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Report

# Relapse Induced by Cues Predicting Cocaine Depends on Rapid, Transient Synaptic Potentiation

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## SUMMARY

**Cocaine addiction is characterized by long-lasting vulnerability to relapse arising because neutral environmental stimuli become associated with drug use and then act as cues that induce relapse. It is not known how cues elicit cocaine seeking, and why cocaine seeking is more difficult to regulate than seeking a natural reward. We found that cocaine-associated cues initiate cocaine seeking by inducing a rapid, transient increase in dendritic spine size and synaptic strength in the nucleus accumbens. These changes required neural activity in the prefrontal cortex. This is not the case when identical cues were associated with obtaining sucrose, which did not elicit changes in spine size or synaptic strength. The marked cue-induced synaptic changes in the accumbens were correlated with the intensity of cocaine, but not sucrose seeking, and may explain the difficulty addicts experience in managing relapse to cocaine use.**

## INTRODUCTION

Understanding the neurobiology of relapse to drug use will facilitate the development of pharmacotherapies to treat addiction (Kalivas and Volkow, 2011; Vocci and Ling, 2005). An important feature of the enduring vulnerability to relapse is that neutral environmental stimuli become associated with drug use and act as cues that initiate relapse (Goldstein and Volkow, 2002; See, 2002; Wilson et al., 2004). Presenting cues previously paired with cocaine use initiates craving and drug seeking, which are associated with activating the glutamatergic projection from the prefrontal cortex to the nucleus accumbens (Kalivas, 2009; Koob and Volkow, 2010; Wilson et al., 2004). Given the well-established role of the corticostriatal projection in regulating motivated behavior (Balleine et al., 2007; Lüscher and Malenka, 2011; Miller and Marshall, 2004), it is thought that cocaine-induced changes in this glutamatergic projection enable environmental stimuli associated with cocaine use to act as conditioned cues that elicit uncontrollable motivation to relapse to drug use compared with the more manageable motivation to obtain natural reward (Garavan et al., 2000; Levy et al., 2007).

The neurobiology of relapse to cocaine use is most frequently studied in animal models by measuring long-lasting changes in brain structure and function after experimenter-injected or self-administered cocaine followed by varying periods of withdrawal. A key observation using this approach is that excitatory synapses on medium spiny neurons (MSNs) in the accumbens show evidence of long-term potentiation (LTP), including increased dendrite spine head diameter, elevated AMPA glutamate receptor-mediated synaptic currents, and AMPA receptor surface expression (Boudreau et al., 2007; Conrad et al., 2008; Kourrich and Thomas, 2009; Moussawi et al., 2009; Shen et al., 2009; Wolf and Ferrario, 2010). This LTP-like state is suggested to mediate the enhanced motivation underlying relapse to drug use compared to natural reward (Wolf, 2010). However, it remains unknown how initiating relapse with cocaine-conditioned cues (i.e., absent the pharmacological effects of the drug) affects synaptic physiology and morphology, if synaptic changes are important for initiating relapse, or if cues initiating cocaine seeking produce distinct synaptic changes compared with the same cues initiating seeking of a natural reward.

In order to investigate these synaptic mechanisms contributing to cocaine relapse, we used a "short-access" model of cocaine self-administration and examined the reinstatement of cocaine seeking by a light/tone cue previously associated with cocaine delivery. While this paradigm may not model compulsive drug self-administration (Koob, 2012), it allows investigation of cue-induced cocaine seeking after a period of withdrawal (Epstein et al., 2006; Shaham et al., 2003) and elicits enduring physiological and neurochemical changes in the projection from the prefrontal cortex to nucleus accumbens (Kalivas, 2009; Wolf, 2010). Using this model, we show that presenting cocaine-associated cues simultaneously initiated cocaine seeking and a rapid, transient increase in dendritic spine size and synaptic strength in the nucleus accumbens. The synaptic changes were positively correlated with the intensity of reinstated cocaine seeking and required activity in the prefrontal cortex. Importantly, the increase in spine size and synaptic response did not occur when the same seeking behavior was induced by identical cues paired with a natural reward (sucrose). Our data demonstrate that associating cocaine, but not a natural reward, with environmental cues confers a capacity for these cues to transiently potentiate accumbens excitatory

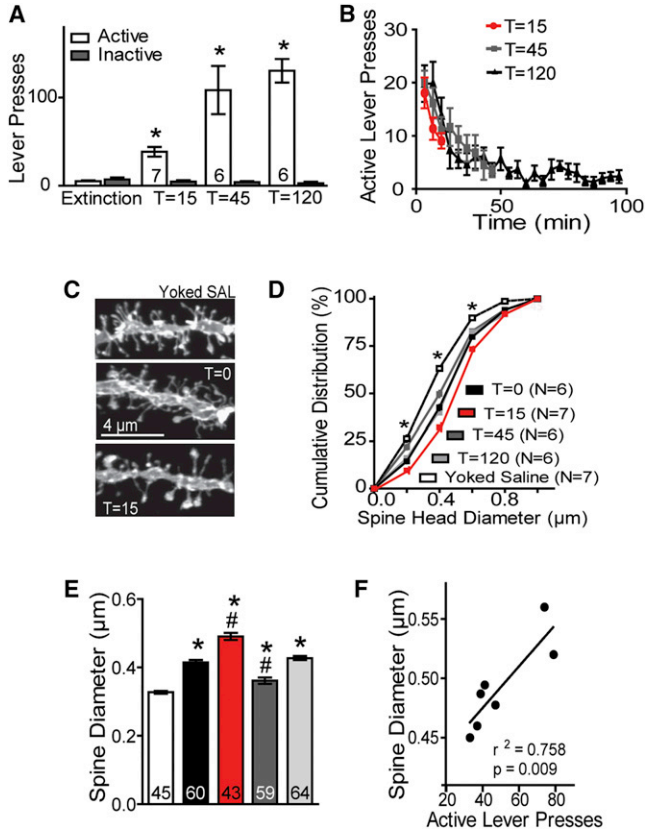


**Figure 1. Cue-Induced Cocaine Seeking Rapidly Enlarges Spine Head Diameter in NAcore MSNs**

(A) Cue-induced reinstatement of cocaine seeking increased active lever pressing over the 15, 45, or 120 min prior to euthanizing rats for morphological or A/N measurements ($F_{(7,92)}$ = 39.93, p < 0.0001). (B) Time course of active lever pressing during the cue reinstatement session. (C) Sample dendrites from NAcore MSNs in yoked-saline ($d_h$ = 0.329 μm) or cocaine-trained rats at T = 0 (0.422 μm); T = 15 (0.528 μm) min after initiating cue-induced reinstatement. (D) Cumulative distribution of spine head diameter reveals changes in $d_h$ between treatment groups (group: $F_{(5,1698)}$ = 8769, p < 0.0001; $d_h$ $F_{(4,1698)}$ = 115.2, p < 0.0001; interaction: $F_{(20,1698)}$ = 21, p < 0.0001). (E) Cocaine self-administration increased mean $d_h$ ($F_{(6,326)}$ = 43.98, p < 0.0001). Spine $d_h$ was elevated at T = 15, decreased below prereinstatement levels at T = 45, and returned to prereinstatement levels at T = 120. (F) The increase in $d_h$ at 15 min was significantly correlated with active lever pressing. N in (D) is the number of rats, and N shown as the number in bars corresponds to either the number of animals (A) or the number of neurons quantified (E). Five to twelve neurons were measured from each rat. Data are shown as mean ± SEM. *p < 0.05, compared to yoked-saline or extinction lever presses; #p < 0.05, compared to T = 0 cocaine.

synaptic transmission. This distinction between cocaine and sucrose may explain the relatively uncontrollable motivation to relapse to cocaine use compared with the more manageable desire for natural reward.

## RESULTS

Rats were trained to self-administer cocaine by pressing one of two levers to receive an intravenous cocaine injection. Rats self-administered cocaine for 2 hr a day over 10 days to achieve stable daily cocaine use, and lever pressing was then extinguished over another 14 days of 2 hr sessions (see Figure S1 available online). A light/tone compound stimulus was paired with cocaine infusions during the self-administration sessions and lever pressing during extinction training yielded neither cocaine nor the light/tone stimulus. A parallel yoked-saline control group was included consisting of rats administered an intravenous infusion of saline when a paired rat self-administered cocaine. Once rats achieved a stable extinguished baseline of lever pressing (Figure S1), the light/tone cue was presented with each press of the lever that previously provided cocaine (active lever), but no cocaine was delivered. Returning the conditioned light/tone cue resulted in a marked reinstatement of lever pressing (Figures 1A and 1B), which was used to model cue-induced relapse (Epstein et al., 2006).

As described above, cocaine self-administration causes stable LTP-like synaptic potentiation in the core subcompartment of the nucleus accumbens (NAcore) that endures for months after discontinuing cocaine use and is proposed to contribute to cocaine relapse. To test whether synaptic alterations initiated in the NAcore by presentation of cocaine-conditioned cues contribute to relapse, we examined animals just prior to beginning cue-induced reinstatement (time [T] = 0) or at 15, 45, or 120 min after beginning the reinstatement trial for two measures of synaptic plasticity. To quantify spine density and $d_h$, we made three-dimensional (3D) confocal images of neurons in the NAcore that were diolistically labeled with the lipophilic dye DiI (Figure 1C) (Shen et al., 2011). Synaptic strength was also estimated by calculating the ratio of AMPA to NMDA currents (A/N) (Malenka and Bear, 2004) using whole-cell patch recordings from MSNs in NAcore tissue slices (Moussawi et al., 2011; Shen et al., 2011).

Conditioned cues reinstated robust active lever pressing compared to inactive lever pressing or to active lever pressing during extinction (Figure 1A). The increase in lever pressing was maximal during the first 10 min of the reinstatement session and progressively decreased thereafter for the remainder of the session (Figure 1B). At T = 0, $d_h$ was increased in rats extinguished from cocaine self-administration (0.415 ± 0.007 μm) compared to yoked-saline controls (0.327 ± 0.004 μm) (Figures 1C–1E). Cue-induced reinstatement further increased $d_h$ at 15 min after the cue was presented (0.491 ± 0.009 μm). By 45 min after initiating reinstatement, $d_h$ decreased below the resting (T = 0) cocaine levels (0.361 ± 0.009 μm) and had returned to prereinstatement levels by 120 min after initiating the reinstatement session (0.427 ± 0.011 μm). Importantly, the amount of reinstated active lever pressing at 15 min was positively correlated with the increase in $d_h$ (Figure 1F). No difference in spine density was found between groups (Figure S2A).

To test whether cue-induced morphological plasticity was specific for reinstating lever pressing for cocaine and not for a natural reward, we trained rats to self-administer sucrose pellets and we paired pellet delivery with the same light/tone stimulus used for cocaine training. While the sucrose-trained rats showed robust cue-induced reinstatement of lever pressing (Figure 2A), no change in mean $d_h$ (Figure 2B) or spine density (Figure S2B) was measured at 15 or 45 min after the cue

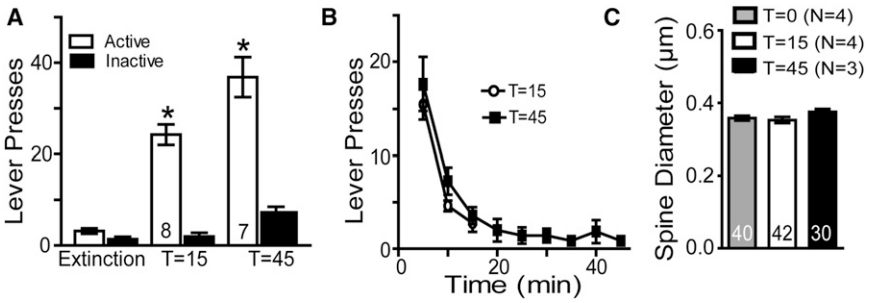


**Figure 2. Sucrose-Trained Rats Do Not Show Increased Spine Head Diameter during Cue-Induced Reinstatement**

(A) Sucrose-trained rats show significant cue-induced reinstatement ($F_{(3,31)} = 86.981$, $p < 0.001$). (B) Lever responding for contingent cues was maximal during the first 10 min of the session and decreased thereafter. (C) Sucrose reinstatement was not accompanied by a change in $d_h$. N is the number in bars and corresponds to either the number of animals (A) or the number of neurons quantified (C). Five to twelve neurons were measured from each rat. Data are shown as mean ± SEM. *$p < 0.05$, compared to extinction lever presses.

(Figure 2C). To further test that the cue-induced increase in $d_h$ depended on a contingent association between the cocaine-paired lever and the light/tone cue, we exposed rats to the chamber either without presenting cues or when cues were presented independent of lever pressing, and these animals also showed no increase in $d_h$, spine density, or lever pressing at 15 min after beginning the session (Figures S3A–S3C). Taken together, the morphological measurements show that withdrawal from daily cocaine self-administration causes a resting enlargement of $d_h$ and that cue-induced reinstatement of cocaine, not sucrose, seeking is accompanied by a further rapid, transient enlargement of the spines that is significantly correlated with reinstated behavior.

Whole-cell patch recordings revealed parallel evidence for rapid synaptic potentiation during cue-induced reinstatement (Figures 3A and 3B). Withdrawal from self-administered cocaine increased A/N compared to yoked-saline rats. The A/N was further increased 15 min after initiating cue-induced reinstatement and returned to prereinstatement levels after 120 min. In contrast with the decrease in $d_h$ (Figure 1F), the A/N remained elevated at T = 45. Similar to $d_h$, the increase of A/N at 15 min was significantly correlated with the number of reinstated active lever presses (Figure 3C), and cue-induced reinstatement of sucrose did not show a change in A/N at 15 or 45 min after initiating the reinstatement session (Figure 3D).

The NAcore receives glutamatergic input from various sources, and increased release of glutamate from the prelimbic cortex (PL) into the NAcore is required for reinstating drug seeking (LaLumiere and Kalivas, 2008; McFarland et al., 2003). We found that neural activity in the PL is also critical for the cue-induced synaptic changes in the NAcore. Inhibiting the PL by microinjecting GABA agonists (baclofen plus muscimol) prior to the reinstatement session prevented cue-induced increases in $d_h$ and A/N in NAcore MSNs, as well as blocked reinstated active lever pressing (Figure 4; Figure S4 for histology).

## DISCUSSION

We show here that the reinstatement of cocaine seeking by conditioned cues, but not the reinstatement of seeking a natural reward, was accompanied by rapid, transient synaptic potentiation in NAcore MSNs. The rapid potentiation contrasts with previous reports showing that cocaine use reduces the ability of prefrontal input to induce classical forms of synaptic plasticity, such as LTP and LTD (Martin et al., 2006; Moussawi et al., 2009). Thus, while cocaine use diminishes the capacity of stimuli not associated with drug use to induce synaptic plasticity, LTP-like plasticity is readily induced by stimuli paired with cocaine use. The importance in relapse of synaptic plasticity selectively coded by cocaine-associated cues was supported by a significant correlation between the intensity of cocaine seeking and both morphological and electrophysiological measures of synaptic potentiation.

Changes in spine density and/or head diameter ($d_h$) are a structural substrate for synaptic plasticity, with larger $d_h$ being associated with LTP and reduced $d_h$ with LTD (Carlisle and Kennedy, 2005; De Roo et al., 2008; Yang and Zhou, 2009). Consistent with previous reports (Kourrich et al., 2007; Moussawi et al., 2011; Shen et al., 2009), withdrawal from investigator- or self-administered cocaine increased $d_h$ and A/N compared to yoked-saline rats. The $d_h$ and A/N were further increased 15 min after initiating cue-induced reinstatement and returned to prereinstatement levels after 120 min. Although there was a decrease in $d_h$ at T = 45, the A/N remained elevated. The slower normalization of the A/N is consistent with previous in vitro studies indicating that although both $d_h$ and A/N are reliable markers of synaptic plasticity, they are regulated in part by distinct signaling pathways (Fukazawa et al., 2003; Henley et al., 2011). For example, inhibiting protein phosphatase 1 prevents electrophysiological measures of LTP without affecting enlargement of dendritic spines (Zhou et al., 2004). Conversely, inhibiting actin polymerization reduces spine size in cultured neurons (Gu et al., 2010) but inhibits only enduring LTP (>1 hr), leaving intact short-term synaptic potentiation that is akin to what we show here being initiated by cocaine-conditioned cues (Fukazawa et al., 2003; Krucker et al., 2000; Ramachandran and Frey, 2009).

A link between reinstated cocaine seeking and the rapid LTP-like plasticity was also indicated by inactivating the PL and showing necessary involvement of this region of the PFC in cue-induced increases in $d_h$ and A/N. It is likely that the glutamatergic projection from the PL to the NAcore is contributing to the effects of inactivation since double-dissociation pharmacological inactivation and more selective optogenetic inhibition show that this pathway is necessary for reinstated cocaine seeking (McFarland and Kalivas, 2001; Stefanik et al., 2013). This mechanism is also consistent with in vivo recordings showing

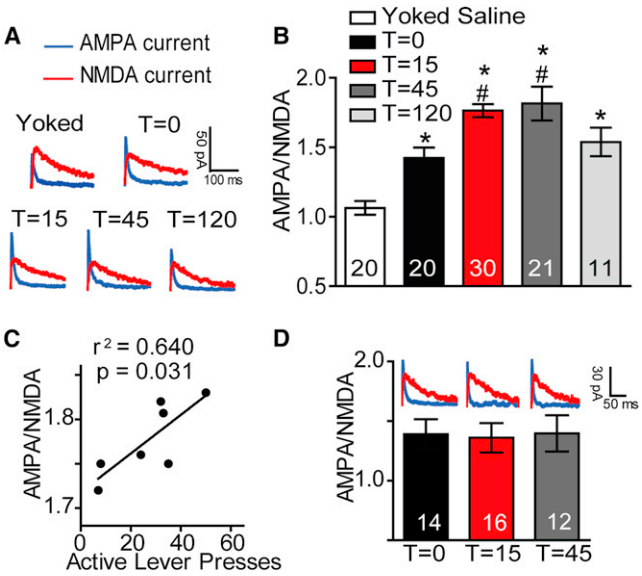


**Figure 3. Synaptic Potentiation Initiated by Cue-Induced Cocaine Seeking**

(A) Sample AMPA and NMDA current traces from each group. (B) AMPA to NMDA ratios (A/N) were significantly elevated in animals withdrawn with extinction training from cocaine self-administration (1.423 ± 0.075) compared to yoked-saline animals (1.064 ± 0.050). In addition, the initiation of cue-induced reinstatement further elevated A/N at T = 15 (1.780 ± 0.060). Ratios remained elevated at T = 45 (1.815 ± 0.122) and returned to prereinstatement levels by T = 120 (1.538 ± 0.103) ($F_{(4,101)}$ = 14.45, p < 0.001). (C) The increase in A/N at 15 min was significantly correlated with the number of active lever presses. (D) Cue-induced reinstatement of sucrose seeking did not alter A/N. Two to five neurons were recorded from each animal. Data are shown as mean ± SEM. *p < 0.05, compared to yoked-saline animals at T = 0 (white bar); #p < 0.01, compared to T = 0 (black bar).

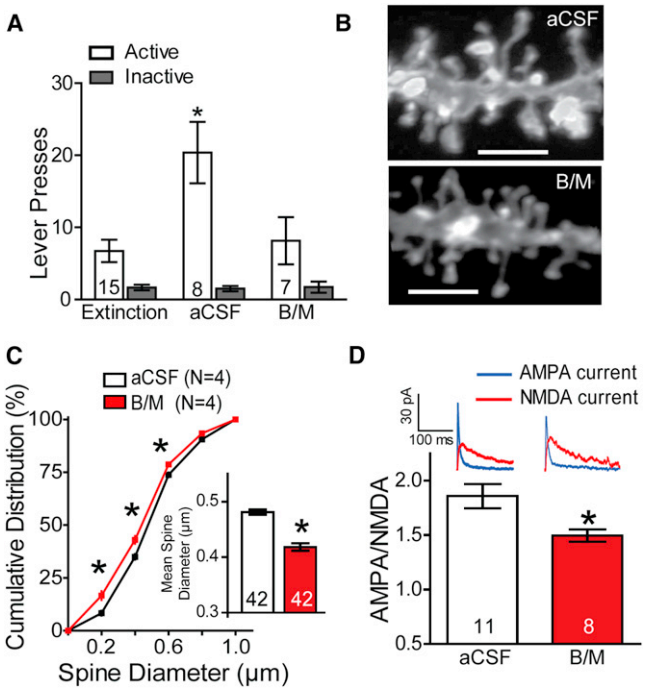


**Figure 4. Inactivation of the PL Prevents Cue-Induced Reinstatement and the Increase in $d_h$ and A/N in NAcore**

(A) B/M infusions into PL inhibited cue-induced reinstatement (T = 15; $F_{(5,59)}$ = 11.971, p < 0.001; N is shown in bars). (B) Sample dendrites of animals receiving either aCSF or B/M into PL prior to initiating cue-induced reinstatement and sacrificed 15 min later. (C) B/M into PL inhibited the mean increase in $d_h$ ($t_{(82)}$ = 7.504, p < 0.001; see inset) and shifted the cumulative distribution to the left. (D) B/M into PL inhibited the increase in A/N ($t_{(17)}$ = 2.554, p = 0.021). Data are shown as mean ± SEM. *p < 0.05, comparing aCSF to B/M.

increased activation of NAcore neurons in response to cocaine-conditioned cues after a period of extinction training (Hollander and Carelli, 2007) and with neuron culture studies indicating that glutamate induces LTP-like synaptic changes (Shepherd and Huganir, 2007). In addition, the lack of rapid LTP-like plasticity accompanying reinstated sucrose seeking supports a role for PL glutamatergic input, since cocaine reinstatement requires a marked rise in the release of synaptic glutamate from the PL into the NAcore, but reinstated sucrose seeking does not induce measurable glutamate release (McFarland et al., 2003). However, it is possible that PL projections to other brain regions innervating the NAcore known to regulate reinstated behavior may also play a role, such as dopamine projections from the ventral tegmental area or glutamatergic input from the basolateral amygdala (Koob and Volkow, 2010). A role for dopaminergic afferents is supported by the fact that in cocultured prefrontal and accumbens neurons, D1 receptor stimulation facilitates trafficking of AMPA receptors to the surface and costimulation of NMDA receptors promotes D1 synaptic insertion (Sun et al., 2008). In this regard, it will be of interest in future studies to determine whether the changes identified here are selective for D1 or D2 receptor-expressing MSNs.

Cocaine addiction is defined in part by the unmanageable motivation to take cocaine and differs markedly from relative control over engaging natural reward. The lack of change in $d_h$ and A/N after cue-induced sucrose seeking indicates that associating cues with cocaine delivery is conferring neuroadaptations that are not occurring when the identical cues are associated with sucrose delivery. This supports the possibility that the rapid, transient synaptic potentiation may be a biomarker for a cocaine seeking neuropathology and poses the possibility that countermanding the synaptic potentiation may selectively disrupt the vulnerability to relapse to cocaine use without affecting the motivation to seek natural reward.

## EXPERIMENTAL PROCEDURES

### Animal Housing and Surgery

Male Sprague-Dawley rats (250 g; Charles River Laboratories) were individually housed with a 12:12 hr dark/light cycle. 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 experiment. Rats were allowed 1 week to acclimate to the vivarium before inducing anesthesia and implanting indwelling jugular catheters, and in some experiments, microinjection guide cannula were also implanted in the PL (surgical details in Supplemental Experimental Procedures). 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

Seven days after surgery, rats began daily 2 hr cocaine self-administration sessions, in which one response on the active lever yielded one intravenous cocaine infusion (0.2 mg/infusion, followed by a 20 s timeout period), paired with a white cue light above the active lever and a discrete tone cue. An inactive lever was also available throughout each session. After ten consecutive sessions of self-administration (greater than or equal to ten infusions a 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 ($\leq$25 active lever responses for a minimum of two sessions). Reinstatement was elicited by cues (tone plus light delivery after an active lever press).

### Microinfusion Procedures and Histology

Rats were stereotaxically implanted immediately after catheterization with bilateral guide cannulae aimed above PL (see Supplemental Experimental Procedures for surgical details). Obturators were placed into the guide cannulae and were removed during bilateral injection of 0.3 μl baclofen/muscimol cocktail (0.3/0.03 nmol, $GABA_B$/$GABA_A$ receptor agonists, respectively) over 1 min (McFarland and Kalivas, 2001). Rats were placed in the operant chamber 10 min after removal of injection cannulae and replacement of the obturators. Rats were sacrificed at various times for either dendritic spine or electrophysiological quantification. When appropriate, coronal slices (100 μm thick) of PL were mounted and stained via cresyl violet to verify guide cannulae placement (Figure S4).

### Quantification of Dendritic Spines

All dendritic spine quantification procedures have been described previously (Shen et al., 2009). Briefly, a confocal microscope was used to image DiI-labeled sections, and DiI was excited using the Helium/Neon 543 nm laser line. Images of DiI-labeled dendrites (see Figure 1C) were acquired via optical sectioning using a 63× 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. Images were deconvoluted prior to analysis, and a 3D perspective was rendered by the Surpass module of Imaris software package (Bitplane). Only spines on dendrites beginning at >75 μm and ending at $\leq$200 μm distal to the soma and after the first branch point were quantified from cells localized to the NA core (see Table S1). The length of quantified dendrites was 45–55 μm. Five to twelve neurons were analyzed from each animal, and the minimum end segment diameter (spine head) was set at $\geq$0.143 μm.

### Slice Preparation and Whole-Cell Recordings

Rats were anesthetized with ketamine and decapitated, and coronal accumbens brain slices were collected into a vial containing artificial cerebrospinal fluid (aCSF). All recordings were collected at 32°C in the dorsomedial NAcore, where the prefrontal inputs are most dense (Gorelova and Yang, 1997). Inhibitory synaptic transmission was blocked with picrotoxin (50 μM), and AMPA and NMDA currents were recorded in whole-cell patch-clamp configuration. Glass microelectrodes (1–2 MΩ) were filled with cesium-based internal solution. To evoke postsynaptic currents, we placed a bipolar stimulating electrode ~300 μm dorsomedial of the recorded cell to maximize chances of stimulating PL afferents. The stimulation intensity chosen evoked an ~50% of maximal AMPA current. Recordings were collected every 20 s and begun >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 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.

### Statistics

All spine density and $d_h$ data were statistically analyzed after 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). A/N data were analyzed using ANOVA. Behavioral data were analyzed using repeated-measures ANOVA, and t tests were used to compare $d_h$ and A/N in animals receiving aCSF or B/M. Additionally, linear regression was used to determine the association between magnitude of reinstated lever pressing and $d_h$ or A/N. Post hoc comparisons were conducted using Bonferroni-corrected t tests.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.01.005.

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