ONLINE METHODS

Animals

We used adult male c57bl6j mice (Jackson) and Long-Evans rats (Charles River). Animals were habituated in our facility 1 week prior to experimentation and housed on a 12 hr light-dark cycle with access to food and water *ad libitum*. All animal experiments were approved by IACUC's of Mount Sinai and UT Southwestern.

Drugs

For chronic cocaine (Sigma) experiments, we used a standard cocaine injection paradigm[2, 5-7]: 7 daily intraperitoneal (IP) injections of 20 mg/kg cocaine. For acute experiments, mice were injected for 6 days with saline before a cocaine injection on day 7. Controls were injected with saline. For spine analysis, we used an established injection schedule known to increase spine density—consisting of five 20 mg/kg injections. This protocol is shortened to coincide with the duration of HSV-mediated overexpression which wanes 6 days post-surgery[23, 42].

For methionine (MP Biochemicals) experiments, animals were injected subcutaneously with 0.78 g/kg L-methionine 2x/day for 10 days. This was timed to ensure that the minimum dose and injection duration previously shown to increase DNA methylation in the striatum[19], would coincide with the beginning of CPP training. During training, animals were injected with methionine 2-3 hrs prior to behavioral experiments.

For RG108 (Sigma) experiments, we used 7-10 days of continuous (0.25 μl/hr) intra-NAc delivery of 100μM RG108 dissolved in 5% hydroxypropyl β-cyclodextrin vehicle (Trappsol). This dose of RG108 was found to decrease DNA methylation *in vivo* and showed no detectable neurotoxicity as assessed by activated caspase-3 staining. RG108 was chosen over other Dnmt inhibitors, such as 5-aza-cytidine, because: 1) it inhibits methylation without incorporation into DNA which has been shown to be linked to cytotoxicity[43] and 2) it is chemically stable with a mean 37°C t½ of 20 days, whereas the 37°C t½ of 5-aza-cytidine is on the order of hr[20]. Osmotic delivery was performed as previously described[1, 5, 7]

For fluoxetine (Tocris) experiments, we injected mice with 20 mg/kg fluoxetine IP for 14 days, as described previously[1].

Cocaine self-administration

Self-administration was performed as previously described[44, 45]. Animals had 3 hr daily access to cocaine (0.75 mg/kg/infusion) under a fixed ratio-1 (FR1) reinforcement schedule. For the 24 hr withdrawal experiment, rats had 13 days of cocaine intake; for the 28 day withdrawal experiment, rats had 3 weeks of intake.

Herpes simplex and adeno-associated virus injections

We used the bi-cistronic p1005+ HSV vector, which expressed GFP alone or GFP with Dnmt3a. In this system, HSV infection occurs selectively in neurons. GFP expression is driven under the human immediate early cytomegalovirus (CMV) promoter, while the gene of interest, Dnmt3a, is driven by the IE4/5 promoter[46]. We used a previously cloned mouse Dnmt3a-1 plasmid[47]. AAV-GFP or AAV-CreGFP was exactly used as described[5]. Stereotaxic surgery was performed based on published methods[5, 7].

Conditioned place preference

We used a standard, unbiased CPP procedure[6, 7, 23]. In brief, before experimental manipulation, animals were pretested for 20 min in a photo-beam monitored box with free access to environmentally distinct chambers. The mice were then arranged into control and experimental groups with equivalent pretest scores. After experimental manipulation, mice underwent four 30 min training sessions (alternating cocaine and saline pairing). On the test day, mice had 20 min of unrestricted access to all chambers, and a CPP score was ultimately assigned by subtracting time spent in the cocaine-paired chamber minus time spent in the saline-paired chamber.

Cocaine-induced locomotor sensitization

We used a standard locomotor sensitization procedure[17]. In brief, mice (saline habituated) were injected daily with cocaine (IP) 30 min after being placed in standard plastic cages similar to their home cages. Total locomotor activity was measured via photobeam breaks for 30 min following their injection (20mg/kg).

Social defeat stress

Experimental C57bl6j mice were subjected to 10 days of social defeat stress as previously described[26, 27]. In summary, mice are exposed daily to an unfamiliar aggressive CD1 mouse for 5 min. For the remaining 24 hr, the defeated mouse was housed in a ‘protected’ compartment of the same cage where it endured chronic stress

from the CD1. On the 11th day, mice underwent a social interaction test to verify defeat-induced social avoidance[26, 48]. As previously published, control mice spend more time interacting with a social "target" as compared to "no target", whereas chronically defeated mice spend significantly less time interacting with the target mouse compared to "no target"[26, 48] .

For RG108 and fluoxetine experiments, we selected mice displaying robust social avoidance—showing "Day 11" interaction times <40 sec. Then, using baseline interaction times, we divided animals into 3 similar groups as follows – Controls: intra-NAc vehicle (75.1 ± 3.1 sec), intra-NAc RG108 (75.4 ±4.1 sec), or systemic fluoxetine (75.5 ± 6.1 sec); Defeats: intra-NAc vehicle (20.5 ± 3.3 sec), intra-NAc RG108 (20.4 ±3.6 sec), fluoxetine (20.1 ± 3.1 sec).

To test for a pro-depressive phenotype, we performed a submaximal defeat experiment as previously described[26, 27]: 3 days following HSV surgery, naïve mice are subjected to 3 consecutive 5 min defeat episodes interspersed by 15 min rest periods. Mice underwent the social interaction test the following day. In this paradigm, these stressors are not sufficient to cause social avoidance in HSV-GFP mice as indicated by a significant increase in "target" interaction time versus "no target" interaction time[26, 27].

Forced swim test

Immobility measures were obtained using a 2-day forced-swim test procedure commonly used in rats[27, 28]. Three days following surgery, Sprague-dawley rats were forced to swim for 15 min in plastic cylinders (20 × 45 cm). The following day, rats were tested under identical conditions for 5 min. All sessions were videotaped and scored by a blinded observer. Latency to immobility was defined as the time that the rat first initiated a stationary posture that did not reflect attempts to escape.

Novel object recognition and object habituation tests

Tests were performed according to previous publications with minor modifications[49]. Briefly, for the novel object recognition test, mice were placed in an open field area to habituate (15 min). After 3 min of rest, they were exposed to 3 objects for 5 min in the same arena. Each object was placed in opposite corners of the open field arena. Mice were reexposed two additional times to the same objects in the same locations and on the final trial, one object was switched with a novel object. A normal mouse with intact memory is expected to spend more time with this novel object as

compared to the familiar objects. For habituation to a novel object, we exposed mice to a single object for 150 sec and the re-exposure time was extended to one hr. Here, as mice are re-exposed to this object, they are expected to become disinterested in the object, as measured by number of object investigations.

RNA isolation, reverse transcription, quantitative PCR, and primers

Bilateral NAc punches were obtained and the tissue homogenized in TriZol (Invitrogen). RNA was purified with RNeasy Micro columns (QIAGEN). Reverse transcription of RNA was carried out using iScript (Biorad). qPCR was run using approximately 5 ng of cDNA per reaction. Each reaction was run in triplicate and quantified using the ΔΔCt method as previously described[48]. A complete list of primers is given in Supplemental Table 2.

Global DNA methylation analysis

We used Epigentek's Methylamp Global DNA Methylation Quantification Ultra Kit and followed manufacturer's instructions. This method is ideally suited for our experiments on NAc tissue because of its ability to use small amounts of input DNA and for high degree of sensitivity. Raw values were colorimetrically quantified and total methylation levels estimated by generating a standard curve from Epigentek's methylated DNA standard. Values are then represented as methylation % relative to vehicle control.

ChIP promoter analysis

Chromatin from NAc tissue punches was immunoprecipitated with an antibody against me3K4H3 (Abcam ab1012) as described previously without modifications[2, 6, 25]. For the 24 hr experiment, samples were amplified using GenomePlex whole genome amplification kit (Sigma), labeled with Cy3 (input) or Cy5 (me3K4H3 enriched), and hybridized to NimbleGen MM8 mouse promoter arrays with 3 biological replicates used per condition. Each replicate consisted of bilateral NAc punches pooled from 10 mice. ChIP-chip analysis was performed as described previously without modification[6, 25]. Based on these analyses, the Dnmt3a promoter was found to have significantly reduced binding at approximately -500 bp upstream of the transcriptional start site. ChIP's were then performed on additional sets of 4 and 24 hr cocaine-injected mice and PCRs were performed using primers designed around this region as well as -2000 bp upstream.

Dendritic spine analysis (GFP immunostaining and single-cell dye filling)

For viral-based (GFP immunostaining) spine analysis (**Fig. 4a**), mice were perfused with 4% PFA 4 hr following the final cocaine injection and brains sectioned at 100 µm using a Leica vibratome. Slices were immunostained with an anti-GFP antibody (Millipore). Sections were mounted, coded, and confocal imaging (Zeiss LSM 710) was performed with the rater blind to experimental conditions. We imaged secondary and tertiary dendrites of NAc medium spiny neurons under a 100x oil-immersion objective at a resolution of 0.027 µm x-axis x 0.027 µm y-axis x 0.3 µm z. Approximately 7-10 neurons were imaged per animal (average total dendritic length 400 µm/animal, n=5-6). Dendritic length was measured using NIH ImageJ software and spine numbers were counted manually by a trained observer who was also blind to experimental conditions. To account for any possible surgical or sampling bias, we annotated anterior/posterior or NAc core/shell location of all images and found no significant differences in location or region.

Because HSV-GFP (like other HSV encoded transgenes) is no longer expressed after 7 days, we performed single-cell dye filling experiments (**Fig. 4b,c**). As previously described[50], random cells in NAc shell were impaled with a micropipette containing 5% Lucifer Yellow (Molecular Probes) and injected with 1-10 nA of current. Slices were washed in PBS, mounted and imaged on the confocal microscope using the same imaging parameters as the above GFP experiment. On average, for each animal 12 dendrites from 6 neurons were imaged (average total dendritic length of 500 µm/animal, n=4-8). Spine density and spine type analysis were performed using the semi-automated software, NeuronStudio (http://research.mssm.edu/cnic/tools-ns.html)[24]. Of note, NeuronStudio analysis is not amenable to other dendritic images, such as GFP, Golgi, or diolistics. NeuronStudio analyzes single-cell filled deconvolved images in 3 dimensions (3D) and, as an extra advantage, this software uses an algorithm of voxel clustering and Rayburst Sampling to ultimately classify spines into the 3 major morphologic types—thin, mushroom, and stubby. After NeuronStudio processing, a human operator verifies that all spines have been appropriately identified and manually corrects any errors in spine characterization. For the operator, the manual aspect of analysis takes about 10 min per dendritic image. Due to the labor intensive nature of 3D analysis, we had a separate NeuronStudio operator for each of the 3 cell-filling experiments. For each experiment, the NeuronStudio operator randomly analyzed coded images. Upon finishing analysis,

the spine density (as well as spine type) was calculated by dividing the total number of spines present by the dendritic length of the segment. Human-operated NeuronStudio was previously shown to have a range of 68% to 90% inter-operator variability in spine density counts[24]. Similarly, we found a high degree of inter-operator variability in our 3 datasets and, therefore, to control for such variability and to allow for comparison across experiments, we normalized the spine density and spine type data from each experiment with respect to its own control. Data are expressed as % change in spine density relative to saline controls.

Statistical analysis

Statistical significance was measured using an unpaired two-tailed Student *t*-test when comparing two groups. One-tailed Student *t*-tests were used for pharmacological verification of changes in global methylation and 7day-4hr spine density analysis since values are expected to fall in an expected sampling distribution. For mRNA analysis, HSV-GFP dendritic spine analysis, submaximal defeat susceptibility, and antidepressant tests in defeated mice, two-way ANOVA's were performed since experiments contained multiple groups. Repeated measures ANOVA was used for locomotor sensitization analysis. For ANOVAs, post-hoc comparisons were performed when appropriate. All error bars represent sem.