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Prolonged withdrawal from cocaine self-administration affects prefrontal cortex- and basolateral amygdala-nucleus accumbens core circuits but not accumbens GABAergic local interneurons

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Abstract

Withdrawal from extended-access cocaine self-administration leads to progressive intensification ('incubation') of cocaine craving. After prolonged withdrawal (1–2 months), when craving is high, expression of incubation depends on strengthening of excitatory inputs to medium spiny neurons (MSN) of the nucleus accumbens (NAc). These excitatory inputs interact with the intra-NAc GABAergic 'microcircuit', composed of MSN axon collaterals and GABAergic interneurons. Here, we investigated whether the increased glutamatergic neurotransmission observed after prolonged withdrawal is accompanied by altered GABAergic neurotransmission, focusing on NAc core. Rats self-administered cocaine or saline (6 h/day) and then underwent >40 days of withdrawal. First, we investigated parvalbumin positive (PV+) interneurons, GABAergic fast-spiking interneurons that regulate MSN activity. Immunohistochemical studies revealed no significant change in PV signal intensity or the number of PV+ cells in cocaine rats versus saline controls. We then screened PV and other interneuron markers using immunoblotting. We detected no changes in levels of PV, calretinin, calbindin, or neuronal nitric oxide synthase. Since expression of these markers is activity-dependent, our results suggest no marked changes in interneuron activity. Finally, we utilized local field potential recording, which can detect GABA-mediated alterations at the circuit level, to investigate potential changes in two circuits implicated in cocaine craving: prelimbic prefrontal cortex to NAc core and basolateral amygdala to NAc core. We detected differential adaptations in these circuits, some of which may involve GABA. Overall, our results suggest that alterations in GABA transmission may accompany incubation of cocaine craving, but they are circuit-specific and less pronounced than alterations in glutamate transmission.

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Authors contribution

Purgianto and Wolf designed experiments, conducted data analysis and prepared the manuscript. Purgianto conducted electrophysiological and immunohistochemistry experiments, with assistance from Weinfeld. Purgianto conducted immunoblotting experiments. All authors reviewed the content and approved the final version of the manuscript.

Keywords

Cocaine self-administration; GABA; Incubation of craving; Interneurons; Local field potential recording; Nucleus accumbens

Introduction

The nucleus accumbens (NAc), a critical structure for cocaine addiction, is composed mainly (~95%) of medium spiny neurons (MSN), which are GABAergic projection neurons that send both intra-NAc axon collaterals and efferent projections to regions outside the NAc (Meredith, 1999). The other ~5% of NAc neurons are interneurons, which are primarily GABAergic but include cholinergic neurons as well. The GABAergic ‘microcircuit’, composed of MSN axon collaterals and GABAergic interneurons, plays a critical role in regulating striatal function (Tepper et al., 2004; Wilson, 2007; Silberberg & Bolam, 2015).

The GABAergic interneurons can be classified based on protein expression and electrophysiological properties into the parvalbumin-positive (PV+) fast spiking interneurons (FSI), calretinin-positive (CR+) interneurons, or calbindin-positive (CB+) interneurons that express neuropeptide Y (NPY+) (Hussain et al., 1996; Hussain & Totterdell, 1994; Figueredo-Cardenas et al., 1996; Tepper et al., 2010). NPY+ interneurons can be further subdivided into somatostatin (SOM) and nitric oxide synthase (NOS) positive (NPY/SOM/NOS+), also referred to as low-threshold spiking (LTS) interneurons, or neurogliaform (NPY-NGF) interneurons (Ibanez-Sandoval et al., 2011). There is also a subset of fast-spiking interneurons in the NAc that expresses the cannabinoid 1 receptor (CB1R; Winters et al., 2012). PV, CR, and CB are calcium-binding proteins and their expression defines the functional characteristics of the interneurons (Schwaller, 2010).

A wealth of knowledge has been generated about adaptations in NAc glutamatergic transmission in animal models of cocaine addiction (Wolf & Tseng, 2012). These adaptations vary according to the cocaine regimen (e.g., Purgianto et al., 2013). In the ‘incubation of craving’ model, cue-induced cocaine craving progressively intensifies (incubates) over the first month of withdrawal from extended-access cocaine self-administration and then remains high for several more months (Pickens et al., 2011). Incubation also occurs in human drug users and is potentially important for maintaining persistent vulnerability to cue-induced relapse (Wolf, 2016). We are particularly interested in neuroadaptations that maintain high levels of craving after incubation has plateaued. During prolonged withdrawal (30–90 days), we have documented stable elevation of calcium-permeable AMPA receptors (CP-AMPA) in NAc core synapses (Wolf & Tseng, 2012). This increases responsiveness of MSN to excitatory drive (Purgianto et al., 2013) and is necessary for the expression of incubated craving (Wolf, 2016).

Despite the importance of the GABA microcircuit, studies of cocaine’s effects on GABA transmission in the NAc lag behind studies of glutamate and dopamine. While the majority of such studies have evaluated effects of non-contingent cocaine (e.g., Maguire et al., 2014), evidence is emerging to indicate changes in GABA release (Wydra et al., 2013) and GABAergic synaptic transmission (Otaka et al., 2013) in the NAc following cocaine self-

administration. To our knowledge, however, no studies have examined the effect of abstinence following extended-access cocaine self-administration on GABA function in the NAc. Since the NAc GABAergic microcircuit can influence the response to incoming stimuli (Pennartz & Kitai, 1991; Koos & Tepper, 1999; Gruber et al., 2009; Gittis et al., 2010), we are interested in whether changes in this microcircuit accompany the increased excitatory drive that can be demonstrated in the NAc after prolonged withdrawal from cocaine self-administration (see above).

We took two approaches to investigating this question. First, we assessed interneuron activity using marker proteins whose expression is activity-dependent (see below). A main question was whether the activity of PV+ interneurons is altered after incubation of craving. Anatomical studies have shown that these neurons receive cortical input and synapse with MSN (e.g., Bennett & Bolam, 1994). Confirming anatomical results, electrophysiological studies show that PV+ interneurons elicit inhibitory postsynaptic potentials in MSN (Taverna et al., 2007) and play a major role in the GABA microcircuit (Koos & Tepper, 1999; Gruber et al., 2009). Furthermore, immunohistochemical studies have shown that they are affected by non-contingent cocaine exposure (Todtenkopf et al., 2004). The level of PV expression correlates with the activity of these interneurons (Behrens et al., 2007; Kinney et al., 2006). Therefore, we used immunohistochemistry to measure PV immunoreactivity in the NAc core and thereby assess possible changes in activity of PV+ interneurons after prolonged cocaine withdrawal. We also used immunoblotting to measure expression of PV, CR, CB and nNOS in the core. Expression of these proteins is also modulated by the activity of the interneuron (Zettel et al., 2001; Philpot et al., 1997; Marty & Onteniente, 1997; Chaudhury et al., 2008; Aguila et al., 2011).

As another way to assess the GABA microcircuit, we utilized a local field potential (LFP) recording protocol that has been previously used to distinguish altered GABA components at a circuit level (Thomases et al., 2013; Cass et al., 2013; Jayasinghe et al., 2015). We focused on two circuits that are implicated in cue-induced cocaine craving after prolonged withdrawal (Wolf, 2016): prelimbic (PL) medial prefrontal cortex (mPFC) to NAc core and basolateral amygdala (BLA) to NAc core. To investigate whether these circuits have GABA components that are altered after cocaine craving has 'incubated', we recorded the LFP response of the NAc core after single and train stimulation of the mPFC or BLA.

Materials & Methods

Cocaine self-administration

All procedures have been described previously (Purgianto et al., 2013). Adult male Sprague-Dawley rats (250–275 g on arrival; Harlan, Indianapolis, IN) were maintained on a reverse light cycle with food and water available *ad libitum*. After a week to acclimate, a jugular catheter (PlasticsOne, Roanoke, VA) was implanted under ketamine-xylazine anesthesia (80–10mg/kg, i.p., respectively). Rats also received the analgesic flunixin meglumine (2mg/kg, s.c.; Henry Schein, Melville, NY) before surgery. After surgery, rats were singly housed. Immediately after surgery and each day during 5–7 days of recovery, intravenous antibiotic was administered (Cefazolin; 100mg/ml, 0.15ml; Moore Medical, Farmington, CT) and the catheters were flushed with sterile saline solution to ensure patency. After

recovery, rats began self-administration training (during dark cycle) in operant chambers (MED Associates, St Albans, VT). Nose-poking in the inactive hole had no consequences. Nose-poking in the active hole delivered an infusion of saline or cocaine (0.5 mg/kg in a 100 μ l/kg volume over 3 s), paired with a 20 s light cue inside the nose-poke hole. All procedures were performed according to the USPHS Guide for Care and Use of Laboratory Animals, and were approved by our Institutional Animal Care and Use Committee.

Immunocytochemistry

Methods were adapted from those described previously (Cass et al., 2013). After 52–60 days of withdrawal, rats were perfused with 150ml of ice-cold 0.9% saline followed by 150ml of ice-cold 4% paraformaldehyde (PFA). Brains were fixed in 4% PFA for 24 h and then switched to 30% sucrose. After >3 days, the brains were sliced at 50 μ m thickness and stored in 0.1% azide solution. Ten sections of the NAc (100 μ m apart) were sampled per animal. Sections were mounted on Superfrost plus slides (VWR, Radnor, PA). They were treated with 1% glycine (1h), blocked with 5% normal donkey serum (1h; Jackson ImmunoResearch, West Grove, PA), and incubated with 1° anti-PV antibody overnight (1:2500; Swant, Switzerland). They were then incubated with anti-rabbit Alexa-488 antibody (1:500; Invitrogen, Waltham, MA). Slides were treated with Fluoromount (F4680; Sigma-Aldrich, St. Louis, MO) and cover-slipped. Digital images were taken under 20x magnification with a Nikon E400 microscope (Nikon Instruments, Tokyo, Japan) connected to an ORCA-AG deep-cooled digital camera (Hamamatsu, Shizuoka, Japan). We analyzed the number of PV+ cells and the intensity of PV immunofluorescence at three rostral-to-caudal levels of the NAc (from Bregma: $\sim$ +2.0mm to +1.8mm; $\sim$ +1.7mm to +1.5mm; $\sim$ +1.4mm to +1.1mm). Cell counting was done manually by two observers (one was blinded to experimental condition) using ImageJ software (NIH, Bethesda, MD) and the numbers averaged. For each section, all PV+ cells in the NAc core were counted and the numbers were averaged between left and right sides. To obtain the average for the entire NAc core, numbers for all sections were averaged (i.e., from Bregma: +2.0mm to +1.1mm). Immunofluorescence intensity was quantified using the same images and software and corrected by subtracting the background immunofluorescence signal defined using the anterior commissure. The volume of the NAc is not affected by our cocaine regimen (Li et al., 2013), indicating that quantification of PV staining in the two groups was not influenced by volume changes.

SDS-PAGE and immunoblotting

Animals were decapitated after 48 days of withdrawal and brains were rapidly removed. The NAc (mainly core, but including a portion of lateral shell) was punched from a 2 mm coronal section obtained using a brain matrix. Tissue was processed for SDS-PAGE and immunoblotting as described previously (Conrad et al., 2008). Using the SNAP-ID 2.0 protein detection system (EMD Millipore, Billerica, MA), PVDF membranes were blocked in 0.5% non-fat milk and 1% goat serum in TBS-Tween20 (TBS-T) for 10 min, followed by incubation with primary antibodies to parvalbumin (1:4000; PV25; Swant), calretinin (1:8000; CR7699/3H; Swant), calbindin (1:5000; CB38; Swant), or nNOS (1:160; ab2801; ABCAM, Cambridge, UK) for 30 min. Membranes were then washed 4 times with TBS-T, followed by incubation with secondary antibodies (HRP-conjugated anti-rabbit or anti-

mouse; 1:3000; Invitrogen, Carlsbad, CA). Membranes were then washed 4 times with TBS-T and immersed in chemiluminescence (ECL) detecting substrate (GE Healthcare, Piscataway, NJ). Images were acquired with an Amersham Imager 600 (GE Healthcare) and quantified with TotalLab software (TotalLab, Newcastle, UK). Data were excluded if there were problems that interfered with band analysis such as bubbles. Diffuse densities for bands of interest were normalized to either total protein in the lane as determined by Ponceau staining (P7170-1L; Sigma-Aldrich) or a loading control (GAPDH; CB1001; EMD Millipore)

Local field potential recording

Electrophysiological experiments were performed as described previously (Cass et al., 2013; Thomases et al., 2013; Jayasinghe et al., 2015). Briefly, after 40–80 days of withdrawal, rats were anesthetized using chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). A recording electrode (SNE-100 × 50mm; Rhodes Medical Instrument, Santa Barbara, CA) was lowered into the NAc (from Bregma at 12° angle: AP +1.4mm; ML +3.0mm; DV -7.0mm). Signals from the recording electrode were amplified (500x; ER-1; Cygnus Technology, Delaware Water Gap, PA), filtered (1–100Hz bandwidth), and digitized (Digidata 1440A; Molecular Devices, Sunnyvale, CA). A stimulating electrode (NE-100 × 50mm; Rhodes Medical Instrument) was lowered into the mPFC (from Bregma: AP +2.7mm; ML -0.7mm; DV -4.5mm) or the BLA (from Bregma: AP -2.7mm; ML +4.8mm; DV -8.2mm) ipsilateral to the recording electrode. The stimulating electrode was connected to a DC power source and regulated using a computer-controlled pulse generator (Master 8; AMPI, Jerusalem, Israel). For all stimulations, the duration for each pulse was 300μs. Fifteen minutes after both electrodes were in place, single stimulation at 0.25, 0.50, 0.75, or 1.00mA was delivered to measure the input/output (I/O) curve. Each intensity was repeated 10–15 times with a 15s inter-pulse interval. Train stimulation was delivered at 5, 10, 20, and 40Hz with intensity between 0.50–0.65mA; each train consisted of 10 pulses and each frequency was repeated 15–20 times. Patterns of stimulation intensity and train stimulation were counter-balanced (i.e., one experiment starts with 5Hz and progresses to 40Hz, while the next would be 40Hz to 5Hz). Traces were analyzed off-line using Clampfit software. Changes in voltage were measured from the onset to the peak amplitude of the evoked response. For microinfusion experiments, a cannula was placed immediately adjacent to and 1mm ventral to the tip of the recording electrode. Artificial cerebrospinal fluid (aCSF) containing 100uM picrotoxin (Sigma-Aldrich) or vehicle (0.1% DMSO) was infused at a rate of 0.1μL/min for 10 min (1μL total volume of infusion). Baseline recordings were obtained for 24 min, followed by I/O and train stimulation protocols described above.

Statistical analysis

Results are expressed as mean ± SEM. Two-tail unpaired t-tests were used to assess group differences (cocaine vs. saline) in overall PV immunoreactivity, PV+ cell count, and immunoblotting experiments. Differences between NAc subregions with respect to PV immunoreactivity and PV+ cell count were analyzed using two-way ANOVA. Group differences in I/O and train stimulation experiments were analyzed using two-way ANOVA.

Results

For all studies, rats self-administered cocaine or saline (6 h/day for 10 days) and were killed at times ranging from withdrawal day (WD) 40 to WD80. This is a period of withdrawal in which cue-induced cocaine craving is maximal (Pickens et al., 2011) and the underlying enhancement of glutamate transmission in the NAc is stably expressed (Wolf and Tseng, 2012).

Our first study focused on PV+ interneurons, whose role in regulating NAc activity has been described in the Introduction. Based on the activity-dependent nature of PV expression, we assessed the activity level of PV+ interneurons by measuring PV immunoreactivity using immunohistochemistry. This approach has been used to detect cocaine-induced alterations in PV+ interneurons in the mPFC (Cass et al., 2013). Rats were perfused on WD56–60 and slices were prepared that encompassed the entire rostral-caudal extent of the NAc (see Methods). When we assessed the NAc as a whole, by combining data from all of these sections, we found no difference between cocaine and saline groups with respect to PV immunostaining intensity, suggesting that prolonged withdrawal from cocaine self-administration does not alter PV+ interneuron activity (Fig. 1a,b). When we sub-divided the NAc into three rostral-to-caudal levels, we also failed to observe group differences in PV immunostaining intensity (Fig. 1c–e). Furthermore, there was no significant difference between cocaine and saline groups in the number of PV+ cells in the NAc as a whole (Fig. 2a) or in each sublevel (Fig. 2b–d), although a trend towards an increase was observed in the cocaine group when data from all sublevels were combined (see legend to Fig. 2).

To assess the expression of other interneuron-specific proteins, and to confirm our immunohistochemical data for PV, separate groups of saline and cocaine rats were generated and killed on WD48. Immunoblotting was used to measure protein levels of PV, CR, CB, and nNOS in the NAc. Consistent with our immunohistochemistry results, the cocaine group did not show alterations in PV expression (Fig. 3a); expression of CR, CB, and nNOS was also unaffected (Fig. 3b–d).

As an alternative way to test for cocaine-induced alterations in GABA transmission, we performed LFP recordings in two circuits implicated in cue-induced cocaine craving (see Introduction). Different types of striatal interneurons are preferentially engaged by different patterns of cortical stimulation (Beatty et al., 2015); therefore, an alteration at a specific frequency may suggest an alteration of a specific group of neurons. For example, in striatum, PV+ interneurons generate action potentials primarily at input frequencies in the gamma range, so a difference between groups when train stimulations are conducted at 40 Hz may be suggestive of altered activity of PV+ interneurons; changes in the delta range (1–5 Hz) and beta range (10–30 Hz) may indicate effects on cholinergic and LTS interneurons, respectively (Beatty et al., 2015; Jayasinghe et al., 2015).

We first investigated the mPFC-NAc core pathway, by recording LFP in NAc core after stimulation in the PL portion of the mPFC (Fig. 4b). We detected no alteration in the I/O curve (Fig. 4c), suggesting no alteration in basal synaptic strength. We also analyzed the NAc LFP response after several different frequencies of train stimulation. We observed a

potentiation of the LFP, relative to the first pulse in the train, in response to 5 Hz and 10 Hz train stimulations that was of similar magnitude in the cocaine and saline groups (Fig. 4d, e). Potentiation of the NAc LFP response was also observed after train stimulation at 20 Hz, but at this frequency the magnitude of the potentiation was significantly reduced in cocaine animals compared to saline animals (Fig. 4f). Stimulation at 40Hz frequency led to a depression of the NAc response that was equal in magnitude between cocaine and saline groups (Fig. 4g). For all the train stimulation experiments described above, the baseline intensity (i.e., raw LFP magnitude of the first pulse) was similar between saline and cocaine groups; therefore, ceiling or floor effects can be ruled out.

The lack of between-group difference at 40 Hz suggests that PV+ interneurons were not altered by prolonged cocaine withdrawal, consistent with our immunohistochemistry and immunoblotting data. However, we were still interested to explore whether the difference we observed at 20Hz involved GABA neurotransmission. To test this possibility, we infused picrotoxin (100 μ M) or vehicle (0.1% DMSO) into the NAc adjacent to the recording electrode and repeated the stimulation protocols. Using single stimulation at different frequencies to determine the I/O relationship, we found that neither DMSO nor picrotoxin injection significantly altered basal synaptic responsiveness in the mPFC-NAc circuit (Supp. Fig. 1, $p>0.05$). Unfortunately, in train stimulation experiments, we observed a vehicle effect at 20 Hz and 40 Hz frequencies, which precluded clear interpretation of picrotoxin effects at those frequencies (Supp. Fig. 2c,d,g,h). Interestingly, at lower frequencies where no vehicle effect was observed (5 and 10 Hz), we observed a reduction in the LFP response after picrotoxin infusion in the saline group (Supp. Fig. 2a,b). This picrotoxin-induced reduction of the LFP response was not seen in the cocaine group (Supp. Fig. 2e,f), potentially suggesting that the lower frequency train stimulations recruited a GABA component in the saline group but not the cocaine group (see Discussion).

For the BLA-NAc core circuit, we did not observe a significant difference in I/O curve between the cocaine and saline groups (Fig. 5c). Train stimulation at 5Hz did not elicit potentiation or depression of the NAc LFP relative to the first pulse (Fig. 5d). Train stimulation at 10Hz resulted in a significantly greater LFP response in the cocaine group compared to saline controls, although neither group exhibited significant potentiation relative to the first pulse (Fig. 5e). In contrast to the effect seen in the mPFC-NAc circuit, 20Hz train stimulation in the BLA led to depression of the NAc LFP with no difference observed between saline and cocaine groups (Fig. 5f). Finally, 40Hz stimulation resulted in depression of in the BLA due to the observation of vehicle effects after picrotoxin infusion into the mPFC (Supp. Fig. 2c,d,g,h).

Discussion

In this study, we explored whether the persistent enhancement in AMPAR transmission that occur after prolonged withdrawal from extended-access cocaine self-administration is accompanied by alteration in the pre-synaptic component of the GABA microcircuit. We are interested in understanding whether GABA interneurons participate in maladaptive changes that lead to craving or in compensatory mechanisms that limit craving.

Immunohistochemical and immunoblotting studies

Our immunohistochemistry and immunoblotting studies found no significant alteration in the expression of PV, an indirect measure of the activity of PV+ interneurons, after >40 days of cocaine withdrawal. While we did observe a trend towards an increase in the number of PV+ interneurons when results from the entire NAc were combined ($p = 0.07$), this trend was less evident at individual rostral-caudal levels. Taken together, these results argue against a cocaine-induced change in PV+ interneurons, although we cannot rule out this possibility altogether. Previously, others have documented cocaine-induced alterations in PV immunoreactivity and the number of PV+ cells in the mPFC (Cass et al., 2013) and dorsal striatum (Todtenkopf et al., 2004), but these studies used non-contingent cocaine exposure and short withdrawal times. Different results in our study could reflect our use of contingent cocaine exposure (see Introduction) or the age of the rats. Thus, results of Cass et al. (2013) suggest that alterations in GABA neurotransmission, especially those mediated by PV+ interneurons, are more likely to occur during adolescence. In our adult rats, the GABA system may be less plastic; this could contribute to an imbalance towards plasticity of excitatory transmission after prolonged cocaine withdrawal. In addition, we acknowledge that there are other structures in the NAc that are PV+, such as the axon terminals of GABAergic afferents from the ventral pallidum (Kuo & Chang, 1992). Given the sparse amount of PV+ interneurons in the NAc, it is possible that the background PV immunoreactivity from these structures may mask any alterations in PV immunoreactivity expressed by interneurons. Finally, results obtained in the NAc shell after non-contingent cocaine exposure leave open the possibility of changes in a subset of fast-spiking interneurons that expresses the CB1R (Winters et al., 2012).

The expression of other interneuron-specific proteins, CR and CB, was also not altered after incubation of cocaine craving. Likewise, we detected no change in expression of nNOS in the NAc, although we note that its activity has been implicated in neuroadaptations produced by non-contingent cocaine exposure (e.g., Nasif et al., 2011; Selvakumar et al., 2014). Taken together with results on PV expression, our findings suggest that prolonged withdrawal from cocaine self-administration has minimal effect on GABAergic interneurons. It is still possible, however, that the GABAergic microcircuit is altered by the component we did not explore: changes in GABA release from MSN axon collaterals. It is also important to note that our method of assessing interneuron activity was indirect. This is because the low number of each type of interneuron makes it very challenging to record their activity using electrophysiological methods.

Circuit level analysis using LFP recordings

LFP recordings were used to investigate possible modulation by GABA of the NAc response to incoming stimulation. This technique has proven effective in teasing out GABA components in other brain areas, and demonstrating changes in the GABA component after experimental manipulations (Thomases et al., 2013; Cass et al., 2013; Jayasinghe et al., 2015).

In experiments in which we applied single pulse stimulation to the PL mPFC and recorded the LFP response in NAc core, we found no change in basal synaptic strength (I/O curve) in

this circuit. Previously, in studies that did not distinguish between specific glutamate inputs to NAc MSN, we observed an increase in CP-AMPA contribution to synaptic transmission in NAc core MSN from rats subjected to the same cocaine regimen and withdrawal time (Conrad et al., 2008), leading to a greater response of MSN to excitatory drive (Purgianto et al., 2013). Together, these results may suggest that CP-AMPA receptors are not inserted postsynaptic to glutamate projections originating from the PL mPFC, but rather postsynaptic to projections originating from different regions. In the only study to examine specific pathways into the NAc core after a regimen leading to incubation of cocaine craving, Ma et al. (2014) observed an increase in silent synapses in the PL mPFC to NAc core pathway during early withdrawal; however, by WD45, these synapses were unsilenced through addition of GluA2-containing AMPARs not CP-AMPA receptors. Whether this resulted in a strengthening of this pathway relative to the pre-cocaine baseline could not be determined from their design.

The NAc LFP response to train stimulation of the PL mPFC was not different between saline and cocaine groups, except at the 20 Hz train frequency, potentially suggesting differential engagement of LTS interneurons (Beatty et al., 2015). We attempted to determine if the difference observed at 20 Hz was due to a difference in GABA transmission by repeating the experiment in the presence of picrotoxin (Supp. Fig. 1). This strategy has previously revealed that differences in GABA transmission underlie differential responding of striatal neurons from control and 6-hydroxydopamine lesioned rats in response to 10, 20 and 40 Hz stimulation (Jayasinghe et al., 2015). Unfortunately, at higher frequency train stimulation (20 and 40 Hz), but not lower frequency (5 or 10 Hz), the vehicle used to dissolve the picrotoxin produced its own effect on the LFP. Therefore, we were not able to interpret the results of picrotoxin infusion at the higher frequencies. Interestingly, we found that picrotoxin attenuated potentiation of the NAc LFP during 5 Hz and 10 Hz trains in the saline group. This suggests that a GABA component is recruited after PL stimulation at lower frequencies. In contrast, picrotoxin had no effect on the response to 5 Hz and 10 Hz train stimulation in the cocaine group, suggesting lack of recruitment of a GABA component. The source of alteration in GABA function between saline and cocaine groups during 5 and 10 Hz train stimulation remains unknown. The present results on expression levels of interneuron markers argue against changes in interneuron activity (Figs. 1–3). It is possible that GABA release from MSN or a subset of GABAergic interneurons that we did not screen (NPY-NGF or CB1R positive) was the source. Picrotoxin infusion also failed to alter basal synaptic strength (I/O relationship) in the PL mPFC to NAc core circuit in either the saline or cocaine group, suggesting that a single stimulation in the PL results in a NAc response that has a minimal GABA component. This finding, together with our data showing that picrotoxin alters NAc responses to 5 and 10 Hz stimulation in the saline group, suggests that a more substantial GABA component is recruited during train stimulations. This seems to fit the notion of the role of GABA in the NAc as mediating feedforward/feedback inhibition, where subsequent stimulations to the NAc are modulated by GABA.

In experiments in which we applied single pulse stimulation to the BLA and recorded the NAc core LFP response, we found no change of basal synaptic strength (I/O curve) in this circuit. Previously, Lee et al. (2013) found that CP-AMPA receptors were inserted into silent synapses postsynaptic to BLA afferents to the NAc shell in conjunction with incubation of

cocaine craving. Another study examined NAc shell MSN postsynaptic to BLA inputs after a regimen leading to incubation of cocaine craving and found increased CP-AMPA receptors in MSN that expressed the D2 dopamine receptor, but not MSN expressing the D1 dopamine receptor (Terrier et al., 2015). Since both of these studies recorded MSN in the NAc shell but not the core, it is possible that increased CP-AMPA levels occur postsynaptic to BLA projections to shell but not to core, which would be consistent with our failure to observe enhancement of the I/O curve in the BLA-NAc core pathway. Alternatively, given substantial differences in the electrophysiological methods used in these studies versus the present study, it is possible that our electrophysiological approach failed to recruit sufficient afferents from the BLA to elicit a statistically significant difference in the NAc. It is also possible that our local stimulation in the BLA also activates GABAergic interneurons in the BLA, which would suppress BLA projection neurons (Muller et al., 2006).

Responses to train stimulation in the saline group were different in the BLA-NAc core circuit compared to the mPFC-NAc core circuit. With the stimulating electrode in the mPFC, potentiation of the LFP response in the NAc core was observed at 5, 10 and 20 Hz and depression at 40 Hz (Fig. 4). In contrast, BLA stimulation produced no significant potentiation or depression at 5 Hz or 10 Hz, while depression was observed at 20 Hz and 40 Hz (Fig. 5). And, whereas cocaine and saline groups differed only with 20 Hz stimulation of the mPFC (Fig. 4f), after stimulating the BLA we observed a significantly enhanced NAc response in the cocaine group compared to the saline group after 10 Hz train stimulation (Fig. 5e) and a significantly attenuated depression in the cocaine group after 40 Hz train stimulation (Fig. 5g). Alterations at higher frequencies may be attributed to the PV+ fast spiking interneurons (Beatty et al., 2015), since this type of interneuron can fire at high frequency without adaptation (Kawaguchi, 1993). Furthermore, in mPFC, alterations in PV immunoreactivity may be linked to LFP changes at 20–40 Hz (Cass et al., 2013). However, our failure to observe significant alteration in PV immunoreactivity after cocaine suggests that the cocaine-saline group difference we observed at 40 Hz may be due to mechanisms unrelated to PV+ interneurons. Since we did not conduct picrotoxin experiments for this circuit, due to our finding of substantial vehicle effects in mPFC experiments, we are unable to determine whether the alterations we observed in the BLA-NAc core circuit were mediated by GABA or not. However, comparing the results between mPFC-NAc and BLA-NAc circuits, it appears that withdrawal from cocaine exposure leads to differential adaptations in these two circuits. Other results also indicate circuit-specific adaptations after withdrawal from cocaine self-administration (e.g., Suska et al., 2013; Lee et al., 2013; Ma et al., 2014; Terrier et al., 2015).

Conclusions

Based on the expression level of interneuron-specific proteins, our results suggest no marked alteration in the activity of GABAergic interneurons in the NAc after prolonged withdrawal from extended-access cocaine self-administration. Combined with our findings showing no alterations in GABA_A receptor expression in the NAc on WD25 or WD48 after the same cocaine regimen (Purgianto et al., 2016), this may suggest failure by the GABAergic microcircuit to compensate for the enhancement in glutamatergic input that occurs in the NAc core of rats that have undergone incubation of cocaine craving. We note, however, that

dynamic changes in the balance between GABA and glutamate transmission have been found in the NAc shell over 21 days of abstinence from limited-access cocaine self-administration (Otaka et al., 2013). Even if the GABAergic microcircuit in NAc core is not substantially altered after prolonged withdrawal from extended-access cocaine self-administration, it may be of interest to explore whether pharmacological enhancement of GABA transmission can compensate for the enhancement in glutamate transmission and thereby attenuate cue-induced cocaine seeking. We also discovered that cocaine exposure leads to differential adaptations in mPFC-NAc core and BLA-NAc core circuits. We were not able to characterize the mechanisms underlying most of the adaptations we observed. However, our picrotoxin infusion experiments suggested reduced recruitment of GABA transmission during 10 Hz train stimulation in the cocaine group in the mPFC-NAc core circuit. The source of this alteration in GABA tone was not conclusively determined, but some possibilities are GABA release from MSN or from a subset of GABA interneurons that we did not screen. Indeed, a microdialysis study in NAc shell detected reduced baseline GABA efflux during cocaine self-administration; this was normalized during extinction training, but the effect of abstinence was not studied (Wydra et al., 2013). Given growing interest in neuroadaptations during cocaine abstinence and their link to craving (Wolf, 2016) and findings of alterations in both GABA and glutamate transmission in other brain regions during prolonged abstinence from cocaine self-administration (Keralapurath et al., 2015), more work on long-lasting adaptations in NAc GABA transmission and their relationship to relapse vulnerability is needed.

Supplementary Material

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References

- Aguila FA, Oliveira-Pelegrin GR, Yao ST, Murphy D, Rocha MJA. Anteroventral third ventricle (AV3V) lesion affects hypothalamic neuronal nitric oxide synthase (nNOS) expression following water deprivation. *Brain Res.* 2011; 86(3–4):239–245.
- Beatty JA, Song SC, Wilson CJ. Cell-type-specific resonances shape the responses of striatal neurons to synaptic input. *J Neurophysiol.* 2015; 113(3):688–700. [PubMed: 25411465]
- Behrens MM, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL, Dugan LL. Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Science.* 2007; 318(5856):16454–1647.
- Bennett BD, Bolam JP. Synaptic input and output of parvalbumin-immunoreactive neurons in the neostriatum of the rat. *Neuroscience.* 1994; 62(3):707–719. [PubMed: 7870301]
- Cass DK, Thomases DR, Caballero A, Tseng KY. Developmental disruption of gamma-aminobutyric acid function in the medial prefrontal cortex by noncontingent cocaine exposure during early adolescence. *Biol Psychiatry.* 2013; 74(7):490–501. [PubMed: 23558299]

- Chaudhury S, Nag TC, Wadhwa S. Calbindin D-28k and parvalbumin expression in embryonic chick hippocampus is enhanced by prenatal auditory stimulation. *Brain Res.* 2008; 1191:96–106. [PubMed: 18096144]
- Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature.* 2008; 454(7200):118–121. [PubMed: 18500330]
- Figueredo-Cardenas G, Morello M, Sancesario G, Bernardi G, Reiner A. Colocalization of somatostatin, neuropeptide Y, neuronal nitric oxide synthase and NADPH-diaphorase in striatal interneurons in rats. *Brain Res.* 1996; 735(2):317–324. [PubMed: 8911672]
- Gittis AH, Nelson AB, Thwin MT, Palop JJ, Kreitzer AC. Distinct roles of GABAergic interneurons in the regulation of striatal output pathway. *J Neurosci.* 2010; 30(6):2223–2234. [PubMed: 20147549]
- Gruber AJ, Powell EM, O'Donnell P. Cortically activated interneurons shape spatial aspects of cortico-accumbens processing. *J Neurophysiol.* 2009; 101(4):1876–1882. [PubMed: 19176610]
- Hussain Z, Johnson LR, Totterdell S. A light and electron microscopic study of NADPH-diaphorase-, calretinin-, and parvalbumin-containing neurons in the rat nucleus accumbens. *J Chem Neuroanat.* 1996; 10(1):19–39. [PubMed: 8703362]
- Hussain Z, Totterdell S. Calbindin-28k immunoreactive neurons form two populations in the rat nucleus accumbens: a compartmental study. *Brain Res.* 1994; 656(1):191–198. [PubMed: 7804837]
- Ibanez-Sandoval O, Tecuapetla F, Unal B, Shah F, Koos T, Tepper JM. A novel functionally distinct subtype of striatal neuropeptide Y interneuron. *J Neurosci.* 2011; 31(46):16757–16769. [PubMed: 22090502]
- Jayasinghe VR, Flores-Barrera E, West AR, Tseng KY. Frequency-dependent corticostriatal disinhibition resulting from chronic dopamine depletion: Role of local striatal cGMP and GABA-AR Signaling. *Cereb Cortex* 2015. 2015 Oct 27. pii: bhv241 Epub ahead of print.
- Kawaguchi Y. Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *J Neurosci.* 1993; 13(11):4908–4923. [PubMed: 7693897]
- Keralapurath MM, Briggs SB, Wagner JJ. Cocaine self-administration induces changes in synaptic transmission and plasticity in ventral hippocampus. *Addict Biol* 2015. 2015 Dec 22. Epub ahead of print. doi: 10.1111/adb.12345
- Kinney JW, Davis CN, Tabarean I, Conti B, Bartfai T, Behrens MM. A specific role for NR2A-containing NMDA receptors in the maintenance of parvalbumin and GAD67 immunoreactivity in cultured interneurons. *J Neurosci.* 2006; 26(5):1604–1615. [PubMed: 16452684]
- Koos T, Tepper JM. Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nature Neuroscience.* 1999; 2(5):467–472. [PubMed: 10321252]
- Kuo H, Chang HT. Ventral pallido-striatal pathway in the brain: a light and electron microscopic study. *J Comp Neurol.* 1992; 321(4):626–636. [PubMed: 1380522]
- Lee BR, Ma YY, Huang YH, Wang X, Otaka M, Ishikawa M, Neumann PA, Graziane NM, Brown TE, Suska A, Guo C, Lobo MK, Sesack SR, Wolf ME, Nestler EJ, Shaham Y, Schluter OM, Dong Y. Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nat Neurosci.* 2013; 16(11):1644–1651. [PubMed: 24077564]
- Li X, DeJoseph MR, Urban JH, Bahi A, Dreyer JL, Meredith GE, Ford KA, Ferrario CR, Loweth JA, Wolf ME. Different roles of BDNF in nucleus accumbens core versus shell during the incubation of cue-induced cocaine craving and its long-term maintenance. *J Neurosci.* 2013; 33(3):1130–1142. [PubMed: 23325250]
- Ma YY, Lee BR, Wang X, Guo C, Liu L, Cui R, Lan Y, Balcita-Pedicino JJ, Wolf ME, Sesack SR, Shaham Y, Schluter OM, Huang YH, Dong Y. Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projection. *Neuron.* 2014; 83(6):1453–1467. [PubMed: 25199705]
- Maguire EP, Macpherson T, Swinny JD, Dixon CI, Herd MB, Belelli D, Stephens DN, King SL, Lambert JJ. Tonic inhibition of accumbal spiny neurons by extrasynaptic $\alpha 4\beta 8$ GABAA receptors modulates the action of psychostimulants. *J Neurosci.* 2014; 34(3):823–838. [PubMed: 24431441]

- Marty S, Onteniente B. The expression pattern of somatostatin and calretinin by postnatal hippocampal interneurons is regulated by activity-dependent and -independent determinants. *Neuroscience*. 1997; 80(1):79–88. [PubMed: 9252222]
- Meredith GE. The synaptic framework for chemical signaling in nucleus accumbens. *Annals of the NY Acad Sci*. 1999; 877:140–156.
- Muller JF, Mascagni F, McDonald AJ. Pyramidal cells of the basolateral amygdala: synaptology and innervation by parvalbumin-immunoreactive neurons. *J Comp Neurol*. 2006; 494(4):635–650. [PubMed: 16374802]
- Nasif FJ, Hu XT, Ramirez OA, Perez MF. Inhibition of neuronal nitric oxide synthase prevents alterations in medial prefrontal cortex excitability induced by repeated cocaine administration. *Psychopharmacology*. 2011; 218(2):323–330. [PubMed: 21125397]
- Otaka M, Ishikawa M, Lee BR, Liu L, Neumann PA, Cui R, Huang YH, Schlüter OM, Dong Y. Exposure to cocaine regulates inhibitory synaptic transmission in the nucleus accumbens. *J Neurosci*. 2013; 33(16):6753–6758. [PubMed: 23595733]
- Paxinos, G., Watson, C. The rat brain in stereotaxic coordinates. 6. San Diego: Elsevier Academic Press; 2007.
- Pennartz CMA, Kitai ST. Hippocampal inputs to identified neurons in an in vitro slice preparation of the rat nucleus accumbens: evidence for feed-forward inhibition. *J Neurosci*. 1991; 11(9):2838–2847. [PubMed: 1679123]
- Philpot BD, Lim JH, Brunjes PC. Activity-dependent regulation of calcium-binding proteins in developing rat olfactory bulb. *J Comp Neurol*. 1997; 387:12–26. [PubMed: 9331168]
- Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. *Trends Neurosci*. 2011; 34(8):411–420. [PubMed: 21764143]
- Purgianto A, Scheyer AF, Loweth JA, Ford KA, Tseng KY, Wolf ME. Different adaptations in AMPA receptor transmission in the nucleus accumbens after short vs. long access cocaine self-administration regimens. *Neuropsychopharm*. 2013; 38(9):1789–1797.
- Purgianto A, Loweth JA, Miao JJ, Milovanovic M, Wolf ME. Surface expression of GABA_A receptors in the rat nucleus accumbens is increased in early but not late withdrawal from extended-access cocaine self-administration. *Brain Research* 2016. 2016 Apr 6. pii: S0006–8993(16)30207–4. Epub ahead of print. doi: 10.1016/j.brainres.2016.04.014
- Schwaller B. Cytosolic Ca²⁺ buffers. *Cold Spring Harb Perspect Biol*. 2010; 2(11):a004051. [PubMed: 20943758]
- Selvakumar B, Campbell PW, Milovanovic M, Park DJ, West AR, Snyder SH, Wolf ME. AMPA receptor upregulation in the nucleus accumbens shell of cocaine-sensitized rats depends upon S-nitrosylation of stargazin. *Neuropharmacology*. 2014; 77:28–38. [PubMed: 24035918]
- Silberberg G, Bolam JP. Local and afferent synaptic pathways in the striatal microcircuitry. *Curr Opin Neurobiol*. 2015; 33:182–187. [PubMed: 26051382]
- Suska A, Lee BR, Huang YH, Dong Y, Schlüter OM. Selective presynaptic enhancement of the prefrontal cortex to nucleus accumbens pathway by cocaine. *Proc Natl Acad Sci USA*. 2013; 110(2):713–718. [PubMed: 23267100]
- Taverna S, Canciani B, Pennartz CM. Membrane properties and synaptic connectivity of fast-spiking interneurons in rat ventral striatum. *Brain Res*. 2007; 1152:49–56. [PubMed: 17459351]
- Tepper JM, Koos T, Wilson CJ. GABAergic microcircuit in the neostriatum. *Trends Neurosci*. 2004; 27(11):662–669. [PubMed: 15474166]
- Tepper JM, Tecuapetla F, Koós T, Ibáñez-Sandoval O. Heterogeneity and diversity of striatal GABAergic interneurons. *Front Neuroanat*. 2010; 4:150. [PubMed: 21228905]
- Terrier J, Lüscher C, Pascoli V. Cell-type specific insertion of GluA2-lacking AMPARs with cocaine exposure leading to sensitization, cue-induced seeking and incubation of craving. *Neuropsychopharmacology* 2015. 2015 Nov 20. Epub ahead of print. doi: 10.1038/npp.2015.345
- Thomases DR, Cass DK, Tseng KY. Periadolescent exposure to the NMDA receptor antagonist MK-801 impairs the functional maturation of local GABAergic circuits in the adult prefrontal cortex. *J Neurosci*. 2013; 33(1):26–34. [PubMed: 23283319]

- Todtenkopf MS, Stellar JR, Williams EA, Zahm DS. Differential distribution of parvalbumin immunoreactive neurons in the striatum of cocaine sensitized rats. *Neuroscience*. 2004; 127(1):35–42. [PubMed: 15219666]
- Wilson CJ. GABAergic inhibition in the neostriatum. *Prog Brain Res*. 2007; 160:91–110. [PubMed: 17499110]
- Winters BD, Krüger JM, Huang X, Gallaher ZR, Ishikawa M, Czaja K, Krueger JM, Huang YH, Schlüter OM, Dong Y. Cannabinoid receptor 1-expressing neurons in the nucleus accumbens. *Proc Natl Acad Sci USA*. 2012; 109(40):E2717–2725. [PubMed: 23012412]
- Wolf ME, Tseng KY. Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: when, how, and why? *Front Mol Neuro*. 2012; 5:75.doi: 10.3389/fnmol.2012.00072
- Wolf ME. Synaptic mechanisms underlying persistent cocaine craving. *Nat Rev Neurosci*. 2016; published online ahead of print May 6. doi: 10.1038/nrn.2016.39
- Wydra K, Golembiowska K, Zaniowska M, Kaminska K, Ferraro L, Fuxe K, Filip M. Accumbal and pallidal dopamine, glutamate, and GABA overflow during cocaine self-administration and its extinction in rats. *Addict Biol*. 2013; 18(2):307–324. [PubMed: 23311632]
- Zettel ML, O'Neill WE, Trang TT, Frisina RD. Early bilateral deafening prevents calretinin up-regulation in the dorsal cortex of the inferior colliculus of aged CBA/CaJ mice. *Hear Res*. 2001; 158(1–2):131–138. [PubMed: 11506945]

Figure 1.

PV+ immunoreactivity in the NAc core was not altered by cocaine self-administration and prolonged withdrawal (WD56–60). (a) Illustration of areas sampled and representative images from saline and cocaine groups. Dotted box indicates the image area, but immunoreactivity was quantified only in the NAc core as defined by Paxinos & Watson (2007). (b) When the entire rostrocaudal extent of the NAc core was analyzed by combining data from all sections within +2.0mm to +1.1mm from Bregma, we found no significant difference in PV staining intensity between saline and cocaine groups (unpaired t-test, 2-tailed: $t_{17} = 1.53$, $p = 0.14$). (c–e) We also failed to find group differences in PV immunostaining when we analyzed the NAc core at specific rostro-caudal levels (unpaired t-tests, 2-tailed: rostral $t_{16} = 0.09$, $p = 0.93$; middle $t_{17} = 0.63$, $p = 0.19$; caudal $t_{17} = 1.40$, $p = 0.18$).

Figure 2.

The number of PV+ cells in the NAc core was not altered by cocaine self-administration and prolonged withdrawal (WD56–60). (a) When the entire rostrocaudal extent of the NAc core was analyzed (from +2.0mm to +1.1mm from Bregma), we found no difference in PV+ cell counts between saline and cocaine groups (unpaired t-test, 2-tailed: $t_{17} = 0.79$, $p = 0.44$). (b–d) We also failed to find group differences in PV+ cell counts when we analyzed specific rostro-caudal levels of the NAc core (unpaired t-tests, 2-tailed: rostral $t_{15} = 1.46$, $p = 0.17$; middle $t_{17} = 1.56$, $p = 0.14$; caudal $t_{17} = 1.13$, $p = 0.27$), although there was a trend towards elevation in PV+ cell count in the cocaine group when results from all sections were combined (two-way ANOVA; $p = 0.07$).

Figure 3.

Levels of activity-dependent proteins expressed by various GABAergic interneurons were not altered after cocaine self-administration and prolonged withdrawal (WD48). (a) Supporting our immunohistochemistry data shown in Fig. 1, no alteration in PV protein levels was detected in the cocaine group (unpaired t-test, 2-tailed; $t_{18} = 0.55$, $p = 0.59$). (b–d). Other interneuron markers (Calbindin, Calretinin, and nNOS) were also unaffected (Calbindin: $t_{19} = 0.31$, $p = 0.76$; Calretinin: $t_{19} = 0.32$, $p = 0.75$; nNOS, $t_{19} = 0.06$, $p = 0.96$). Representative blots show data from 4 different animals: 2 cocaine animals and 2 saline animals.

Figure 4.

Cocaine self-administration and prolonged withdrawal (WD40–80) does not alter basal synaptic strength (I/O curve) but leads to frequency-specific alterations in the NAc response to train stimulation of the mPFC. (a) Experimental timeline. (b) Representative stimulating electrode placements in the PL region of mPFC and recording electrode placements in the core of NAc (saline, $n = 10$; cocaine, $n = 9$; n indicates number of animals recorded for all panels of this figure, except where noted). For some animals, both left and right sides were recorded; the average of the two sides was used for these animals. (c) I/O curve showing no alteration after the incubation regimen. (d) 5 Hz train stimulation led to facilitation of the LFP in both cocaine and saline groups (main effect of pulse number: $F_{(9,170)} = 2.676$; $*p < 0.01$), with no difference between these groups. For this frequency, $n = 9$ for the saline group. (e) Similarly, 10 Hz train stimulation led to facilitation of the LFP in both saline and cocaine groups (main effect of pulse number: $F_{(9,180)} = 4.446$; $***p < 0.0005$). (f) Although 20 Hz train stimulation facilitated the LFP response in the saline group (vs. 1st pulse, least significant difference after significant two-way ANOVA: $*p < 0.05$; $**p < 0.005$; $***p < 0.0005$), similar facilitation did not occur in the cocaine group. Furthermore, the LFP response of the cocaine group was significantly attenuated compared to the saline group (main effect of treatment: $F_{(1,180)} = 22.14$; $###p < 0.0005$). (g) The LFP response showed depression after 40 Hz stimulation in both groups, with no difference between the groups (main effect of pulse number: $F_{(9,180)} = 18.86$; $***p < 0.0005$). Representative traces showing PFC-evoked NAc LFP from both cocaine and saline groups. Calibration bars: (c) 40ms, 1mV; (d) 200ms, 2mV; (e) 100ms, 2mV; (f) 100ms, 1mV; (g) 40ms, 1mV.

Figure 5.

Cocaine self-administration and prolonged withdrawal (WD40–80) does not alter basal synaptic strength (I/O curve) but leads to frequency-specific alterations in the NAc response to train stimulation of the BLA. (a) Experimental timeline. (b) Representative stimulating electrode placements in the BLA and recording electrode placements in the core of NAc. White boxes indicate saline group (n=6). Black boxes indicate cocaine group (n=6). (c) Although the I/O curve appears to show a trend toward enhanced LFP response in the cocaine group, no significant difference between the groups was found ($p > 0.05$). (d) 5 Hz train stimulation did not lead to potentiation or depression of the LFP response. (e) At 10 Hz train stimulation, animals who underwent prolonged withdrawal from cocaine exhibited a significantly enhanced LFP response compared to saline controls (main effect of treatment: $F_{(1,100)} = 5.53$; $\#p < 0.05$); however, neither group demonstrated significant potentiation

compared to the first pulse. (f) 20 Hz train stimulation led to depression of the LFP response in the NAc (main effect of pulse number: $F_{(9,100)} = 4.18$; *** $p < 0.0005$), with no group difference. (g) Finally, the LFP response showed depression after 40 Hz stimulation in both groups (main effect of pulse number: $F_{(9,100)} = 54.66$; *** $p < 0.0005$), but this depression was attenuated in the cocaine group compared to the saline group (main effect of treatment: $F_{(1,100)} = 40.14$; ### $p < 0.0005$). Representative traces showing BLA-evoked NAc LFP from both cocaine and saline groups. Calibration: (c) 40ms, 1mV; (d) 200ms, 1mV; (e) saline: 100ms, 1mV; cocaine: 100ms, 2mV; (f) 100ms, 1mV; (g) 40ms, 1mV.