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.
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 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 $t_{(10)} = 0.814$, $p > .05$. S = Yoked Saline, Reinst = Reinstated

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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.
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.
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 $F_{(2,9)} = 0.12$, $p < .05$. 
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 $F_{(2, 22)} = 0.02$, $p > .05$. 

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