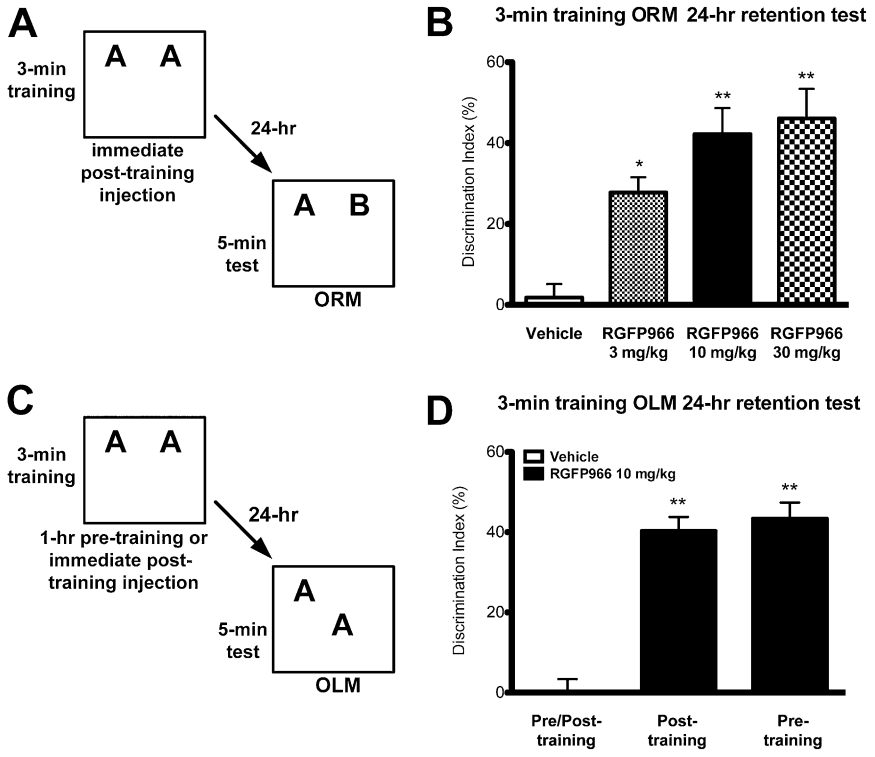

Fig. S2. HDAC3 inhibition enhances ORM. (*A*) Schematic of subthreshold novel ORM training, with immediate posttraining treatment and testing. (*B*) RGFP966 delivered immediately after training enhances ORM. Vehicle, $n = 6$; RGFP966 3 mg/kg, $n = 5$; RGFP966 10 mg/kg, $n = 9$; RGFP966 30 mg/kg, $n = 5$. (*C*) Schematic of subthreshold OLM training, with 1 h pretraining or immediate posttraining treatment, and testing. (*D*) RGFP966 pretraining or posttraining enhances OLM. Vehicle pretraining/posttraining, $n = 7$; RGFP966 10 mg/kg posttraining, $n = 6$; RGFP966 10 mg/kg pretraining, $n = 7$. Data are expressed as mean ± SEM. *Significantly different from vehicle; $P < 0.05$.

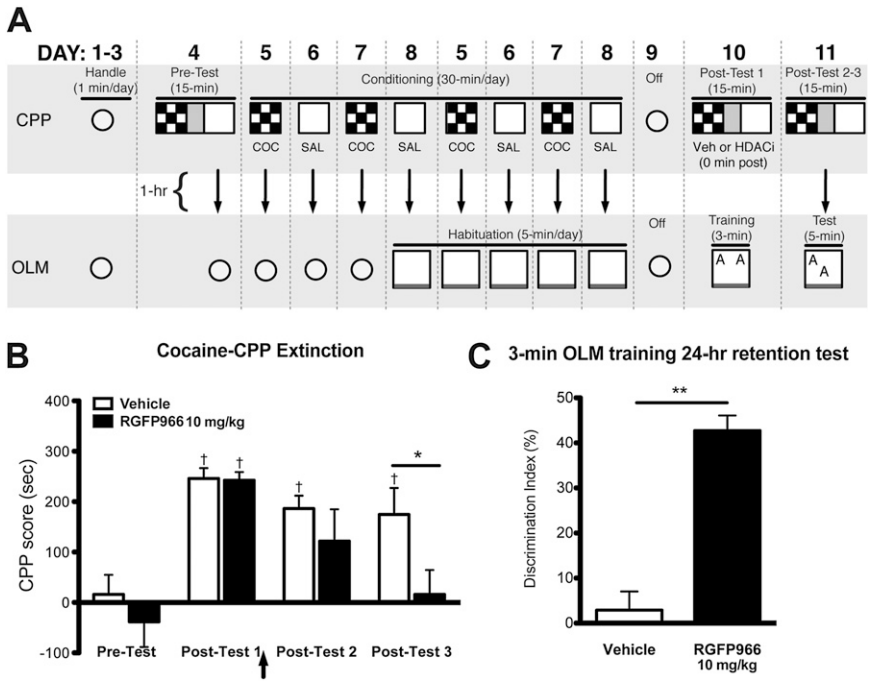

Fig. S3. HDAC3 inhibition facilitates the rate and persistence of extinction after robust cocaine-CPP training. (*A*) Schematic of extended cocaine-CPP training and extinction, followed in parallel by OLM training. RGFP966 is administered immediately after extinction training, 1 h before OLM training in the same mice; vehicle, $n = 6$; RGFP966 10 mg/kg, $n = 6$. (*B*) RGFP966 (10 mg/kg) immediately after a nonreinforced exposure results in loss of CPP by posttest 3. (*C*) RGFP966 treatment results in significantly enhanced object location memory. Data are expressed as mean ± SEM. †Significantly different from pretest; *significantly different from vehicle; $P < 0.05$.