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Synaptic plasticity mediating cocaine relapse requires matrix metalloproteinases

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Relapse to cocaine use necessitates remodeling excitatory synapses in the nucleus accumbens and synaptic reorganization requires matrix metalloproteinase (MMP) degradation of the extracellular matrix proteins. We found enduring increases in MMP-2 activity in rats after withdrawal from self-administered cocaine and transient increases in MMP-9 during cue-induced cocaine relapse. Cue-induced heroin and nicotine relapse increased MMP activity, and increased MMP activity was required for both cocaine relapse and relapse-associated synaptic plasticity.

Vulnerability to relapse is a defining characteristic of drug addiction, and controlling relapse is a primary therapeutic goal in treating addiction[1]. The inability to control drug use is associated with neuropathologies in cortical regulation of the striatal circuitry, including constitutive potentiation of cortical glutamatergic synapses in the nucleus accumbens core (NAcore)[2,3], and further transient synaptic potentiation (t-SP) when relapse is initiated by cocaine injection or cocaine-associated cues[4,5]. Although these studies have shown that synaptic potentiation at glutamatergic synapses in NAcore is required for relapse to cocaine seeking, it is not understood how the long-lasting potentiation after withdrawal is stabilized or how relapse-associated t-SP is initiated.

Synaptic remodeling depends on the extracellular matrix (ECM), which is a proteinacious network ensheathing synapses that is regulated by Zn^{2+}-dependent endopeptidases called MMPs[6]. MMP-2 and MMP-9 make up the gelatinase subfamily[7] that regulates synaptic structure and physiology by proteolytically processing ECM glycoproteins to initiate glutamate receptor trafficking and actin polymerization[6,8]. Using a relapse model of cocaine, heroin and nicotine

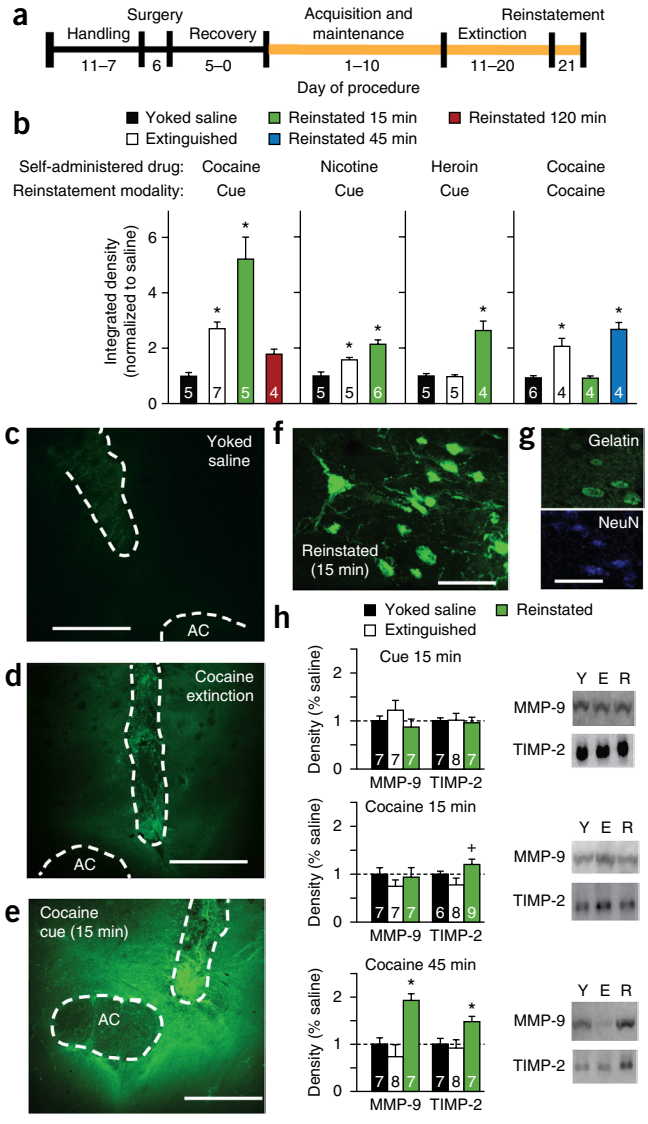

Figure 1 Cocaine extinction and reinstatement elevated MMP activity in the NAcore. (**a**) Outline of the self-administration/reinstatement protocol. (**b**) Gelatinase activity was increased following extinction from cocaine and nicotine self-administration, and was further increased 15 min following cue-induced cocaine ($F_{3,17} = 17.80$, $P < 0.001$), nicotine ($F_{2,13} = 19.70$, $P < 0.001$) or heroin reinstatement ($F_{2,11} = 25.19$, $P < 0.001$), or 45 min after cocaine-induced reinstatement ($F_{3,14} = 23.42$, $P < 0.001$). (**c**–**e**) Examples of FITC-gelatin fluorescence in NAcore of yoked-saline, extinguished and cue-reinstated rats. Dashed line outlines the anterior commissure (AC) and injection site that were masked-out for quantification (data are average of four NAcore slices per rat). Scale bars represent 500 µm. (**f**,**g**) Representative micrographs showing MMP activity over neurons (NeuN, neuronal marker). Scale bars represent 100 µm. (**h**) The level of TIMP-2 was elevated in cocaine-reinstated rats compared with rats after extinction at 15 min ($F_{2,20} = 3.76$, $P < 0.05$), and both MMP-9 and TIMP-2 were elevated compared with extinguished and yoked-saline rats at 45 min (TIMP, $F_{2,19} = 4.306$, $P < 0.05$; MMP-9, $F_{2,19} = 10.35$, $P < 0.001$). Full-length gels are shown in **Supplementary Figure 9**. Data shown as mean ± s.e.m. $^{*}P < 0.05$ compared with yoked-saline using a Newman-Kuels test for multiple comparisons, $^{+}P < 0.05$ compared with extinction.

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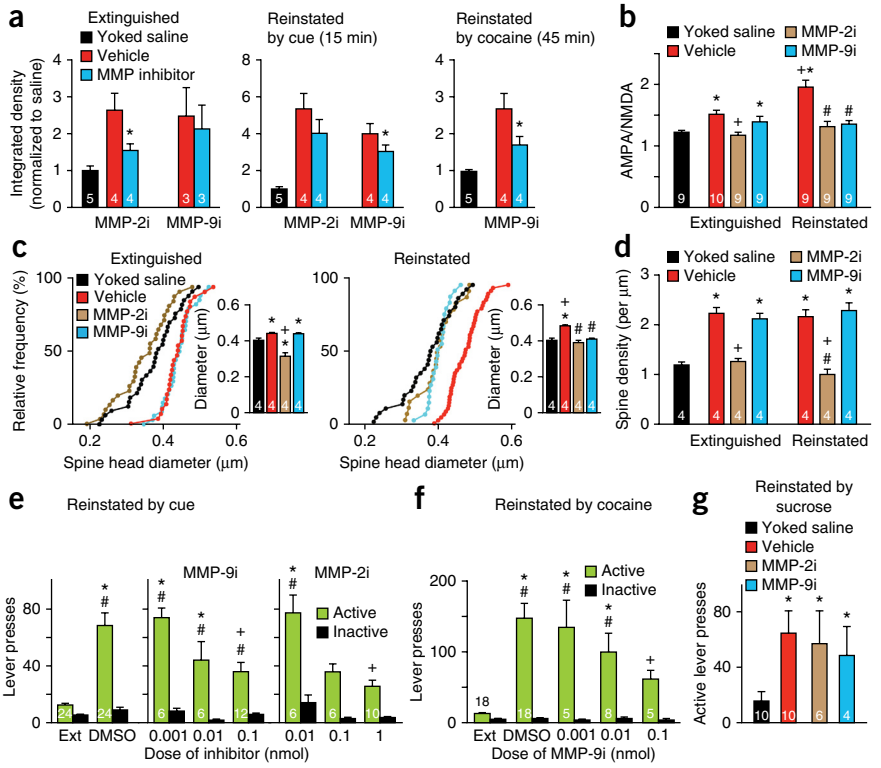

Figure 2 Constitutively induced MMP-2 following extinction and transient increases in MMP-9 by reinstatement mediate t-SP. (**a**) MMP-2i (1 nmol per side) decreased gelatinase activity following extinction compared with vehicle injection into the contralateral NAcore ($t_3 = 3.72$, $P = 0.034$), whereas MMP-9i (0.1 nmol per side) had no effect. Yoked-saline data shown for comparison are from **Figure 1b**. MMP-9i, but not MMP-2i, reduced gelatinase activity 15 min following cue-induced reinstatement ($t_3 = 3.47$, $P = 0.040$). MMP-9 inhibition reduced fluorescence induced 45 min after a cocaine-priming injection ($t_3 = 3.77$, $P = 0.037$). The numbers in the bars represent the number of rats. $^*P < 0.05$ comparing vehicle with inhibitor using a paired Student's *t* test. (**b**) A/N elevated after cocaine extinction was reduced by MMP-2i, whereas transiently increased A/N during reinstatement was reduced by either MMP-2i or MMP-9i. The numbers in the bars represent the number of neurons recorded from >3 rats in each condition ($F_{6,57} = 13.08$, $P < 0.001$). (**c**) The enduring elevation of d_h by cocaine extinction was reduced by MMP-2i, whereas transient increases in d_h during reinstatement were reduced by either MMP-2i or MMP-9i. The numbers in the bars represent the number of rats quantified (6–12 neurons per rat; $F_{8,27} = 11.68$, $P < 0.001$). (**d**) The increase in spine density produced in rats after extinction was blocked by MMP-2i, but not MMP-9i ($F_{8,27} = 13.47$, $P < 0.001$). $^*P < 0.05$ compared with yoked-saline vehicle, using a *post hoc* Newman-Keuls test, $^+P < 0.05$ compared with extinguished vehicle, $^\#P < 0.05$ compared with reinstated vehicle. (**e**) Bilateral microinjection of MMP-2i or MMP-9i into NAcore decreased active lever pressing in response to cocaine-conditioned cues over a 2-h reinstatement session. A randomized crossover design over three sessions was used and three reinstatement sessions yielded equivalent active lever pressing (interaction, $F_{7,172} = 8.02$, $P < 0.001$; **Supplementary Fig. 10**). (**f**) Bilateral microinjection of MMP-9i decreased active lever pressing in response to a cocaine priming injection (interaction, $F_{4,57} = 11.28$, $P < 0.001$). (**g**) Intra-NAcore microinjection of either MMP-2i (1 nmol per side) or MMP-9i (0.1 nmol per side) failed to reduce cue-induced reinstatement of sucrose seeking (Kruskal-Wallis$_{(4,30)} = 10.61$, $P = 0.014$). The numbers in the bars represent the number of rats. Data are presented as mean ± s.e.m. $^*P < 0.05$ compared with extinction, $^+P < 0.05$ compared with vehicle, $^\#P < 0.05$ compared with paired inactive responding.

self-administration and reinstatement in rats, we tested the hypothesis that MMP-2 and MMP-9 are required for both cue-induced reinstatement and associated synaptic plasticity.

MMP-2 and MMP-9 proteolytic activity in the NAcore was quantified using a FITC-quenched gelatin peptide that fluoresces following cleavage by MMP-2 or MMP-9 (ref. 9) in a linear manner over 60 min (**Supplementary Fig. 1**). Rats were trained to self-administer cocaine, heroin or nicotine and lever pressing was extinguished (**Fig. 1a** and **Supplementary Fig. 2**). FITC-gelatin was microinjected into NAcore before or at various times after initiating drug-seeking by restoring drug-associated conditioned cues (tone/light) to reinstate active lever pressing, and rats were killed 15 min later (**Supplementary Fig. 2**). Gelatinase activity was increased in NAcore of cocaine-extinguished compared with yoked-saline control rats, and 15 min of cue-induced reinstatement caused a further increase (**Fig. 1b**–**e**). The increase in MMP activity returned to pre-reinstatement levels by 120 min (**Fig. 1b**). Rats trained to self-administer nicotine showed constitutively increased MMP activity after extinction, and both nicotine- and heroin-trained rats showed increases after 15 min of cued-reinstatement. When cocaine-trained rats were reinstated using a noncontingent cocaine injection, the constitutive increase in MMP activity was eliminated at 15 min after injection, but rebounded by 45 min. The increase in fluorescence was localized to the soma and dendrites of NAcore neurons (**Fig. 1f**,**g**). We found no increases in MMP activity in the dorsal striatum or accumbens shell after 15 min of cue-induced reinstatement (**Supplementary Fig. 3a**,**b**).

Neither the enduring increase in gelatinase activity after extinction nor the increase elicited by 15 min of cue-induced reinstatement were accompanied by a change in NAcore protein content of MMP-2, MMP-9 or the MMP-2/9 inhibitory protein TIMP-2, although TIMP-2 was elevated in reinstated rats compared with rats after extinction (**Fig. 1h** and **Supplementary Fig. 4a**). Furthermore, no difference was detected in *Mmp2* or *Mmp9* mRNA between yoked-saline and reinstated rats (**Supplementary Fig. 4b**), indicating that the increase in MMP-2 and MMP-9 activity likely results from protein activation rather than protein synthesis[6]. In contrast, both MMP-9 and TIMP-2 protein content were elevated 45 min after reinstating cocaine-seeking with an acute injection of cocaine (**Fig. 1h**).

We used pharmacological inhibitors of MMP-2 and MMP-9 to determine which MMP mediated the increased fluorescence[10]. The constitutive increase in fluorescence in rats after extinction was abolished by intra-NAcore microinjection of an MMP-2 inhibitor (MMP-2i), but not MMP-9 inhibitor (MMP-9i; **Fig. 2a**). Conversely, the increase in fluorescence after 15 min of cue-induced reinstatement was reduced by an MMP-9i, but not an MMP-2i (**Fig. 2a**). Increased MMP activity after 45 min after cocaine-induced reinstatement was also prevented by an MMP-9i (**Fig. 2a**). This pattern of gelatinase expression is consistent with work showing that the brain

constitutively expresses MMP-2 activity, whereas MMP-9 is transiently induced by external stimuli[11].

Withdrawal from cocaine self-administration is associated with constitutive synaptic potentiation in NAcore excitatory synapses[12–14], and after 15 min of reinstated lever pressing, NAcore synapses undergo t-SP[4,5]. We assessed synaptic potentiation morphologically, as spine density and head diameter (d_h), and electrophysiologically, as the ratio of AMPA to NMDA currents (A/N). Whole-cell patch-clamp measurement of A/N in medium spiny neurons (MSNs) revealed that, following vehicle microinjection into the NAcore, A/N in cocaine-extinguished rats was elevated compared with yoked-saline rats and was further elevated 15 min after initiating cue-induced reinstatement (**Fig. 2b**). The increase in A/N in rats after extinction was restored to yoked-saline levels by MMP-2i, but not MMP-9i. However, either inhibitor prevented the elevated A/N initiated by 15 min of reinstatement (**Supplementary Fig. 5**). Diolistic labeling of MSNs with the lipophilic carbocyanine dye DiI (**Supplementary Fig. 6a–c**) revealed that, following vehicle injection into the NAcore, d_h was increased after extinction from cocaine self-administration compared with yoked-saline and was further increased 15 min after cue-induced reinstatement of cocaine seeking (**Fig. 2c**). The constitutive increase in d_h in rats after extinction depended on MMP-2 activity, and the increase after 15 min of reinstatement depended on both MMP-2 and MMP-9 activity. Spine density was elevated in cocaine-extinguished compared with yoked-saline rats, but no further elevation was produced by cue-induced reinstatement (**Fig. 2d** and **Supplementary Fig. 6d**). Spine density in both extinguished and reinstated rats was normalized to yoked-saline levels by MMP-2i, but not MMP-9i. Combined with the measure of d_h, these data indicate that reinstatement is associated with transiently increasing the size (d_h) of existing spines rather than creating new spines and that MMP-2 activity supports the increase in spine number in rats after extinction that are enlarged by MMP-9 activity during cued reinstatement. Neither MMP inhibitor affected A/N, d_h or spine density in yoked-saline rats (**Supplementary Fig. 7**).

Given that reinstated behavior requires synaptic potentiation in accumbens MSNs[5], and the dependence of synaptic potentiation on MMP-2 and MMP-9 activity, we hypothesized that MMP-2i and MMP-9i would reduce cue-induced reinstatement. Microinjection of either inhibitor into the NAcore dose dependently reduced cue-induced reinstatement compared with control (**Fig. 2e** and **Supplementary Fig. 8**). MMP-9i also reduced cocaine-induced reinstatement (**Fig. 2f**). Consistent with the lack of synaptic potentiation in the NAcore during cue-induced sucrose reinstatement[5], neither inhibitor reduced cue-induced reinstatement of lever pressing for sucrose pellets (**Fig. 2g**).

A role for MMPs in addiction is indicated by *MMP9* gene expression being altered in the brain of cocaine addicts[15] and the serum of heroin addicts[16], and an *MMP9* gene polymorphism being associated with alcohol dependence[17]. In addition, intra-cerebroventricular injection of nonselective MMP inhibitors reduces drug seeking in animal models[18,19]. Our data show a specific and necessary role for MMP-2 and MMP-9 in the enduring vulnerability to relapse and suggest the study of ECM signaling as a research theme for understanding and treating addiction.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

A.C.W.S. conducted the behavioral and zymography studies, analyzed data and wrote the manuscript. Y.M.K. conducted the electrophysiological experiments. M.D.S. conducted the western blotting and contributed to the spine analysis. C.D.G. contributed to spine analysis. A.W. developed the *in vivo* zymography assay to generate preliminary data for this study. C.A.T. performed surgeries and conducted behavioral experiments. P.W.K. oversaw the project, analyzed data and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animal housing and surgery. Male Sprague-Dawley Rats (250g; Charles River) were individually housed with a 12:12-h dark/light cycle. All experimentation occurred during the dark phase, and animals were allowed to acclimate to the vivarium environment for 4 d before surgery. Rats were ~65 d old when they were anesthetized with a combination of ketamine HCl and xylazine, and received ketorolac for analgesia. All rats received intrajugular catheters, and rats for microinjection experiments received intracranial cannula targeted 2 mm above the NAcore, dorsolateral striatum or NA shell[20]. Rats were food restricted to 25 g of rat chow per d. All methods used complied with the US National Institutes of Health Guide for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina.

Drugs used. Cocaine HCl was supplied by the National Institute of Drug Abuse. Inhibitors used included the MMP-2 inhibitor Oleoyl-N-Hydroxylamide (OA-Hy) (EMD4BioScience; MMP-2, K_i = 1.07 μM; MMP-9, K_i > 50 μM,) and MMP-9 inhibitor $C_{27}H_{33}N_3O_5S$ (EMD4Bioscience; MMP-9, K_i = 5 nM; MMP-1, K_i = 1.05 mM; MMP-13, K_i = 113 nM[21]). The MMP inhibitors were dissolved in a maximum concentration of 2% DMSO (vol/vol).

Drug self-administration and reinstatement. After 5 d of recovery from surgery, rats underwent one overnight (12 h) food training session. The next day, animals began daily 2-h self-administration (SA) sessions for either cocaine or nicotine, or 3-h SA session for heroin[5,22,23]. During SA, drug was delivered using an FR1 schedule with a 20-s timeout following each infusion. Active lever presses that resulted in cocaine (cocaine hydrochloride, 0.2 mg per infusion; donated by the National Institute on Drug Abuse), heroin (heroin-hydrochloride, 100 μg per infusion for days 1 and 2, 50 μg per infusion for days 3 and 4, 25 μg per infusion for days 5–10; donated by National Institute on Drug Abuse) or nicotine (0.02 mg per kg of body weight per infusion) infusion simultaneously resulted in presentation of a compound light (above the active lever) and tone (2,900 Hz) conditioning stimulus. An inactive lever was also provided to control for non-motivated responding. Following 10 SA sessions at ≥10 infusions per day, rats began extinction training, during which all programmed consequences were removed from lever pressing. Extinction training lasted at least 10 d, or until two consecutive days with ≤25 active lever presses. Reinstatement was induced by presentation of light/tone cues following an active lever press. Microinjections of an MMP inhibitor or vehicle were given 15 min before beginning reinstatement in most cases, or 15 min before gel infusion for zymography experiments. For behavioral experiments (**Fig. 2e**,**f**), a within-subjects crossover design was used. In this procedure, each rat received each condition (MMP-2i, MMP-9i or vehicle) according to a Latin square design. Rats were required to meet extinction criteria before each reinstatement test. Reinstatement sessions lasted 120 min in the behavioral experiment, and for zymography, spine morphology, and A:N experiments reinstatement sessions were 15, 45, or 120 min long, at which point rats were killed for further measurements. When rats were assigned to different drug versus yoked-saline groups, they were randomly assigned. When rats were assigned to extinguished or reinstated groups they were assigned in order to maintain equal variance and mean number of drug infusions during the last 3 d of self-administration.

***In vivo* zymography.** Because MMPs are secreted in inactive pro-forms and catalytically activated in the ECM, activity assays are preferable to immunoblotting for protein content for assessing changes in MMP function[24]. We used an *in vivo* zymography assay to directly measure MMP activity. Dye-quenched gelatin is an MMP-2/9 substrate containing intra-molecularly quenched FITC fluorophores that cannot fluoresce until proteolytically processed by MMP-2 or MMP-9 (ref. 9). The amount of fluorescence produced forms a linear relationship with incubation time and MMP activity (**Supplementary Fig. 1**). Dye-quenched FITC-Gelatin (Molecular Probes) was reconstituted in phosphate-buffered saline (PBS) at 1 mg ml^{-1} pH 7.2–7.4. 3.0 μl of gel (1.5 μl per side) was microinjected 15 min prior to administering an overdose of pentobarbital (100 mg per kg, intraperitoneal) and beginning transcardial perfusion of 4% paraformaldehyde (PFA, vol/vol). Brains were removed, placed in 4% PFA for 90 min for additional fixation, a vibratome was used to obtain 50-μm sections through the NAc. Sections were mounted and coverslipped. Fluorescence was excited with a 488-nm argon laser, emissions were filtered to 515–535 nm, and images were obtained through a 10× objective with a 0.3 numerical aperture (Leica confocal microscope). Only slices in which the injection site and anterior commissure could be visualized in the same frame were imaged. ImageJ (US National Institutes of Health) was used to quantify images. All quantified images contained the anterior commissure, which was masked to prevent being quantified, but provided a landmark for the NAcore. MMP activity is induced as part of the acute inflammatory response to tissue damage from the microinjector, and thus the microinjector tract was readily visible in all quantified sections due to equivalent high fluorescence in all treatment groups (**Fig. 1**). This tract was also masked to eliminate quantifying any MMP activity caused by microinjection-induced acute damage. Fluorescence was quantified bilaterally as integrated density from four sections per rat, and the integrated densities were averaged within each rat and normalized to yoked-saline control values. Quantification of density was conducted by an individual blinded to the treatment group.

Western blotting. Rats were rapidly decapitated after extinction of cocaine self-administration or yoked-saline, or following 15 or 45 min after cued or cocaine-primed reinstatement. The NAcore was dissected and homogenized in RIPA lysis buffer containing 1.0% SDS (vol/vol) and protease/phosphatase inhibitors. Homogenate was centrifuged at 4 °C for 5 min at 10,000 *g*. Supernatant was collected and protein concentration was determined via a biconchinic acid assay (Thermo Scientific). 30 μg of protein was added to each lane of 10% Bis-Tris gels (Bio-Rad) and transferred to nitrocellulose membranes via the Invitrogen iBlot transfer system. Primary antibodies were used for MMP-2 (1:1,500, Abcam ab79781), MMP-9 (1:500, Millipore AB6001) and TIMP2 (1:1,000, Abcam ab53730) and HRP-conjugated goat secondary antibody to rabbit was used at 1:10,000. GAPDH was used as a loading control for MMP-2 and MMP-9, and Calnexin was used for TIMP-2. A Kodak Image Station was used to visualize and quantify protein expression. Each western blot was repeated twice.

Semi-quantitative RT-PCR. NAcore brain tissue was dissected from cocaine or saline animals killed on the final day of extinction training. Total RNA was extracted from NAcore tissue using the Qiagen RNeasy mini kit with Qiashredder homogenization (Qiagen). Reverse transcription was performed at 37 °C for 1 h using 1μg of total RNA for each sample in 20-μl reactions using the High Capacity RNA-to-cDNA kit (Applied Biosystems). PCR reactions were assembled in taq PCR master mix (Qiagen) with 35 pmol of each primer set detailed below and 6 μl of cDNA as template yielding a final reaction volume of 20 μl. PCR reactions were run in a MyCycler thermal cycler (Bio-Rad) with a protocol consisting of a single 94 °C step for 3 min followed by 35 repetitions of 94 °C for 30 s, 51 °C for 30 s and 72 °C for 1 min cycle, ending with a final 5-min extension at 72 °C. 35 cycles was selected following optimization experiments performed before mRNA measurement indicating that 35 cycles resulted in levels of product formation that remained in the linear range for each set of primers and thus was suitable for semiquantitative RT-PCR (data not shown). The following primers were used[25]: MMP2 forward 5′-GATCTGCAAGCAAGACATTGTC TT-3′, MMP2 reverse 5′-GCCAAATAAACCGATCCTTGAA-3′, MMP9 forward 5′-GTAACCCTGGTCACCGGACTT-3′, MMP9 reverse 5′-ATACG TTCCCGGCTGATCAG-3′, TIMP-2 forward 5′-AGGGAAGGCGGAAGGAG AT-3′, TIMP-2 reverse 5′-CCAGGGCACAATAAAGTCACAGA-3′, TIMP-3 forward 5′- AGCATCAGCAATGCCACAGA-3′, and Cyclophilin forward 5′-GGGGAGAAAGGATTTGGCTA-3′, Cyclophilin reverse 5′-ACATG CTTGCCATCCAGCC-3′ (ref. 26). PCR reactions were separated by 1.5% agarose gel electrophoresis and densitometry values were determined using Fiji (imageJ Version 1.47) software. During each PCR run no template negative controls were run and contained no products, in addition minus RT samples were also run and show no product formation (data not shown). Relative amounts of MMP2, MMP9, TIMP-2 and TIMP3 mRNA were calculated as a ratio of the density value of amplicon for MMP2, MMP9, TIMP-2 or TIMP3 that of the corresponding cyclophilin control amplicon.

Quantification of dendritic spine head morphology. Rats were deeply anesthetized with ketamine HCl (87.5 mg per kg, intraperitoneal) and xylazine (5 mg per kg, intraperitoneal). Transcardial perfusions with PBS followed by 1.5% PFA in PBS. Brains were removed and post-fixed in the same fixative for 30 min, then coronally sectioned at 200 μm in PBS on a vibratome. Tungsten particles

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(1.3-µm diameter, Bio-Rad) were coated with DiI (Invitrogen). DiI-coated particles were delivered diolistically into the tissue at 80 PSI using a Helios Gene Gun system (Bio-Rad) fitted with a polycarbonate filter with a 3.0-µm pore size (BD Biosciences). DiI was allowed to diffuse along neuron axons and dendrites in PBS for 24 h at 4 °C, and then fixed again in a 4% PFA for 1 h at 25 °C. After a brief PBS wash, tissue was mounted onto slides in aqueous medium Prolong Gold (Invitrogen).

Spine morphology was performed as described previously[27]. Briefly, images of DiI-labeled sections were taken on a confocal microscope (Zeiss) using a helium/neon 543-nm laser line. Optimal sampling frequency was calculated using the Nyquist-Shannon sampling theorem. Images of dendrites were taken through a 63× oil immersion objective (Plan-Apochromat, Zeiss; NA = 1.4, WD = 90 µm) with pixel size of 0.07 µm in the XY plane and 0.10-µm intervals along the Z axis at 0.1-µm intervals. Images were deconvoluted via Autoquant before analysis (Media Cybernetics), and a three-dimensional perspective was rendered by the Surpass module of Imaris software package (Bitplane). The smallest quantifiable diameter spine head was 0.143 µm. Only spines on dendrites beginning >75 µm and ending <200 µm distal to the soma and after the first branch point were quantified on cells localized to the NAcore. The length of quantified segments was 45–55 µm. One segment from each neuron was quantified, and the minimum spine head diameter was set at 0.15 µm. Between 6 and 12 neurons were imaged in each animal (the number of neurons per treatment group: yoked saline/vehicle, 32 neurons; yoked saline/MMP-2i, 34 neurons; yoked saline/MMP-9i, 29 neurons; extinguished/vehicle, 29 neurons; extinguished/MMP-21, 33 neurons; extinguished/MMP-9i, 33 neurons; reinstated/vehicle, 48 neurons; reinstated/MMP-2i, 25 neurons; reinstated/MMP-9i, 25 neurons). Morphological measurements were conducted by an individual unaware of the treatment groups.

Slice preparation for electrophysiology. Rats were anesthetized with ketamine HCl (100 mg per kg Ketaset, Fort Dodge Animal Health) and decapitated. The brain was removed from the skull and 220-µm-thick coronal NAc sections were obtained using a vibratome (VT1200S Leica vibratome, Leica Microsystems). Slices were immediately placed into a vial containing artificial cerebrospinal fluid (126 mM NaCl, 1.4 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 2.5 mM KCl, 2.0 mM sodium pyruvate, 0.4 mM ascorbic acid, bubbled with 95% O_2 and 5% CO_2) and a mixture of 5 mM kynurenic acid and 50 µM D-(–)-2-amino-5-phosphonopentanoic acid (D-AP5). Slices were incubated at 25 °C until recording.

***In vitro* whole-cell recording.** All recordings were collected at 32 °C (controlled by TC-344B, Warner Instrument) in the dorsomedial NAcore. Inhibitory synaptic transmission was blocked with picrotoxin (50 µM). Multiclamp 700B (Axon Instruments) was used to record excitatory postsynaptic currents (EPSCs) in whole cell patch-clamp configuration. Glass microelectrodes (1–2 MΩ) were filled with cesium-based internal solution (124 mM cesium methanesulfonate, 10 mM HEPES potassium, 1 mM EGTA, 1 mM MgCl2, 10 mM NaCl, 2.0 mM MgATP, 0.3 mM NaGTP, 1 mM QX-314, pH 7.2–7.3, 275 mOsm). Data were acquired at 10 kHz, and filtered at 2 kHz using AxoGraph X software (AxoGraph Scientific). To evoke EPSCs, a bipolar stimulating electrode (FHC) was placed ~300 µm dorsomedial to the recorded cell to maximize chances of stimulating prelimbic afferents. The stimulation intensity was set to evoke an EPSC of 200–500 pA that was usually 30–70% of maximal EPSC. Recordings were collected every 20 s. Series resistance (Rs) measured with a 2-mV hyperpolarizing step (10 ms) given with each stimulus and holding current were always monitored online. Recordings with unstable Rs, or when Rs exceeded 10 MΩ were aborted.

Measuring the AMPA/NMDA ratio. Recordings started no earlier than 10 min after the cell membrane was ruptured to allow diffusion of the internal solution into the cell. AMPA currents were first measured at −80 mV to ensure stability of response. The membrane potential was then gradually increased to +40 mV. Recording of currents resumed 5 min after reaching +40 mV to allow stabilization of cell parameters. Currents composed of both AMPA and NMDA currents were then obtained. Then D-AP5 was bath-applied (50 µM) to block NMDA currents and recording of AMPA currents at +40 mV was started after 2 min. NMDA currents were obtained by subtracting the AMPA currents from the total current at +40 mV. Electrophysiological measurements were conducted by an individual unaware of the treatment groups.

Statistical analysis. Sample size for each experiment was determined by power analysis by G*Power and from analysis of power in similar experiments previously published from our laboratory. All statistics were done using GraphPad Prism Version 6. Zymography measurements were analyzed using either a one-way ANOVA followed by a Newman-Kuels *post hoc* test (**Fig. 1**) or paired Student's *t* test (**Fig. 2**) when comparisons were made between different treatments in each brain hemisphere. Protein (**Fig. 1h** and **Supplementary Fig. 4a**) and mRNA (**Supplementary Fig. 4b**) measurements were made using a one-way ANOVA followed by a Newman-Kuels *post hoc* test or an unpaired Student's *t* test, respectively. Electrophysiological data (**Fig. 2b** and **Supplementary Figs. 5** and **7**) and dendritic spine data (**Fig. 2c**,**d** and **Supplementary Figs. 6** and **7**) were analyzed using a one-way ANOVA with a Newman-Kuels *post hoc* test. Reinstatement of cocaine seeking behavior in **Figure 2e**,**f** was analyzed via a two-way ANOVA using lever and dose of inhibitor as factors and a Newman-Kuels *post hoc*. In contrast, sucrose reinstatement behavior in **Figure 2g** that was compared using a Kruskal-Wallis nonparametric test because the data were not normally distributed according to a D'Agostino-Pearson omnibus normality test.

A **Supplementary Methods Checklist** is available.

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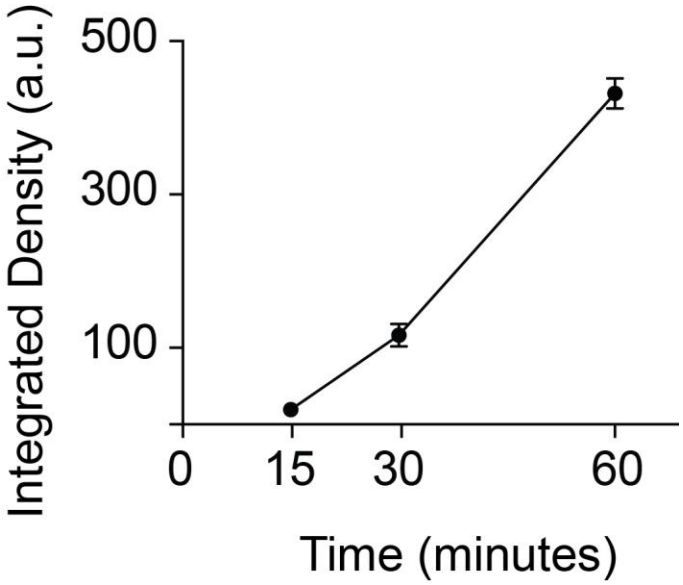
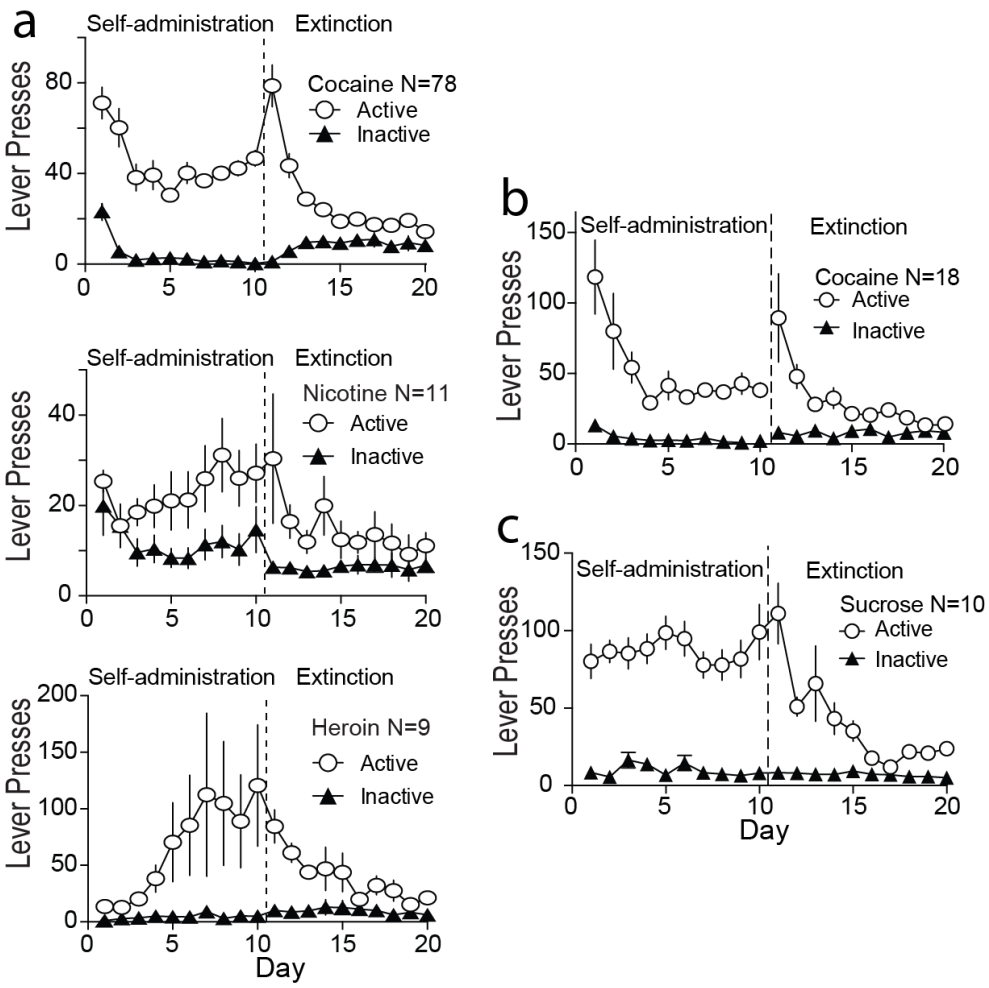


Supplementary Figure 1

Control FITC-gelatin substrate experiments show linearity of fluorescence over time after injection.

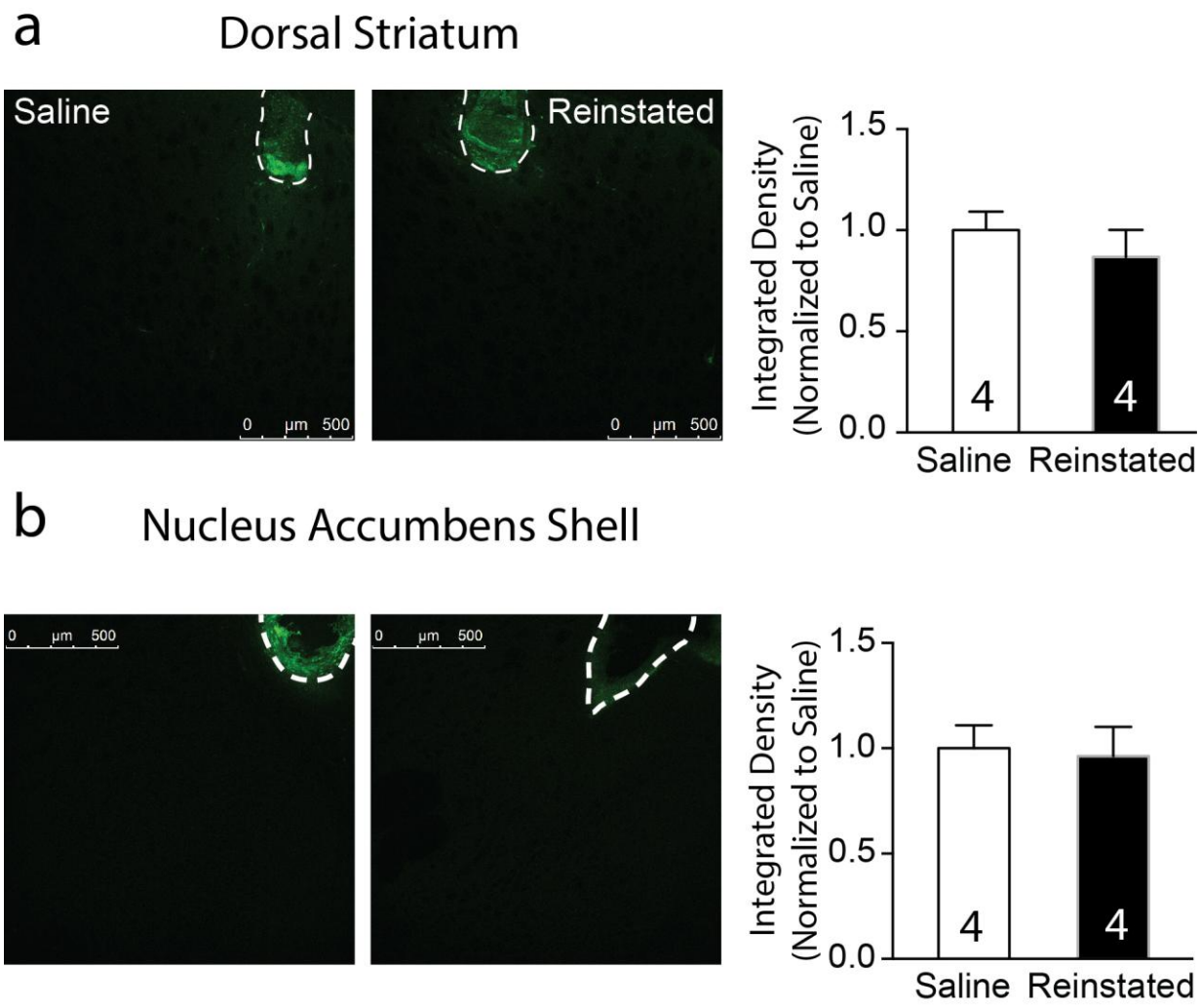
The catabolism of the FITC-gelatin substrate shows linear increases in gelatinolytic fluorescence over 15 - 60 minutes incubation *in vivo*. Injections were made into the dorsal hippocampus due to relatively higher constitutive MMP activity compared with the NAcore or striatum. N= 3 at each time point.



Supplementary Figure 2

Lever pressing during self-administration and extinction of cocaine, nicotine and heroin.

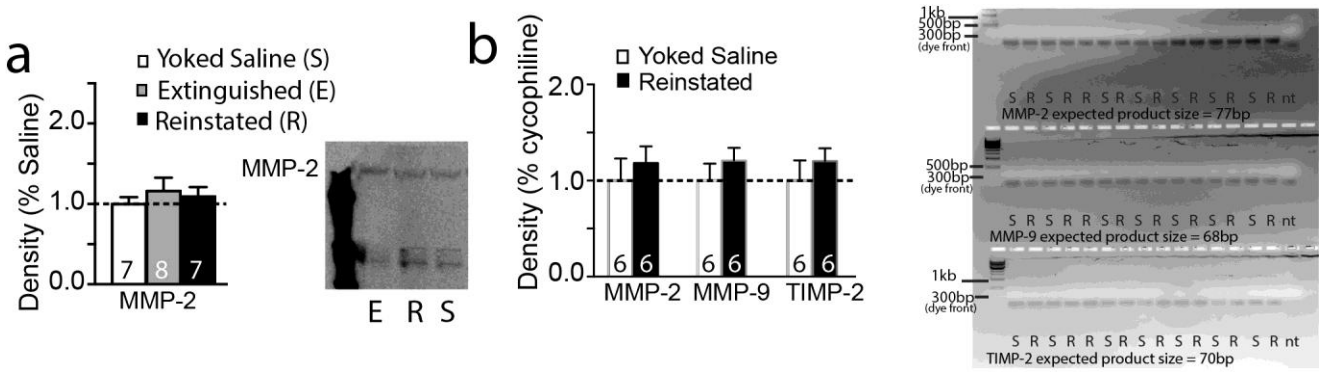
a) Active and inactive lever pressing data for animals that were used for in vitro measurements of zymography, Western blotting, electrophysiology or dendrite morphology in figures 1 and 2. b) Training data for rats used in cocaine studies in figure 2e,f. c) Training data for rats used in sucrose studies in figure 2g.



Supplementary Figure 3

Lack of increased MMP-2 and MMP-9 activity in the dorsal striatum or accumbens shell following cue-induced reinstatement of cocaine seeking.

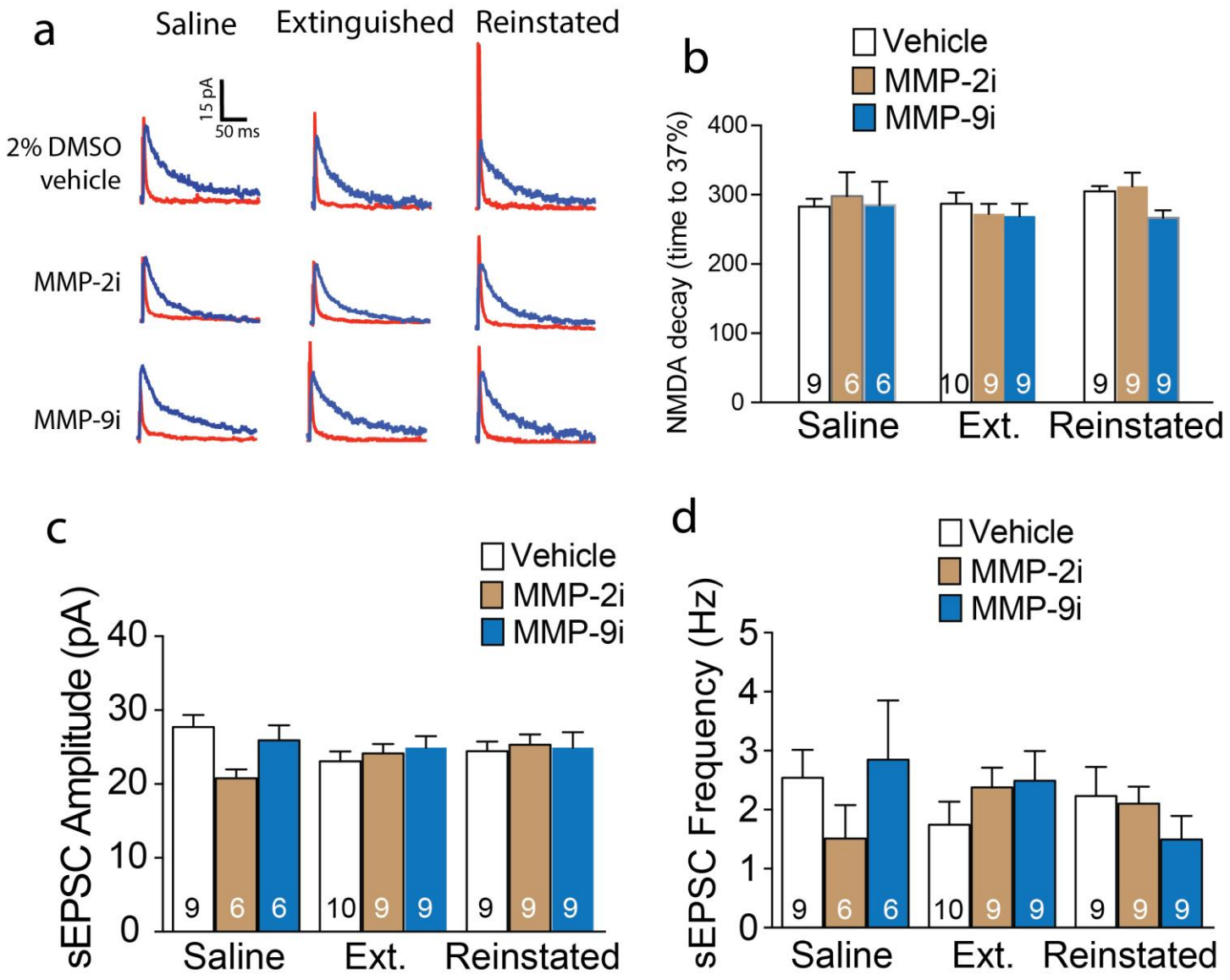
There was no change in gelatinolytic fluorescence in either the dorsal striatum or the nucleus accumbens shell between yoked-saline controls and animals that underwent 15 minutes of cue-induced reinstatement. This indicates anatomical specificity for MMP-dependent plasticity within within the striatum is largely confined to the NAcore. Dashed lines on the micrographs encompass the injection site that was masked-out for quantification. Unpaired Student's t-test revealed no significant effect of reinstatement on fluorescence, dorsal striatum $t_{(4)} = 1.411$, $p > 0.05$, NAshell $t_{(4)} = 0.2112$, $p > 0.05$.



Supplementary Figure 4

There were no changes in MMP-2 protein or mRNA of each MMP-2, MMP-9 or TIMP-2.

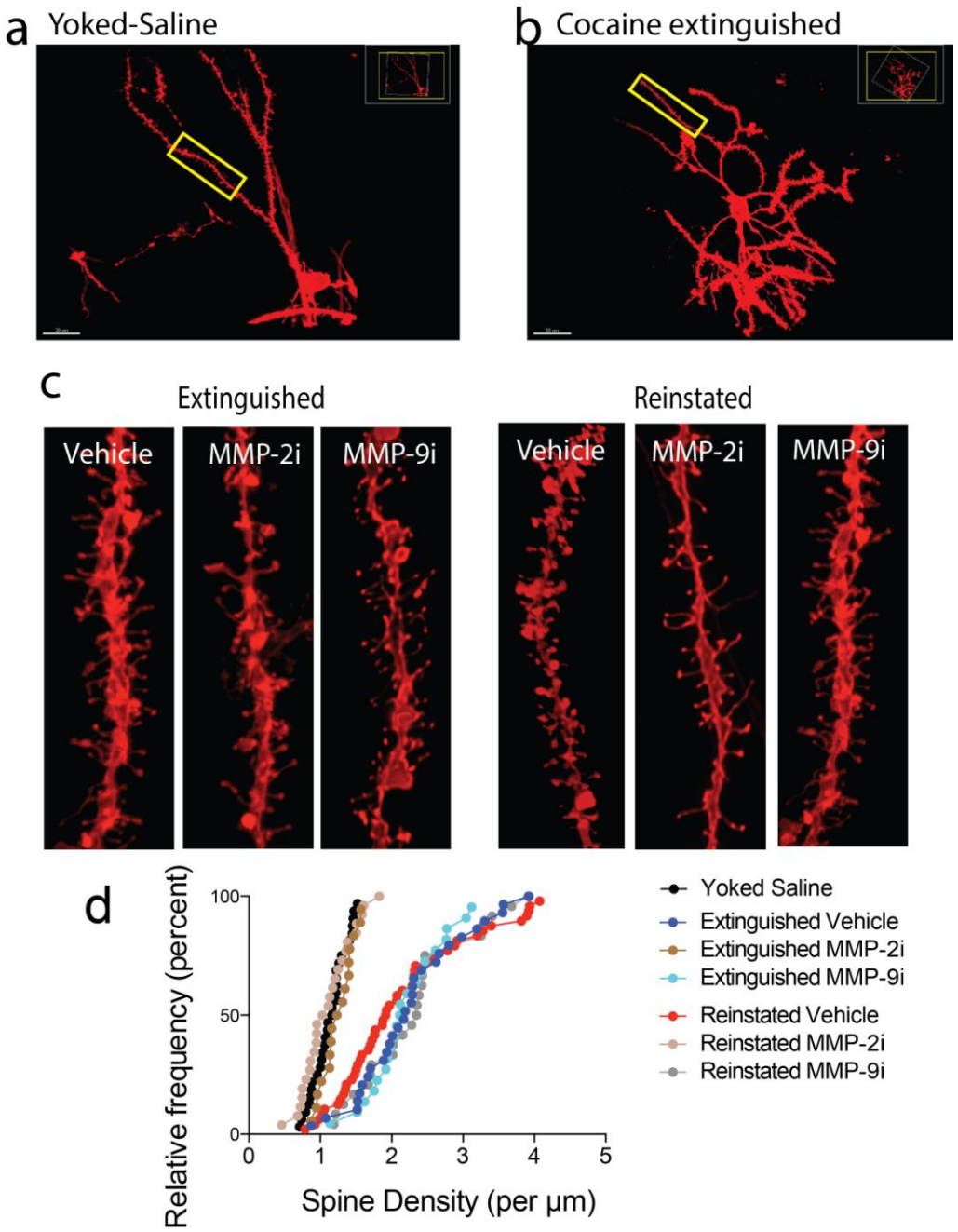
a) MMP-2 protein was quantified in NAcore tissue obtained from yoked saline, cocaine extinguished and after 15 min of cued reinstatement in cocaine-trained rats. There was no difference between treatment groups using a one-way ANOVA. b) mRNA content quantified by PCR. Paired Student's t-tests did not reveal any significant differences between groups. MMP-2 $t_{(10)} = 0.6321$, $p > .05$, MMP-9 $t_{(10)} = 0.934$, $p > .05$, TIMP-2 $t_{(10)} = 0.814$m $p > .05$. S = Yoked Saline, Reinst = Reinstated



Supplementary Figure 5

MMP inhibition did not have an effect on NMDA decay time or sEPSCs.

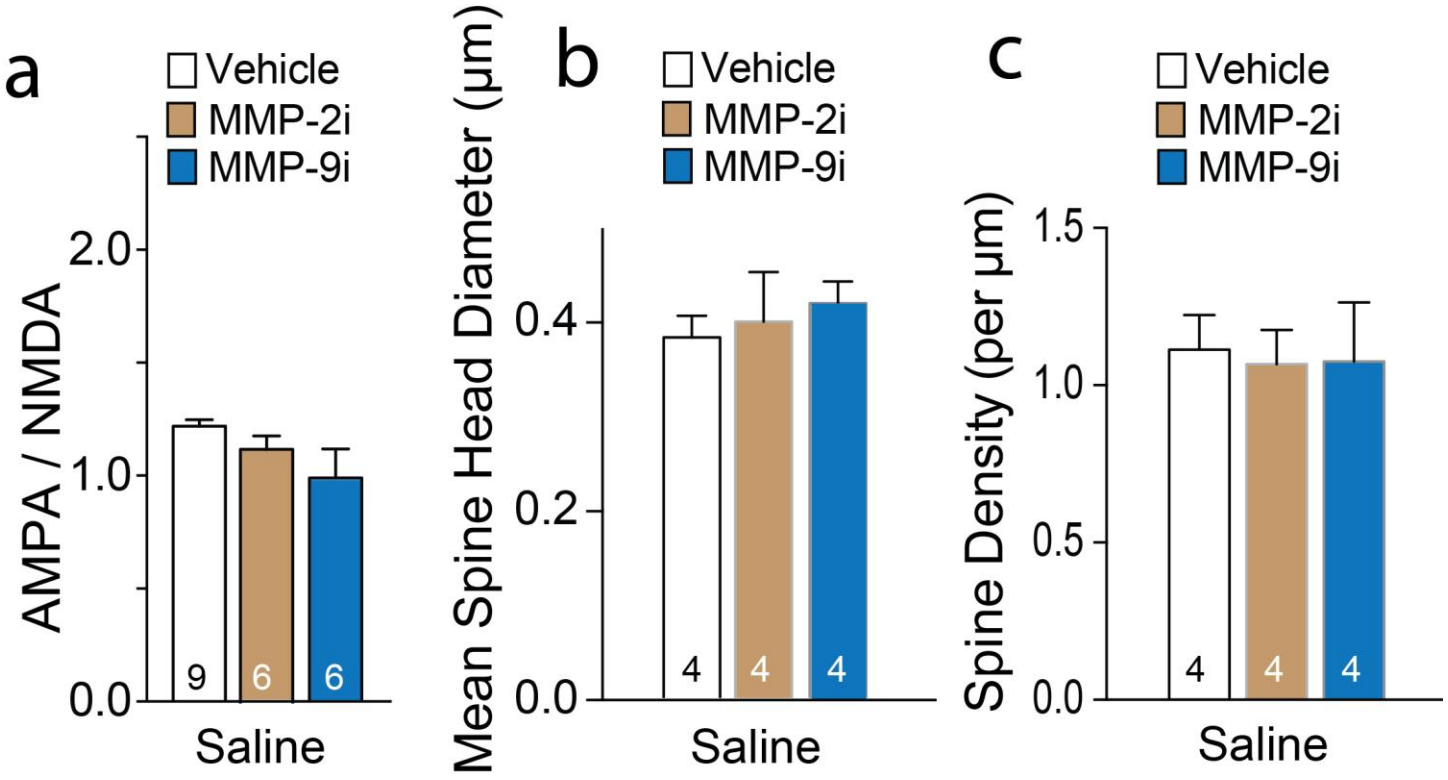
a) Representative traces showing full length AMPA and NMDA current recordings. b) No effect of MMP inhibition on NMDA decay time. One-way ANOVA $F_{(8,67)} = 0.83$, $p > .05$. c) MMP inhibition did not alter sEPSCs amplitude in any condition. One-way ANOVA $F_{(8,67)} = 1.34$, $p > .05$. d) MMP inhibition did not alter sEPSCs frequency in any condition. One-way ANOVA $F_{(8,67)} = 0.88$, $p > .05$. N is shown in bars as number of neurons recorded (panels a, d, e) or animals quantified (panels b, c) with each animal being the average of 6-12 neurons.



Supplementary Figure 6

Representative images and dendritic spine density frequency plot.

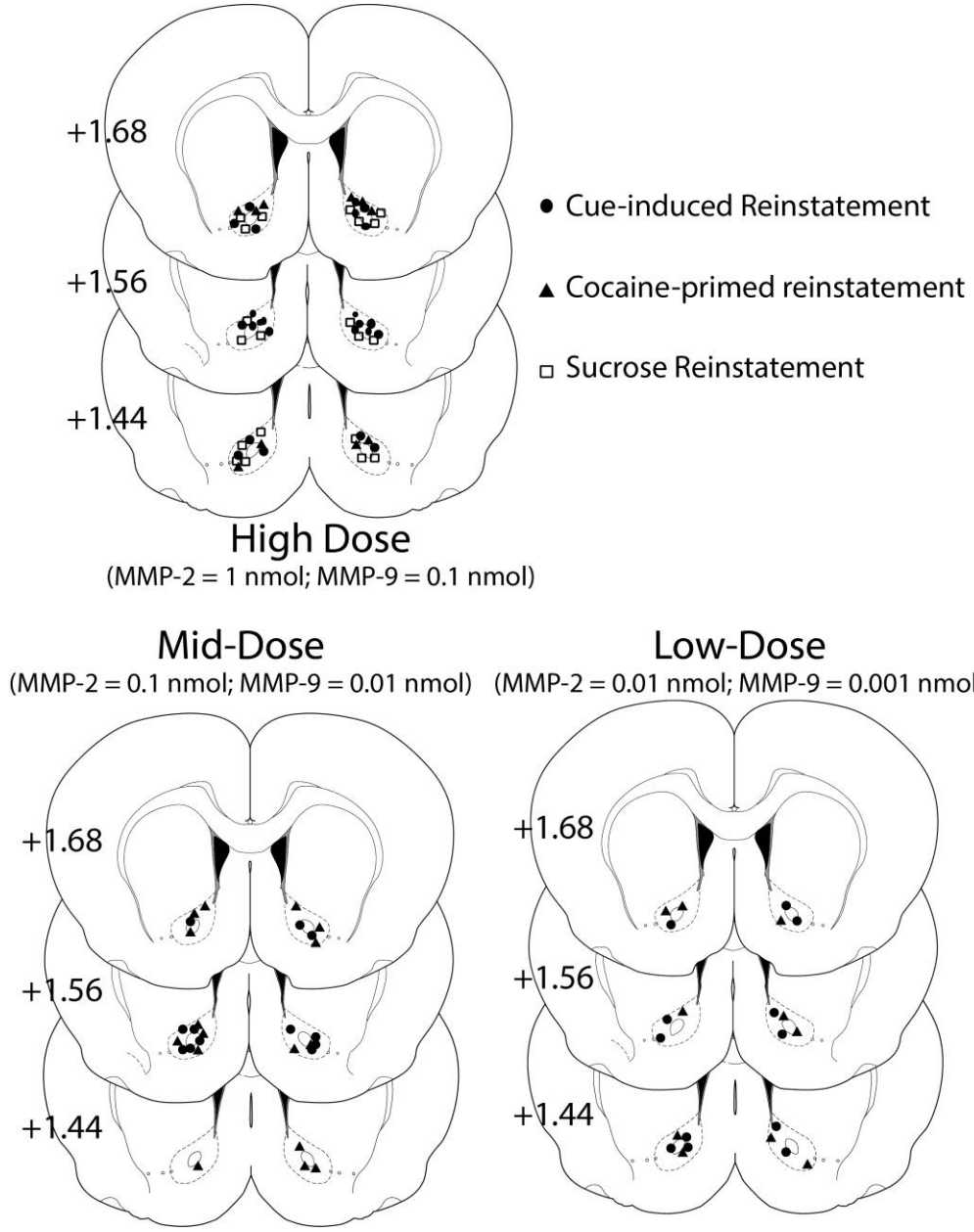
a,b) For dendritic spine analysis, images of entire neurons were taken at 1 µm resolution, and then 45-55 µm segments located between 75-200 µm from the soma and after the first branch point were imaged at 0.1 µm resolution for 3-dimensional reconstruction and morphological analysis. The yellow rectangle indicates the location of the dendritic segment that was imaged from these neurons. c) Representative micrographs of quantified dendritic segments from each group. d) Shows cumulative frequency distribution for dendritic spine density. There was a noticeable shift to the right in extinguished cocaine treated animals relative to yoked saline indicating greater spine density in cocaine-extinguished rats (see statistical analysis of mean values in figure 5). While MMP-2 inhibition reversed this effect in cocaine-extinguished subjects, MMP-9 inhibition was ineffective. Reinstatement did not alter the distribution relative to extinguished vehicle treatment, and MMP-2, but not MMP-9 inhibition returned the reinstated cocaine distribution to yoked saline distribution.



Supplementary Figure 7

MMP inhibition did not affect synaptic strength in yoked saline animals.

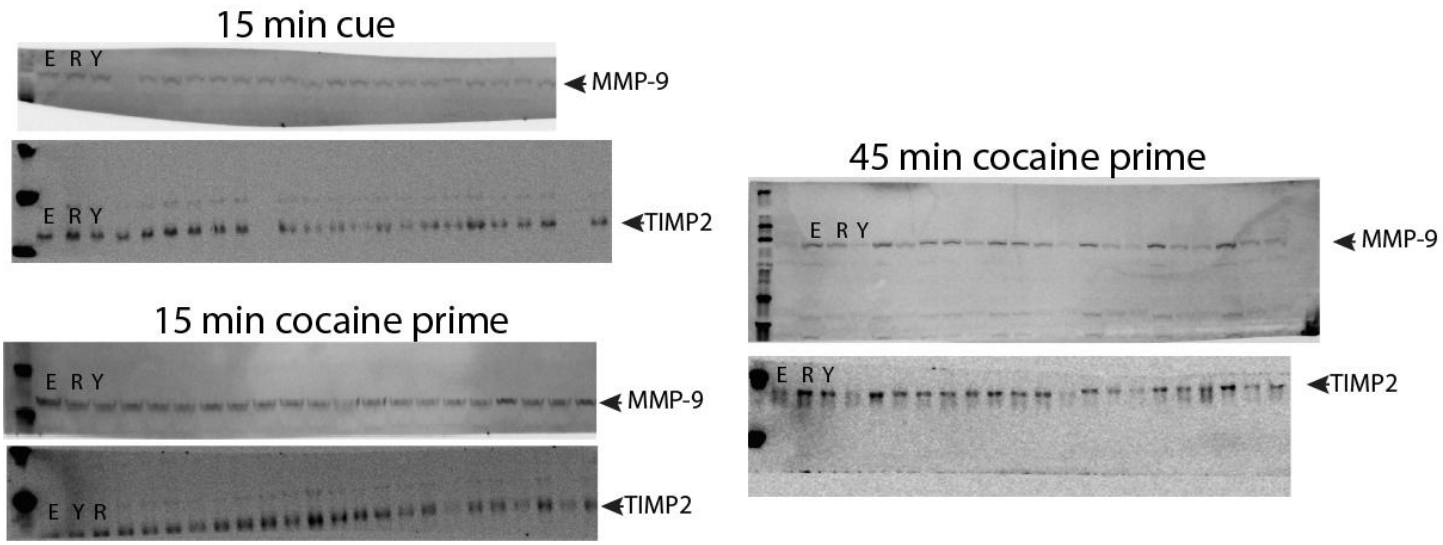
MMP inhibition did not affect synaptic strength in yoked saline animals. a) Shows A/N following vehicle, MMP-2 or MMP-9 inhibition in yoked-saline controls. One-way ANOVA revealed $F_{(2,19)}$= 3.41, $p > .05$. b) Neither MMP-2 nor MMP-9 inhibition affected spine head diameter in yoked-saline controls. One-way ANOVA revealed $F_{(2,9)} = 1.01$, $p > .05$. c) Neither MMP-2 nor MMP-9 inhibition affected spine density in yoked-saline controls. One-way ANOVA $F_{(2,9)} = 0.12$, $p < .05$.



Supplementary Figure 8

Histological verification of microinjection sites for animals microinjected with vehicle or MMP inhibitors prior to reinstatement in Figure 2.

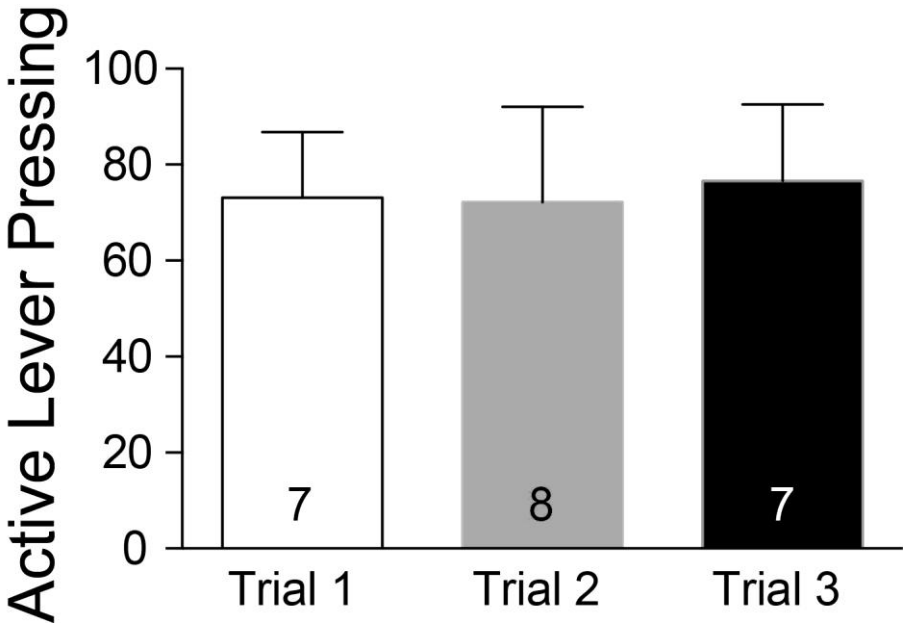
Rats with injection cannula outside of the NAcore were excluded from behavioral analysis.



Supplementary Figure 9

Full-length western blots corresponding to truncated blots shown in Figure 1h.

ERY pattern of spotting the gel repeated across the gel. E- extinguished, R- reinstated, Y- yoked saline



Supplementary Figure 10

Lever pressing following vehicle microinjection in dose-response analysis in Figure 2 was stable across three reinstatement trials.

The experiments in figures 2e-f were conducted as a within-subject crossover design consisting of 3 reinstatement trials per animal (unless a microinjection cannula became clogged, in which case only 2 trials were conducted. These data show the response to vehicle when it was randomly given in the first, second or third trial, and that there is no difference in vehicle reinstatement across 3 trials. The data argue against the possibility that the data if figure 6 were influenced by the order of drug injection across trials. One-Way ANOVA revealed $F_{(2, 22)} = 0.02$, $p > .05$.