Repeated Cocaine Administration Promotes Long-Term Potentiation Induction in Rat Medial Prefrontal Cortex

Although drug-induced adaptations in the prefrontal cortex (PFC) may contribute to several core aspects of addictive behaviors, it is not clear yet whether drugs of abuse elicit changes in synaptic plasticity at the PFC excitatory synapses. Here we report that, following repeated cocaine administration (15 mg/kg/day intraperitoneal injection for 5 consecutive days) with a 3-day withdrawal, excitatory synapses to layer V pyramidal neurons in rat medial prefrontal cortex (mPFC) become highly sensitive to the induction of long-term potentiation (LTP) by repeated correlated presynaptic and postsynaptic activity. This promoted LTP induction is caused by cocaine-induced reduction of γ-aminobutyric acid (GABA)A receptor-mediated inhibition of mPFC pyramidal neurons. In contrast, in slices from rats treated with saline or a single dose of cocaine, the same LTP induction protocol did not induce significant LTP unless the blockade of GABA(A) receptors. Blockade of the D1-like receptors specifically prevented the cocaine-induced enhancement of LTP. Repeated cocaine exposure reduced the surface GABA(A) receptor α1 subunit expression in mPFC slices from repeated cocaine-treated rats. These findings support an important role for cocaine-induced enhancement of synaptic plasticity in the PFC in the development of drug-associated behavioral plasticity.

Keywords: addiction, cocaine, GABA(A) receptor, long-term potentiation, medial prefrontal cortex

Introduction

Neural circuits and their elements are not fixed and immutable but rather vary in response to their environment (Burron and Murthy 2003). Drug addiction is a remarkable example of this concept. Compelling recent evidence has shown that neurotransmitter receptors or receptor-mediated signaling, intrinsic neuronal excitability, neuronal morphology, synaptic strength, and gene expression can be substantially altered in response to single or repeated exposure to addictive drugs (Hyman and Malenka 2001; Nestler 2002; Thomas and Malenka 2003). Drug-induced adaptations do not occur globally, but within specific critical brain circuits, among which is the mesocorticolimbic dopamine (DA) system (Everitt and Wolf 2002). This system consists of the ventral tegmental area (VTA) neurons that innervate the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC), which receives a dense DA innervation from the VTA and sends glutamatergic efferents to the VTA and NAc (Oades and Halliday 1987). Furthermore, a growing body of evidence indicates that excitatory synaptic transmission within this system may also be a critical neurochemical substrate for drug addiction (Wolf 1998; Steketee 2003).

Recent studies examining the role of altered excitatory synaptic transmission in mediating the development of cocaine addiction have yielded provocative results. Several studies have revealed that the initiation of cocaine-induced behavioral sensitization is prevented by intra-VTA administration of N-methyl-D-aspartate (NMDA) receptor antagonists (Kalivas and Alesdatter 1993; Vezina and Queen 2000). In addition, electrophysiological studies have demonstrated an increase in the responsiveness of VTA DA neurons to excitatory effects of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) after single or repeated cocaine or amphetamine exposure (White et al. 1995; Zhang et al. 1997; Ungless et al. 2001). Furthermore, in vivo microdialysis measures have shown that repeated amphetamine administration increases the ability of VTA AMPA receptors to regulate DA and glutamate transmission both locally in the VTA and downstream in the NAc (Giorgetti et al. 2001). Thus, potentiation of excitatory synaptic transmission in the VTA could account for the transient increase in DA neuron activity thought to be critical in transferring sensitization to forebrain regions such as the NAc that are important in its maintenance and expression (Wolf 1998).

Although most addiction studies have focused on the VTA rather than other mesocorticolimbic systems, recent studies have implicated an important role for the PFC in the development of addictive behaviors. For instance, direct electrical stimulation of the PFC leads to rewarding effects and sensitization to cocaine, whereas lesions of the PFC or impairment of mesocorticolimbic glutamatergic transmission prevent the development of cocaine-induced behavioral sensitization (Li et al. 1997; Tzschentke 2001). In addition, evidence is accumulating that the alterations of multiple mPFC neurotransmitter systems, including DA, serotonin, glutamate, noradrenaline, acetylcholine, γ-aminobutyric acid (GABA), and peptides, and signaling by receptors activated by these neurotransmitters may contribute to the development of psychostimulant-induced behavioral sensitization and addition (Steketee 2003). However, it is currently unknown whether in vivo administration of psychostimulants affects the induction of long-term synaptic plasticity at mPFC excitatory synapses. Considering that alterations of long-term synaptic plasticity may contribute to circuit modification induced by repeated addictive drug administration (Wolf 2004), we were interested in determining the effect of cocaine treatment on the induction of mPFC long-term potentiation (LTP). In this study, we have demonstrated the first evidence that repeated cocaine administration in vivo promotes LTP induction in mPFC layer V pyramidal neurons by reducing GABA(A) receptor-mediated inhibition to below a critical level.

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Materials and Methods

Animals and Treatment
Male Sprague-Dawley rats (21–32 days old) were housed under a 12-h light-dark cycle (light on at 7 AM) with free access to food and water. After a 1-week acclimation period, animals were assigned randomly to 2 groups that received an intraperitoneal injection of saline (1 mL/kg) or cocaine HCl (15 mg/kg) once per day for 5 consecutive days followed by 3 days of withdrawal prior to the experiments. During this time, they were handled and weighed daily. All comparisons between saline- and cocaine-treated groups were performed by experimenters blind to group assignment. Animal care was consistent with the guidelines set by the Laboratory Animal Center of National Cheng Kung University. All experiments were approved by the National Cheng Kung University Institutional Animal Care and Use Committee governing the participating laboratories.

Cannulation
Rats (28–32 days old) were bilaterally implanted under deep pentobarbital (50 mg/kg, intraperitoneally supplemented as required) anesthesia with 26-gauge guide cannulae (Plastics One, Roanoke, VA) in the mPFC. Medial PFC coordinates were +2.5-mm anterior to bregma, ± 0.5-mm bilateral to midline, and 2.0-mm ventral to brain surface in accordance with the description by Park et al. (2002). The cannulae were fixed to the skull with dental cement. After 3–5 days of recovery, rats received 0.5 μl microinjections in each side over a 1-min period using an infusion pump (CAM/100; CAM Microdialysis, Söna, Sweden). The infusion cannulae were kept in place for an additional 2 min to minimize backflow of the injectant. Dose of KTS720 (0.5 μg per side) was based on published study (Vienna et al. 1999), which has been shown to be effective to block protein kinase A (PKA) activity by 90%. Infusions were administered 30 min before cocaine injection. Rats with injection cannulae placements outside the mPFC or with extensive tissue damage at the injection cannulae site were excluded from the analyses. We never observed any effects of cannulation itself or vehicle artificial cerebrospinal fluid (aCSF) (20% DMSO) injection.

Locomotor Activity
Following each intraperitoneal injection of cocaine or saline, rats were immediately placed in the activity chamber and horizontal locomotor activity was monitored with a video tracking system (Ethovision; Noldus, The Netherlands) for 20 min. Distance traveled was analyzed for estimates of locomotor response. After 2 days of saline injections to habituate the chamber, rats were divided into 2 groups that received 5 daily injections of either cocaine or saline (during 10–12 AM). To assess the persistence of the effect of repeated cocaine administration, following 3 days without injections, both groups received cocaine injections, and locomotor activities were assessed.

Electrophysiology
Slice preparation and whole-cell patch-clamp recordings were conducted as reported previously (Huang and Hsu 2006). Briefly, rats were anesthetized with halothane and decapitated with guillotine, and coronal slices (200 μm thick) containing the prelimbic area of the mPFC (2.0–3.7 mm from the bregma) (Paxinos and Watson 1998) were prepared using a vibrating microtome (Leica VT1000S; Leica, Nussloch, Germany). The slices were placed in a storage chamber of aCSF oxygenated with 95% O2–5% CO2 and kept at room temperature for at least 1 h before recording. The composition of the aCSF solution was (in mM): NaCl, 117; KCl, 4.7; CaCl2, 2.5; MgCl2, 1.2; NaHCO3, 25; NaH2PO4, 1.2; and glucose, 11 at pH 7.3–7.4 and equilibrated with 95% O2–5% CO2. For whole-cell patch-clamp recording, one slice was transferred to a recording chamber of standard design and fixed at the glass bottom of the chamber with a nylon grid on a platinum frame. The chamber consisted of a circular well of low volume (1–2 ml) and was perfused constantly at 32.0 ± 0.5 °C with a rate of 2–3 ml/week. Whole-cell recordings were made using a patch-clamp amplifier (Axopatch 200B, Axon Instruments, Union City, CA) under infrared differential interference contrast microscope. Electrical signals were low-pass filtered at 2 kHz, digitized at 10 kHz using a 12-bit analog-to-digital converter (Digidata 1320, Axon Instruments). An Intel Pentium-based computer with pClAMP software (version 8.0; Axon Instruments) and Mini Analysis 4.3 (Synaptosoft, Leonia, NJ) were used for online acquisition and offline analysis of the data. For presynaptic stimulation, a bipolar stainless steel stimulating electrode placed on layers I-II about 150–200 μm away the apical dendrites of the recorded neurons was used to stimulate excitatory afferents at 0.05 Hz. For recording excitatory postsynaptic potentials (EPSPs), neurons were current clamped at −70 mV. The strength of synaptic transmission was mostly quantified by measuring the initial rising slope of EPSP (2ms period from its onset, mV/μs), which contains only a monosynaptic component. For recording inhibitory postsynaptic currents (IPSCs), neurons were voltage clamped at −20 mV in the presence of 6-cyano-7-nitroquinolinoxaline-2,3-dione (CNQX, 20 μM) and D-(-)-2-amino-5-phosphonopentanoic acid (D-APV, 50 μM). Patch pipettes were pulled from borosilicate capillary tubing and heat polished. The electrode resistance was typically 3–6 MΩ. The composition of intracellular solution was (in mM): K-glucurate, 115; KCl, 20; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10; MgCl2, 2; ethyleneglycol-bis(2-aminoethyl)-ether)-N,N',N'-tetraacetic acid (EGTA), 0.5; Na2ATP, 3; Na3GTP, 0.3, and sucrose to bring osmolality to 290–295 mOsm and pH to 7.3. For recording miniature inhibitory postsynaptic currents (mIPSCs), potassium gluconate was replaced in the KC in the internal solution and tetrodotoxin (TTX, 1 μM), CNQX (20 μM), and D-APV (50 μM) were added to the bath. For recording miniature excitatory postsynaptic currents (mEPSCs), TTX (1 μM) and bicuculline methiodide (BMI, 20 μM) were added to the bath. For recording mIPSCs and mEPSCs, NaCl, N-(2,6-dimethylphenyl)acetamide-2-triethylammonium bromide (QX-314, 5 mM) was added to the intracellular solution. Detection threshold of 5 pA was used for mIPSCs and mEPSCs. Series resistance and input resistance were monitored online throughout the whole-cell recording with a 5-mV depolarizing step given after every afferent stimulus and data were discarded if resistance changed by more than 20%. Only cells with a stable resting membrane potential at or more negative than −60 mV and evoked spikes that overshoot across a 0 mV were used for experiments.

The spike-timing protocol for LTP induction consisted of 10 bursts of EPSP-spike pairs, with each burst consisting of 5 paired stimuli delivered at 50-ms intervals (20 Hz) and an interburst interval of 10 s. The postsynaptic spikes were evoked by injection of depolarizing current pulses (1–2 nA, 2–4 ms), with the onset of EPSPs preceding the peak of postsynaptic spikes by 10 ms. Evoked EPSPs were sampled at 0.05 Hz before and after LTP induction.

Biochemical Measurement of Surface-Expressed Receptors
The procedure was similar to that described previously (Zhao et al. 2002). mPFC slices were incubated with aCSF containing 1 mg/ml Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) for 20 min on ice. Unreacted biotinylation reagent was washed once with ice-cold aCSF and quenched by 2 successive 20-min washes in aCSF containing 100 mM glycine, followed by 2 washes in ice-cold TBS (50 mM Tris, pH 7.5, 150 mM NaCl). The slices were then lysed in ice-cold homogenate buffer (50 mM Tris-HCl, 100 mM NaCl, 15 mM sodium pyrophosphate, 50 mM sodium fluoride, 5 mM EGTA, 5 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, 0.5% Triton X-100, 2 mM benzamidine, 60 μg/ml aprotinin, and 60 μg/ml leupeptin) and ground with a pellet pestle. Samples were sonicated and spun down at 14,000 x g for 15 min at 4 °C. A total of 15 μg of protein was removed to measured total GABA_A receptor subunit. For surface protein, 150 μg of protein was incubated with 100 μl of 50% Neutravidin agarose (Pierce) for 2 h at 4 °C to measure the isolated biotinylated proteins. After the Neutravidin agarose was washed 5 times with homogenate buffer, bound proteins were eluted with SDS sample buffer by boiling for 15 min. Total protein and isolated biotinylated proteins were analyzed by quantitative immunoblotting with polyclonal anti-α1 subunit C-terminal (1:1000, Upstate Biotechnology, Lake Placid, NY). It was then probed with horse radish peroxidase–conjugated secondary antibody for 1 h and developed using the enhanced chemiluminescence immunoblotting detection system. Immunoblots were quantified by densitometric measurement.

Drug Treatment
Cocaine HCl (15 mg/kg), SCH23390 (0.5 mg/kg), raclopride (0.5 mg/kg), and RS102221 (2 mg/kg) were dissolved in 0.9% NaCl and
administered intraperitoneally. Drug doses were selected on the basis of published studies (Dong et al. 2004, 2005; Conductier et al. 2005) and on pilot experiments in our laboratory. SCH23390, raclopride, zolpidem, CNQX, CGP55845, RS102221, KT5720, D-APV, and TTX were purchased from Tocris Cookson (Bristol, UK); cocaine HCl and BMI were obtained from Sigma (St Louis, MO).

Data Analysis
All data are expressed as means ± standard error of mean, and unless stated otherwise, the statistic significance was determined using a Mann-Whitney U-test. Number of animals used is indicated by n. Probability values of P < 0.05 were considered to represent significant differences. mIPSC or mEPSC of 3 min was used for obtaining cumulative distribution plots of the amplitude. Statistical comparisons of the synaptic currents were made using the Kolmogorov-Smirnov test. Distributions were considered different using a conservative critical probability level of P < 0.01.

Results

Locomotor Sensitization to Repeated Cocaine Administration
To induce behavioral sensitization, animals were administered daily injection of saline (1 ml/kg) or cocaine (15 mg/kg) intraperitoneally for 5 consecutive days, followed by a 3-day withdrawal period. When both groups of rats were challenged with cocaine (15 mg/kg) 3 days after the last injection, the cocaine group exhibited a much greater locomotor response to cocaine than did saline-treated rats (cocaine, 5968 ± 396, n = 8; saline, 3218 ± 485 cm; n = 8; P < 0.01) when assayed immediately after injection (Supplementary Fig. S1). These results indicate that the initial 5-day exposure to cocaine caused behavioral sensitization that lasted for at least 3 days after termination of cocaine treatment.

Repeated Cocaine Administration Promotes mPFC LTP Induction
In electrophysiological experiments, rats received the same injection protocol as above but did not receive a cocaine challenge injection. On the third day of withdrawal, mPFC slices were prepared to determine the effect of repeated cocaine administration on LTP induction in mPFC layer V pyramidal neurons. Extracellular stimulation was applied to the layers I–II of the mPFC, and the evoked EPSPs were monitored by whole-cell recordings from layer V pyramidal neurons at −70 mV. Because EPSPs were completely abolished by CNQX (20 μM) plus D-APV (50 μM), they were predominantly mediated by ionotropic glutamate receptors (data not shown).

To induce LTP, we used a spike-timing protocol consisting of bursts of EPSP-spike pairs, with the onset of EPSPs preceding the peak of the postsynaptic spike by 10 ms (Fig. 1A). This pattern of stimulation was used to mimic bursting activity observed in PFC neurons in vivo (Fuster 1998; Kitano et al. 2002). This paired stimulation protocol induced a strong and long-lasting increase of the slope of EPSPs in mPFC layer V pyramidal neurons in slices obtained from rats treated cocaine for 5 days (135.3 ± 6.8% of baseline, n = 12; P < 0.05) (Fig. 1C) but not from rats treated with a single injection of cocaine (1 day, 115.6 ± 7.8% of baseline, n = 8; P > 0.05) (Fig. 1E). No change in synaptic efficacy was observed in slices from rats that were given time-matched saline injections (5 days, 108.6 ± 5.1% of baseline, n = 10; P > 0.05; 1 day, 111.5 ± 6.2% of baseline, n = 17; P > 0.05) (Fig. 1B,D). Although repeated cocaine administration modestly increased input resistance (saline, 22.5 ± 4.5 MΩ, n = 16; cocaine, 104.5 ± 3.7 MΩ, n = 18; P = 0.03), there were no significant differences in baseline EPSP slope between the 2 groups. These results suggest that repeated but not single cocaine administration promotes LTP induction in mPFC pyramidal neurons.

Effect of GABA-Mediated Inhibition on mPFC LTP Induction
How might in vivo cocaine exposure lead to the enhancement of LTP induction in mPFC pyramidal neurons? Given that GABA-mediated inhibition is known to be an important regulatory factor during induction of LTP at many excitatory synapses (Wigstrom and Gustafsson 1983; Paulsen and Moser 1998; Meredith et al. 2003; Liu et al. 2005), one possibility is that repeated cocaine administration promotes LTP induction by reducing GABA-mediated inhibition of mPFC pyramidal neurons. To determine whether the presence of GABA-mediated inhibition is responsible for the absence of LTP induction in mPFC layer V pyramidal neurons, we then examined LTP induction in the presence of the GABAA receptor antagonist BMI (20 μM). We found that EPSP-spike pairing stimulation induced robust LTP in mPFC pyramidal neurons in slices from rats treated with saline for 5 days (141.5 ± 7.5% of baseline, n = 8; P < 0.05), to a similar extent as that observed for rats treated with cocaine for 5 days (145.3 ± 6.5% of baseline, n = 8) (Fig. 2A,B). Note that the presence of BMI did not further increase the extent of LTP in slices from rats treated with cocaine for 5 days (Fig. 2B). In addition, we found that in the presence of BMI, the pairing stimulation also induced LTP on mPFC neurons in slices from rats given one injection of saline or cocaine (saline, 138.6 ± 6.4% of baseline, n = 6; P < 0.05; cocaine, 141.6 ± 7.2% of baseline, n = 6; P < 0.05) (Fig. 2C,D). Furthermore, administration of zolpidem, a benzodiazepine agonist known to enhance the activation of GABAA receptors (Depoortere et al. 1986), prevented the induction of LTP in slices from 5-day cocaine-treated rats at a concentration of 1 μM (112.5 ± 7.5% of baseline, n = 6) but not at 0.1 μM (142.5 ± 8.6% of baseline, n = 6) (Fig. 2F). No LTP was induced in slices from 5-day saline-treated rats in the presence of zolpidem (0.1 μM, 109.6 ± 6.5% of baseline, n = 4; 1 μM, 107.9 ± 5.6% of baseline, n = 4) (Fig. 2E). The effect of zolpidem on LTP induction was completely abolished by BMI coapplication (data not shown). Thus, the induction of associative LTP in mPFC pyramidal neurons is tightly controlled by GABAA receptor-mediated inhibition.

There is accumulating evidence suggesting a role for presynaptic GABAA receptors in regulating the induction of LTP in multiple brain regions (Staubli et al. 1999; Shaban et al. 2006). In addition, it has been recently reported that the GABAA receptor–positive modulators attenuate cocaine-induced hyperlocomotion (Lhuillier et al. 2006). To further test the idea that whether repeated cocaine exposure in vivo facilitates LTP induction through an alteration of GABAA receptor function, we also examined LTP induction in the presence of the specific GABAA receptor antagonist CGP55845. We found that, in the presence of CGP55845 (10 μM), the paired stimulation protocol failed to induce a reliable LTP in slices from rats treated with saline for 5 days (106.5 ± 4.5% of baseline, n = 4; P > 0.05) (Supplementary Fig. S2A). In addition, CGP55845 did not affect the extent of LTP induction in slices from 5-day cocaine-treated rats (138.6 ± 5.4% of baseline, n = 3; P > 0.05 when compared with slices from 5-day cocaine treatment in the absence of CGP55845, unpaired Student’s t-test) (Supplementary Fig. S2B). These findings suggest the possibility that the observed cocaine-induced
enhancement of LTP induction in the mPFC is not caused by the change of GABAA receptor–mediated function.

The lack of additive effects of BMI and cocaine on the extent of potentiation induced by the EPSP-spike pairs is consistent with the idea that some of the mechanisms responsible for these enhancements of LTP induction on mPFC pyramidal neurons are shared. To investigate whether the increased susceptibility to LTP induction after repeated cocaine treatment is caused by reduced GABA<sub>A</sub> receptor–mediated inhibition, we compared IPSCs recorded in mPFC layer V pyramidal neurons in slices from saline- and cocaine-treated rats. Monosynaptic IPSCs were evoked while holding neurons in voltage clamp at –20 mV in the presence of CNQX (20 μM) and D-APV (50 μM). Figure 3A depicts the relationship between stimulation intensity and IPSC amplitude. Five-day cocaine-treated rats showed a significant decrease in the amplitude of IPSCs, compared with rats treated with saline for 5 days. In contrast, no significant change in the IPSC amplitude was observed in rats treated with cocaine for 1 day. The cocaine-induced reduction in IPSC amplitude is probably of postsynaptic origin because 5-day cocaine-treated rats showed a significant reduction of the amplitude of mIPSCs (mIPSCs: 10.7 ± 2.3 pA, n = 12, P < 0.01), compared with 5-day saline-treated controls (25.7 ± 3.5 pA, n = 10) (Fig. 3B). The mean frequency of mIPSCs was not significantly different between saline- (2.8 ± 0.3 Hz, n = 10) and cocaine-treated rats (2.2 ± 0.3 Hz, n = 12). However, we found no difference between 1-day cocaine- and saline-treated rats in either the frequency (saline, 2.7 ± 0.3 Hz, n = 6; cocaine, 2.4 ± 0.3 Hz, n = 6) or the amplitude of mIPSCs (saline, 26.5 ± 3.2 pA, n = 6; cocaine, 21.6 ± 3.2 pA, n = 6). Taken together, these results suggest that the enhancement of LTP induction by repeated cocaine administration in mPFC pyramidal neurons is primarily attributable to

Figure 1. Repeated cocaine administration promotes LTP induction in mPFC pyramidal neurons. (A) Left, the protocol for LTP induction consists of 10 bursts of EPSP-spike pairs. Right, a typical postsynaptic response during one burst of paired stimuli. (B, C) Example (top) and summary (bottom) of normalized EPSP slope before and after paired stimulation in slices from rats treated with saline (B) or cocaine (C) for 5 days. Arrows indicate application of the LTP induction protocol. Representative traces of EPSPs were taken at the time indicated by number. Input resistance (R) was monitored throughout the experiment (middle). (D, E) Summary of experiments showing the effect of paired stimulation on EPSP slope in slices from rats treated with a single dose of saline (D) or cocaine (E) for 1 day. Total neurons examined is indicated by n.
a reduction of postsynaptic GABA<sub>A</sub> receptor responsiveness, whereas GABA<sub>A</sub> receptor function is not altered by single cocaine exposure.

Consistent with the reduced GABA-mediated inhibition of mPFC pyramidal neurons, we found that the firing activity of these neurons by quantifying the number of action potentials in response to postsynaptic depolarizing current injection (1 nA, 500 ms) was increased after repeated cocaine exposure (12.1 ± 2.3 spikes, n = 12; P < 0.01), compared with saline-treated controls (7.2 ± 0.9 spikes, n = 10). BMI itself increased the firing rate (16.2 ± 2.1 spikes, n = 10; P < 0.01), and the cocaine-induced increase in firing rate was significantly diminished in the presence of BMI (14.9 ± 1.9 spikes, n = 10) (Fig. 4). No significant changes on membrane potential, action potential threshold, or kinetics were observed in mPFC pyramidal neurons from repeated cocaine exposure rats, compared with saline-treated controls.

**Surface GABA<sub>A</sub> Receptors Are Lost Following Repeated Cocaine Administration**

How might repeated cocaine administration reduce postsynaptic GABA responsiveness in mPFC pyramidal neurons? A straightforward hypothesis is that repeated cocaine administration stimulates postsynaptic endocytotic removal of GABA<sub>A</sub> receptor from the plasma membrane, resulting in a persistent reduction of GABA-mediated synaptic transmission. To test this idea, we made surface biotinylation to measure levels of surface GABA<sub>A</sub> receptor α1 subunits in mPFC slices. In slices from rats
treated with cocaine for 5 days, we found that there was a significant reduction of surface expression of GABAA receptor α1 subunits, with no change in the total α1 subunit (Fig. 5A).

However, neither rats treated with single injection of cocaine nor saline-treated rats significantly affect the surface expression of GABAA receptor α1 subunits (Fig. 5B). One possible mechanism that might account for the alteration of postsynaptic GABA responsiveness is the changes in GABAA receptor subunit composition (Gingrich et al. 1995). To explore this possibility, we compared the kinetics of the evoked IPSCs and mIPSCs in mPFC pyramidal neurons. We found no significant difference between cocaine- and saline-treated rats in rise time and decay time constant of both evoked IPSCs (saline—rise time: 2.3 ± 0.4 ms, decay time: 22.8 ± 3.1 ms, n = 5; cocaine—rise time: 2.2 ± 0.3 ms, decay time: 23.4 ± 3.5 ms, n = 6) and mIPSCs (saline—rise time: 2.1 ± 0.3 ms, decay time: 20.8 ± 3.5 ms, n = 10; cocaine—rise time: 2.0 ± 0.3 ms, decay time: 21.4 ± 2.9 ms, n = 12), suggesting that repeated cocaine exposure does not affect GABAA receptor subunit composition.

Recent evidence indicates that a single exposure to cocaine in vivo results in an increase in the ratio of AMPA receptor- to NMDA receptor-mediated synaptic response in the VTA DA neurons in midbrain slices (Ungless et al. 2001; Saal et al. 2003). To investigate whether AMPA receptor function was modified in rats following repeated cocaine administration, we examined mEPSCs. The mEPSCs were recorded at a holding potential of −70 mV.
Cocaine Promotes LTP Induction Via Activation of D1-like Receptors

Considering that cocaine blocks the DA reuptake transporter and thus acutely increases local DA concentrations in brain areas receiving dopaminergic inputs (Hyman 1996), it is therefore possible that activation of DA receptors in critical brain areas is required for the aforementioned cocaine-induced enhancement of LTP induction. Based on gene sequence and pharmacological profile, DA receptors could be divided into 2 families, D1- and D2-like receptors (Civelli et al. 1993). To examine the role of these DA receptor subtypes, we administrated specific D1- or D2-like receptor antagonists before cocaine injection. As shown in Figure 6A–C, neither the D1 receptor antagonist SCH23390 (0.5 mg/kg) nor the D2 receptor antagonist raclopride (0.5 mg/kg) had a significant effect on pairing stimulation when administered with saline (SCH23390, 108.6 ± 5.8% of baseline, n = 4; raclopride, 106.5 ± 6.2% of baseline, n = 4). When administered with cocaine, SCH23390, but not raclopride, prevented LTP induction in slices from 5-day cocaine-treated rats (SCH23390, 112.8 ± 6.5% of baseline, n = 6; raclopride, 143.5 ± 6.9% of baseline, n = 6).

It has been shown that administration of SCH23390 into the mPFC prevents the expression of 3,4-methylenedioxymethamphetamine-induced behavioral sensitization through the activation of 5-HT₂C, but not of D1 receptors (Ramos et al. 2005). To exclude a role for the 5-HT₂C receptor activation in the development of cocaine-induced enhancement of LTP induction, we administrated specific 5-HT₂C receptor antagonist RS102221 15 min before cocaine injection. However, in contrast to SCH23390, RS102221 did not significantly affect the induction of LTP in slice from 5-day cocaine-treated rats (136.5 ± 5.3% of baseline, n = 4) (Fig. 6F). RS102221 also had no significant effect on paired stimulation when administered with saline (105.5 ± 4.5% of baseline, n = 3) (Fig. 6F). Taken together, these findings indicate that the activation of D1-like receptors is required for the facilitatory effect of repeated cocaine administration on LTP induction in mPFC pyramidal neurons. Furthermore, the cocaine-induced reduction in the amplitude of mIPSC and surface GABA<sub>A</sub> receptor α1 subunits was also blocked by administrating SCH23390 (Fig. 5F).

It has been reported that D1-like receptors may play an important role in the development of cocaine-induced behavioral sensitization (Fontana et al. 1993; McCracken and Marsden 1993; Tell 1994; Xu et al. 2000; Zhang et al. 2000). If the cocaine-induced enhancement of LTP induction in the mPFC is related to the development of behavioral sensitization, administration of SCH23390 with cocaine should block cocaine-induced behavioral sensitization. Consistent with this prediction, pretreatment with SCH23390 (0.5 mg/kg) 15 min before each of the 5 daily cocaine injections not only prevented the acute psychomotor stimulant effects of cocaine but also reduced the development of sensitization when tested after a 3-day withdrawal (Supplementary Fig. S4). Raclopride (0.5 mg/kg) also significantly attenuated the acute locomotor activation produced by cocaine but failed to influence the development of sensitized locomotion after repeated cocaine administration.

The best-characterized consequence of D1-like receptor stimulation is the activation of adenyl cyclases, formation of cAMP, and activation of PKA (Stoof and Kebabian 1981). We next wondered whether D1-like receptor/cAMP/PKA plays a critical role in the cocaine-induced reduction in the surface GABA<sub>A</sub> receptor α1 subunit. To test this idea, the highly selective PKA inhibitor KT5720 was bilaterally infused into...
**Figure 5.** Repeated cocaine treatment reduces the surface expression of GABA<sub>Δ</sub> receptor α1 subunit. (A, B) Top, representative immunoblot showing the surface GABA<sub>Δ</sub> receptor α1 subunit in slices from rats receiving saline or cocaine pretreatment for 5 days (A) or 1 day (B). Bottom, densitometric quantification revealed the reduction of surface GABA<sub>Δ</sub> receptor α1 subunit in slices from rats receiving cocaine pretreatment for 5 days, which was blocked by coadministration of SCH23390 (0.5 mg/kg) but not raclopride (0.5 mg/kg) with cocaine. Neither a single injection of cocaine nor time-matched saline injection alters the surface expression of GABA<sub>Δ</sub> receptor α1 subunit in mPFC slices. The total number of slices examined is shown in parentheses. Results are shown as means ± standard error of mean. Asterisk indicates significant difference from saline group at *P < 0.05.*

the mPFC before cocaine injection. We found that KT5720 blocks the cocaine-induced reduction in the surface expression of GABA<sub>Δ</sub> receptor α1 subunit (Fig. 7).

**Discussion**

Repeated exposure to psychostimulants results in a progressive and enduring augmentation in the locomotor activating and reinforcing effects of these drugs, a phenomenon known as behavioral sensitization. The neuronal plasticity underlying behavioral sensitization has been suggested to model some of the core features of addiction (Robinson and Berridge 1993; Wolf 1998), as well as the development of drug-elicited psychosis (Vanderschuren and Kalivas 2000). Over the last few years, evidence has accumulated that modifications in excitatory neural circuits in the mPFC may be important in the development of sensitization (Li et al. 1997; Tzschentke 2001; Steketee 2003). However, adaptations of excitatory synaptic transmission following repeated drug exposure have not been examined directly. In this study, we measured LTP induction at the excitatory synapses in slices of the mPFC prepared from animals in which behavioral sensitization was induced by repeated in vivo treatment with cocaine. We found that cocaine treatment facilitates LTP induction in mPFC layer V pyramidal neurons, and this occurred only in repeated cocaine exposure but not a single exposure. Consistent with the idea that cocaine promotes LTP induction by reducing GABA-mediated inhibition, we found that the amplitude of GABA<sub>Δ</sub> receptor-mediated synaptic currents and levels of surface GABA<sub>Δ</sub> receptor α1 subunit was significantly reduced in the cocaine-treated rats. Changes in glutamate-mediated synaptic response and in the probability of transmitter release were not detected. Together, these findings suggest that this cocaine-induced enhancement of LTP induction in the mPFC may be important for drug-induced behavioral plasticity.

The finding that modulation of GABA-mediated inhibition is able to control the induction of LTP in the mPFC is consistent with previous studies in the hippocampus, indicating that LTP induction is facilitated by blockade of GABA<sub>Δ</sub> receptors (Wigstrom and Gustafsson 1983) or by GABA<sub>Δ</sub> autoreceptors on feedforward interneurons (Davies et al. 1991). In addition, GABA-mediated inhibition has also been shown to suppress LTP induction at many central excitatory synapses, including in the visual cortex (Huang et al. 1999), the lateral amygdala (Bissiere et al. 2003), and the VTA (Liu et al. 2005). Our results further show that repeated cocaine exposure in vivo, by reducing GABA<sub>Δ</sub> receptor-mediated inhibition, could result in enhanced neuronal excitability and promote LTP induction. Rather, the observed cocaine-induced neuronal adaptations are persistent and remain several days after termination of cocaine treatment. A similar adaptation has been observed in VTA DA neurons, in which, after inducing behavioral sensitization, using the similar injection protocol described here, GABA<sub>Δ</sub> receptor-mediated synaptic transmission could reflect a decrease in the presynaptic release of GABA, or a decrease in postsynaptic GABA<sub>Δ</sub> receptor responsiveness, or a combination of them. The present study has revealed that repeated cocaine exposure selectively reduced the mIPSC amplitude in mPFC pyramidal neurons (Fig. 3B), suggesting that this synaptic modification is of postsynaptic origin. The rise and decay kinetics of evoked IPSCs and mIPSCs are unaffected by repeated cocaine administration, arguing against changes in GABA<sub>Δ</sub> receptor composition. Further experiments using surface biotinylation to measure levels of surface GABA<sub>Δ</sub> receptor α1 subunits in mPFC slices indicated that the cocaine-induced decrease in GABA-mediated synaptic transmission is due to a postsynaptic reduction in surface expression of GABA<sub>Δ</sub> receptors on the cell membrane (Fig. 5A). In accordance with this notion, a previous study has also demonstrated that repeated cocaine treatment leads to a decrease in benzodiazepine binding site, a part of the GABA<sub>Δ</sub> receptor complex, in the mPFC.
In contrast, in amphetamine-induced behavioral sensitization study, Gruen et al. (1999) found no changes in GABAA receptor binding in the mPFC following repeated exposure to amphetamine. The reasons for this discrepancy are unclear. One possible explanation is the use of different psychostimulants (cocaine vs. amphetamine) in these studies resulting in the activation of different cellular processes that may vary in their model of action and produce different types of behavioral sensitization effects (Steketee 2003).

In addition to GABAA receptors, GABAB receptors have been shown to regulate the induction of LTP in many brain areas (Staubli et al. 1999; Shaban et al. 2006). A recent study has implicated an important role for the GABA B receptors in regulating molecular and behavioral effects of cocaine (Lhuiller et al. 2006). Moreover, it has been shown that early withdrawal from repeated cocaine exposure is associated with a decrease in GABAB receptor responsiveness in the mPFC (Jayaram and Steketee 2004). Here, we found that CGP55845 did not affect the cocaine-induced enhancement of LTP induction. In addition, CGP55845 failed to facilitate the induction of LTP in slices from saline-treated rats. Thus, it is unlikely that the increased susceptibility to LTP induction after repeated cocaine administration seen in the present study is caused by the alteration of GABAB receptor function.

Cocaine blocks the reuptake of DA, norepinephrine, and serotonin (Koe 1976; Heikkila et al. 1979). Although there is (Goeders 1991). In contrast, in amphetamine-induced behavioral sensitization study, Gruen et al. (1999) found no changes in GABAA receptor binding in the mPFC following repeated exposure to amphetamine. The reasons for this discrepancy are unclear. One possible explanation is the use of different psychostimulants (cocaine vs. amphetamine) in these studies resulting in the activation of different cellular processes that may vary in their model of action and produce different types of behavioral sensitization effects (Steketee 2003).

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Cocaine blocks the reuptake of DA, norepinephrine, and serotonin (Koe 1976; Heikkila et al. 1979). Although there is
some evidence that norepinephrine and serotonin are also involved in the development of cocaine-induced behavioral sensitization (Cunningham et al. 1992; Drouin et al. 2002), an extensive literature of cocaine-induced behavioral sensitization studies has revealed a fundamental role for mesocorticolimbic DA systems. Here, we further show that the effect of repeated cocaine exposure on the induction of LTP and behavioral sensitization were likely mediated by the activation of D1-like receptors. This conclusion is mainly based on the observation that the cocaine-induced enhancement of LTP induction and sensitized locomotion were prevented after pharmacological blockade of D1-like but not of D2-like receptors (Fig. 6 and supplementary Fig. S4). Furthermore, the cocaine-induced reduction in mIPSC amplitude was also blocked by D1-like receptor antagonist. Although these experiments do not allow us to determine where in the brain D1-like receptors are required to trigger the effects of cocaine, our results are consistent with the recent findings showing that the D1-like receptor play an essential role in the development and/or expression of cocaine-induced behavioral sensitization (Fontana et al. 1993; McCready and Marsden 1993; Tella 1994; Xu et al. 2000; Zhang et al. 2000). Similarly, Dong et al. (2004) also demonstrated that activation of D1-like receptor is required for the cocaine-induced synaptic potentiation in VTA DA neurons. Nevertheless, there are several studies demonstrating that pharmacological blockade of D1-like receptors does not prevent the development of behavioral sensitization to cocaine (Mattingly et al. 1994, 1996; Steketee 1998; White et al. 1998). Additionally, a previous study has demonstrated that D1-like receptor activation in the mPFC prevents the expression of cocaine sensitization (Sorg et al. 2001). The reason for this discrepancy is not clear. It remains to be determined if the use of different ages of animals and the withdrawal time has a role in explaining in conflicting results. It is also possible that SCH23390 did not prevent cocaine-induced behavioral sensitization but appeared to mask or delay the induction of cocaine-induced behavioral sensitization (White et al. 1998). Further work is needed to assess this possibility.

An intriguing question arises as to what intracellular signaling pathways might give rise to D1-like receptor to regulate GABA<sub>A</sub> receptor function and hence to promote LTP induction in mPFC pyramidal neurons. Because it is well documented that D1-like receptor couples to stimulatory G-proteins (G<sub>s</sub>) thereby activating adenyl cyclase and increasing cAMP formation (Stoof and Kebabian 1981), we speculate that the cocaine-induced regulation of GABA<sub>A</sub> receptor function might be through the activation of PKA. Given that the cocaine-induced reduction of GABA<sub>A</sub> receptor-mediated synaptic transmission is correlated with the loss of surface GABA<sub>A</sub> receptor α1 subunit expression and intra-mPFC infusion of KT5720 prevents cocaine-induced reduction in the surface expression of GABA<sub>A</sub> receptor α1 subunit, it will be important for future studies to delineate whether D1-like receptors regulate GABA-mediated signaling through a mechanism by increasing phospho-dependent endocytosis of GABA<sub>A</sub> receptors. Although the relative dynamics of delivery, removal, and recycling as factors determinate the steady-state number of surface GABA<sub>A</sub> receptors, controlling the stability of GABA<sub>A</sub> receptors in the inhibitory synapses by anchoring proteins may also affect the abundance of GABA<sub>A</sub> receptors (Kittler et al. 2000; Herring et al. 2003). We could not exclude the possibility that the GABA<sub>A</sub> receptor anchoring proteins may also be the target molecule for D1-like receptor signaling to regulate the abundance of GABA<sub>A</sub> receptors during repeated cocaine treatment and after cocaine withdrawal.

Interestingly, a very recent study showed that administration of SCH23390 into the mPFC prevents the expression of 3,4-methylenedioxymethamphetamine-induced behavioral sensitization through the activation of 5-HT<sub>2C</sub> but not of D1 receptors (Ramos et al. 2005). Given that SCH23390 also exhibits a high affinity to 5-HT<sub>2C</sub> receptors (K<sub>c</sub> = 0.3 nM for D1 receptor vs. K<sub>c</sub> = 6.3 nM for 5-HT<sub>2C</sub> receptors) (Millan et al. 2001), it might be thought that the antagonizing effect of SCH23390 on cocaine seen in the present study is in part through its action on 5-HT<sub>2C</sub> receptors. However, this possibility seems unlikely because the selective 5-HT<sub>2C</sub> receptor antagonist RS102221 did not affect the cocaine-induced enhancement of LTP induction (Fig. 6F).

Another interesting observation made in our study is that the AMPA receptor function in mPFC neurons was not changed after repeated cocaine exposure (Supplementary Fig. S3), suggesting that enhancement of LTP induction in these neurons is probably not attributable to altering the quality of excitatory transmission. Other investigators also demonstrated that repeated cocaine exposure produces no change in the AMPA receptor to NMDA receptor ratio in the VTA DA neurons (Borgland et al. 2004; Liu et al. 2005), although this ratio was significantly increased after a single exposure to cocaine.

In conclusion, our data indicate that repeated cocaine administration in vivo promotes LTP induction in mPFC pyramidal neurons by reducing GABA-mediated inhibition. We also observe that D1-like receptors are required to trigger the cocaine-induced enhancement of LTP induction and behavioral sensitization, in agreement with previous studies (Fontana et al. 1993; McCready and Marsden 1993; Tella 1994; Xu et al. 2000; Zhang et al. 2000). These findings provide a major advance in establishing correlation and possible links between the cocaine-induced synaptic plasticity in the mPFC and the development of behavioral sensitization to cocaine. Moreover, the current results could lead to the development of pharmacological therapies based on the enhancement of GABA-mediated inhibition in the mPFC that may be useful in reducing cocaine addiction.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

**Notes**

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