Repeated Cocaine Administration Suppresses HVA-Ca\(^{2+}\) Potentials and Enhances Activity of K\(^{+}\) Channels in Rat Nucleus Accumbens Neurons

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Hu, Xiu-Ti, Somnath Basu, and Francis J. White. Repeated cocaine administration suppresses HVA-Ca\(^{2+}\) potentials and enhances activity of K\(^{+}\) channels in rat nucleus accumbens neurons. J Neurophysiol 92: 1597–1607, 2004; 10.1152/jn.00217.2004. The nucleus accumbens (NAc) is an important forebrain area involved in sensitization, withdrawal effects, and self-administration of cocaine. However, little is known about cocaine-induced alterations in the neuronal excitability and whole cell neuroplasticity in this region that may affect behaviors. Our recent investigations have demonstrated that repeated cocaine administration decreases voltage-sensitive sodium and calcium currents (VSSCs and VSCCs, respectively) in freshly dissociated NAc neurons of rats. In this study, current-clamp recordings were performed in slice preparations to determine the effects of chronic cocaine on evoked Ca\(^{2+}\) potentials and voltage-sensitive K\(^{+}\) currents in NAc neurons. Repeated cocaine administration with 3–4 days of withdrawal caused significant alterations in Ca\(^{2+}\) potentials, including suppression of Ca\(^{2+}\)-mediated spikes, increase in the intracellular injected current intensity required for generation of Ca\(^{2+}\) potentials (rheobase), reduced duration of Ca\(^{2+}\) plateau potentials, and abolishment of secondary Ca\(^{2+}\) potentials associated with the primary Ca\(^{2+}\) plateau potential. Application of nickel (Ni\(^{2+}\)), which blocks low-voltage activated T-type Ca\(^{2+}\) channels, had no impact on evoked Ca\(^{2+}\) plateau potentials in NAc neurons, indicating that these Ca\(^{2+}\) potentials are high-voltage activated (HVA). In addition, repeated cocaine pretreatment also hyperpolarized the resting membrane potential, increased the amplitude of afterhyperpolarization in Ca\(^{2+}\) spikes, and enhanced the outward rectification observed during membrane depolarization. These findings indicate that repeated cocaine administration not only suppressed HVA-Ca\(^{2+}\) potentials but also significantly enhanced the activity of various K\(^{+}\) channels in NAc neurons. They also demonstrate an integrative role of whole cell neuroplasticity during cocaine withdrawal, by which the subthreshold membrane excitability of NAc neurons is significantly decreased.

INTRODUCTION

Repeated cocaine administration causes various behavioral changes in both humans and experimental animals. In cocaine addicts, drug dependence is accompanied by withdrawal symptoms, including anergia, anhedonia, depression, anxiety, and cocaine craving (Gawin 1991). Many of these changes are also found in cocaine-sensitized animal models (Carroll and Lac 1987; Kokkinidis and McCarter 1990; Markou and Koob 1991; Mutschler and Miczek 1998). In addition, the changes in dopamine receptor (DA-R) modulation within the mesoaccumbens and mesocortical DA system have been attributed to induction and expression of cocaine-induced sensitization and withdrawal effects (for reviews, see Kalivas et al. 1998; Pierce and Kalivas 1997; White and Kalivas 1998; White et al. 1995a, 1997), suggesting a functional involvement of the nucleus accumbens (NAc) and the prefrontal cortex in cocaine addiction.

Our previous studies have demonstrated that the behavioral changes in cocaine-sensitized animals are correlated with neuroadaptation in both pre- and postsynaptic aspects of DA and glutamate transmission, leading to alterations in the basal activity and neuronal responses to dopaminergic and glutamatergic agonists (Henry and White 1991, 1995; Henry et al. 1989, 1998; Li et al. 1999; White et al. 1995a,b; Zhang et al. 1997). Increased inhibitory responses to DA D1 class receptor (D1R) agonists (Henry and White 1991, 1995; Henry et al. 1989) and decreased excitatory responses to glutamate receptor agonists (White et al. 1995b) strongly suggest that the membrane excitability of medium spiny neurons (MSNs) is reduced in the cocaine-sensitized NAc. Although the cellular and molecular mechanisms underlying chronic cocaine-induced changes in neuronal excitability are not fully understood, various alterations in membrane ion channel function and intracellular signaling pathways have been observed in NAc neurons (Hu et al. 2003; Zhang et al. 1998, 2002).

The membrane excitability of NAc neurons is predominantly regulated and controlled by Na\(^{+}\), Ca\(^{2+}\), and different K\(^{+}\) channels (Higashi et al. 1989; Nicola and Malenka 1997; North and Uchimura 1989, O’Donnell and Grace 1993; Uchimura et al. 1990). Our recent studies have demonstrated that repeated cocaine administration renders NAc neurons much less excitable in response to depolarizing influences via depressing whole cell voltage-sensitive sodium and calcium currents (VSSCs and VSCCs) (White et al. 1995b; Zhang et al. 1998, 2002). More importantly, these changes in Na\(^{+}\) and Ca\(^{2+}\) channel function are found to be associated with increased activity of the D1R/Gi/cAMP/protein kinase A (PKA) signaling pathway (Hu et al. 2003; Nestler 1997; Self and Nestler 1995; Zhang et al. 2002) and decreased function of calcineurin (protein phosphatase 2B, PP-2B), suggesting that an increased phosphorylation and decreased dephosphorylation may both contribute to the mechanism that underlies cocaine-induced whole cell neuroplasticity in the NAc (Hu et al. 2003). However, it is unknown how the reduced VSSCs dynamically affect the subthreshold membrane excitability and generation of Ca\(^{2+}\) potentials in NAc neurons. In addition, it is also unknown whether potassium channels in NAc neurons are functionally involved in the altered membrane excitability observed after repeated cocaine pretreatment. Based on our

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previous findings, we hypothesized that chronic cocaine-induced $I_{\text{Ca}}$ reduction would result in a marked decrease in the subthreshold excitability and suppress generation of $Ca^{+2}$ potentials in NAc neurons. To further investigate the effects of repeated cocaine administration on the subthreshold membrane excitability, which is primarily modulated by membrane $Na^{+}$, $Ca^{+2}$, and $K^{+}$ channels, the present study was performed to determine how the voltage-sensitive $Ca^{+2}$ potentials and whether $K^{+}$ channel activity were affected, respectively, in cocaine-withdrawn NAc neurons.

**Methods**

**Animals and pretreatments**

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) initially weighing 175–200 g were housed in groups of two to three in a temperature and humidity-controlled vivarium under a 12-h light/dark cycle. Food and water were freely available. The animals were randomly assigned to two groups and received daily repeated intraperitoneal injections of saline (0.9% NaCl) or cocaine HCl (15 mg/kg) for 5 consecutive days. All recording experiments were conducted on the third or fourth day after cessation of pretreatment.

**Preparation of brain slices**

All procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23) and were approved by the Institutional Animal Care and Use Committee of our school. Rats were decapitated under isoflurane anesthesia and brains were removed and sectioned in the coronal plane (400 μm) with a vibratome. Brain slices were transferred to a holding chamber and incubated at room temperature in oxygenated (95% O2 - 5% CO2) ACSF for 1 h. After washing, slices were transferred to a holding chamber and incubated at room temperature in oxygenated (95% O2 - 5% CO2) ACSF for up to 60 min. Slices were then transferred to an interface-type recording chamber where they were perfused continuously for 60 min with warm (34–35°C) oxygenated ACSF (flow rates of 1–2 ml/min) before recording.

**Intracellular current-clamp recordings**

Both “sharp” and patch electrodes were used in this study. The patch electrodes were used specifically for internal dialysis of $K^{+}$ channel blockers. Sharp electrodes were made with borosilicate glass capillaries (1.0/0.58 mm OD/ID), pulled with a tip diameter <1 μm and filled with 3 M potassium acetate (resistance: 70–150 MΩ). Current-voltage ($I-V$) relationships were studied by injecting step constant current pulses through recording electrode using an axon bridge circuit of an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). Electrical activity was first amplified by a head stage located near the slice before being sent to the amplifier, which was connected to an oscilloscope and to a computer running pClAMP software (Version 9.0) through an interface (Axon Instruments). In this study, all recordings were made from the core region of the NAc (and the effects of chronic cocaine on the shell region of NAc neurons are currently under investigation in a separate experiment). The resting membrane potential (RMP) was originally measured in the absence of current injection prior to the initiation of other experiments. Sodium action potentials (APs) were generated by injecting step depolarizing current pulses of 0.1-nA increments into NAc neurons. Calcium potentials were evoked by depolarizing current pulses with external and/or internal application of different $K^{+}$ channel blockers (see following text). The characteristics of $Ca^{+2}$ potential were obtained from the initial spike evoked by the minimal depolarizing current pulse (rheobase) in each NAc neuron. The amplitude of $Ca^{+2}$ potential was measured from the threshold of spike to its peak. The half-amplitude duration of $Ca^{+2}$ plateau potentials was measured at the amplitude level that one-half of the peak of plateau potential was reached. The amplitude (deepness) of afterhyperpolarization (AHP) was measured from the equipotential point of the spike threshold to the maximum deflection of the hyperpolarization following the end of $Ca^{+2}$ potentials. In addition, to determine the possible differences in the $I-V$ relationship between saline- and cocaine-pretreated NAc neurons, RMP was held at −80 mV for each NAc neuron by injecting a small constant current through manual control.

To detect the possible influence of $K^{+}$ channels, particularly the voltage-gated $K^{+}$ channels (delayed rectifiers), in generation of $Ca^{+2}$ potentials, patch electrodes were used in some NAc neurons. They were pulled from Corning 7056 glass capillary tubes using a micropipette puller (Sutter Instrument, Novato, CA; resistance: 3–5 MΩ) and filled with cesium (Cs⁺)-based internal pipette solution to block all $K^{+}$ channels from the inside of membrane [where CsOH (in mM) 140 Cs gluconate, 10 HEPES, 2 MgCl₂, 2.4 CaCl₂, 10 and d-glucose, pH 7.4 ± 0.05] and sectioned in the coronal plane (400 μm) with a vibrating microtome. Brain slices were transferred to a holding chamber and incubated at room temperature in oxygenated (95% O₂-5% CO₂) ACSF for ≥60 min. Slices were then transferred to an interface-type recording chamber where they rested on nylon mesh and were superfused continuously for 60 min with warm (34–35°C) oxygenated ACSF (flow rates of 1–2 ml/min) before recording.

**Drug application**

The effects of different ion channel blockers on the membrane properties and evoked action potentials were studied in NAc neurons of saline- or cocaine-pretreated rats. Drugs used in this study were applied externally via bath solution and/or internally dialyzed from the pipette solution. Bath solutions with ion channel blockers were made with ACSF immediately before use and were applied via a pump. Such ion channel blocker solutions included the selective $Na^{+}$ channel blocker tetrodotoxin (TTX, 1 μM), the $Ca^{+2}$ channel blocker cadmium (Cd²⁺, 200 μM), the relatively selective A-type $K^{+}$ channel blocker 4-aminopyridine (4-AP) at a low concentration (500 μM), and the $K^{+}$ channel blocker tetraethylammonium chloride (TEA, 20 mM). Termination of the effects of these drugs was achieved by washout using drug-free ACSF. In some cases, another $K^{+}$ channel blocker Cs⁺ was internally applied into NAc neurons via dialyzing from recording pipette (see preceding text). Under this circumstance, some NAc neurons were studied by injecting step constant current pulses (20- to 40-ms duration, 0 to +2.0 nA with 0.05-nA increments) through the recording electrode (SEC-05L/H single-electrode amplifier, npi Electronic Systems, Tamm, Germany). Electrical activity was also collected using Clampex 9.0 software (pClAMP, Axon Instruments). The same measurements were used for characterization of $Ca^{+2}$ plateau potentials as described above. In addition, the whole “area” of the $Ca^{+2}$ plateau potential was also measured by integrating the area between the peak of $Ca^{+2}$ potential and the end of the $Ca^{+2}$ potential while keeping RMP as the baseline (mV × ms; Origin 7.0).
activated (LVA-) Ca\(^{2+}\) channels in neurons. However, the evoked Ca\(^{2+}\) plateau potentials were not significantly affected by such Ni\(^{2+}\) treatment (see the results in the following text), indicating that the Ca\(^{2+}\) potentials evoked by depolarizing currents in NAc neurons were high-voltage activated (HVA).

**Statistical analysis**

Comparisons between the I-V curves were made with a two-way ANOVA (ANOVA) with repeated measures on one variable (depolarizing currents). Other comparisons were made with either Student’s t-test or \(\chi^2\) tests.

**RESULTS**

**Characterization of different forms of HVA-Ca\(^{2+}\) potentials in NAc neurons**

All NAc neurons were recorded within the core region (O’Donnell and Grace 1993; Zahm and Brog 1992) with stabilized RMP at the levels of \(-85\) to \(-60\) mV. There was no spontaneous activity in NAc neurons under the stabilized RMP. Various ion channel blockers were applied externally and/or internally to determine their effects on evoked Ca\(^{2+}\) potentials and other membrane properties. Injection of depolarizing current pulses evoked a Na\(^{+}\) action potential (AP), which was blocked by TTX (Fig. 1A). With blockade of Na\(^{+}\) currents and 4-AP-sensitive A-type K\(^+\) currents (\(I_{\text{A}}\)) (Nakajima 1966; Thompson 1977) by TTX and 4-AP (500 mM) respectively, depolarizing currents evoked a slow-developing, small Ca\(^{2+}\) spike (\(n = 8/8\)), which was blocked by Cd\(^{2+}\) (400 \(\mu\)M, \(n = 4/4\); Figs. 1B and 3A). With application of TEA (20 mM), depolarizing currents evoked a Na\(^{+}\) AP immediately followed by a Ca\(^{2+}\) plateau potential (\(n = 12/12\) cells; Figs. 1C, 2A, and 3B), which were blocked by combined application of TTX and Cd\(^{2+}\) (\(n = 4/4\); Fig. 1C). With concurrent blockade of K\(^+\) and Na\(^{+}\) currents by TEA plus TTX, a typical Ca\(^{2+}\) plateau potential was evoked (Fig. 1D). In addition, additional blockade of TEA-insensitive \(I_{\text{A}}\) by internally applied Cs\(^{+}\) (Fig. 3C) markedly prolonged the duration of Ca\(^{2+}\) plateau potentials (comparing the saline-pretreated cells in Figs. 1D and 3C and note the difference in the time scales). Nevertheless, the afterhyperpolarization, which was produced by activation of the Ca\(^{2+}\)-activated K\(^+\) currents \(I_{\text{K(Ca)}}\) and generally found in the slow-developing Ca\(^{2+}\) spike without blockade of \(I_{\text{K(Ca)}}\) (Fig. 3C), was never observed in Ca\(^{2+}\) plateau potentials with complete blockade of all types of K\(^+\) currents from the inside and outside of the membrane (comparing saline-pretreated NAc cells in Fig. 3, A and C).

With blockade of TEA-sensitive K\(^+\) currents but preserved Na\(^{+}\) currents, we also observed a small membrane depolarization (the secondary Ca\(^{2+}\) potential) following the evoked primary Ca\(^{2+}\) plateau potentials in all saline-pretreated NAc neurons (\(n = 12/12\), Figs. 2A and 3B). This secondary membrane depolarization could trigger additional Na\(^{+}\) APs (\(n = 2\) cells; Fig. 2B). More interestingly, with complete blockade of both TEA-sensitive and -insensitive K\(^+\) currents, the small Ca\(^{2+}\) potential could be converted to a secondary Ca\(^{2+}\) plateau, from which multiple Ca\(^{2+}\) plateau potentials were generated (\(n = 3\) cells; Fig. 2, C and D). In addition, the secondary Ca\(^{2+}\) potential might also be remarkably enlarged and there-

![FIG. 1. Representative traces illustrating various action potentials generated by injection of depolarizing current pulses in the absence or presence of different ion channel blockers. A: Na\(^{+}\)-dependent action potential was evoked by injection of a depolarizing current pulse in the absence of any ion channel blockers. This Na\(^{+}\) spike was blocked by bath application of TTX. B: with concurrent application of TTX (1 \(\mu\)M) and 4-AP (500 \(\mu\)M), a slow-developing Ca\(^{2+}\) spike was evoked by a depolarizing current pulse and was then blocked by application of Cd\(^{2+}\). C: with application of TEA (20 mM), an evoked Na\(^{+}\) action potential was immediately followed by a Ca\(^{2+}\) plateau potential. The combined Na\(^{+}\) and Ca\(^{2+}\) potentials were blocked by concurrent application of TTX and Cd\(^{2+}\) (400 \(\mu\)M). D: with concurrent application of TTX and TEA, a separate Ca\(^{2+}\) plateau potential was evoked without a preceding Na\(^{+}\) action potential. This potential was then completely blocked by bath application of Cd\(^{2+}\).](http://jn.physiology.org/Downloadedfrom/fig1.png)
observed in cocaine-withdrawn neurons although they were sensitive K channels. Under this condition, the rheobase for evoking Ca$^{2+}$ plateau potential was capable to occasionally evoke additional Na$^+$ spikes (n = 2 cells). C and D: blockade of Na$^+$ currents by TTX did not eliminate the secondary Ca$^{2+}$ potential. In some cases, concurrent blockade of voltage-sensitive sodium currents (VSSCs) and TEA-sensitive as well as TEA-insensitive K$^+$ currents (TTX + 4-AP + TEA), resulted in small Ca$^{2+}$ potentials that were occasionally enlarged and converted to a secondary Ca$^{2+}$ plateau, from which multiple Ca$^{2+}$ plateaux could be generated (n = 3 cells). Arrows indicate the locations of the secondary Ca$^{2+}$ potential and its effects.

FIG. 2. Secondary Ca$^{2+}$ potentials modulate the generation of Na$^+$ spike and Ca$^{2+}$ plateau potentials. A: after blockade of TEA-sensitive K$^+$ channels, a small membrane depolarization (the secondary Ca$^{2+}$ potential) was observed at the end of an initially evoked Ca$^{2+}$ plateau potential in saline-pretreated nucleus accumbens (NAc) neurons (n = 12/12). B: this secondary Ca$^{2+}$ potential was capable to occasionally evoke additional Na$^+$ spikes (n = 2 cells). C and D: blockade of Na$^+$ currents by TTX did not eliminate the secondary Ca$^{2+}$ potential. In some cases, concurrent blockade of voltage-sensitive sodium currents (VSSCs) and TEA-sensitive as well as TEA-insensitive K$^+$ currents (TTX + 4-AP + TEA), resulted in small Ca$^{2+}$ potentials that were occasionally enlarged and converted to a secondary Ca$^{2+}$ plateau, from which multiple Ca$^{2+}$ plateaux could be generated (n = 3 cells). Arrows indicate the locations of the secondary Ca$^{2+}$ potential and its effects.

22.3 ± 2.6 vs. 34.6 ± 3.2 mV, t = 2.9643, **P < 0.01; Figs. 3A and 4A). These changes were accompanied with a significantly hyperpolarized RMP (saline vs. cocaine group: −79.0 ± 1.0 vs. −84.0 ± 1.0 mV, t = 4.7412, **P < 0.001; Table 1). In addition, a tendency toward a decrease of the input resistance, increase of the threshold, and reduction in the amplitude and the duration of this spike were also observed in cocaine-withdrawn neurons although they were not statistically significant (Table 1).

Differing from the slow-developing Ca$^{2+}$ spikes, the Ca$^{2+}$ plateau potential was evoked with blockade of TEA-sensitive K$^+$ channels. Under this condition, the rheobase for evoking Ca$^{2+}$ plateau potential was also significantly increased, and the duration of Ca$^{2+}$ plateau potentials measured at the one-half-amplitude was significantly reduced in cocaine-pretreated NAc neurons (saline vs. cocaine group: 0.6 ± 0.1 vs. 1.0 ± 0.2 nA, t = 2.2378, *P < 0.05 and 145.7 ± 24.7 vs. 55.5 ± 11.8 ms, t = 3.0844, **P < 0.01, respectively; Figs. 3B and 4B). In addition, the secondary Ca$^{2+}$ potential, which might represent back-propagation of Na$^+/Ca^{2+}$-dependent APs in saline-pretreated NAc neurons (see DISCUSSION), was abolished in the majority of cocaine-withdrawn cells (saline vs. cocaine group: 100% or n = 12/12 cells vs. 40% or n = 4/10 cells, χ$^2$ = 10.0768, **P < 0.01; Figs. 3B and 4B). These changes were also accompanied with a significant hyperpolarization of RMP (saline vs. cocaine group: −76.0 ± 1.1 vs. −83.0 ± 1.4 mV, t = 4.8912, **P < 0.01; Table 1). Because of the difficulty in determining the threshold of these Ca$^{2+}$ plateau potentials without blockade of VSSCs and because the amplitude of Ca$^{2+}$ plateau potentials appeared to be unchanged after repeated cocaine administration, data with respect to the two issues were not collected (but see following text).

Similar alterations in the membrane properties and characteristics of Ca$^{2+}$ plateau potentials were observed in cocaine-pretreated NAc neurons with concurrent block of VSSCs and all types of K$^+$ currents. With combined application of external TTX, TEA and internal Cs$^+$ (Figs. 3C and 4C), a significant increase in the rheobase, decrease in the duration of Ca$^{2+}$ plateau potential measured at the one-half-amplitude, and reduction in the total area of the Ca$^{2+}$ plateau potential were observed in cocaine-withdrawn NAc neurons (saline vs. cocaine group: 0.9 ± 0.1 vs. 1.2 ± 0.1 nA, t = 2.422, *P < 0.05;
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315.4 ± 24.3 vs. 210.1 ± 16.3 ms, $t = 3.674$, **$P < 0.01$; and 38.016 ± 2.889 vs. 25.502 ± 2.042 ms, $t = 3.612$, **$P < 0.001$, respectively; Figs. 3C and 4C). Moreover, internally applied Cs$^+$ eliminated the hyperpolarizing effects of chronic cocaine on RMP. Therefore there was no significant difference in the RMP between saline- and cocaine-pretreated NAc cells with internal presence of Cs$^+$ ($-68.7 ± 1.2$ vs. $70.1 ± 0.7$ mV, $P > 0.05$; Table 1). In addition, Cs$^+$-induced depolarization in RMP was also observed in saline-pretreated NAc neurons (Table 1). These results indicate that Cs$^+$-induced blockade of TEA-insensitive K$^+$ currents, including $I_{KCa}$ and $I_k$ in this case, plays an important role in modulating RMP and Ca$^{2+}$ plateau potentials, respectively. The cocaine-induced alterations in the characteristics of Ca$^{2+}$ plateau potentials are summarized in Fig. 4 and Table 1.

Although HVA-Ca$^{2+}$ currents are thought to make a major contribution for generation of the plateau potential in rat striatal neurons (Bargao et al. 1991b, 1994), a recent report suggests that some LVA T-type Ca$^{2+}$ channels are expressed in the striatum of newborn and juvenile rats (McRory et al. 2001). To determine whether these LVA-Ca$^{2+}$ channels are present and functionally involved in generation of Ca$^{2+}$ plateau potentials in NAc neurons of adult rats, a relatively low concentration of Ni$^{2+}$ (100 μM) was used in this study to block any possible effects of LVA T-type Ca$^{2+}$ channels. However, application of Ni$^{2+}$ affected neither the duration of Ca$^{2+}$ plateau potential.

**TABLE 1.** Repeated cocaine administration in vivo altered the membrane properties and characteristics of HVA-Ca$^{2+}$ potentials recorded in vitro

<table>
<thead>
<tr>
<th>Group</th>
<th>Saline pretreated (cells/rats)</th>
<th>Cocaine pretreated (cells/rats)</th>
</tr>
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<tbody>
<tr>
<td><strong>Passive membrane properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP, mV</td>
<td>$-79.0 ± 1.0$</td>
<td>$-84.0 ± 1.0$**</td>
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<tr>
<td>$R_m$, mΩ</td>
<td>47.6 ± 5.6</td>
<td>41.5 ± 2.5</td>
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<td>$t_m$, ms</td>
<td>6.5 ± 1.0</td>
<td>4.3 ± 1.6</td>
</tr>
<tr>
<td><strong>Active membrane properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rho, nA</td>
<td>$-6.9 ± 0.2$</td>
<td>$1.7 ± 0.3^*$</td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>$-39.3 ± 2.0$</td>
<td>$-32.4 ± 2.6$</td>
</tr>
<tr>
<td>Amplitude of slow Ca$^{2+}$ spike, mV</td>
<td>24.3 ± 3.7</td>
<td>19.5 ± 2.5</td>
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<tr>
<td>Duration of slow Ca$^{2+}$ spike, ms</td>
<td>8.4 ± 1.2</td>
<td>6.1 ± 0.7</td>
</tr>
<tr>
<td>AHP of slow Ca$^{2+}$ spike, mV</td>
<td>22.3 ± 2.6</td>
<td>34.6 ± 3.2*</td>
</tr>
<tr>
<td>Ca$^{2+}$ Spike evoked in neurons, %</td>
<td>100 (8/8)</td>
<td>45.5 (5/11)*</td>
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<tr>
<td><strong>RMP</strong></td>
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<tr>
<td>Passive membrane properties</td>
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<tr>
<td>RMP, mV</td>
<td>$-76.0 ± 1.1$</td>
<td>$-83.0 ± 1.4**</td>
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<td>N/A +</td>
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<td>N/A +</td>
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<tr>
<td><strong>Active membrane properties</strong></td>
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<tr>
<td>Rho, nA</td>
<td>$0.6 ± 0.1$</td>
<td>$1.0 ± 0.2^*$</td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>N/A +</td>
<td>N/A +</td>
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<tr>
<td>Amplitude of Ca$^{2+}$ plateau potential, ms</td>
<td>N/A + +</td>
<td>N/A + +</td>
</tr>
<tr>
<td>Duration at 1/2 amplitude, ms</td>
<td>145.7 ± 24.7</td>
<td>55.5 ± 11.8**</td>
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<td><strong>Ca$^{2+}$</strong></td>
<td>100% (12/12)</td>
<td>40% (4/10)*</td>
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<td>Passive membrane properties</td>
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<tr>
<td><strong>Active membrane properties</strong></td>
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<tr>
<td>Rho, nA</td>
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<td>$1.2 ± 0.1^*$</td>
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<td>Threshold, mV</td>
<td>$-14.2 ± 1.3$</td>
<td>$-11.6 ± 1.5$</td>
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<tr>
<td>Amplitude of Ca$^{2+}$ plateau potential, ms</td>
<td>58.2 ± 1.8</td>
<td>54.5 ± 1.6</td>
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<td>Duration at 1/2 amplitude, ms</td>
<td>315.4 ± 24.3</td>
<td>210.1 ± 16.3**</td>
</tr>
<tr>
<td>Total area of plateau potential, mV × ms</td>
<td>38.016 ± 2.889</td>
<td>25.502 ± 2.042**</td>
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$+$: Not available due to the use of short duration of current pulse (20 ms) + +: Not available due to difficulty in measuring the threshold with TEA but without TTX. Values are means ± SE. 4-AP, 4-aminoopyridine; RMP, resting membrane potential; $R_m$, input resistance; $t_m$, time constant. *$P < 0.05$, **$P < 0.01$, t-test, $P < 0.05$, $P < 0.001$.
plateau potentials ($n = 5/5$; control vs. Ni$^{2+}$: 276.77 ± 55.65 vs. 307.35 ± 53.47 mV, $P > 0.05$) nor other characteristics in saline-pretreated NAc neurons (Fig. 5), indicating that the evoked Ca$^{2+}$ plateau potential was generated primarily by HVA-Ca$^{2+}$ currents in NAc neurons of these young adult rats.

Repeated cocaine administration enhances the activity of various K$^+$ channels

As described in the preceding text in saline-pretreated NAc cells, depolarizing current pulses induced a slowly developed, subthreshold membrane depolarization that gradually reached the threshold and eventually evoked a Na$^+$ AP (Fig. 6A1). TTX inhibited the Na$^+$-dependent action potential by suppressing the subthreshold depolarization, which therefore revealed an outward rectification represented by a downward deflection of membrane potential traces in response to increment of depolarizing current pulses (Fig. 6A2). This outward rectification was initiated near the membrane potential levels around −60 mV (Fig. 6C), at which $I_K$ channels (including the somatic $I_K$) began to be activated (Nisenbaum and Wilson 1995a,b). There was a significant difference in the $I$-$V$ curves of saline-pretreated NAc neurons during membrane depolarization before and after TTX application [SAL vs. SAL/TTX group, $n = 10$ cells, $F(1,18) = 24.936$, $P < 0.001$; Fig. 6C].

Repeated cocaine administration mimicked the effects of TTX in suppressing the subthreshold depolarization and inhibiting Na$^+$ AP. It also caused a similar outward (downward) rectification in the $I$-$V$ curve during membrane depolarization. There was a significant difference in the $I$-$V$ curve between saline- and cocaine-pretreated NAc neurons [SAL vs. COC, $F(1,18) = 31.266$, $P < 0.001$]. In addition, the rheobase for evoking Na$^+$ spike was also markedly increased (comparing Fig. 6, A1 and B1). Under these circumstances, application of TTX failed to produce further significant changes in the outward rectification (COC vs. COC/TTX, $n = 10$ cells, $P > 0.05$).

![Image](http://jn.physiology.org/)

**FIG. 6.** Repeated cocaine administration enhanced outward rectification in NAc neurons during membrane depolarization: blockade of VSSCs. A: in a saline-pretreated NAc neuron, TTX suppressed the increase in subthreshold membrane excitability (the "ramp" potentials) induced by depolarizing current pulses and the generation of a Na$^+$-dependent action potentials (A1). These changes revealed an outward rectification indicated by a downward deflection of membrane potential traces during membrane depolarization (A2). B: repeated cocaine administration caused a similar inhibition in the subthreshold membrane excitability and suppression of Na$^+$ action potentials during membrane depolarization (B1). Note that higher depolarizing current was needed for generation of Na$^+$ action potentials in cocaine-pretreated NAc neurons as compared with that in saline-pretreated cells. TTX did not produce further downward deflection of membrane potentials during membrane depolarization (B2). C: the current-voltage ($I$-$V$) curves indicate alterations in the $I$-$V$ relationship of NAc neurons following repeated cocaine administration. In saline-pretreated NAc neurons, membrane depolarization initially induced an inward rectification (● SAL, $n = 10$), which was represented by an upward reflection in the $I$-$V$ curve. This inward rectification was suppressed by application of TTX (○ SAL/TTX, $n = 10$ cells). There was a significant difference in the $I$-$V$ curve between the SAL/control and SAL/TTX-treated NAc neurons (● SAL vs. ○ SAL/TTX, $F(1,18) = 24.936$, $P < 0.001$). Repeated cocaine administration mimicked the effects of TTX on the $I$-$V$ relationship. There was a significant difference in the $I$-$V$ curve between saline- and cocaine-pretreated NAc neurons (● SAL vs. ○ SAL/TTX, $F(1,18) = 31.266$, $P < 0.001$). However, this effect of cocaine on the $I$-$V$ curve was not significantly affected by TTX (● COC vs. ○ COC/TTX, $n = 10/10$ cells, $P > 0.05$). In addition, it is noted that in either SAL/TTX-treated NAc neurons (○) or COC-withdrawn cells (● or ○), the inward rectification was converted to an outward rectification represented by a downward deflection in the $I$-$V$ curve. This outward rectification was initiated at the subthreshold potential levels (approximately −70 to −60 mV) during membrane depolarization, in which the voltage-gated delayed rectifiers (D2) were activated at the time point during the depolarizing current pulses where the membrane potentials were measured.

![Image](http://jn.physiology.org/)

**FIG. 5.** Application of Ni$^{2+}$ did not affect evoked Ca$^{2+}$ plateau potentials in saline-pretreated NAc neurons. Although the concentration of Ni$^{2+}$ (100 μM) utilized in the current study usually blocked the low-voltage-activated (LVA) T-type Ca$^{2+}$ channels in other types of neurons, representative Ca$^{2+}$ plateau potentials recorded from a saline-pretreated NAc neuron exhibited overlapping traces either with or without Ni$^{2+}$. All neurons recorded under these circumstances were also treated with internally dialyzed Cs$^+$ and externally applied TEA plus TTX. There was no significant difference either in the duration of Ca$^{2+}$ plateau potential measured at a half-amplitude (insertion, $n = 5/5$ cells; control vs. Ni$^{2+}$: 276.77 ± 55.65 vs. 307.35 ± 53.47 ms, $P > 0.05$) or in the threshold and amplitude of Ca$^{2+}$ plateau potentials (not shown) between control and nickel-treated NAc neurons, indicating that the Ca$^{2+}$ plateau potential evoked in NAc neurons is high voltage activated (HVA).
An enhanced outward rectification during membrane depolarization was also observed in cocaine-pretreated NAc neurons with blockade of both VSSCs and HVA-Ca\textsuperscript{2+} currents. Cocaine-pretreated neurons showed a marked increase in outward rectification in their I-V curves in response to increment of depolarizing current pulses with application of both TTX and Cd\textsuperscript{2+} compared with saline-pretreated NAc neurons (Fig. 7, A and B). Again, there was a significant difference in the I-V curves regarding the outward rectification between saline- and cocaine-pretreated neurons during membrane depolarization with application of TTX and Cd\textsuperscript{2+} [SAL vs. COC, n = 14/16 cells, F(1,28) = 9.84, *P < 0.05; Fig. 7C]. The integrated effects of repeated cocaine administration on the activity of VSSCs, HVA-Ca\textsuperscript{2+} currents and a variety of K\textsuperscript{+} currents including $I_{K\text{rest}}$, $I_K$, and presumably $I_K(Ca)$ (see DISCUSSION), which leads to decrease of membrane excitability of NAc neurons, are summarized in Fig. 8.

**DISCUSSION**

The present study demonstrates that repeated cocaine administration significantly altered the characteristics of HVA-Ca\textsuperscript{2+} potentials and the activity of a variety of K\textsuperscript{+} currents in NAc neurons. These findings are consistent with our previous studies demonstrating decreased whole cell VSCCs and VSSCs in chronic cocaine-treated rats (Zhang et al. 1998, 2002). These effects of chronic cocaine could not be attributed to its direct action because the experiments were performed 3–4 days after the last injection of cocaine and acutely applied cocaine failed to produce identical responses. In addition, a local anesthetic effect of cocaine on VSSCs, which might affect generation and propagation of Ca\textsuperscript{2+} potentials, is also ruled out because the brain concentrations of cocaine (<3 μM) induced by the regimen (Pettit et al. 1990) used in our study are considerably lower than that (25–50 μM) needed to interrupt VSSCs and alter membrane potentials (Reith et al. 1986; Wheeler et al. 1993; Zimanyi et al. 1989).

**Repeated cocaine administration inhibits HVA-Ca\textsuperscript{2+} potentials in NAc neurons**

The major finding in the present study is that repeated cocaine administration inhibited HVA-Ca\textsuperscript{2+} potentials in NAc neurons. Significant alterations in the characteristics of HVA-Ca\textsuperscript{2+} potentials include the suppression of Ca\textsuperscript{2+} spikes, increased rheobase, and most remarkably, reduced duration of Ca\textsuperscript{2+} plateau potentials in cocaine-pretreated cells. These effects of chronic cocaine on Ca\textsuperscript{2+} potentials are expected and primarily attributed to reduced N- and R-type Ca\textsuperscript{2+} currents (Zhang et al. 2002). In addition, the increased rheobase should also be attributed to the reduced subthreshold excitability in which the activity of voltage-sensitive Na\textsuperscript{+} (Zhang et al. 1998) and K\textsuperscript{+} currents (see following text) were decreased and enhanced, respectively. It is well known that in rat striatal...
MSNs, plateau potentials are generated by VSCCs (Bargas et al. 1994, 1999; Cherubini and Lanfumey 1987; Dunia et al. 1996; Hernandez-Lopez et al. 1997; Higashi et al. 1988; Surmeier et al. 1995) after primarily activation of HVA-Ca\(^{2+}\) channels (Bargas et al. 1994; Hoehn et al. 1993; Surmeier et al. 1995). Given that stimulation of DA D1Rs indirectly decreases VSCCs via dephosphorylation of N-, R-, and P/Q-type channels by protein phosphatase 1 (PP-1) (Surmeier et al. 1995; Zhang et al. 2002) and that repeated cocaine administration upregulates the D1R/G/AC/cAMP/PKA pathway (Nestler 1997; Self and Nestler 1995), our findings indicate that suppression of HVA-Ca\(^{2+}\) potentials in cocaine-withdrawn NAc neurons is mediated by the upregulated D1R/G/AC/cAMP/PKA/PP1 pathway.

Another interesting finding in this study is that repeated cocaine administration abolished the secondary Ca\(^{2+}\) potential that is usually associated with the primary Ca\(^{2+}\) plateau potentials. “Active” propagation of APs from the axosomatic region to dendrites (known as backpropagation) as well as that from the dendrites to soma (known as “forward” propagation) has been demonstrated in different types of neurons (Hanson et al. 2004; Jaffe et al. 1992; Magee 2000; Magee and Carruth 1999; Stuart and Sakmann 1994). In these processes, voltage-gated dendritic Na\(^{+}\) and Ca\(^{2+}\) channels are activated by back- or forward-propagation of spikes (Debarbieux et al. 2003; Kavalali et al. 1997; Reuveni et al. 1993; Yuste et al. 1994), thereby eliciting VSCC activation in dendrites (Markram and Sakmann 1994; Schiller et al. 1995; Spruston et al. 1995). Such alterations in the activity of dendritic Na\(^{+}\) and Ca\(^{2+}\) channels provide a prolonged inward current that is capable of generating multiple APs in the soma (Magee and Carruth 1999). Similar to these findings, backpropagation of APs is also found in striatal neurons (Bargas et al. 1991a, 1994; Hernandez-Lopez et al. 1997). Although the previous recording methods do not allow us to make conclusions about dendritic events, our studies examining the secondary Ca\(^{2+}\) potential, as well as additional Na\(^{+}\) spikes, secondary Ca\(^{2+}\) plateau, and multiple Ca\(^{2+}\) plateau potentials evoked by it, suggest that both backpropagation and forward propagation of Na\(^{+}/Ca\(^{2+}\) potentials may be present in NAc neurons. However, this secondary Ca\(^{2+}\) potential was abolished after repeated cocaine administration, suggesting that the assumed dendritic Na\(^{+}/Ca\(^{2+}\) influx were also reduced in cocaine-withdrawn NAc neurons.

Repeated cocaine administration enhances multiple K\(^{+}\) currents in NAc neurons

Repeated cocaine administration also apparently alters the activity of somatic K\(^{+}\) channels in NAc neurons, evidenced by the hyperpolarized RMP, enhanced outward rectification during membrane depolarization, and deepened amplitude of afterhyperpolarization. Previous studies have determined the existence of various K\(^{+}\) currents in dorsal striatal and NAC MSNs, including but not limited to the inward rectifiers (I\(_{KIR}\)) (Galarreta et al. 1994; Nisenbaum and Wilson 1995a,b; Pacheco-Cano et al. 1996; Uchimura et al. 1989), delayed rectifiers (I\(_{K}\)) (Nisenbaum et al. 1994; Surmeier and Kitai 1993, 1997), and Ca\(^{2+}\)-activated K\(^{+}\) currents (I\(_{K(Ca)}\)), which flow through the large (BK) and small (SK) conductance K\(^{+}\) channels, respectively (Bargas et al. 1999; Köhler et al. 1996). Given that one of the usual functions of I\(_{KIR}\) is to maintain RMP by carrying outward K\(^{+}\) current at the membrane potential levels slightly above the E\(_{K}\) (Hille 2001), it is not surprising that blockade of I\(_{KIR}\) by internal Cs\(^{+}\) depolarized the RMP of NAc cells. In contrast, the RMP of NAc neurons was hyperpolarized after repeated cocaine treatment, suggesting that I\(_{KIR}\) was enhanced in cocaine-withdrawn NAc neurons.

Associated with a hyperpolarized RMP, there was an enhanced outward rectification during membrane depolarization. Previous studies have demonstrated that there are at least three types of I\(_{K}\) in striatal MSNs involved in the regulation of membrane excitability (Nisenbaum and Wilson 1995a,b; Nisenbaum et al. 1994; Surmeier et al. 1991, 1992). Among them, the slowly inactivating A current (I\(_{KA}\)) and noninactivating K\(^{+}\) current (persistent K\(^{+}\) current, I\(_{Kper}\)) are activated at the subthreshold levels (−60 − 75 mV, respectively) to restrict the amplitude of APs and to dampen repetitive firing. In contrast, the fast-inactivating A current (I\(_{KA}\)) is activated near suprathreshold levels (approximately −40 mV) to assist membrane repolarization. Given that all NAc neurons in the I-V relationship study were initially held at the RMP level of −80 mV and that the outward rectification during membrane depolarization was activated at the subthreshold levels and was significantly enhanced in cocaine-withdrawn NAc neurons, our results indicate that repeated cocaine administration enhanced the voltage-gated I\(_{K}\) in NAc neurons. This finding is consistent with previous studies that have demonstrated that repeated cocaine administration upregulates PKA activity (Nestler 1997; Self and Nestler 1995), while PKA-induced phosphorylation increases not only I\(_{K}\) (Hille 2001) but also the open probability of I\(_{A}\) (Koh et al. 1996). Therefore we propose that the enhanced outward rectification in cocaine-pretreated NAc neurons during membrane depolarization is caused most likely by increased I\(_{K}\) (I\(_{KA}\) and/or I\(_{Kper}\)), but not I\(_{KA}\) because the later is downregulated by PKA-induced phosphorylation (Yuan et al. 2002). Moreover, the deepened AHP observed with blockade of I\(_{A}\) also implies an enhanced I\(_{K(Ca)}\) in cocaine-sensitized NAc neurons. Although the subtype of I\(_{K(Ca)}\) responsible for this enhancement is currently unknown, previous investigations suggest involvement of Ca\(^{2+}\)-activated BK rather than SK because PKA-induced phosphorylation increases BK but shuts down SK in different types of cells (Bringmann et al. 1997; Köhler et al. 1996).

In addition to the somatic K\(^{+}\) channels mentioned in the preceding text, repeated cocaine administration, which eliminates the secondary Ca\(^{2+}\) potentials, may also alter the activity of dendritic K\(^{+}\) currents because dendritic signal propagation is also modulated by I\(_{A}\). For instance, activation of a high density of I\(_{A}\) channels prevents initiation of APs, limits backpropagation of APs, and therefore reduces excitation-synaptic events in dendrites (Hoffman et al. 1997). In contrast, downregulation of dendritic I\(_{A}\) increases the amplitude of backpropagating APs (Yuan et al. 2002). Combined with these previous findings, our results, particularly the abolished secondary Ca\(^{2+}\) potentials, suggest that repeated cocaine administration not only reduces dendritic Na\(^{+}/Ca\(^{2+}\) influx but might also enhance I\(_{A}\) during dendritic membrane depolarization. Taken together, it is likely that chronic cocaine treatment disrupts the function of multiple ion channels critically in-
volved in regulating the backpropagation and forward propagation of APs in NAc neurons.

Recurrent cocaine administration decreases membrane excitability of NAc neurons via disruption of normal signal transduction

We have recently demonstrated the remarkable alterations in the activity of membrane ion channels of NAc neurons occurred after repeated cocaine administration (Zhang et al. 1998, 2002; Nestler et al. 1990; Striplin and Kalivas 1993; Terwilliger et al. 2000). Functional alterations beyond the DA-R have to be considered. In fact, recent investigations have suggested that repeated cocaine administration increases D1R-stimulated activity in cAMP/PKA cascade (Nestler 1997; Self and Nestler 1995), and attenuates the function of calcineurin but not the function of membrane ion channels in NAc neurons. These findings indicate that cocaine-induced alterations in the function of membrane ion channels in NAc neurons are based on the integrated changes in different signaling pathways and gene expression.

In summary, the present study demonstrates that repeated cocaine administration not only suppresses somatic HVA-Ca\(^{2+}\) potentials but also enhances the activity of a variety of K\(^+\) currents [e.g., I\(_{K\text{frest}}\), I\(_{A}\) and I\(_{K\text{Ca}}\)] in rat NAc MSNs. It also suggests that dendritic propagation of Na\(^+\)/Ca\(^{2+}\) potentials is suppressed by repeated cocaine administration. The integrated changes in the membrane ion channel function leads to decreased membrane excitability during cocaine withdrawal (see Fig. 8 for summarization). Given that most addictive substances affect DA neurotransmission in the mesoaccumbens and mesocortical DA system, this study provides a useful model for further investigation of whole cell intrinsic neuroplasticity in addictive drug-induced sensitization and withdrawal effects.

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