Brief Communication

Repeated Cocaine Administration Increases Voltage-Sensitive Calcium Currents in Response to Membrane Depolarization in Medial Prefrontal Cortex Pyramidal Neurons

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The medial prefrontal cortex (mPFC) plays a critical role in cocaine addiction. However, evidence to elucidate how the mPFC is functionally involved in cocaine addiction remains incomplete. Recent studies have revealed that repeated cocaine administration induces various neuroadaptations in pyramidal mPFC neurons, including a reduction in voltage-gated K^+ currents (VGKCs) and a possible increase in voltage-sensitive Ca^{2+} currents (I_{Ca}). Here, we performed both current-clamp recordings in brain slices and voltage-clamp recordings in freshly dissociated cells to determine whether I_{Ca} is altered in mPFC pyramidal neurons after chronic cocaine treatment with a short-term or long-term withdrawal. In addition, a critical role of VGKCs in regulating the generation of Ca^{2+} plateau potential was also studied in mPFC neurons. Repeated cocaine administration significantly prolonged the duration of evoked Ca^{2+} plateau potentials and increased the whole-cell I_{Ca} in mPFC neurons after a 3 d withdrawal. Selective blockade of L-type Ca^{2+} channels by nifedipine not only significantly increased the threshold but also reduced the duration and amplitude of Ca^{2+} plateau potentials in both saline- and cocaine-withdrawn mPFC neurons. However, there was no significant difference in the increased threshold, reduced duration, and decreased amplitude of Ca^{2+} potentials between saline- and cocaine-withdrawn neurons after blockade of L-type Ca^{2+} channels. Moreover, an increase in amplitude was also observed, whereas the prolonged duration persisted, in Ca^{2+} potentials after 2–3 weeks of withdrawal. These findings indicate that chronic exposure to cocaine facilitates the responsiveness of I_{Ca}, particularly via the activated L-type Ca^{2+} channels, to excitatory stimuli in rat mPFC pyramidal neurons.

Key words: **cocaine; medial prefrontal cortex; psychostimulant; Ca^{2+} current; Ca^{2+} plateau potential; patch clamp**

Introduction

The medial prefrontal cortex (mPFC) is an important structure in the mesocorticolimbic dopamine (DA) system, which is functionally implicated in several neurological disorders, including cocaine addiction (for review, see Tzschentke, 2001). In rodents, lesions of the mPFC abolish neuroadaptations in the mesoaccumbens DA system and prevent increased behavioral responses to cocaine (Li et al., 1999), suggesting that the glutamatergic output from the mPFC plays a critical role in the development of behavioral sensitization, an established model of drug addiction (Pierce et al., 1998; Wolf, 1998). Despite evidence indicating involvement of the mPFC in cocaine addiction, little is known about whether and how chronic exposure to cocaine affects the activity of pyramidal mPFC neurons. Recent investigations reveal that repeated cocaine administration alters ion channel function in mPFC neurons, leading to an increase in evoked firing frequency and a decrease in voltage-gated K^+ currents (VGKCs) in cocaine-withdrawn mPFC neurons (Dong et al., 2005; Nasif et al., 2005).

Neuronal excitability is primarily controlled and regulated by Na^+, Ca^{2+}, and K^+ channels (Hille, 2001). It is possible that repeated cocaine administration may not only affect VGKCs but also alter the activity of Ca^{2+} and Na^+ currents in mPFC neurons. Although it is currently unknown whether Na^+ channel function is changed after repeated cocaine administration, a possible increase in I_{Ca} in cocaine-withdrawn mPFC neurons has been suggested (Nasif et al., 2005). It is well established that cortical neurons express various subtypes of Ca^{2+} channels (Brown et al., 1993; Ye and Akaike, 1993; Lorenzon and Foehring, 1995), which are modulated by DA receptors (Young and Yang, 2004). Based on those findings, we hypothesize that chronic cocaine-induced alterations in DA neurotransmission may change the activity of whole-cell I_{Ca}, thereby increasing the membrane excitability in rat pyramidal mPFC neurons, particularly in response to certain stimuli. The present study was performed to determine whether repeated cocaine administration facilitates Ca^{2+} channel function in mPFC pyramidal neurons after a short- or long-term withdrawal.

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Materials and Methods

Animals and pretreatment. Male Sprague Dawley rats (~4 weeks of age) were group housed in a vivarium under a 12 h light/dark cycle. Food and water were available *ad libitum*. Rats received repeated administration of saline (0.1 ml) or cocaine (15 $mg \cdot kg^{-1} \cdot d^{-1}$, i.p.) for 5 consecutive days, followed by a 3 d (short-term) or a 2–3 week (long-term) withdrawal. After the pretreatment with saline or cocaine, rats were ~5–6 weeks of age. In electrophysiological experiments, technical limitations usually restrict voltage-clamp recordings in freshly dissociated neurons from rats >6 weeks of age. A prolonged time period for tissue digestion and increased physical force for cell dissociation from older tissues with increased density of fibers could cause damage in dissociated cells that would make the recording extremely difficult. All experimental procedures were in strict accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals* and were approved by our Institutional Animal Care and Use Committee.

Current-clamp recordings in brain slices. Rats were decapitated under halothane anesthesia, and the brain was immediately excised and immersed in ice-cold artificial CSF (aCSF) containing the following (in mM): 124 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $MgCl_2$, 2 $CaCl_2$, and 10 glucose, pH 7.4 (310 mOsm/l). Coronal sections (300 μm) containing the mPFC were sliced and incubated in oxygenated (95% O_2/5% CO_2) aCSF for 1 h at room temperature before recording. Slices were anchored in a recording chamber and perfused with oxygenated aCSF. All current-clamp recordings in slice preparations were performed at 34°C. Recording glass pipettes were pulled with a horizontal pipette puller, measured with a resistance of 3–5 MΩ, and filled with internal recording solution (in mM): 120 K^+-gluconate, 10 HEPES, 0.1 EGTA, 20 KCl, 2 $MgCl_2$, 3 Na_2ATP, and 0.3 Na_2GTP. Long Ca^{2+} plateau potentials were generated using the following internal solution (in mM): 140 Cs^+-gluconate, 10 HEPES, 2 $MgCl_2$, 3 Na_2ATP, and 0.3 Na_2GTP. Recordings were performed in visually identified mPFC pyramidal neurons within the layers V–VI using differential interference contrast microscopy (Stuart et al., 1993). After whole-cell configuration was formed, voltage-clamp mode was changed to current-clamp recording. The signals were amplified, digitized, and distributed to a computer. Ca^{2+} plateau potentials were generated with depolarizing current pulses after blockade of Na^+ and K^+ channels (Hu et al., 2004). Characteristics of the Ca^{2+} potential were obtained from the initial spike evoked by the minimal depolarizing current (rheobase) in each mPFC neuron recorded. After the membrane depolarization caused by internal application of Cs^+, mPFC pyramidal neurons with stable resting membrane potential (RMP) were recorded and used for analysis.

Voltage-clamp recordings in freshly dissociated mPFC neurons. The brain tissues were immersed in ice-cold high-sucrose solution (in mM: 25 NaCl, 2.5 KCl, 5 HEPES, 11 D-glucose, 210 sucrose, 2 $CaCl_2$, and 2 $MgSO_4$, pH 7.40) and cut to coronal sections (350 μm). As described in our previous study (Zhang et al., 2002), slices were incubated in holding solution, digested with protease (type XIV; 1.5 mg/ml), and rinsed with a low Ca^{2+}, HEPES-buffered saline. The tissue was then dissected, and cells were mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The suspension was placed into a Petri dish containing 2 ml of HEPES-buffered HBSS, which was mounted under an inverted microscope. Dissociated cells were allowed to settle, and the solution bathing them was changed to the external solution. Electrodes were pulled from Corning (Corning, NY) 7056 glass capillaries and fire-polished before use. Voltage-sensitive I_{Ca} was isolated by using an internal solution consisting of (in mM) 180 *N*-methyl-glutamine, 40 HEPES, 4 $MgCl_2$, 0.1 BAPTA, 12 phosphocreatine, 2 Na_2ATP, 0.2 Na_2GTP, and 0.1 leupeptin, pH 7.3 (270–275 mOsm/l) and an external solution consisting of (in mM) 135 NaCl, 20 CsCl, 1 $MgCl_2$, 10 glucose, 10 HEPES, 0.001 TTX, and 5 $BaCl_2$, pH 7.4 (300–305 mOsm/l). Ba^{2+} was used as a charge carrier and K^+ channel blocker (Lorenzon and Foehring, 1995). Recordings were performed using an amplifier and controlled/monitored with a personal computer. After seal rupture, the series resistance (<10 MΩ) was compensated (70–80%) and periodically monitored. I_{Ca}, with leak subtraction, was activated by stepping the voltage from the holding potential (−90 mV) to various membrane potentials (up to 40 mV). Voltage-clamp experiments were performed at room temperature (20–22°C).

Drug application and statistics. During current-clamp recordings, TTX (1 μM), tetra-

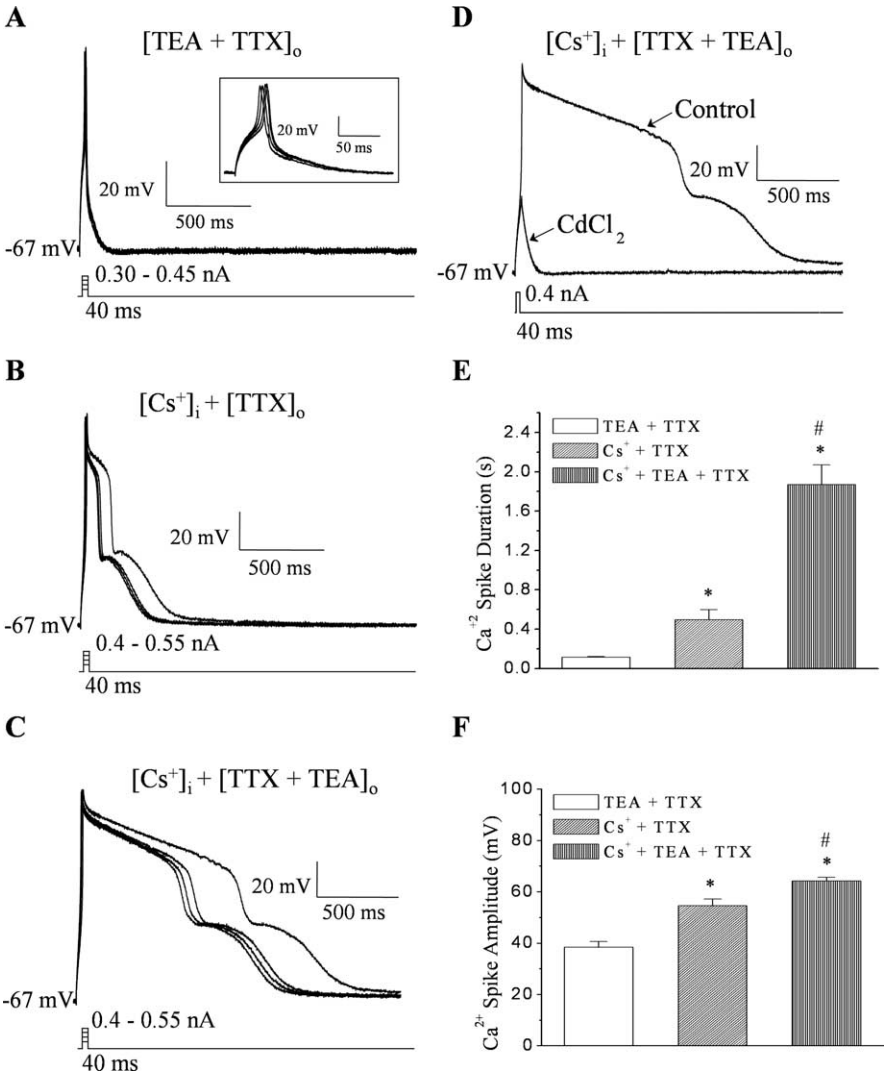

Figure 1. Characterization of Ca^{2+} potentials in mPFC pyramidal neurons. ***A***, Representative traces show that Ca^{2+} spikes, with a short duration but without a stepwise repolarization, were evoked with blockade of Na^+ channels and TEA-sensitive K^+ channels in saline-pretreated neurons (inset indicates extended durations). ***B***, With internal Cs^+ and bath-applied TTX, Ca^{2+} potentials exhibited a longer duration and apparent stepwise repolarization. ***C***, With application of TTX, TEA, and Cs, typical Ca^{2+} plateau potentials were evoked. ***D***, The plateau potential was blocked by Cd^{2+}. ***E***, ***F***, Bar graphs indicate an increase in the spike duration and amplitude, respectively, with blockade of different K^+ channels (*$p < 0.05$ compared with TEA plus TTX; #$p < 0.05$ compared with Cs^+ plus TTX).

ethylammonium chloride (TEA) (20 mM), $CdCl_2$ (200 μM), and the selective L-type Ca^{2+} channel blocker nifedipine (5 μM) were applied externally in the bath solution. Cs^+-gluconate (140 mM) was applied internally in the cytosol. The glutamate receptor antagonist kynurenic acid (2.5 mM) and the $GABA_A$ receptor antagonist 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)-pyridazinium bromide (4 μM) were bath applied in all experiments. Statistical comparisons between cocaine- and saline-pretreated rats were made using either paired and unpaired Student's *t* tests or two-way ANOVA with repeated measures.

Results

Ca^{2+} plateau potentials were initially characterized using current-clamp recordings in mPFC neurons of saline-pretreated rats (Fig. 1). Because the mean RMP was approximately −67 mV (Nasif et al., 2005), all mPFC neurons were held at this RMP level during slice recordings. To determine whether K^+ channels affected the generation of Ca^{2+} potentials in mPFC neurons, different K^+ channel blockers were applied externally and/or internally. With the application of TTX and TEA, depolarizing current pulses (0.3–0.55 nA) evoked Ca^{2+} spikes with a very short duration (Fig. 1*A*). With internally applied Cs^+ (140 mM), which blocked both TEA-sensitive and TEA-insensitive K^+ channels, an increased duration in Ca^{2+} potentials was observed (Fig. 1*B*). It was also noted that an apparent stepwise repolarization, with a primary (first) and a smaller secondary membrane depolarization, occurred in Ca^{2+} potentials. Blockade of I_{Na} by TTX and VGKCs (internally by Cs^+ and externally by TEA) evoked long-lasting Ca^{2+} plateau potentials (>1.5 sec) in saline-withdrawn mPFC neurons (Fig. 1*C*), indicating that complete blockade of all types of K^+ channels was required for the development of Ca^{2+} plateau potentials (Hu et al., 2004). Cd^{2+} eliminated this potential (Fig. 1*D*). Bar graphs indicate that, with the application of different K^+ channel blockers (Fig. 1*E*,*F*), there was a significant increase in the durations (TEA plus TTX vs Cs^+ plus TTX, 113.90 ± 8.24 vs 496.47 ± 102.92 ms, $n = 9$ vs 7 cells, $t = 4.574$, $^*p < 0.05$; TEA plus TTX vs Cs^+ plus TEA plus TTX, 113.90 ± 8.24 vs 1867.82 ± 203.64 ms, $n = 9$ vs 10 cells, $t = 8.582$, $^*p < 0.05$; Cs^+ plus TTX vs Cs^+ plus TEA plus TTX, 496.47 ± 102.92 vs 1867.82 ± 203.64 ms, $n = 7$ vs 10 cells, $t = 5.573$, $^{\#}p < 0.05$) (Fig. 1*E*) and the amplitudes (TEA plus TTX vs Cs^+ plus TTX, 36.39 ± 2.22 vs 54.57 ± 2.60 mV, $n = 9$ vs 7 cells, $t = 5.083$, $^*p < 0.05$; TEA plus TTX vs Cs^+ plus TEA plus TTX, 36.39 ± 2.22 vs 64.20 ± 1.34 mV, $n = 9$ vs 10, $t = 10.782$, $^*p < 0.05$; Cs^+ plus TTX vs Cs^+ plus TEA plus TTX, 54.57 ± 2.60 vs 64.20 ± 1.34 mV, $n = 9$ vs 10 cells, $t = 3.840$, $^{\#}p < 0.05$) (Fig. 1*F*) of Ca^{2+} potentials when K^+ channels were fully blocked.

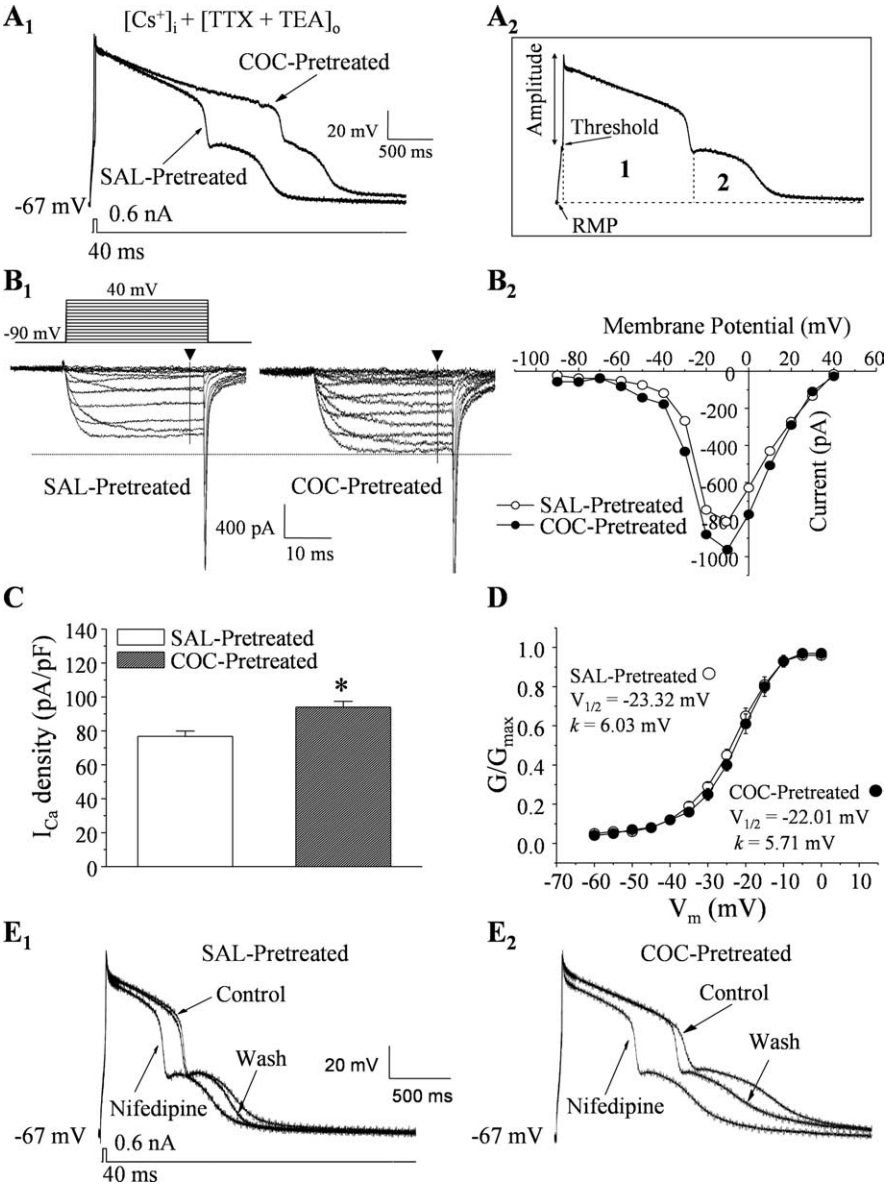

Figure 2. Repeated cocaine administration increased Ca^{2+} channel function in mPFC neurons after a 3 d withdrawal. ***A*****1**, ***B*****1**, ***B*****2**, Repeated cocaine pretreatment prolonged the duration of Ca^{2+} plateau potential (***A*****1**) and increased I_{Ca} (***B*****1**, ***B*****2**) in mPFC neurons after a 3 d withdrawal. ***B*****1**, Triangles indicate the time point at which the Ca^{2+} currents were measured. ***A*****2**, Two components of an evoked Ca^{2+} plateau potential (the duration and area under the trace were measured from the threshold to the end of each step, and RMP was used as the baseline of the spike area). ***C***, Repeated cocaine administration significantly increased the I_{Ca} density in dissociated mPFC neurons ($^*p < 0.05$). Error bars reprsent SEM. ***D***, There was no significant difference in the activation curve between saline- and cocaine-pretreated neurons. Data represent means ± SEM. ***E*****1**, ***E*****2**, Representative traces showing that application of nifedipine (NIF; 5 μM) markedly reduced the duration and area of the first, but not the second, step of Ca^{2+} plateau potentials in both saline- and cocaine-withdrawn mPFC neurons. The reduced durations and amplitudes of Ca^{2+} potentials after blockade of L-type Ca^{2+} channels were comparable in the two groups of neurons ($p > 0.05$). COC, Cocaine; SAL, saline; Wash, washout.

Repeated cocaine administration significantly increased the duration and "area" of the primary (first), but not the secondary, component of Ca^{2+} plateau potentials after a 3 d withdrawal (Fig. 2A_1, Table 1). Figure 2A_2 indicates the characteristics of the two components in a Ca^{2+} plateau potential and how they were measured. Voltage-clamp recordings in dissociated mPFC pyramidal neurons revealed that whole-cell I_{Ca} was enhanced during membrane depolarization after chronic exposure to cocaine (Fig. 2*B*). Bar graphs show that the density of I_{Ca} was significantly increased in cocaine-withdrawn cells (saline- vs cocaine-pretreated, 76.75 ± 3.16 vs

Table 1. Repeated cocaine administration altered Ca^{+2} plateau potentials in mPFC pyramidal neurons after both 3 d and 2–3 weeks of withdrawal

	Saline	Cocaine
3 d of withdrawal		
Number of neurons	20	21
Current to generate Ca^{2+} spikes (nA)	0.63 ± 0.05	0.65 ± 0.03
Spike threshold (mV)	−20.19 ± 0.67	−20.54 ± 0.81
Spike amplitude (mV)	64.02 ± 0.96	64.74 ± 0.93
Spike duration − one-half amplitude (ms)	697 ± 43	808 ± 26*
Spike duration, step 1 (ms)	1349 ± 125	1938 ± 131*
Spike duration, step 2 (ms)	681 ± 95	640 ± 101
Whole spike duration (ms)	2002 ± 147	2578 ± 89*
Spike area, step 1	110,629 ± 9616	151,601 ± 8310*
Spike area, step 2	18,209 ± 2853	16,855 ± 3088
Whole spike area	126,230 ± 9946	168,456 ± 6849*
2–3 weeks of withdrawal		
Number of neurons	15	12
Current to generate Ca^{2+} spikes (nA)	0.46 ± 0.04	0.41 ± 0.02
Spike threshold (mV)	−22.73 ± 0.75	−23.69 ± 0.73
Spike amplitude (mV)	69.03 ± 1.27	75.15 ± 1.05*
Spike duration − one-half amplitude (ms)	695 ± 60	885 ± 46*
Spike duration, step 1 (ms)	1282 ± 141	1661 ± 68*
Spike duration, step 2 (ms)	664 ± 50	787 ± 71
Whole spike duration (ms)	1945 ± 163	2448 ± 83*
Spike area, step 1	103,047 ± 11,061	137,542 ± 5756*
Spike area, step 2	17,084 ± 1897	20,494 ± 2405
Whole spike area	120,131 ± 11,640	158,036 ± 5542*

The properties of Ca^{2+} plateau potentials, measured by whole-cell current-clamp recordings, of mPFC pyramidal neurons from brain slices from saline- and cocaine-pretreated rats are shown. Values represent the mean ± SEM for the number of neurons indicated (*$p < 0.05$; Student's *t* test compared with the saline group).

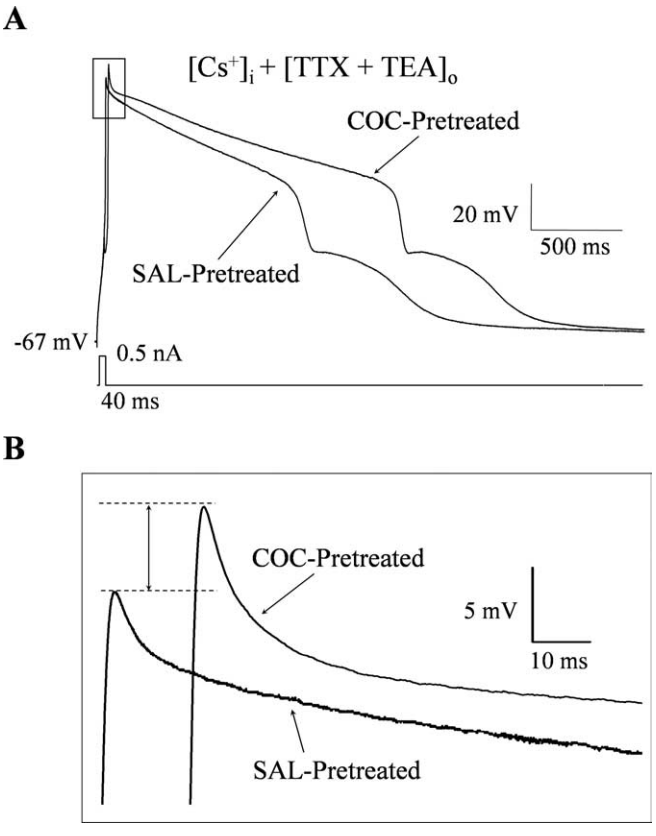

Figure 3. Increased Ca^{2+} channel function persisted after 2–3 weeks of withdrawal. ***A***, Repeated cocaine (COC) administration increased the duration and amplitude of Ca^{2+} potential in mPFC neurons after 2–3 weeks of withdrawal. ***B***, Amplification of the boxed area in ***A*** to show the increased amplitude. SAL, Saline.

93.96 ± 3.48 pA/pF; $n = 10$ vs 14 cells; $t = 3.774$; $^*p < 0.05$) (Fig. 2*C*). There was no significant change in the activation curve between saline- and cocaine-pretreated neurons (Fig. 2*D*), suggesting that the increased I_{Ca} might not be attributable to alterations in voltage dependence of activation.

To determine whether L-type Ca^{2+} channels were functionally involved in the increased duration of Ca^{2+} plateau potentials, the selective L-type blocker nifedipine was applied in bath during recording. Nifedipine (5 μM; ~10 min) significantly reduced the duration of evoked Ca^{2+} plateau potentials in both saline-withdrawn (whole duration, 13.02 ± 4.20%; whole area, 23.30 ± 3.44%; $n = 7$ cells; $p < 0.05$; paired *t* test) and cocaine-withdrawn (whole duration, 27.62 ± 5.28%; whole area, 37.89 ± 4.44%; $n = 8$ cells; $p < 0.05$; paired *t* test) neurons. These changes resulted primarily from the reduction in the first component of the duration and area of Ca^{2+} potentials in saline-withdrawn (duration, 27.81 ± 2.69%; area, 29.95 ± 3.11%; $n = 7$ cells; $p < 0.05$; paired *t* test) (Fig. 2E_1) and cocaine-withdrawn (duration, 43.10 ± 3.41%; area, 43.62 ± 3.48%; $n = 8$ cells; $p < 0.05$; paired *t* test) neurons (Fig. 2E_2). No significant change was found in the second component of Ca^{2+} potentials. The nifedipine-induced reduction in the first and whole component of Ca^{2+} potentials was significantly greater in cocaine-withdrawn mPFC neurons compared with that in the saline group (saline-pretreated vs cocaine-pretreated: whole duration, 13.02 ± 4.20 vs 27.62 ± 5.28%; whole area, 23.30 ± 3.44 vs 37.89 ± 4.44%; $n = 7$ vs 8 cells; both $p < 0.05$; *t* test). However, there was no significant difference in the duration and area of the first component of Ca^{2+} potentials between saline- and cocaine-withdrawn neurons after blockade of L-type Ca^{2+} channels (duration in the saline/nifedipine group vs the cocaine/nifedipine group: 542 ± 70.00 vs 658.83 ± 98.02 ms; area in saline/nifedipine vs cocaine/nifedipine: 42,628 ± 5265 vs 522,008 ± 8144; $n = 7$ vs 8 cells; both $p > 0.05$; *t* test). In addition, the threshold of Ca^{2+} plateau potentials was significantly increased to more depolarized membrane potential levels after application of nifedipine in both saline- and cocaine-withdrawn mPFC neurons (saline-pretreated control vs nifedipine, −22.07 ± 1.67 vs −18.93 ± 1.80 mV, $n = 7$ cells; cocaine-pretreated control vs nifedipine, −21.44 ± 0.97 vs −18.81 ± 0.98 mV, $n = 8$ cells; both $p < 0.05$; paired *t* test). Moreover, the amplitude of the Ca^{2+} plateau potential was also significantly decreased with blockade of L-type Ca^{2+} channels in both saline and cocaine groups (saline-pretreated control vs nifedipine, 65.26 ± 1.91 vs 56.41 ± 2.93 mV; $n = 7$ cells; cocaine-pretreated control vs nifedipine, 63.88 ± 1.48 vs 53.81 ± 2.36 mV, $n = 8$ cells; both $p < 0.05$; paired *t* test). Similar to that observed in the reduced durations, there was also no significant difference in either the amplitude or the threshold of Ca^{2+} plateau potentials between saline- and cocaine-withdrawn neurons after selective blockade of L-type Ca^{2+} potentials (both $p > 0.05$; *t* test).

After 2–3 weeks of withdrawal, the prolonged duration in Ca^{2+} plateau potentials was still evident in cocaine-withdrawn mPFC neurons compared with saline-pretreated controls (Fig.

3*A*, Table 1). Moreover, a significant increase in the amplitude of the Ca^{2+} potential also occurred in cocaine-withdrawn neurons (Fig. 3*B*, Table 1). Technical difficulties restricted us from performing voltage-clamp recording in mPFC neurons of the "aged" rats in either group.

Discussion

The major finding of this study is that repeated cocaine administration increases the responsiveness of voltage-sensitive Ca^{2+} channels to membrane depolarization in rat mPFC pyramidal neurons. The prolonged duration of Ca^{2+} plateau potentials and increased whole-cell I_{Ca} observed in the present study confirms our previous hypothesis (see Introduction), stating that the increased membrane excitability in cocaine-withdrawn mPFC neurons should be attributed to a facilitation in I_{Ca} activity, along with a reduction in voltage-gated K^+ currents.

The increased I_{Ca} is likely related to the L-type current. It is well known that I_{Ca} plays an important role in modulating the action potential of mPFC neurons. In addition to generation of Ca^{2+} potentials, increased I_{Ca} activates Ca^{2+}-dependent K^+ currents, thereby shaping the repolarization, afterhyperpolarization, and frequency adaptation of Na^+ spikes (Meech, 1978). Although some high-voltage-activated Ca^{2+} channels (e.g., N- and P/Q-type) apparently control afterhyperpolarization in cortical neurons (Pineda et al., 1998), L-type channels may regulate the inward currents that modulate the interspike interval during repetitive firing (Pineda et al., 1998). More importantly, recent investigations indicate that repeated cocaine administration increases protein kinase A (PKA) activity in the mPFC (Dong et al., 2005), whereas activation of PKA via stimulation of DA D_1-class receptors enhances a subthreshold L-type Ca^{2+} potential in PFC pyramidal neurons (Young and Yang, 2004).

Moreover, the present findings also indicate that repeated cocaine administration only enlarged the primary (first), but not the secondary, component of Ca^{2+} plateau potentials, suggesting that the Ca^{2+} channels affected by chronic cocaine were probably located within and/or nearby the soma. Previous findings have determined that the stepwise repolarization in Ca^{2+} potentials should be attributed to the different sites for Ca^{2+} electrogenesis: the first (primary) component represents I_{Ca} activated in the soma or dendrites proximal to the soma region, whereas the secondary component reflects I_{Ca} evoked distally in the dendrites (Reuveni et al., 1993). Because L-type Ca^{2+} channels are primarily distributed around the soma region (Westenbroek et al., 1990; Hell et al., 1993) and regulate the first component of Ca^{2+} plateau potentials (Young and Yang, 2004), whereas N- and P/Q-type Ca^{2+} currents are mainly located in the dendrites (Hillman et al., 1991; Usowicz et al., 1992; Westenbroek et al., 1992; Mills et al., 1994), it is possible that the L-type I_{Ca} might play a major role in the prolonged duration and increased amplitude of Ca^{2+} plateau potentials in cocaine-withdrawn mPFC neurons. In fact, the data obtained from saline- and cocaine-withdrawn mPFC neurons with blockade of L-type Ca^{2+} channels provide additional evidence in support of our hypothesis. First, nifedipine significantly reduced the first component of the plateau potential without affecting the second component in both saline- and cocaine-withdrawn neurons. Second, no significant difference in the duration, the amplitude, or the area of the first components of Ca^{2+} plateau potentials was found between saline- and cocaine-withdrawn neurons after blockade of L-type Ca^{2+} channels. Therefore, these results, along with the previous ones, clearly indicate that the chronic cocaine-induced facilitation of voltage-sensitive Ca^{2+} channel function in mPFC pyramidal neurons results from an increased activity of L-type Ca^{2+} channels.

Nevertheless, although the secondary component of Ca^{2+} plateau potentials was found to be unaffected, our findings would not rule out any possible changes in the function of Ca^{2+} and K^+ channels located in the dendrites of cocaine-withdrawn pyramidal mPFC neurons. Investigation using proper recording techniques in the dendrites of those neurons should provide additional information for this issue.

Another interesting finding in this study is that both TEA-sensitive and TEA-insensitive VGKCs functionally modulate the generation of Ca^{2+} potentials in mPFC neurons of drug-naive rats during membrane depolarization. Our results indicate that blockade of TEA-sensitive K^+ (and Na^+) channels alone was not adequate for evoking Ca^{2+} plateau potentials with a "full-length" duration in pyramidal mPFC neurons. In contrast, concurrent application of Cs^+ and TEA, which blocks both TEA-sensitive and TEA-insensitive VGKCs (from the inside and outside of the membrane, respectively) is necessary and critical for the generation of a "full-size" Ca^{2+} plateau potential. Under this circumstance, typical Ca^{2+} plateau potentials could be reliably and constantly evoked by depolarizing current pulses in every cell recorded. This result is in agreement with our previous finding in medium spiny neurons within the rat nucleus accumbens (NAc) (Hu et al., 2004), suggesting a general character of evoked Ca^{2+} potentials, which explains why various Ca^{2+} spikes may be generated with different potential forms in neurons in response to membrane depolarization. In addition, this finding also reveals the role of VGKCs in "fine tuning" the function of voltage-sensitive Ca^{2+} channels in pyramidal mPFC neurons.

The present study provides novel evidence indicating that chronic cocaine treatment induces an enduring facilitation in I_{Ca} in rat mPFC neurons in response to excitatory stimuli. This change in Ca^{2+} entry, which occurs after increased activity of L-type I_{Ca}, and a resulting increase in the membrane excitability, could be translated to an enhanced glutamatergic output to the ventral tegmental area and NAc (Smith et al., 1995; Reid et al., 1997), particularly in cocaine-sensitized rats (Pierce et al., 1996; Kalivas and Duffy, 1998) when they are challenged with the same drug after a short-term withdrawal (Williams and Steketee, 2004). More importantly, the alterations in Ca^{2+} channel function appeared to be enduring, because they persisted for at least 2–3 weeks after the termination of chronic cocaine administration. These findings suggest that, although the basal neuronal activity within the withdrawn mPFC significantly decreases after chronic exposure to cocaine, such as that shown in functional image study (for review, see Volkow et al., 2004), the responsiveness of pyramidal mPFC neurons to certain stimuli including, but not limited to, environmental cues or an additional (challenge) dose of psychostimulants, may be markedly increased. Therefore, these changes in Ca^{2+} and DA signaling within the mesocortical and mesoaccumbens DA systems may ultimately contribute to the development of behavioral sensitization and the withdrawal effects of chronic cocaine.

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