Supplementary Data

Table S1. Distance from soma of neuron segments used in spine analysis for all the data shown in figure 1. Average distance from the soma (mean ± SEM) did not significantly differ between groups. Average was determined as the distance (μm) from the start of the dendrite at the soma to the middle of the dendritic segment being measured. Number in parentheses is the number of dendrites measured at each time point. Data are shown as mean ± SEM.

Distance from Soma (μm)				
Yoked SAL	T=0	T=15	T=45	T=120
118.8±4.9 (45)	127.1±4.2 (60)	122.0±4.6 (43)	120.2±4.0 (59)	124.8±3.9 (64)

Fig. S1: Training for cocaine self-administration and extinction for all animals used in figures 1-4 of the text. Cocaine self-administration and extinction training to a stable baseline prior to reinstatement testing from animals shown in figures 1-3 of the manuscript (n= 57). Data are shown as mean ± SEM.

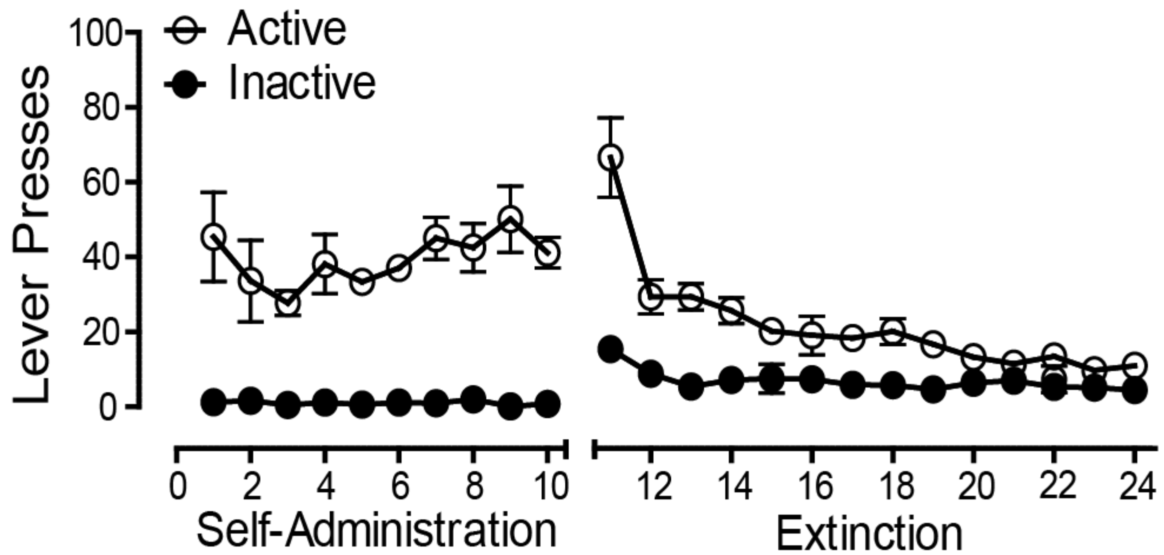


Fig. S2: Spine density in the NAcore was not altered by (a) cue-induced reinstatement of cocaine seeking, (b) reinstating sucrose seeking, or (c) by inhibiting neuronal activity in the PL with a microinjection of baclofen/muscimol (B/M). These data correspond to the experiment in figures 1, 2 and 4 showing effects on spine head diameter. The number of neurons quantified in each group is indicated in the bar, and 5-12 neurons were quantified in each animal. Data are shown as mean ± SEM.

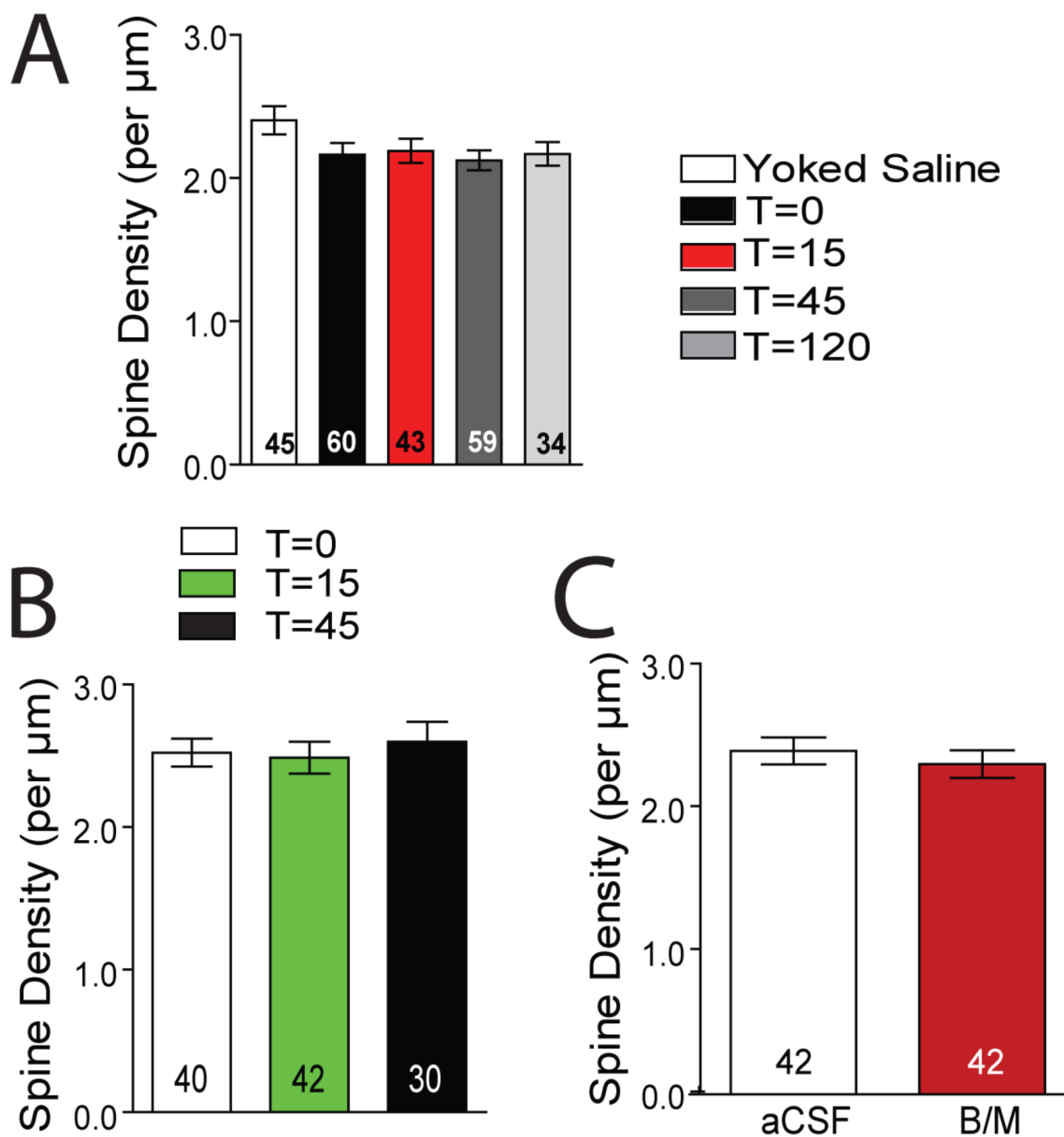


Fig. S3: Spine head diameter or spine density are not affected by exposure to the operant chamber or to non-contingent conditioned cues previously paired with cocaine; further validation of the specificity of the spine changes shown in figure 1. **(a)** Non-contingent (NC) cues did not reinstate cocaine-seeking behavior above extinction levels at T=15 (p> 0.05), consistent with previous findings (Grimm, 2000) (Ext at T = 120 represents the session prior to either T=15 NC cue or extinction). **(b)** Representative segments from animals receiving either 15 min of NC cues or extinction. **(c)** A basal enlargement of spine head diameter following withdrawal with extinction training from cocaine self-administration was found in T=0, T=15 NC cue, and T=15 extinction conditions compared to saline-yoked animals ($F_{(7,299)}$ = 438.542, p< 0.001). However, compared to T=0, NC cues and extinction did not further enlarge spine head diameter. No change in spine density was found (p> 0.05). Data are shown as mean ± SEM.

*p< 0.05 compared to yoked saline animals at T=0 (white bar); † p< 0.05 compared to yoked saline at T=15 NC cue (light gray bar).

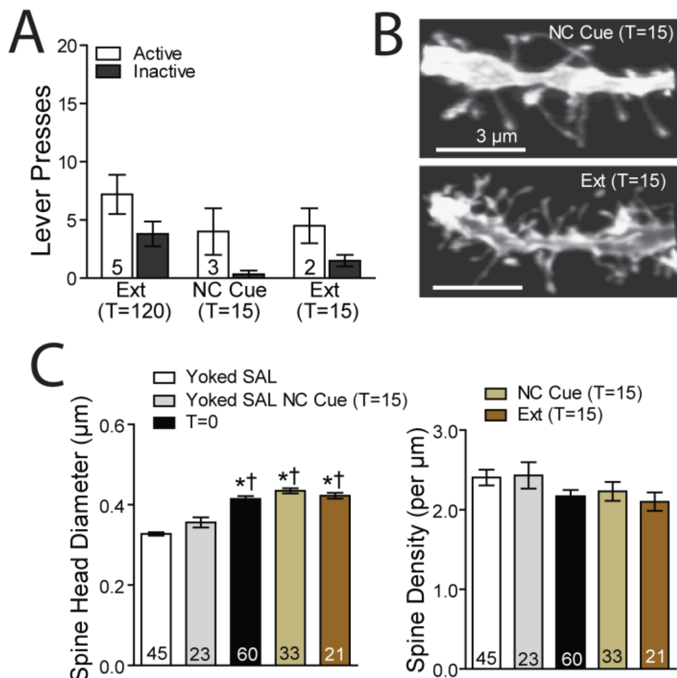
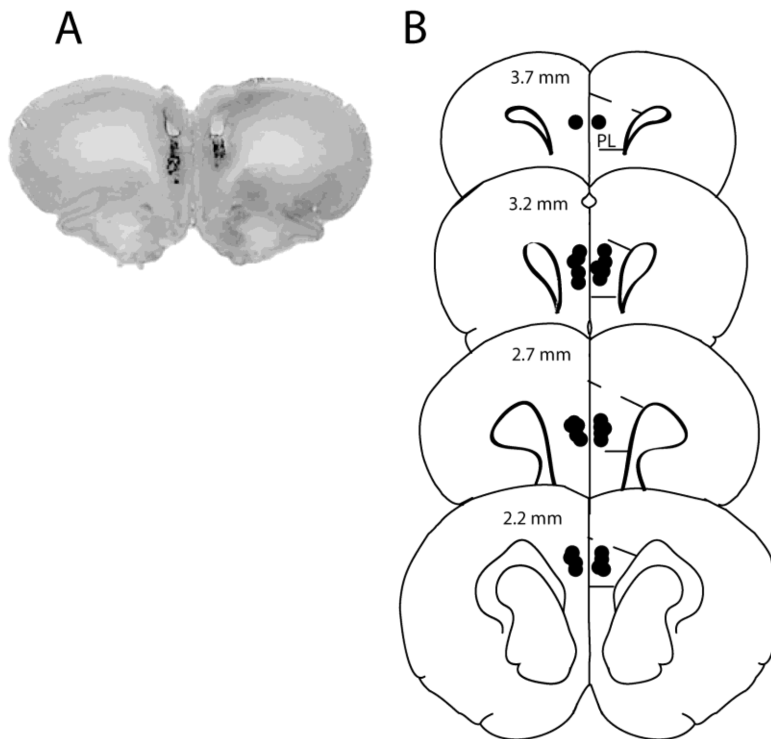


Fig. S4: Histological verification of guide cannula placement for the experiment shown in Figure 4. **(a)** A representative slice of microinjection sites in the PL. Note the hole indicating the tip of the chronic indwelling guide cannula above the darkly stained neuropil corresponding to the glial scar resulting from the acute penetration of the injection needle through the guide cannula. **(b)** Circles drawn on the modified stereotaxic atlas (Paxinos and Watson, 1986) indicate the location of the tip of the microinjector (2 mm below the end of the guide cannula). Dotted lines demarcate boundaries of PL; numbers refer to mm rostral to Bregma.



Supplemental Experimental Procedures

Animal housing and surgery. Male Sprague Dawley rats (250 g; Charles River Laboratories, Wilmington, MA) were individually housed in a temperature- and humidity-controlled environment with a 12:12 hr dark/light cycle (lights on at 6:00 P.M.). All experimentation occurred in the dark cycle. Rats received food ad libitum until the day prior to behavioral training, after which food restriction (20 g of rat chow per day) was implemented and maintained throughout the duration of the experiment. Rats were allowed 1 week to acclimate to the vivarium before inducing anesthesia and implanting indwelling jugular catheters (surgical details have been described previously(Knackstedt et al., 2010; LaLumiere and Kalivas, 2008)). All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Assessment and Accreditation of Laboratory Animal Care.

Cocaine self-administration procedures. Rats were allowed to recover for 7 days after surgery, prior to the beginning of the experimental protocol. Animals then began daily 2-hr cocaine self-administration sessions, in which one response (fixed-ratio 1) on the active lever yielded one intravenous cocaine infusion (0.2 mg/infusion, followed by a 20-sec timeout period), paired with a white cue light above the active lever and a discrete tone cue (2900 Hz tone). An inactive lever was also available throughout the duration of each session to control for nonspecific responding. Following 10 consecutive sessions of self-administration (≥ 10 infusions/day), rats were placed into daily extinction training sessions (no cocaine delivery or cues) for at least 14 sessions, or until extinction criteria were met (≤ 25 active lever responses for a minimum of 2 sessions). Reinstatement was elicited by cues (tone + light delivery following an active lever press). A computer controlled the experimental sessions using Med-IV software (Med-Associates).

Microinfusion procedures and histology. Rats were stereotaxically implanted immediately after catheterization with bilateral guide cannulae aimed above PL (AP +3.0, ML ±0.6, DV -3.3(Paxinos and Watson, 1986)). Intracranial cannulae were secured to the skull using jeweler's screws and dental acrylic. Obturators were placed into the guide cannulae during the experimental protocol to prevent outside debris from entering the cannulae, and were removed during microinfusions. Bilateral injection cannulae were lowered into the final site through implanted guide cannulae both the day prior to injection (sham) and the day of injection where 0.3 μl baclofen/muscimol cocktail (0.3/0.03 nmole, $GABA_B$/$GABA_A$ receptor agonists, respectively) was infused over 1 min using a syringe pump. Injectors were kept in place for 1 min following injection to allow for diffusion (McFarland and Kalivas, 2001). Rats were placed in the operant chamber 10 min following removal of injection cannulae. Obturators were again placed in the guides following injection. At T=15, rats were sacrificed for either dendritic spine quantification or electrophysiological procedures. Coronal slices (100 μm thick) of PL were mounted and stained via cresyl violet to verify guide cannulae placement (see fig S3).

Quantification of dendritic spines. All dendritic spine quantification procedures have been described previously (Shen et al., 2009). Briefly, a confocal microscope (Zeiss LSM 510, Thornwood, NY) was used to image DiI-labeled sections, and DiI was excited using the Helium/Neon 543 nm laser line. The micrograph of DiI-labeled dendrite (see Fig. 1) was acquired via optical sectioning by a 63x oil immersion objective (Plan-Apochromat, Zeiss; NA = 1.4, WD = 90 μm) with pixel size 0.07 μm at XY plane and 0.1 μm intervals along the z-axis. A saturation limit of 5% along the dendrite was used. Images were deconvoluted by Autoquant prior to analysis (Media Cybernetics,

Bethesda, MD), and then a 3-D perspective was rendered by the Surpass module of Imaris software package (Bitplane; Saint Paul, MN). Only spines on dendrites beginning at >75 μm and ending at ≤ 200 μm distal to the soma and after the first branch point were quantified from cells localized to the NA core (see Table S1 for mean ±SEM segment distance from soma of each group). As well, only spines that did not have saturation at the head were included in analysis. Any spine head that showed saturation was manually deleted from analysis. The length of quantified dendrites was 45-55 μm. For each of the animals examined in each group, 5-12 neurons were analyzed. A protocol that quantifies spine density and head diameter based on the Filament module of Imaris was used. The minimum end segment diameter (spine head) was set at ≥0.143 μm. We determined this sampling interval in the x/y/z based on the minimum size of a diffraction-limited spot of light, which can be estimated using the Rayleigh criteria (Wallace et al., 2001). A concern is that the measurement of small structures such as spine heads is near the resolution limits of the confocal microscope. The specifications of the optics used in these experiments however, according to the Rayleigh criteria, allow a theoretical lateral resolution of a minimum diameter of 0.21 μm (Inoué, 1995). With the use of confocal microscopy in a small field of view within the confocal plane, however, this limit can be augmented by a factor of two (Wilson, 1984), thus allowing a lateral resolution in the range of 0.1 μm (Vecellio et al., 2000; Wallace and Bear, 2004; Wallace et al., 2001).

Slice preparation and whole cell recordings. Rats were anesthetized with ketamine HCl (1 mg/kg Ketaset, Fort Dodge Animal Health, Iowa) and decapitated. When necessary, head caps were removed first. The brain was removed from the skull and coronal accumbens brain slices (220 μm) (VT1200S Leica vibratome; Leica Microsystems, Wetzlar, Germany) were collected into a vial containing artificial

cerebrospinal fluid (aCSF) (in mM: 126 NaCl, 1.4 NaH_2PO_4, 25 $NaHCO_3$, 11 glucose, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 2.5 KCl, 2.0 NaPyruvate, 0.4 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 32°C for 30-40 minutes and then stored at room temperature.

All recordings were collected at 32˚C (TC-344B, Warner Instrument Corporation, Hamden, CT) in the dorsomedial NAcore, where the prefrontal inputs are most dense(Gorelova and Yang, 1997; Voorn et al., 2004). Neurons were visualized with an Olympus BX51WI microscope (Olympus America, Center Valley, Pennsylvania). Inhibitory synaptic transmission was blocked with picrotoxin (50 μM). Multiclamp 700B (Axon Instruments, Union City, CA) was used to record AMPA and NMDA currents in whole cell patch-clamp configuration. Glass microelectrodes (1-2 MΩ) were filled with cesium-based internal solution (in mM: 124 cesium methanesulfonate, 10 HEPES potassium, 1 EGTA, 1 $MgCl_2$, 10 NaCl, 2.0 MgATP, and 0.3 NaGTP, 1 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, Sydney, Australia). To evoke postsynaptic currents, a bipolar stimulating electrode (FHC, Bowdoin, Maine) was placed ~300 μm dorsomedial of the recorded cell to maximize chances of stimulating prelimbic afferents. The stimulation intensity chosen evoked a ~50% of maximal AMPA current. Recordings were collected every 20 sec. Series resistance (Rs) measured with a 2 mV depolarizing 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.

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. Then the membrane potential was gradually increased until +40 mV. Recording of currents was resumed 5 min after reaching +40

mV to allow stabilization of cell parameters. Currents composed of both AMPA and NMDA components were then obtained. Then (2*R*)-amino-5-phosphonovaleric acid (D-AP5), an NMDA receptor antagonist, was bath-applied (50 μM) to block the 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.

Statistics. All spine density and diameter data was statistically analyzed by averaging the values for all the neurons in each animal. The number of determinations in each group was established using an analysis of statistical power based on previous morphological data from our laboratory (Shen et al., 2009). Behavioral data was analyzed using appropriate repeated-measures analyses of variance (ANOVA), and *t* tests were used to compare spine head diameter and AMPA/NMDA ratio in animals receiving aCSF or B/M. Additionally, linear regression was used to determine the association between magnitude of reinstatement behavior and morphological changes in spines, as well as changes in AMPA/NMDA ratio. Post-hoc comparisons were conducted using Bonferroni-corrected *t* tests. All statistical tests were conducted using Graphpad or SPSS software packages. Rats that did not meet criteria for acquisition of cocaine self-administration and/or extinguished lever pressing were eliminated.

Supplemental References

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