Prelimbic Cortex and Ventral Tegmental Area Modulate Synaptic Plasticity Differentially in Nucleus Accumbens During Cocaine-Reinstated Drug Seeking

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Addictive drug use causes long-lasting changes in synaptic strength and dendritic spine morphology in the nucleus accumbens that might underlie the vulnerability to relapse. Although activity in mesocorticolimbic circuitry is required for reinstating cocaine seeking, its role in reinstatement-associated synaptic plasticity is not well characterized. Using rats extinguished from cocaine self-administration, we found potentiated synaptic strength (assessed as the AMPA/NMDA current amplitude ratio) and increased spine head diameter in medium spiny neurons in the accumbens core (NAcore). The basal changes in synaptic strength and morphology in cocaine-extinguished animals were further augmented during cocaine-induced reinstatement. Two NAcore afferents contributing to cocaine reinstatement are glutamatergic inputs from the prelimbic prefrontal cortex (PL) and dopamine from the ventral tegmental area (VTA). Pharmacological inhibition of either PL or VTA prevented cocaine-primed reinstatement. However, inhibiting the PL further potentiated AMPA/NMDA and spine head diameter, while inactivating the VTA or the combined systemic administration of dopamine D1 and D2 antagonists prevented the increase in AMPA/NMDA and spine diameter induced by cocaine priming. These data indicate that neuronal activity in the VTA and associated dopamine receptor stimulation is necessary for the synaptic potentiation in the NAcore during cocaine-induced reinstatement. Although activity in the PL was necessary for reinstatement, it inhibited synaptic potentiation initiated by an acute cocaine injection. Thus, although the PL and VTA differentially regulate the direction of synaptic plasticity induced by a cocaine-priming injection, coordinated synaptic potentiation by both NAcore afferents is necessary for cocaine-induced relapse.

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INTRODUCTION

Drug addiction causes enduring functional impairments in neural adaptation, including modified neuroplasticity at glutamatergic synapses in the nucleus accumbens (Luscher and Malenka, 2011). For example, there is a loss of excitatory long-term potentiation (LTP) or depression (LTD) in medium spiny neurons (MSNs) measured *in vivo* and in brain slices containing shell or core (NAcore) of the accumbens after withdrawal from self-administered cocaine. (Kasanez *et al*, 2010; Martin *et al*, 2006; Moussawi *et al*, 2009). Changes in excitatory neuroplasticity are also revealed by probing AMPA receptor trafficking, the strength of AMPA relative to NMDA-mediated currents (A/N; Luscher and Malenka, 2011; Pierce and Wolf, 2013; Wolf and Ferrario, 2010), and dendritic spine remodeling (Robinson and Kolb,

2004; Russo *et al*, 2010; Shen *et al*, 2009) in animal models of cocaine addiction.

In addition to prefrontal cortical and allocortical glutamatergic afferents, the NAcore GABAergic MSNs receive dense dopaminergic innervation from the ventral tegmental area (VTA) (Fallon *et al*, 1978). Both glutamatergic and dopaminergic afferents to NAcore MSNs regulate various motivated behaviors thought to model aspects of drug addiction, including locomotor sensitization, conditioned place preference, intravenous self-administration, and reinstated drug seeking (Feldenstein and See, 2008; Robinson and Berridge, 2000; Sesack and Grace, 2010; Stewart *et al*, 1984; Stuber *et al*, 2011). Specifically relevant to the experiments described herein, pharmacological or optogenetic inactivation of neuronal activity in either prelimbic cortical (PL) or VTA afferents to the NAcore prevents cocaine- or cue-induced reinstatement of cocaine seeking (McFarland and Kalivas, 2001; McLaughlin and See, 2003; Stefanik *et al*, 2013a; Stefanik *et al*, 2013b; Sun *et al*, 2005).

Recently, we used whole-cell patch recordings to obtain a ratio of AMPA and NMDA glutamate currents (A/N), combined with measures of dendritic spine morphology to show that cue- or context-induced reinstatement of cocaine

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or nicotine seeking induces rapid, reversible LTP-like synaptic potentiation in glutamatergic synapses on MSNs in the NAc core that required activity in PL afferents (Gipson *et al*, 2013a; Gipson *et al*, 2013b; Stankeviciute *et al*, 2013). In addition to drug-associated cues and context, using cocaine can trigger a desire for drug use in human addicts (O'Brien and Gardner, 2005). However, cocaine-induced craving in humans and reinstated drug seeking in animals is associated with acute pharmacological drug effects, such as enduring increases in dopamine transmission, that may differentially affect reinstatement-associated neuroadaptations (Ito *et al*, 2002; Volkow *et al*, 2006). To determine whether reinstatement-associated LTP may differ between cocaine-induced reinstatement compared with cue- or context-induced reinstatement, rats were extinguished from daily cocaine self-administration and lever pressing reinstated by an acute, noncontingent injection of cocaine. We measured A/N and spine morphology in MSNs from the NAc core during cocaine-induced reinstatement and how this plasticity was regulated by neuronal activity in the VTA or prelimbic prefrontal cortex (PL). We found that, akin to cue and context, reinstating cocaine seeking with an acute cocaine injection produced transient LTP-like synaptic potentiation. We then found that pharmacological inactivation of the VTA or PL prevented cocaine-induced reinstated cocaine seeking but differentially prevented or potentiated, respectively, reinstatement-associated synaptic potentiation.

MATERIALS AND METHODS

Animals and Surgery

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. Male Sprague Dawley rats (250 g on arrival; Charles River Laboratories, Wilmington, MA) were individually housed in a temperature- and humidity-controlled environment with a 12-h dark/light cycle (1800 hours lights on). Experiments were conducted during the rats' dark cycle. Rats received food *ad libitum* until 1 day before behavioral training, after which food restriction procedures (20 g of rat chow per day) were implemented and maintained throughout the duration of the experiment. Rats were allowed 1 week to acclimate to the vivarium before inducing anesthesia and implanting jugular and intracranial cannula. The surgical details have been described in a previous study (Shen *et al*, 2011). Intracranial guide cannulas (26 gauge) were stereotactically implanted bilaterally into the PL (anteroposterior (AP), +3.2 mm; mediolateral (ML), ± 0.5 mm; dorsoventral (DV), -3.5 mm from bregma) or VTA (AP, +5.2; ML, ± 0.5 ; DV, -8.0).

Cocaine Self-Administration Procedures

All self-administration experiments occurred in standard operant chambers with two retractable levers, a house light, and a cue light and tone (78 dB, 4.5 kHz) generator (Med Associates, Fairfield, VT). During 2-h sessions on 10 consecutive days, rats were trained to press the active lever on a fixed ratio 1 schedule with 20-s timeout for an infusion of cocaine (200 μ g in 50 μ l sterile saline; cocaine hydro-

chloride was provided by the National Institute on Drug Abuse). Concurrent with the drug infusion, a cue tone and light immediately above the active lever turned on. All animals in the study met the criterion for acquisition of cocaine self-administration, which was a minimum of 10 infusions per day over three consecutive daily self-administration sessions. Then rats began extinction training. Active lever presses produced no drug infusion or light/tone cues. Animals continued under extinction conditions for a minimum of 14 days and until <15 active lever presses were made per session for 3 consecutive days. For cocaine-primed reinstatement, rats received a single injection of cocaine (10 mg/kg, ip) to initiate a reinstatement session. The reinstatement session was identical to an extinction session (ie, there was no programmed consequence of active lever pressing).

Drug Administration

A mixture (B/M) of the GABA_B agonist baclofen (0.1 nmol) and the GABA_A agonist muscimol (0.01 nmol) was dissolved in artificial cerebrospinal fluid (aCSF). Immediately before reinstatement testing, animals were microinjected bilaterally with either aCSF or B/M in a volume of 0.3 μ l per side over 60 s, and the injectors were left in place for 60 s to permit diffusion from the injection site. Five minutes later, cocaine (10 mg/kg, ip) or saline, i.p., was administered, and lever pressing was quantified. In one experiment, a combination of D1 (SCH-23390; 0.03 mg/kg, ip) and D2 (raclopride; 0.3 mg/kg, ip) dopamine receptor antagonists was administered 10 min before cocaine-induced reinstatement.

Whole-Cell Recordings in Acute Brain Slices

Rats were anesthetized with ketamine HCl (87.5 mg/kg, i.p.), and then brains were rapidly removed into ice-cold aCSF. Coronal slices (230 μ m thick) containing the nucleus accumbens were cut in ice-cold aCSF with a vibratome and incubated in 32 °C aCSF (in mM: 126 NaCl, 2.5 KCl, 1.4 NaH₂PO₄, 1.2 MgCl₂, 2.4 CaCl₂, 25 NaHCO₃, and 11 glucose) for at least 45 min before returning to room temperature. Picrotoxin (50 μ M) was added to block GABA_A receptor-mediated IPSCs during recording. Cells were visualized using infrared-differential interference contrast optics. To assess excitatory synaptic transmission, neurons were voltage clamped at -80 mV using a Multiclamp 700 A amplifier (Molecular Devices, Foster City, CA). Patch electrodes (1.5–2 M Ω) were filled with internal solution containing (in mM) 124 Cesium Methanesulfonate, 10 NaCl, 1 MgCl₂, 10 HEPES, 1 EGTA, 1 QX-314, 2 MgATP, and 0.3 MgGTP (275–285 mOsm, pH 7.2–7.4). Series resistance (<20 M Ω) and input resistance were monitored online with a 5-mV depolarizing step (100 ms) given with each stimulus. Afferents were stimulated at 0.05 Hz by a concentric bipolar electrode placed about 150 μ m dorsomedial to the recorded neurons. The intensity of stimulation was chosen to evoke a 200–300 pA EPSC. Glutamatergic afferents were electrically stimulated, and the AMPA and NMDA currents were pharmacologically isolated by bath applying the NMDAR antagonist D-AP5 (50 μ M) at $+40$ mV clamping voltage. The synaptic strength was determined by the ratio of AMPAR- to NMDAR-mediated peak current (A/N ratio).

For the measurement of AMPAR rectification, the internal solution contained 0.1 mM spermine. The rectification index was calculated from the current–voltage (I–V) curve as a ratio of the slope at negative holding potentials (–80 mV to reversal potential) divided by the slope at positive holding potentials (reversal potential to +40 mV) (Clem and Haganir, 2010). All holding potentials were corrected for liquid junction potential. Data were filtered at 2 kHz, digitized at 5 kHz, and collected and analyzed using AxoGraph X software (AxoGraph Scientific, Sydney, Australia).

Diolistic Labeling and Spine Morphology Quantification

Animals were administered an overdose of pentobarbital (100 mg/kg, i.p.) and perfused through the heart with phosphate-buffered saline (PBS) followed by 2% paraformaldehyde in PBS. MSNs in the NAcore were labeled with DiI as described in detail elsewhere (Shen *et al*, 2011). Labeled neurons were imaged using a confocal microscope, and spine density and head diameter (d_h ; diameter of the vertex at the spine terminal point) were quantified following three-dimensional rendering of the imaged neuron using the Imaris software package (Bitplane), as described in detail elsewhere (Shen *et al*, 2011). The images were deconvoluted by Autoquant (Media Cybernetics). Spine quantification commenced on dendrites beginning at >75 μ m from the soma and after the first branch point. Measurements were made out to a maximum of 200 μ m from the soma, and the length of dendrite quantified was 40–55 μ m in length. At least 12 neurons per animal were analyzed.

Statistics

All spine density and diameter data were statistically analyzed after averaging multiple dendrite segment measurements for each neuron. When two groups were compared, the data were statistically analyzed using a two-tailed unpaired Student's *t*-test. For comparing the multiple measurements in the same experiment, the data were analyzed using a one-way or two-way ANOVA. A Tukey's *post hoc* test was applied for multiple comparisons and $p < 0.05$ was considered statistically significant. All statistical tests are conducted using the SPSS (Chicago, IL) or Prism (Graphpad, La Jolla, CA) software.

RESULTS

Acute Cocaine Priming Potentiated Synaptic Strength without Changing AMPA Current Rectification

To investigate synaptic adaptations after extinction training and cocaine-induced reinstatement, animals underwent 2–3 weeks of extinction training immediately after 10 days of cocaine self-administration, and yoked saline animals were used as a control group (Figure 1a). Interestingly, although a relatively short-access paradigm (ie, daily 2-h cocaine self-administration training over 10 days), animals demonstrated escalated lever pressing for cocaine, likely due to the absence of previous food training (one-way ANOVA with repeated measures over the first 10 days, $F_{(9,558)} = 16.72$, $p < 0.001$). Figure 1b shows that an acute cocaine injection (10 mg/kg, i.p.) reinstated active lever pressing during the first 45 min of

the reinstatement session in cocaine-extinguished rats (one-way ANOVA, $F_{(4,58)} = 38.84$, $p < 0.001$). However, in the cohort of animals killed at 15 min after cocaine injection, lever pressing was not yet significantly reinstated.

Either just before or 15 min, 45 min or 24 h after acute cocaine administration, coronal brain slices were prepared, and whole-cell patch recordings were made from MSNs in the NAcore. A one-way ANOVA revealed significant differences between the treatment groups ($F_{(5,75)} = 8.52$, $p < 0.001$; Figure 1c). A Tukey's *post hoc* multiple comparison analysis revealed that the basal A/N (ie, rats killed 24 h after the last extinction session but before injecting acute cocaine) was greater in rats previously trained to self-administer cocaine compared with yoked-saline rats. Although acute cocaine injection reinstated lever pressing only in rats extinguished from cocaine self-administration, the A/N was elevated in both self-administration and yoked-saline groups at 45 min after injection. A more complete time course analysis in rats trained to self-administer cocaine revealed that acute cocaine did not increase A/N by 15 min after injection, and the increase at 45 min after injection returned to the cocaine baseline by 24 h after injection. We did not replicate previous studies showing an LTD-like decrease in A/N or AMPA surface receptor expression at 24 h after acute cocaine administration in rats withdrawn from daily cocaine administration (Boudreau *et al*, 2007; Kourrich *et al*, 2007; Rothwell *et al*, 2011). This difference may result from the fact that the other studies utilized noncontingent, daily injections of cocaine, while the present study utilized the self-administration model. Alternatively, the NAcore may not show the LTD-like changes found in the NAcshell (Kourrich and Thomas, 2009).

The increase in A/N can result from substitutions among AMPA receptor subunits (Liu and Zukin, 2007), and some studies find that the basal increase in A/N after self-administered cocaine results from an increase in GluA2-lacking, Ca^{2+} -permeable AMPA receptors in VTA dopamine neurons or accumbens MSNs (Bellone and Luscher, 2006; Conrad *et al*, 2008; McCutcheon *et al*, 2011). Therefore, we investigated whether the enhanced A/N at 45 min after acute cocaine administration was accompanied by a change in AMPA receptor subtype. Evoked EPSCs were measured using a range from –80 to +40 mV clamping voltage in a spermine-containing bath that endows GluA2-lacking AMPAR with inward rectification (Bowie and Mayer, 1995; Kamboj *et al*, 1995). We observed that neither cocaine training nor acute cocaine administration induced changes in the AMPA rectification index (two-way ANOVA, training $F_{(1,67)} = 0.15$, $p = 0.699$; priming $F_{(1,67)} = 0.044$, $p = 0.834$; interaction $F_{(1,67)} = 0.744$, $p = 0.391$; Figure 1d). It is possible that extinction training may have eliminated the enhanced inward rectification previously observed in MSNs of cocaine-trained rats withdrawn but not undergoing daily extinction training (Conrad *et al*, 2008; McCutcheon *et al*, 2011).

PL and VTA Afferents Differentially Regulate Cocaine-Reinstatement Associated Increases in A/N

Previous studies have shown that neuronal activity in the PL or VTA is necessary for cocaine-induced reinstatement (McFarland and Kalivas, 2001). Figure 2a shows that

Figure 1 Acute cocaine administration potentiated A/N without changing the AMPA receptor rectification. (a) Active lever pressing in the cocaine and yoked-saline administration/extinction training protocol used for all experiments. (b) Acute cocaine reinstates active lever pressing in cocaine-extinguished rats. Histogram shows the active lever pressing over 15 or 45 min after acute cocaine administration (mean $\pm$ SEM). Before cocaine refers to the last day of extinction pressing in rats that were killed 24 h later but before initiating a cocaine reinstatement session, and Cocaine SA refers to rats extinguished from cocaine self-administration. N shown in panel b corresponds to the number of rats assigned in electrophysiological and morphological experiments in panels c and d and Figure 3b. * $p < 0.05$ comparing active lever presses at 45 min after cocaine injection between the yoked-saline and cocaine groups using a Tukey's *post hoc*. (c) In the absence of an acute injection of cocaine, cocaine-extinguished rats exhibited higher A/N in NAc core MSNs than saline-yoked controls. Acute cocaine administration augmented the A/N in both Yoked-saline and Cocaine SA rats at 45 min after injection. * $p < 0.05$ compared with the Yoked-saline/Before cocaine group using a Tukey's *post hoc*. + $p < 0.05$, compared with Before cocaine within the Cocaine SA group. Traces illustrate the dual-component EPSC (black) and the pharmacologically isolated AMPA (red) and NMDA (green) currents from MSNs in each group. N shown in bars is the number of recorded neurons that are from 4–6 rats in each group. (d) Neither cocaine self-administration nor acute cocaine administration changed the AMPA rectification index. Traces are examples of evoked AMPA-mediated currents with holding voltage from -80 to $+40$ mV. The insert shows the current–voltage (I–V) relationship for AMPA EPSCs in each group. N shown in bars is the number of recorded neurons that are from 4–6 rats in each group. S: saline-yoked group; C: cocaine-administered group; Pre-coc: before acute cocaine; Post-coc: 45 min after acute cocaine.

inhibiting the PL or VTA after microinjecting a combination of GABA agonists (baclofen + muscimol; B/M) did not alter active lever pressing in yoked-saline rats. However, Figure 2c shows that inhibiting either the PL or VTA prevented cocaine-induced reinstatement (two-way ANOVA; interaction $F_{(3,41)} = 7.64$, $p < 0.001$). As in Figure 1, Figure 2b shows that 45 min after acute cocaine administration in yoked-saline rats pretreated with aCSF in either the VTA or PL, the A/N was elevated compared with acute saline (two-way ANOVA; interaction $F_{(2,70)} = 3.20$, $p = 0.046$). This increase was prevented by inactivation of the VTA but was unaffected by PL inactivation. VTA inactivation also prevented the increase in A/N elicited by acute cocaine in rats extinguished from cocaine self-administration (Figure 2d; two-way ANOVA; interaction $F_{(3,85)} = 5.43$, $p = 0.002$). In contrast to VTA inactivation, B/M microinjection into the PL facilitated the acute cocaine-induced A/N in rats extinguished from cocaine self-administration. The VTA is rich in dopamine neurons projecting to the NAc core (Fallon *et al*, 1978). Thus, we hypothesized that blocking dopamine receptors would mimic the effect of VTA inactivation. Figure 2c shows that, akin to VTA inactivation, animals pretreated with a combination of the D1 (SCH-23390, 0.03 mg/kg, i.p.) and D2 (raclopride,

0.3 mg/kg, i.p.) dopamine receptor antagonists 10 min before injecting cocaine prevented both reinstated cocaine seeking (Figure 2c) and the increase in A/N (Figure 2d) induced by acute cocaine administration.

Acute Cocaine Induced a VTA-Dependent Increase in Spine Head Diameter (d_h) only in Rats Extinguished from Cocaine Self-Administration

Acute cocaine-induced potentiation in A/N may be associated with a concurrent increase in d_h to accommodate newly inserted AMPA receptors (Collingridge *et al*, 2004; Lamprecht and LeDoux, 2004; McKinney, 2010). Spine density and d_h in NAc core MSNs were quantified following diolistic labeling with the lipophilic dye DiI (Figure 2a shows an example of a DiI-labeled neuron and a dendrite segment to be quantified). There was no difference between the treatment groups in spine density (Yoked-saline, Before cocaine = 2.56 ± 0.07 spines/ μ m, $N = 59$; yoked-saline, 45 min = 2.50 ± 0.07 , $N = 53$; cocaine SA, Before cocaine = 2.62 ± 0.07 , $N = 86$; cocaine SA, 45 min = 2.70 ± 0.09 , $N = 70$; cocaine SA, 24 h = 2.66 ± 0.13 , $N = 66$). However, extinction from cocaine self-administration increased d_h (Figure 3b, one-way ANOVA,

Figure 2 The cocaine-induced A/N is differentially regulated by pharmacological inactivation of the PL and VTA. (a) Previous microinjection GABA receptor agonist (B/M) into the PL or VTA did not affect active lever pressing in yoked-saline animals. N in parentheses corresponds to the number of animals used to generate the A/N data in panel b. (b) Inactivation of the VTA, but not the PL, inhibited acute cocaine-induced potentiation in A/N in yoked-saline rats. N in the bar is the number of cells recorded. (c) Inactivation of the PL or VTA or systemic administration of the dopamine antagonists SCH-23390 (SCH; 0.03 mg/kg, ip) plus raclopride (Rac; 0.3 mg/kg, ip) prevented cocaine-induced reinstatement. N in bars corresponds to the number of animals used to generate the A/N data in panel d. (d) PL inactivation facilitated but VTA inactivation or SCH/Rac injection prevented acute cocaine-induced potentiation in A/N in cocaine-extinguished rats. N in bars is the number of cells recorded. Grouped aCSF refers to a combination of control animals microinjected with aCSF in either the VTA or PL plus animals receiving systemic vehicle injections as controls for the SCH/Rac treatment. The data are shown as mean $\pm$ SEM over the 45 min after acute cocaine or vehicle administration. * $p < 0.05$ comparing the acute cocaine to acute saline within each treatment group within a given panel. + $p < 0.05$ comparing the effect of acute cocaine in the PL, VTA, or SCH/Rac groups to the Grouped aCSF group.

$F_{(1,329)} = 9.55$, $p < 0.001$). Spine head diameter was further increased in cocaine-trained subjects at 45 min after an acute cocaine injection, and d_h returned to basal levels by 24 h after acute cocaine. Interestingly, and in contrast with A/N, acute cocaine administration in yoked-saline rats did not increase d_h . The cumulative distribution plot of d_h for rats extinguished from cocaine self-administration revealed that cocaine-induced reinstatement produced a significant rightward shift in distribution that arose primarily from a significant reduction in spines with $d_h < 0.35 \mu\text{m}$ (two-way ANOVA, interaction $F_{(12,1533)} = 15.77$, $p < 0.001$).

GABA agonists were microinjected to determine whether the cocaine-induced increases in d_h depended on neuronal activity in the VTA or PL. Figure 3c shows that, akin to the increase in A/N, inhibiting activity in the VTA prevented the increase in d_h produced 45 min after acute administration of cocaine in rats extinguished from cocaine self-administration (one-way ANOVA, $F_{(2,206)} = 9.96$, $p < 0.001$). The cumulative distribution analysis showed that the reduction in mean d_h following VTA inhibition resulted from increasing the population of spines with $< 0.35 \mu\text{m}$ diameter. Although *post hoc* analysis revealed that the mean d_h was not affected by inhibiting PL, the cumulative distribution plot showed that B/M microinjection into the PL further increased d_h in spines in the range of $0.35\text{--}0.5 \mu\text{m}$ (two-way ANOVA, interaction $F_{(12,1456)} = 13.98$, $p < 0.001$). There was no difference in spine density between any treatment groups in Figure 3c (Grouped aCSF = 2.65 ± 0.11

spines/ μm , $N = 86$; B/M PL = 2.55 ± 0.06 , $N = 69$; B/M VTA = 2.42 ± 0.08 , $N = 56$).

DISCUSSION

Our data show that reinstating animals extinguished from cocaine self-administration with an acute injection of cocaine induced an LTP-like increase in A/N and d_h . The synaptic potentiation was not present at 15 min after injection but was expressed by 45 min and subsided by 24 h after injection. This time course parallels the behavioral reinstatement that had not begun by 15 min but was strongly manifested by 45 min after injection. Finally, the LTP-like plasticity was found to be dopamine dependent as it was prevented by either pharmacological inactivation of the VTA or by systemic administration of combined D1 and D2 dopamine receptor antagonists.

Association Between Behavioral Reinstatement and LTP-Like Increases in A/N and d_h

The increased A/N and d_h also resembles our recent findings that reinstating cocaine seeking with either a Pavlovian cue or the context that was associated with cocaine self-administration elicits an increase in A/N or d_h in the NAc (Gipson *et al*, 2013a; Stankeviciute *et al*, 2013). In all of these studies, the behavioral time course for

Figure 3 Cocaine reinstatement for 45 min increased d_h in MSNs in rats extinguished from cocaine self-administration (for behavioral data, see Figure 1a and b). (a) Example of neuron and dendritic segment quantified for spine morphology. (b) Left, Cocaine SA rats showed a basal increase in mean d_h compared with Yoked-saline animals, and acute cocaine administration further increased d_h only in Cocaine SA rats. Data are shown as mean $\pm$ SEM. N = number of neurons quantified shown in each bar, obtained from 4–6 rats. Right, the cumulative frequency distribution of spine d_h showing shift to the right 45 min after acute cocaine administration in cocaine-extinguished rats. (c) Left, inactivation of the VTA, but not the PL, prevented the increase in d_h at 45 min after acute cocaine administration in Cocaine SA rats. Right, cumulative distribution plots of d_h demonstrate a shift to the left by inactivation of the VTA and to the right by inactivating the PL compared with the distribution induced by acute cocaine administration in aCSF-microinjected, Cocaine SA rats. Grouped aCSF refers to the pooling of data from animals microinjected with aCSF into either the VTA and PL. $^+p < 0.05$ comparing basal d_h (Before cocaine) between Yoked-saline and Cocaine SA. $*p < 0.05$ compared with basal d_h in Cocaine SA rats.

reinstated behavior paralleled the LTP-like changes. Thus, behavior, A/N, and d_h were all maximal for cue- and context-reinstated behavior at 15 min, while cocaine-induced reinstatement and synaptic potentiation was not present at 15 min but was manifested by 45 min after injection. A similar parallel time course between behavior synaptic potentiation was also measured after cue-induced reinstatement of nicotine seeking (Gipson *et al*, 2013b) and heroin-induced heroin seeking (Shen *et al*, 2011). Taken together, these findings support an argument that regardless of the chemical drug class or modality of stimulus, reinstating operant responding for addictive drugs induces an LTP-like synaptic potentiation at excitatory synapses on MSNs in the NAc.

Acute Cocaine Administration in Control Animals Produces Synaptic Potentiation

Surprisingly, when cocaine was acutely administered to yoked-saline animals there was an equivalent increase in A/N as was elicited in animals extinguished from cocaine self-administration. This is distinct from studies with cue-induced reinstatement where presentation of cocaine- or nicotine-conditioned cues or an injection of heroin to yoked-saline animals did not alter A/N or d_h (Gipson *et al*, 2013a; Shen *et al*, 2011; Stankeviciute *et al*, 2013). Also,

acute cocaine administration in yoked-saline animals did not increase surface expression of GluA1 in the NAc (Anderson *et al*, 2008). However, it is possible that elevated A/N could result from a movement of receptors from the synaptic annulus into the synaptic cleft, which need not require increased surface AMPA receptors. It seems likely that the difference between cue- or heroin-reinstatement and cocaine-induced reinstatement may be related to the more robust increase in dopamine transmission produced in the accumbens by an acute cocaine injection relative to presentation of a reward-associated Pavlovian cue or heroin (Hemby *et al*, 1995; Ikegami *et al*, 2007; Ito *et al*, 2000). Work by Wolf and colleagues demonstrates that D1 receptor stimulation increases in GluA1-containing AMPA receptor surface expression *in vivo* or in cultured neurons, regardless of whether animals have been pretreated with cocaine (Chao *et al*, 2002; Gao *et al*, 2006; Mangiavacchi and Wolf, 2004; Sun *et al*, 2008). However, the same group reported that acute stimulation of D2 dopamine receptors increases endocytosis of accumbens GluA1 and GluA2 receptors (Ferrario *et al*, 2011). In future studies, it will be of interest to use mice selectively expressing D1 or D2 marker proteins to determine whether cocaine increases in dopamine transmission may differentially regulate these two distinct populations of MSN; although differential

changes in two roughly equivalent populations was not indicated by the normal distribution of A/N in our experiments.

It is important to consider that, in addition to affecting afferents or receptors in the NAc, pharmacological inhibition in the VTA and PL, or systemic administration of dopamine antagonists, will also modulate other brain regions that can indirectly affect NAc synaptic transmission. Although optogenetic inactivation of the PL and VTA projections to the NAc supports a specific role for NAc innervation by these afferents in reinstated cocaine seeking (Stefanik *et al*, 2013a; Stefanik *et al*, 2013b), similar optogenetic studies are needed to prove that the effects found in the present study on synaptic plasticity are as selectively regulated. Another consideration is that cocaine blocks serotonin and norepinephrine transport (Ritz *et al*, 1990), and increasing levels of these transmitters may contribute to our findings. However, the fact that inhibiting dopamine cells in the VTA prevented cocaine-induced synaptic plasticity in NAc supports a primary action on dopamine transmission.

Although all of these data are generally consistent with acute cocaine producing a dopamine-dependent increase in A/N regardless of the animals previous exposure to cocaine, it is interesting that an increase in d_h by acute cocaine occurred only in rats previously trained to self-administer cocaine. Similarly, it was previously found that d_h decreased in parallel with cue-reinstated lever pressing at 45 min after initiating the reinstatement session, while the cue-induced increase in A/N endured for up to 120 min (Gipson *et al*, 2013a). Also, the increase in d_h produced during heroin-induced reinstatement transiently decreased with behavior by 120 min after injection, while an LTP-like increase in field potential amplitude persisted for nearly 180 min (Shen *et al*, 2011). Although many studies have shown parallel changes in AMPA currents and spine morphology during LTP and LTD (Gu *et al*, 2010; Lang *et al*, 2004; Passafaro *et al*, 2003; Zhou *et al*, 2004), other studies find that the signaling mechanisms underlying increased AMPA signaling and spine enlargement can diverge (Fukazawa *et al*, 2003; Lu *et al*, 2013). Thus, it is conceivable that D1 receptor stimulation could increase synaptic AMPA signaling without altering spine morphology while reinstating drug seeking, regardless of whether initiated by cue, context, heroin, or cocaine increases both A/N and d_h .

Role for the PL and VTA in Regulating Reinstatement and the Associated LTP-Like Synaptic Plasticity

Neuronal activity in PL glutamatergic afferents to the NAc is necessary to induce reinstatement by cocaine or cues (Park *et al*, 2002; Stefanik *et al*, 2013b). Reinstatement and accumbens synaptic plasticity have also been shown to depend on the activity of neurons in the VTA (Mameli *et al*, 2009; McFarland and Kalivas, 2001). Consistent with these findings, we showed that pharmacological inactivation of either the VTA or PL blocked cocaine-induced reinstatement of active lever pressing. Directly supporting the involvement of dopamine transmission, simultaneous antagonism of D1 and D2 dopamine receptors with systemically administered SCH-23390 and raclopride also prevented cocaine-induced reinstatement. In contrast to

reinstatement, the capacity of VTA or PL inactivation to prevent the LTP-like changes induced by acute cocaine administration diverged. Consistent with dependence on dopamine transmission, VTA inhibition prevented acute cocaine-induced A/N and d_h in yoked-saline and cocaine self-administration rats. However, PL inactivation potentiated cocaine-induced increases in A/N and d_h in rats extinguished from cocaine self-administration and was without effect on the increase in A/N produced by acute cocaine in yoked-saline animals. This demonstrates that the LTP-like plasticity is necessary, but not sufficient, to induce reinstatement. Furthermore, PL and VTA differentially coordinate the potentiation of synaptic strength in the NAc induced by a cocaine priming injection in a manner that is necessary to reinstate behavior. The potentiation by PL inactivation is a surprising contrast to our previous report in which PL inactivation prevented cue-induced increases in A/N and d_h (Gipson *et al*, 2013a). These contradictory findings regarding how PL inactivation alters cue- vs cocaine-induced synaptic potentiation indicates that the role of the PL in regulating reinstatement-associated plasticity may be state-dependent.

Neuroimaging studies reveal that activation of human anterior cingulate (homologue of the rat PL) is associated with a diverse range of tasks involving monitoring and determining the dynamic value of behavior, as well as in selecting appropriate behavioral responses (Bush *et al*, 2000; Goldstein *et al*, 2009; Walton *et al*, 2007). Thus, PL activation can yield seemingly contradictory results when applied to different models of addiction depending on whether responding or not responding is the most adaptive response. For example, optogenetically inhibiting the PL promotes cocaine seeking in model of compulsive operant responding when cocaine delivery is associated with punishment, because the PL is important for devaluing cocaine in the presence of punishment (Chen *et al*, 2013). In contrast, optogenetic or pharmacological inhibition of the PL prevents reinstated cocaine seeking where the PL is necessary for recognizing the value of the cocaine-conditioned cues (McFarland and Kalivas, 2001; Stefanik *et al*, 2013b). Interestingly, both the Chen *et al* (2013) study and the present data indicate that neuronal activity in PL inhibits the effects of cocaine-induced dopamine release, in the former devaluing cocaine reinforcement relative to punishment and in our study reducing acute cocaine-induced, dopamine-dependent synaptic potentiation in NAc MSNs. Together, these studies indicate that PL activity may not only serve to limit the physiological impact of cocaine-induced dopamine transmission in the accumbens to reinforce behavior and induce synaptic potentiation but can also serve to signal cocaine seeking in response to a drug-associated stimulus. Finally, PL inhibition did not alter the increase in A/N produced in yoked-saline animals by acute cocaine administration, indicating that involvement of the PL in regulating cocaine-dependent synaptic plasticity occurs only after repeated cocaine experience.

CONCLUSIONS

Our observations add to a wide literature showing that environmental stimuli induce dendritic spine remodeling

and AMPA receptor trafficking (Fu *et al*, 2012; Holtmaat and Svoboda, 2009; Kessels and Malinow, 2009; Lai *et al*, 2012). In the case of synaptic plasticity elicited by acute cocaine on MSNs in the NAc, we found that cocaine-induced changes in A/N and d_h depend on dopamine transmission. Given that synaptic potentiation occurs in response to a variety of stimuli that reinstate operant responding and in animals trained to self-administer many addictive drugs, including cocaine, nicotine, or heroin (Anderson *et al*, 2008; Gipson *et al*, 2013a; Gipson *et al*, 2013b; Shen *et al*, 2011; Stankeviciute *et al*, 2013), this transient plasticity seems necessary, although not sufficient (see above), for reinstatement to occur. Thus, discerning the cellular processes that uniquely underlie reinstatement-associated synaptic plasticity could be a fruitful avenue of drug development for treating relapse in drug addiction.

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