

Repeated Cocaine Administration Increases Membrane Excitability of Pyramidal Neurons in the Rat Medial Prefrontal Cortex

Fernando J. Nasif, Kyriaki Sidiropoulou, Xiu-Ti Hu, and Francis J. White

Department of Cellular and Molecular Pharmacology, Rosalind Franklin University of Medicine and Science, the Chicago Medical School, North Chicago, Illinois

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ABSTRACT

Although the medial prefrontal cortex (mPFC) plays a critical role in cocaine addiction, the effects of chronic cocaine on mPFC neurons remain poorly understood. Here, we performed visualized current-clamp recordings to determine the effects of repeated cocaine administration on the membrane excitability of mPFC pyramidal neurons in rat brain slices. Following repeated cocaine administration (15 mg/kg/day i.p. for 5 days) with a 3-day withdrawal, alterations in membrane properties, including increased input resistance, reduced intensity of intracellular injected currents required for generation of Na⁺-dependent spikes (rheobase), and an increased number of spikes evoked by depolarizing current pulses were observed in mPFC neurons. The current-voltage relationship was also altered in cocaine-pretreated neurons showing reduced outward rectification during membrane depolarization and decreased inward rectification during membrane hyperpolarization. Application of

the K⁺ channel blocker Ba²⁺ depolarized the resting membrane potential (RMP) and enhanced membrane potential response to injection of hyperpolarizing current pulses. However, the effects of Ba²⁺ on RMP and hyperpolarized membrane potentials were significantly attenuated in cocaine-withdrawn neurons compared with saline-pretreated cells. These findings indicate that repeated cocaine administration increased the excitability of mPFC neurons after a short-term withdrawal, possibly via reducing the activity of the potassium inward rectifiers (K_{ir}) and voltage-gated K⁺ currents. Similar changes were also observed in cocaine-pretreated mPFC neurons after a long-term (2–3 weeks) withdrawal, revealing a persistent increase in excitability. These alterations in mPFC neuronal excitability may contribute to the development of behavioral sensitization and withdrawal effects following chronic cocaine exposure.

The mPFC is involved in several aspects of drug addiction, including the primary rewarding effects of cocaine and mechanisms underlying addiction and craving (for review, see Tzschentke, 2001). In humans, the mPFC is activated during cocaine withdrawal (Volkow et al., 1991, 1996) and also by cue-induced cocaine craving (Grant et al., 1996; Maas et al., 1998; Childress et al., 1999). In rodents, the mPFC is necessary for the development of behavioral sensitization induced by cocaine and other psychostimulants. Lesions of the mPFC prevent the development of cocaine sensitization (Li et al.,

1999), indicating that intact glutamatergic output from the mPFC is necessary for the enduring neuroadaptations related to cocaine addiction (Pierce et al., 1998; Wolf, 1998). Lesions of the mPFC also prevent neuroadaptations in the ventral tegmental area (VTA) and nucleus accumbens, two important brain regions associated with psychostimulant-induced behavioral sensitization (Li et al., 1999). These findings suggest that glutamatergic inputs from the mPFC play a critical role in cocaine-induced neuroadaptations in the reward system. Despite the growing evidence for participation of the mPFC in drug addiction and behavioral sensitization, little is known about how chronic cocaine exposure affects the activity of mPFC pyramidal neurons.

Previous studies suggest that the membrane excitability of pyramidal neurons within the mPFC may be increased and the glutamatergic output from the mPFC may be facilitated following chronic treatment with psychostimulants. In fact, the inhibitory effects of dopamine on mPFC neuronal activity

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ABBREVIATIONS: mPFC, medial prefrontal cortex; VTA, ventral tegmental area; VGKC, voltage-gated outward potassium current; RMP, resting membrane potential; AHP, afterhyperpolarization; aCSF, artificial cerebrospinal fluid; I-V, current-voltage; SAL, saline; COC, cocaine; K_{ir}, potassium inward rectifiers; I_{Kir}, inwardly rectifying K⁺ currents.

is found to be attenuated, whereas glutamate-induced excitation is enhanced following repeated cocaine or amphetamine administration (White et al., 1995; Peterson et al., 2000). Moreover, the density of voltage-gated outward potassium currents (VGKC) is also significantly decreased in a voltage-dependent manner, and repetitive firing evoked by depolarizing current pulses is facilitated in cocaine-pretreated mPFC neurons (Dong et al., 2002). These findings indicate that the glutamatergic outputs from the mPFC are facilitated, particularly in the nucleus accumbens of rats that had developed behavioral sensitization to cocaine (Pierce et al., 1996).

Our previous investigations have demonstrated that repeated cocaine administration induces significant alterations in the membrane properties and activity of a variety of membrane ion channels in nucleus accumbens neurons, leading to a decrease in membrane excitability (Zhang et al., 1998, 2002). These results suggest that alterations in the membrane properties as well as in ion channel activity may also occur in cocaine-pretreated mPFC pyramidal neurons. Based upon our previous findings, we hypothesized that, in contrast to the changes observed in nucleus accumbens neurons, chronic cocaine pretreatment may increase the membrane excitability via changing the activity of different ion currents, including but not limited to decreased VGKC. Since inwardly rectifying K^+ currents (I_{Kir}) play an important role in maintaining the RMP and modulating the inward rectification of mPFC pyramidal neurons during membrane hyperpolarization, the present study was performed to determine whether the membrane properties were affected and whether the activity of the I_{Kir} , in addition to decreased VGKC, was altered in mPFC pyramidal neurons following repeated cocaine administration.

Materials and Methods

Animals and Treatment. Male Sprague-Dawley (Harlan, Indianapolis, IN) rats (4–5 weeks old) were group-housed in a temperature- and humidity-controlled vivarium under a 12-h light/dark cycle. Food and water were freely available. Animals received repeated administration of saline or cocaine (15 mg/kg/day i.p.) for 5 days followed by 3 days (short-term) of withdrawal prior to the experiment. Another group of rats was evaluated after 2 to 3 weeks (long-term) of withdrawal from repeated saline or cocaine administration. All comparisons between saline- and cocaine-pretreated groups were performed by experimenters blind to group assignment.

Preparation of Brain Slices. All procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals and were approved by our Institutional Animal Care and Use Committee. Rats were decapitated under halothane anesthesia, and the brain was immediately excised and immersed in ice-cold artificial cerebrospinal fluid (aCSF) containing: 124 mM NaCl, 2.5 mM KCl, 26 mM NaHCO_3 , 2 mM MgCl_2 , 2 mM CaCl_2 , and 10 mM glucose; pH 7.4; 310 mOsm/l. Coronal slices (300 μm) containing the mPFC were cut with a vibratome (Leica VT1000S) and incubated in oxygenated (95% O_2 /5% CO_2) aCSF for 1 h at room temperature before recording.

Current-Clamp Recordings. Brain slices were anchored in the recording chamber and perfused by gravity-fed oxygenated aCSF (34°C) at a flow rate of 2 to 3 ml/min. Patch recording pipettes (3–5 M Ω) were pulled from Corning 7056 (Corning Glassworks, Corning, NY) glass capillaries with a horizontal pipette puller (Flaming/Brown P-97; Sutter Instrument Company, Novato, CA) and filled with 120 mM K^+ -gluconate, 10 mM HEPES, 0.1 mM EGTA, 20 mM KCl, 2 mM MgCl_2 , 3 mM Na_2ATP , and 0.3 mM Na_2GTP . Whole-cell

patch recordings were initiated in visually identified pyramidal neurons located within layers V–VI of the mPFC using differential interference contrast (DIC) microscopy (Stuart et al., 1993). Some pyramidal neurons within the motor cortex were also recorded as a control. After whole-cell configuration was formed, recordings were converted to current clamp using a SEC-05L npi amplifier (ALA Scientific Instruments, Westbury, NY). Voltage signals were amplified in bridge mode, digitized by a DigiData 1200 Series (Axon Instruments Inc., Union City, CA), and distributed to a computer running pCLAMP 7.01 software (Axon Instruments). The current-voltage (I–V) relationship at negative membrane potentials was studied following injection of hyperpolarizing current pulses (500 ms duration, 0 to -0.8 nA), whereas the I–V curve at positive membrane potentials was made by injecting depolarizing current pulses (0 to $+0.7$ nA) starting after a 5-min perfusion of the specific sodium channel blocker tetrodotoxin (1 μM). Membrane properties were studied in the following manner. RMP was measured in the absence of injected current. Input resistance was determined from linear regression in the linear range (generally ± 10 mV from the RMP) of the voltage-current relationship established by plotting the steady-state voltage change in response to depolarizing and hyperpolarizing current pulses. Time constants were determined by the fit function of pCLAMP software. Na^+ -dependent action potentials were generated by injection of step depolarizing current pulses with 0.05-nA increments. Characteristics of the action potentials were obtained from the initial spike evoked by the minimal depolarizing current pulse in each mPFC neuron recorded. In all cases, the spikes were evoked from the RMP. Action potential amplitude was measured from the spike threshold. Afterhyperpolarization (AHP) amplitude was measured from the equipotential point of the spike threshold to the maximum deflection of the hyperpolarization after the end of the action potential. The action potential duration was measured at one-half amplitude. Whole-cell pipette series resistance was less than 20 M Ω and bridge was compensated. Only cells with a stable RMP at or more negative than -60 mV and evoked spikes that overshoot across a 0-mV level were used for analysis and drug treatment.

Drug Application. Tetrodotoxin (1 μM ; Sigma-Aldrich, St. Louis, MO) and the K^+ channel blocker barium chloride (BaCl_2 , 200 μM ; Sigma-Aldrich) were bath-applied by gravity at a flow rate of 2 to 3 ml/min. During the study of inward rectification, the membrane response to a constant hyperpolarizing current pulse (-0.8 nA) was measured before and during Ba^{2+} application for at least 10 min. When drugs were applied, only one cell per slice was tested.

Statistics. Current-voltage and current-spike relationships were analyzed by analysis of variance with repeated measures and post hoc comparisons carried out by using Newman-Keuls test. The effects of repeated cocaine administration on membrane properties were compared with those from saline-pretreated rats using Student's *t* test. The effects of Ba^{2+} on the membrane properties observed from saline- and cocaine-pretreated groups were also compared using Student's *t* test for unpaired samples, and within-cell comparisons were made using Student's *t* test for paired samples.

Results

Short-Term (3 Days) Withdrawal

Repeated Administration of Cocaine Altered the Membrane Properties and Increased the Evoked Spikes in mPFC Pyramidal Neurons. The passive and active membrane properties of pyramidal neurons located within layer V–VI of the mPFC were first characterized in saline-pretreated rats. Injection of depolarizing and hyperpolarizing current pulses into saline-pretreated mPFC neurons induced depolarization and hyperpolarization of the membrane potential, respectively. When the depolarized mem-

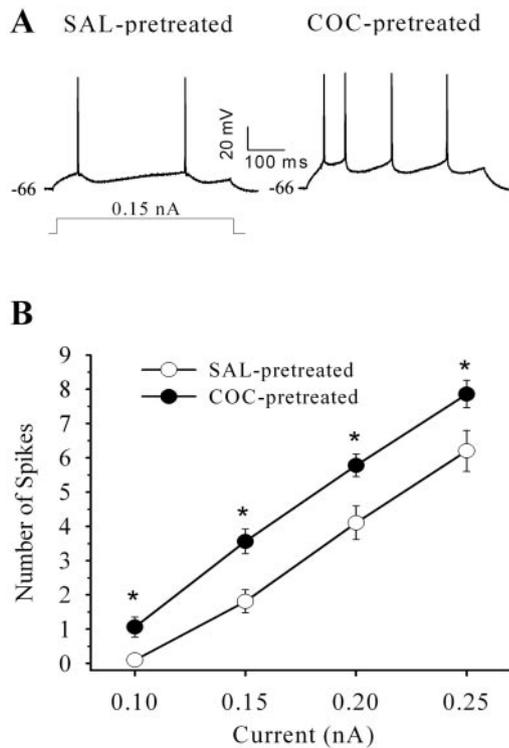


Fig. 1. Repeated COC administration increased Na^+ -dependent action potentials evoked by depolarizing current pulses in rat mPFC pyramidal neurons following a 3-day withdrawal. **A**, representative traces showing action potentials evoked by a depolarizing current pulse (0.15 nA, 500 ms) in a SAL- versus a COC-pretreated pyramidal neuron. **B**, current-spike response curves indicate that the number of evoked spikes in response to depolarizing currents (0.10–0.25 nA) is significantly increased in COC-pretreated mPFC neurons compared with SAL-pretreated cells (SAL versus COC group, $n = 21/18$ cells). Values represent mean \pm S.E.M. *, $p < 0.05$, Newman-Keuls test.

brane potential reached the firing threshold, a sodium-dependent action potential was evoked (Fig. 1A). Increasing the intensity of depolarizing currents generated multiple action potentials (Fig. 1B). The passive and active membrane properties of mPFC neurons were studied and summarized in Table 1. Repeated administration of cocaine induced significant alterations in certain membrane properties, including increased *input resistance* and decreased depolarizing current pulses required for generation of action potentials (rheobase) compared with saline-pretreated mPFC neurons. Moreover, repeated cocaine pretreatment also increased the number of spikes evoked by injection of depolarizing current

TABLE 1

Repeated cocaine administration (15 mg/kg/day) in vivo, followed by 3 days of withdrawal, altered input resistance and current to generate action potentials in mPFC pyramidal neurons

The passive and active membrane properties measured by whole-cell current-clamp recordings of mPFC pyramidal neurons from brain slices from saline- and cocaine-pretreated rats. Values represent the mean \pm S.E.M. for the number of neurons indicated.

Pretreatment	Saline	Cocaine
Number of neurons	21	18
Passive membrane properties		
RMP (mV)	-66.01 ± 0.34	-66.15 ± 0.35
Input resistance (m Ω)	79.60 ± 6.64	$105.28 \pm 7.53^*$
Active membrane properties		
Current to generate action potential (nA)	0.17 ± 0.01	$0.12 \pm 0.01^*$
Action potential threshold (mV)	-43.99 ± 0.93	-43.78 ± 0.96
Action potential amplitude (mV)	89.94 ± 2.02	91.69 ± 1.63
Action potential duration (ms)	0.97 ± 0.05	0.94 ± 0.03
AHP amplitude (mV)	5.61 ± 0.53	4.54 ± 0.63

* $p < 0.05$; Student's *t* test compared with SAL.

pulses (Fig. 1A). There was a significant leftward shift in the current-spike response curves obtained from cocaine- compared with saline-pretreated mPFC neurons, indicating a facilitation in evoked repetitive firing (saline- versus cocaine-pretreated: $n = 21/18$ cells, $F_{1,32} = 7.75$; $p < 0.01$) (Fig. 1B).

Repeated Administration of Cocaine Altered the I-V Relationship. The outward and inward rectification were also studied using the I-V curve based on the alterations in the membrane potential in response to applied current pulses. With application of tetrodotoxin, which blocks generation of Na^+ -dependent action potentials, injection of depolarizing current pulses into saline-pretreated mPFC pyramidal neurons induced an outward rectification, reflected by a downward shift in membrane potential during membrane depolarization (Fig. 2). However, repeated cocaine pretreatment markedly attenuated the outward rectification in mPFC neurons (Fig. 2A). Therefore, the I-V curve was shifted upward to more depolarized levels following repeated cocaine pretreatment (saline- versus cocaine-pretreated: $n = 12/14$, $F_{1,24} = 4.94$; $p < 0.05$) (Fig. 2B), suggesting a decrease in VGKC. On the other hand, repeated cocaine administration also reduced the inward rectification during membrane hyperpolarization. Figure 3A shows that repeated cocaine administration significantly enhanced hyperpolarized membrane potential responses to step-pulse injection of a variety of hyperpolarizing current pulses. Therefore, the I-V curve was significantly shifted downward to more hyperpolarized levels in cocaine-pretreated neurons (saline- versus cocaine-pretreated: $n = 21/18$, $F_{1,37} = 11.93$; *, $p < 0.05$) (Fig. 3B), suggesting that the activity of K_{ir} channels might have been reduced.

Repeated Administration of Cocaine Decreased the Effects of Ba^{2+} on Blocking Inward Rectification. It is well known that although the majority of the I_{Kir} is inactivated at the RMP levels, some K_{ir} channels carry out an outward current [$\text{I}_{\text{Kir}(\text{rest})}$] to maintain the RMP near K^+ equilibrium potential (E_{k}) (Hille, 2001). Therefore, blocking the $\text{I}_{\text{Kir}(\text{rest})}$ should depolarize RMP, whereas blocking other I_{Kir} could enhance the hyperpolarized membrane potential response to membrane hyperpolarization. In addition, it is also possible that these neuronal responses to the blockade of I_{Kir} may be affected by repeated cocaine administration. Bath application of Ba^{2+} (200 μM) was used to block $\text{I}_{\text{Kir}(\text{rest})}$ and I_{Kir} in the present study. It is noted that 1) although Ba^{2+} is not specific for K_{ir} currents, no other K^+ currents are responsible for the inward rectification at the membrane

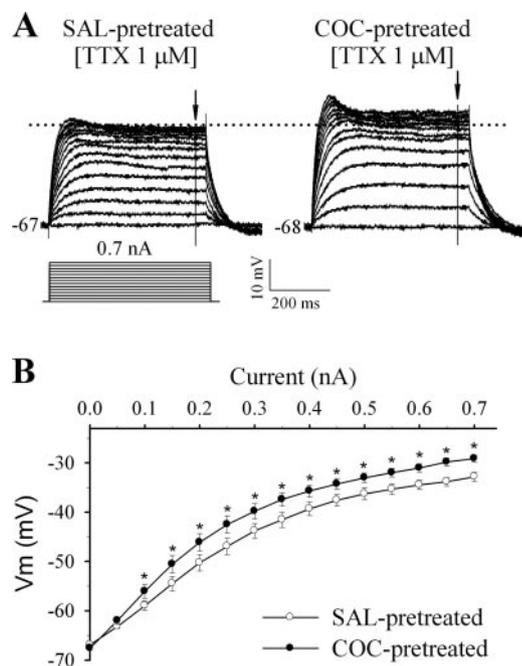


Fig. 2. Repeated cocaine administration decreased the outward rectification during membrane depolarization after a 3-day withdrawal. **A**, representative traces showing the alterations in the membrane voltage responses (V_m) to pulse injection of depolarizing currents (0–0.7 nA, 500 ms) in SAL- and COC-pretreated mPFC neurons. Following blockade of voltage-sensitive Na^+ channels with tetrodotoxin (TTX, 1 μM), the membrane depolarization of mPFC neurons in response to pulse injections of positive currents was significantly enhanced in COC-pretreated neurons. An outward rectification (reflected by the downward membrane potential traces) was observed in both neurons. It is also noted that the membrane depolarization in response to positive current pulses at an early stage (approximately within the initial 0–100 ms) was markedly enhanced in cocaine-withdrawn neurons compared with saline-pretreated cells. Arrows indicate the downward traces and the vertical lines indicate the time at which the membrane potential responses were measured. **B**, the I-V curves indicate the changes in the outward rectification (downward reflection) in mPFC pyramidal neurons during membrane depolarization with blockade of voltage-sensitive Na^+ channels. The I-V relationship is significantly shifted toward more depolarized V_m levels in COC-pretreated neurons compared with SAL-pretreated cells (SAL versus COC: $n = 12/14$ cells). Values represent mean \pm S.E.M. *, $p < 0.05$, Newman-Keuls test.

potential levels more negative than the RMP, and 2) the hyperpolarization-activated cation current (I_h) is insensitive to Ba^{2+} , although it may also contribute to the inward rectification (Funahashi et al., 2003). Although the RMP appeared to be unchanged following repeated cocaine administration (Table 1), application of Ba^{2+} significantly depolarized the RMP in both saline- and cocaine-pretreated mPFC neurons (SAL/Control: -67.46 ± 0.97 mV versus SAL/ Ba^{2+} : -59.83 ± 1.08 mV, $n = 6$ cells; $p < 0.01$; paired t test and COC/Control: -68.39 ± 0.82 mV versus COC/ Ba^{2+} : -64.64 ± 0.73 mV, $n = 6$ cells; $p < 0.01$; paired t test) (Fig. 4, A_1 and A_2). However, the effect of Ba^{2+} on the RMP was markedly reduced after repeated cocaine administration. Therefore, the Ba^{2+} -induced membrane depolarization was significantly attenuated in cocaine-withdrawn neurons compared with saline-withdrawn cells (SAL: 7.63 ± 0.61 mV versus COC: 3.75 ± 0.76 mV, $n = 6$ cells/group, Student's t test, $p < 0.01$) (Fig. 4 A_3).

When the membrane potential was generally hyperpolarized to approximately -110 mV in response to hyperpolarizing current pulses (up to -0.8 nA), a significant change in the

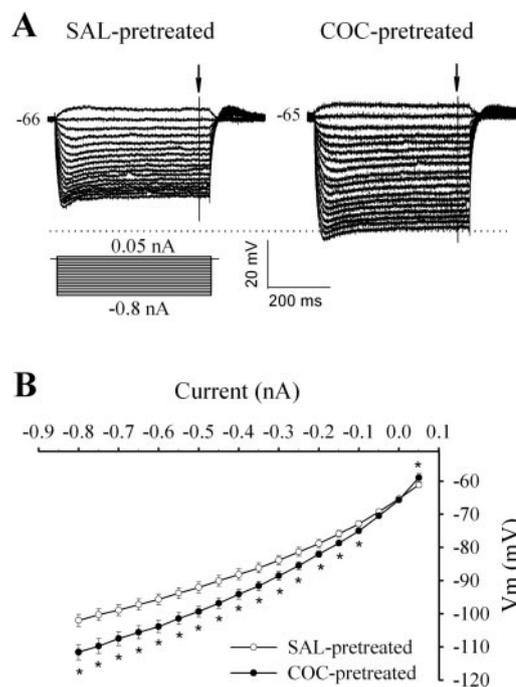


Fig. 3. Repeated cocaine administration decreased the inward rectification during membrane hyperpolarization after a 3-day withdrawal. **A**, representative traces showing the alterations in the membrane potential response of mPFC neurons to pulse injection of hyperpolarizing currents (-0.8 to $+0.05$ nA, 500 ms) following repeated SAL or COC pretreatment. The hyperpolarized V_m in response to negative currents was significantly enhanced in COC- compared with SAL-pretreated mPFC neurons, indicating a reduced inward rectification. Arrows indicate the time at which the voltage responses were measured. **B**, I-V curve was significantly shifted toward a more hyperpolarized direction in COC- compared with SAL-pretreated neurons (SAL versus COC: $n = 21/18$ cells). Values represent mean \pm S.E.M. *, $p < 0.05$, Newman-Keuls test.

I-V curves were observed with application of Ba^{2+} in both saline- and cocaine-withdrawn cells (Fig. 4, B_1 – D_1). To make the I-V curves comparable, the RMP was always held at -66 mV during the experiments, since it was the mean for mPFC neurons (Table 1). With application of Ba^{2+} for 10 min, the voltage response to hyperpolarizing current pulses was significantly enhanced in saline- and cocaine-pretreated mPFC neurons (SAL: control versus Ba^{2+} , $n = 10$ cells, $F_{1,18} = 10.78$, $p < 0.01$ and COC: control versus Ba^{2+} , $n = 10$ cells, $F_{1,18} = 33.07$, $p < 0.01$) (Fig. 4, B_1 – D_1). Under these circumstances, the I-V curve became linear in both saline- and cocaine-withdrawn neurons indicating that the inward rectification was eliminated or significantly reduced (Fig. 4 D_1). Compared with the ones recorded without application of Ba^{2+} , the I-V curves show a transition which was initiated at approximately -90 mV in response to injection of -0.2 to -0.3 nA pulse. From there, the inward rectification (the upward flexing) was gradually lost and the I-V curve became almost a straight line. There was no significant difference in the I-V curves between saline- and cocaine-withdrawn mPFC neurons. These findings suggest that, since no other K^+ channels were responsible for the inward rectification at such hyperpolarized membrane potential levels, elimination of the inward rectification should be mainly attributed to the blockade of K_{ir} by Ba^{2+} . Similar to the action of Ba^{2+} , repeated cocaine administration also significantly reduced the inward rectification (SAL versus COC: $n = 10$ cells/group, $F_{1,18} = 12.29$; $p < 0.05$). Figure 4 D_2 further indicates that the

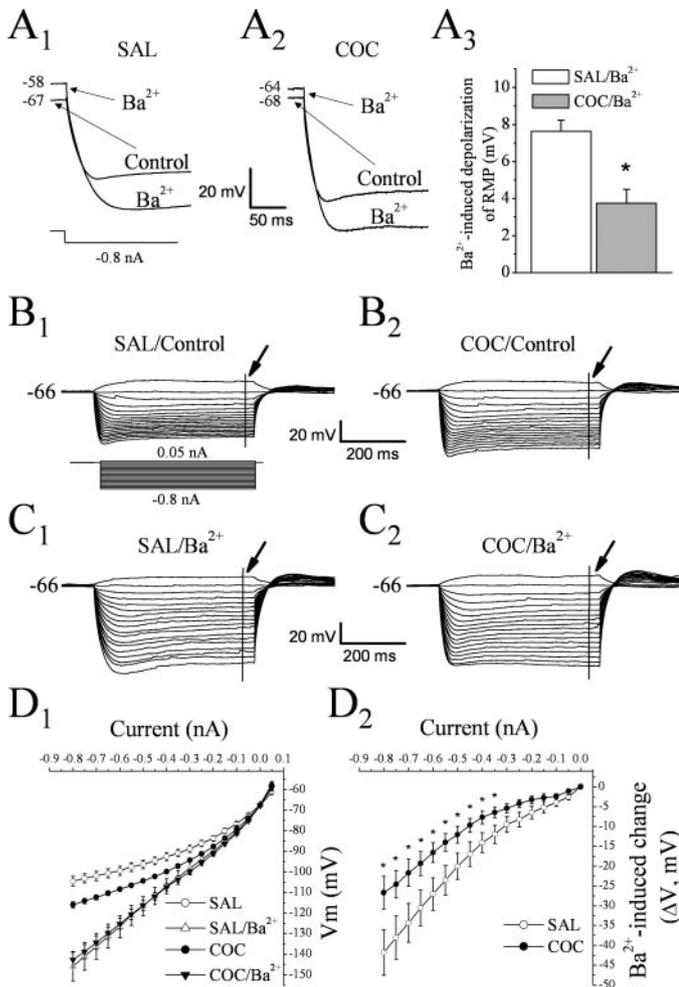


Fig. 4. The effects of Ba²⁺ on depolarizing the RMP and blocking the inward rectification were decreased in short-term cocaine-withdrawn mPFC neurons. **A**, representative traces showing that bath application of Ba²⁺ (200 μM) depolarized the RMP in both SAL- (A₁) and COC-pretreated (A₂) mPFC neurons. However, in COC-pretreated neurons, the Ba²⁺-induced depolarization was markedly attenuated. **A**₃, bar graph shows that the Ba²⁺-induced depolarization of the RMP was significantly decreased in COC- compared with SAL-pretreated mPFC neurons (Student's *t* test; *, *p* < 0.01). Representative traces showing the responses of membrane potentials to a variety of current pulses (-0.8 to 0.05 nA, 500ms) before and after bath application of Ba²⁺ in SAL- and COC-pretreated neurons (B₁, C₁ and B₂, C₂, respectively). Arrow indicates the time point at which the membrane potential was measured. Ba²⁺ also abolished the inward rectification and caused an enhancement in membrane hyperpolarization in both saline- and cocaine-pretreated mPFC neurons (comparing C₁ versus B₁ and C₂ versus B₂). Thus, the I-V curve was significantly shifted downward to a more hyperpolarized direction in both groups (SAL/control versus SAL/Ba²⁺: *p* < 0.01 and COC/control versus COC/Ba²⁺: *p* < 0.01, *n* = 10 cells each group) (D₁). Repeated cocaine administration also reduced the inward rectification (B₂ versus B₁) and significantly shifted the I-V curve downward compared with that in saline-pretreated cells (SAL versus COC: *n* = 10 cells/each, *p* < 0.05) (D₁). D₂, chronic exposure to cocaine also significantly reduced the Ba²⁺-induced change in the membrane hyperpolarization (ΔV) (SAL versus COC: *, *p* < 0.05 Newman-Keuls test). ΔV for each group is: ΔV_{SAL} = ΔV_{SAL/Ba²⁺} - ΔV_{SAL/control} and ΔV_{COC} = ΔV_{COC/Ba²⁺} - ΔV_{COC/control} whereas ΔV_{Ba} and ΔV_{control} were obtained by subtracting the RMP from the hyperpolarized membrane potential in both saline- and cocaine-pretreated groups before and after application of Ba²⁺. Values represent mean ± S.E.M.

Ba²⁺-induced responses (ΔV) in the membrane potentials was significantly reduced in cocaine-withdrawn neurons compared with saline-withdrawn cells during membrane hyperpolarization (SAL versus COC: *F*_{1,17} = 6.12; *p* < 0.05).

Long-Term (2–3 Weeks) Withdrawal

Increase of Evoked Spike Persisted in Cocaine-Pretreated mPFC Neurons. To determine whether the alterations observed in short-term cocaine-withdrawn neurons persist after a long-term withdrawal, the effects of repeated cocaine pretreatment on the passive and active membrane properties of mPFC neurons were evaluated following 2 to 3 weeks of withdrawal. After the long-term withdrawal, rheobase was still found to be significantly lower in cocaine-pretreated neurons than that in saline-pretreated neurons (Table 2). In addition, an increase in the number of evoked spikes was also present in long-term cocaine-withdrawn neurons (Fig. 5A). There was also a significant leftward shift in the current-spike response curves obtained from cocaine-pretreated mPFC neurons compared with saline-pretreated cells (saline- versus cocaine-pretreated: *n* = 16/23, *F*_{1,35} = 6.99; *p* < 0.05) (Fig. 5B), indicating that the facilitation in evoked firing persists after long-term withdrawal.

The I-V Relationship Returned to Control Levels in Cocaine-Pretreated mPFC Neurons. The alterations in the I-V relationship observed in short-term cocaine-withdrawn mPFC neurons appeared to return to the control levels and were no longer observed in cells after the long-term withdrawal. Evaluation of the I-V curves indicate that there was no detectable difference in the membrane voltage response to injection of either depolarizing current pulses (Fig. 6) or hyperpolarizing current pulses (Fig. 7) between saline- and cocaine-pretreated mPFC neurons after 2 to 3 weeks of withdrawal.

Repeated Cocaine Administration Did Not Cause Significant Changes in the Excitability of Pyramidal Neurons within the Motor Cortex. To determine whether the effects of repeated cocaine administration in mPFC pyramidal neurons are regionally specific, we also performed whole-cell recordings in pyramidal neurons located within the motor cortex. It has been established that although motor cortex neurons are morphologically similar to PFC neurons, there is a major anatomical difference between the two brain regions: the PFC receives dense dopaminergic innervations, whereas the motor cortex does not (Lindvall et al., 1978; Carr et al., 1999). Therefore, the recording results obtained from motor cortex neurons could be used as a suitable control in determination of such regional specificity. In contrast to the results from mPFC neurons, repeated cocaine administration did not cause significant alterations in the membrane properties, firing responses to application of depolarizing current pulses, and I-V relationship in pyramidal neurons within the motor cortex after either a 3-day or 2- to 3-week withdrawal (Fig. 8, Table 3). These results not only indicate that the alterations in the neuronal excitability observed in cocaine-withdrawn mPFC pyramidal neurons are regionally specific, but also suggest that the altered dopamine innervation may play a critical role in the neuroadaptation found in mPFC neurons after chronic exposure to cocaine.

Discussion

The present study demonstrates that repeated cocaine administration significantly alters the membrane properties of rat mPFC pyramidal neurons after either a short- or long-term withdrawal. The alterations result, at least partially, from a reduced activity of VGICs and possibly K_v, leading to

TABLE 2

Repeated cocaine administration (15 mg/kg/day) in vivo, followed by 2 to 3 weeks of withdrawal, altered current to generate action potentials in mPFC pyramidal neurons

The passive and active membrane properties measured by whole-cell current-clamp recordings of mPFC pyramidal neurons from brain slices from saline- and cocaine-pretreated rats. Values represent the mean \pm S.E.M. for the number of neurons indicated.

Pretreatment	Saline	Cocaine
Number of neurons	16	22
Passive membrane properties		
RMP (mV)	-68.47 ± 0.86	-66.80 ± 0.68
Input resistance (m Ω)	81.89 ± 6.92	91.60 ± 6.83
Active membrane properties		
Current to generate action potentials (nA)	0.17 ± 0.02	$0.13 \pm 0.01^*$
Action potential threshold (mV)	-43.51 ± 1.10	-43.20 ± 0.70
Action potential amplitude (mV)	95.10 ± 1.85	96.00 ± 1.58
Action potential duration (ms)	1.08 ± 0.06	1.00 ± 0.03
AHP amplitude (mV)	6.74 ± 0.76	8.11 ± 0.60

* $p < 0.05$; Student's t test compared with SAL.

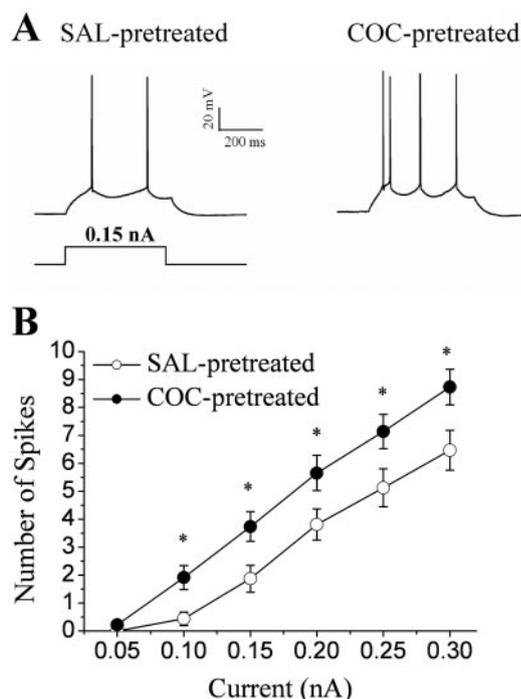


Fig. 5. Increase of evoked sodium spikes persisted in mPFC pyramidal neurons following 2 to 3 weeks of cocaine withdrawal. A, representative traces showing action potentials evoked by a depolarizing current pulse (0.15 nA, 500 ms) in a SAL- versus a COC-pretreated pyramidal neuron in the mPFC. B, current-spoke response curves showing that the number of evoked spikes in response to depolarizing currents was significantly increased in COC- compared with SAL-pretreated neurons (SAL versus COC group, $n = 16/23$ cells; *, $p < 0.05$, Newman-Keuls test). Values represent mean \pm S.E.M.

an increase in neuronal excitability therefore promoting outputs from the mPFC in response to excitatory stimuli. The persistent increase in evoked activity observed in long-term cocaine-withdrawn mPFC neurons could also be important and might be related to some prolonged withdrawal effects during cocaine abstinence, including craving and behavioral sensitization.

Short-Term Withdrawal from Repeated Cocaine Administration. The major finding of this study is that repeated cocaine administration significantly altered the membrane responsiveness of mPFC neurons to excitatory stimuli. Under these circumstances, an upward shift in the I-V curve was observed in cocaine-withdrawn neurons during membrane depolarization. The more depolarized membrane po-

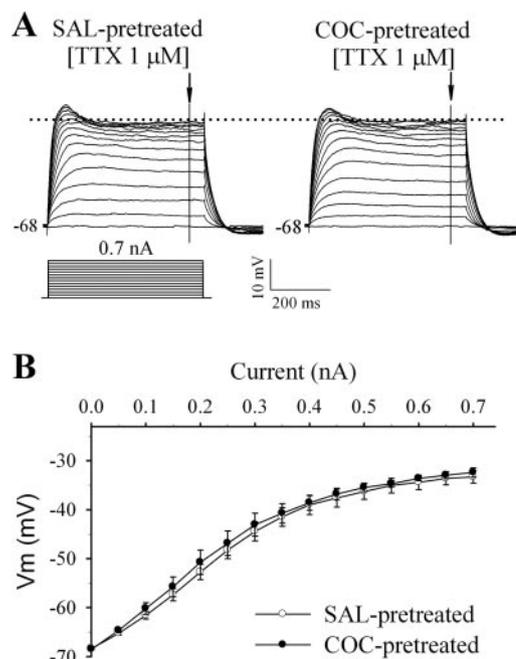


Fig. 6. The outward rectification returned to the control levels after 2 to 3 weeks of withdrawal from repeated cocaine administration. A, representative traces showing the depolarization of membrane potentials of mPFC neurons in response to pulse injection of positive currents (0 to 0.7 nA, 500 ms) following a long-term withdrawal from SAL or COC pretreatment. B, with blockade of voltage-sensitive Na^+ channels (tetrodotoxin, 1 μM), the I-V curves showed no significant difference in the outward rectification between SAL- and COC-pretreated neurons (SAL versus COC: $n = 7/6$ cells, $p > 0.05$). Values represent mean \pm S.E.M. Arrows mark the time point at which the voltage responses were measured.

tential in response to positive current pulses revealed a decrease in the outward rectification, suggesting that the activity of VGKCs was reduced in cocaine-withdrawn mPFC neurons. Previous studies have demonstrated that VGKCs, including A-type K^+ currents (I_A), play an important role in regulating the excitability of cortical neurons (Bekkers, 2000a,b; Kang et al., 2000; Korngreen and Sakmann, 2000). Phosphorylation of A-type K^+ channels by protein kinase A down-regulates I_A in cortical pyramidal neurons (Hoffman and Johnston, 1998; Yuan et al., 2002). Since repeated cocaine administration increases protein kinase A activity (Terwilliger et al., 1991), I_A should be decreased in cocaine-withdrawn cortical neurons. The enhanced membrane depolarization in response to positive current pulses at an early stage (approximately within the initial 0–100 ms) in cocaine-

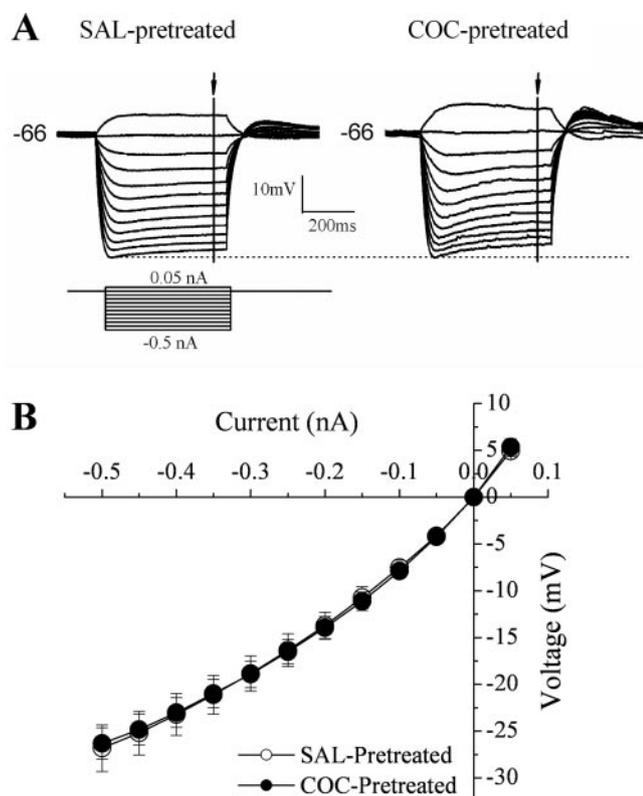


Fig. 7. The inward rectification returned to the control levels after long-term withdrawal from repeated cocaine administration. A, representative traces showing the voltage response of membrane potentials to pulse injection of current pulses (-0.5 to $+0.05$ nA, 500 ms) following repeated SAL or COC pretreatment with 2 to 3 weeks of withdrawal. The responses of V_m during membrane hyperpolarization showed no marked difference in COC- compared with SAL-pretreated mPFC neurons. B, there was no significant difference in the inward rectification in response to hyperpolarizing current pulses between SAL- and COC-pretreated (SAL versus COC: $n = 9/11$ cells, $p > 0.05$) neurons after 2 to 3 weeks of cocaine withdrawal. Values represent mean \pm S.E.M.

withdrawn neurons is in agreement with these findings. It is also consistent with our other findings in which the density of fast-inactivating outward K^+ currents is reduced in mPFC neurons following repeated cocaine administration (Dong et al., 2002). Taken together, these findings reveal that repeated cocaine administration reduces VGKCs, and the increased action potentials evoked by membrane depolarization could be attributed to the decreased I_A and fast-inactivating I_K in cocaine-withdrawn mPFC neurons.

Nevertheless, the decreased outward rectification during membrane depolarization may also be interpreted as an "increased" inward rectification, which would also induce an upward shifting in the I-V curve. An increased inward rectification during membrane depolarization may be induced by enhanced inflowing of voltage-sensitive sodium currents (I_{Na}) and/or calcium currents (I_{Ca}). Our results regarding the increased firings evoked by depolarizing currents in cocaine-withdrawn mPFC neurons also seem to be in agreement with this interpretation. Because I_{Na} was blocked by tetrodotoxin, we cannot assess the role of I_{Na} in the I-V curve studies. However, statistical analysis of the active membrane properties, including the threshold, amplitude, and duration of action potential, provides no evidence for any possible increase in I_{Na} . Therefore, although the current-spike response curves exhibit an increase in the number of evoked action potentials

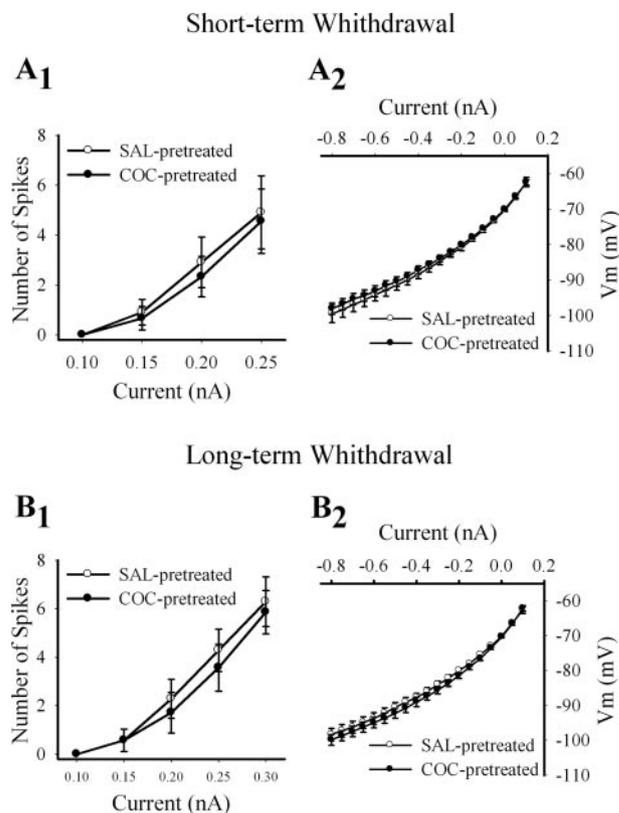


Fig. 8. Repeated COC administration did not cause significant changes in the current-spike response and I-V relationship in pyramidal neurons within the motor cortex following a short- or long-term withdrawal. The number of spikes evoked by increased depolarizing current pulses was not significantly affected after repeated COC pretreatment following either 3 days (SAL versus COC: $n = 11/9$ cells, $p > 0.05$) (A_1) or 2 to 3 weeks (SAL versus COC: $n = 7$ cells each group, $p > 0.05$) (B_1) of withdrawal. The voltage responses to current pulses (-0.08 to 0.1 nA) were also found unchanged in motor cortex neurons following repeated COC administration with a 3-day (SAL versus COC: $n = 9/11$ cells, $p > 0.05$) (A_2) or 2- to 3-week (SAL versus COC: $n = 7$ cells each group, $p > 0.05$) (B_2) withdrawal. Values represent mean \pm S.E.M.

in cocaine-withdrawn mPFC neurons, such change should not be attributed to increased I_{Na} . On the other hand, we have recently found that repeated cocaine administration enhances whole-cell I_{Ca} in mPFC pyramidal neurons (Nasif et al., 2002). The combined changes in I_{Ca} and decreased VGKCs would contribute to an increase in both subthreshold and suprathreshold excitability, leading to enhanced excitatory responses of cocaine-withdrawn mPFC neurons to membrane depolarization.

Another important finding in this study is that repeated cocaine administration diminished the inward rectification during membrane hyperpolarization, showing a more hyperpolarized membrane potential response (downward shift) to negative current pulses in mPFC neurons with short-term cocaine withdrawal. This finding, associated with the increased *input resistance*, suggests that the function of other types of membrane ion channels might also be altered by chronic cocaine treatment. It is well established that the RMP is dynamically regulated and maintained by inward rectifiers such as K_{ir} , which carries some *outward* current at the membrane potential range slightly more positive to E_K (Hille, 2001). Blockade of K_{ir} would not only reduce the inward rectification via blocking $I_{K_{ir}}$ during membrane hyperpolarization, but also preclude the outward K^+ current at

TABLE 3

Repeated cocaine administration (15 mg/kg/day) in vivo does not alter the membrane properties of motor cortex pyramidal neurons

The passive and active membrane properties measured by whole-cell current-clamp recordings of motor cortex pyramidal neurons from brain slices from saline- and cocaine-pretreated rats. Values represent the mean \pm S.E.M. for the number of neurons indicated.

Pretreatment	3 Days of Withdrawal		2 to 3 Weeks of Withdrawal	
	Saline	Cocaine	Saline	Cocaine
Number of neurons	11	9	7	7
Passive membrane properties				
RMP (mV)	-70.34 \pm 0.44	-69.76 \pm 0.24	-70.13 \pm 0.20	-70.39 \pm 0.29
Input resistance (m Ω)	68.03 \pm 8.80	65.58 \pm 4.21	67.66 \pm 8.07	70.23 \pm 6.43
Active membrane properties				
Current to generate action potential (nA)	0.22 \pm 0.02	0.21 \pm 0.02	0.19 \pm 0.01	0.21 \pm 0.02
Action potential threshold (mV)	-41.18 \pm 1.08	-41.16 \pm 1.59	-41.32 \pm 0.80	-39.94 \pm 0.52
Action potential amplitude (mV)	81.75 \pm 1.79	80.53 \pm 1.85	79.70 \pm 1.79	78.64 \pm 1.88
Action potential duration (ms)	1.10 \pm 0.04	1.18 \pm 0.04	1.08 \pm 0.05	1.12 \pm 0.04
AHP amplitude (mV)	2.68 \pm 0.63	3.10 \pm 0.70	4.21 \pm 0.92	4.13 \pm 0.75

the RMP levels ($I_{K_{ir-rest}}$). Such changes in K_{ir} activity could shift the I-V curve downward during membrane hyperpolarization (e.g., the membrane potential would be more hyperpolarized) and depolarize membrane potential from the RMP levels, respectively. These effects are indeed observed in saline-pretreated mPFC neurons following Ba^{2+} -induced blockade of K_{ir} . Similar changes were also found in cocaine-withdrawn neurons, showing a downward shift in the inward rectification and a depolarized RMP. However, this effect of Ba^{2+} on blocking $I_{K_{ir}}$ was attenuated following chronic cocaine exposure. These results suggest that repeated cocaine administration may suppress the activity of $I_{K_{ir}}$ and/or reduce the number of K_{ir} channels blocked by Ba^{2+} . Consequently, both $I_{K_{ir}}$ activated by membrane hyperpolarization and $I_{K_{ir-rest}}$ acting at the RMP level might be suppressed in short-term cocaine-withdrawn mPFC neurons. Under these circumstances, those neurons displayed a significant reduction in the inward rectification during membrane hyperpolarization and became more responsive to excitatory stimuli such as membrane depolarization.

Although the changes in the membrane properties could be attributed to reductions in VGKCs and K_{ir} , involvement of other ion channels should also be considered. Previous investigations have shown that the hyperpolarization-activated cation currents (I_h) also participate in maintaining the RMP and regulating membrane potential at more hyperpolarizing levels in various cells, including cortical pyramidal neurons (Pape, 1996; Berger et al., 2001; Hille, 2001; Lupica et al., 2001; Fernandez et al., 2002; Williams et al., 2002). Since I_h is activated at the membrane voltage range from -40 to -100 mV (DiFrancesco et al., 1986), it cannot be ruled out that the function of I_h channels, either around the RMP or during membrane hyperpolarization, is affected by chronic cocaine treatment. A possible reduction in I_h may also contribute to the increased *input resistance* and the unchanged RMP due to compensation of decreased $I_{K_{ir-rest}}$. Whether I_h is affected in cocaine-withdrawn mPFC neurons remains to be determined.

Long-Term Withdrawal from Repeated Cocaine Administration. The increased number of evoked action potentials and decreased rheobase persisted in long-term (2–3 weeks) cocaine-withdrawn mPFC neurons, suggesting an enduring neuroadaptation. However, although the long-lasting increase in the mPFC neuronal excitability supports previous findings regarding a promoted glutamate output originating from the PFC in cocaine-sensitized rats with a similar

long-term withdrawal (Pierce et al., 1996; Kalivas and Duffy, 1998), the mechanism underlying such increase remains unknown. There are also some other changes in the membrane properties which differ from mPFC neurons with short-term cocaine withdrawal. For instance, alterations in the *input resistance*, outward rectification during membrane depolarization, and inward rectification during membrane hyperpolarization previously observed in mPFC neurons with short-term cocaine withdrawal were no longer found after long-term withdrawal. Both the depolarizing and hyperpolarizing phases of I-V curve returned to the control levels as observed in saline-withdrawn neurons. These results suggest that chronic cocaine-induced reductions in the activity of VGKCs and inward rectifiers might have been compensated and were no longer present after long-term withdrawal. However, our preliminary data show that repeated cocaine administration significantly increases the amplitude and duration of voltage-activated Ca^{2+} plateau potentials in mPFC pyramidal neurons, even after 2 to 3 weeks of withdrawal (Nasif et al., 2003). Combined with the present results, this finding suggests that the increased excitability in long-term cocaine-withdrawn mPFC neurons may be related to an enhanced modulation of voltage-activated Ca^{2+} currents.

The present study provides novel evidence in supporting the view that the mPFC plays an important role in the development of cocaine-induced behavioral sensitization and withdrawal effects. The changes observed in the membrane excitability, which lead to increased responses of cocaine-withdrawn mPFC neurons to excitatory stimuli, are in agreement with previous findings in which enhancement of glutamate output from the mPFC is found in cocaine-sensitized rats (Pierce et al., 1996; Kalivas and Duffy, 1998). The alterations in mPFC excitability and excitatory outputs produce a transient but significant change in the activity of VTA dopamine neurons primarily mediated by α -amino-3-hydroxy-5-methylisoxazol-4-propionic acid (AMPA) receptors (Zhang et al., 1997) leading to a subsequent alteration in dopamine neurotransmission in the mPFC and nucleus accumbens (White et al., 1995; Sorg et al., 1997; Kalivas, 2000). Reduced dopamine neurotransmission in the mPFC and decreased inhibitory responses of mPFC neurons to dopamine have been related to the development of behavioral sensitization induced by repeated administration of psychostimulants, whereas increased dopamine neurotransmission in the nucleus accumbens has been proposed for persistence of behavioral sensitization (Sorg et al., 1997). Furthermore, the alter-

ations in mPFC excitability may also contribute to cocaine-induced long-term plasticity observed at glutamatergic synapses in both nucleus accumbens and VTA neurons (Thomas et al., 2001; Ungless et al., 2001; Saal et al., 2003).

In summary, the present study provides clear evidence for an increase in the membrane excitability of mPFC pyramidal neurons during withdrawal from repeated cocaine administration. These findings suggest that the increased excitability in cocaine-withdrawn mPFC neurons is modulated primarily by a decrease in K_{ir} and VGKCs. These changes would not only facilitate the responsiveness of mPFC pyramidal neurons to excitatory stimuli, but also enhance the mPFC glutamate output to the subcortical areas, including the VTA and nucleus accumbens. These alterations in the mPFC should eventually contribute to the development of sensitization and withdrawal effects after chronic cocaine exposure.

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Address correspondence to: Dr. Xiu-Ti Hu, Department of Cellular and Molecular Pharmacology, Rosalind Franklin University of Medicine and Science, Chicago Medical School, 3333 Green Bay Road, North Chicago, Illinois 60064. E-mail: xiu-ti.hu@rosalindfranklin.edu