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Whole-Cell Plasticity in Cocaine Withdrawal: Reduced Sodium Currents in Nucleus Accumbens Neurons

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The nucleus accumbens is a forebrain region that mediates cocaine self-administration and withdrawal effects in animal models of cocaine dependence. Considerable evidence suggests an important role of dopamine D1 receptors in these effects. Using a combination of current-clamp recordings in brain slices and whole-cell patch-clamp recordings from freshly dissociated neurons, we found that nucleus accumbens neurons are less excitable in cocaine withdrawn rats because of a novel form of plasticity: reduced whole-cell sodium currents. Three days after discontinuation of repeated cocaine injections, nucleus accumbens neurons recorded in brain slices were less responsive to depolarizing current injections, had higher action potential thresholds, and had lower spike amplitudes. Freshly dissociated nucleus accumbens neurons from cocaine-pretreated rats exhibited diminished sodium current density and a depolarizing shift in the voltage-dependence of sodium channel activation. These effects appear to be related to enhanced basal phosphorylation of sodium channels because of increased transmission through the dopamine D1 receptor/cAMP-dependent protein kinase pathway. The effects of repeated cocaine administration were not mimicked by repeated injections of the local anesthetic lidocaine and were not observed in neurons within the motor cortex, indicating that they did not result from local anesthetic actions of cocaine. Because nucleus accumbens neurons are normally recruited to coordinate response patterns of movement and affect, the decreased excitability during cocaine withdrawal may be related to symptoms such as anergia, anhedonia, and depression.

Key words: nucleus accumbens; cocaine addiction; dopamine D1 receptors; sodium channels; patch clamp; whole-cell plasticity

Despite widespread awareness of the addictive potential of cocaine, abuse of this illicit drug remains one of our foremost public health problems (Cornish and O'Brien, 1996). Cocaine dependence is typically associated with cyclical patterns of binge-type abuse and abstinence (Gawin, 1991). During abstinence, there are periods of intense cocaine craving and other withdrawal symptoms, such as anergia, anhedonia, and depression, which play significant roles in relapse (Gawin, 1991; Kleber, 1995). The primary neurochemical action of cocaine is to potentiate synaptic transmission from dopamine (DA) and other monoamine-containing (serotonin and norepinephrine) neurons by preventing those neurons from recapturing released neurotransmitter (for review, see Galloway, 1992). Cocaine prevents reuptake by binding to specific receptor sites on the different monoamine transporter proteins (for review, see Reith, 1992). At higher doses, cocaine can also produce local anesthetic effects by a use-dependent block of voltage-sensitive sodium channels (VSSCs) (Postma and Catterall, 1984; Reith et al., 1986), shifting the voltage dependence of inactivation in the hyperpolarizing direction (Wang and Wang, 1992).

Among the various neurochemical actions of cocaine, blocking the DA transporter is most highly associated with reinforcing (hedonic) efficacy (Ritz et al., 1987). Animal models of cocaine dependence have identified specific DA neuronal systems projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and related limbic and cortical areas as being primarily responsible for cocaine self-administration and at least partially responsible for cocaine withdrawal (for review, see Koob and Bloom, 1988; Koob, 1992; Kuhar and Pilotte, 1996; Wise, 1996). Although several cellular and molecular alterations have been identified within the VTA–NAc neural system both in animals (for review, see Self and Nestler, 1995; Hyman, 1996; Kuhar and Pilotte, 1996) and in humans (Volkow and Fowler, 1995), pharmacotherapies targeted at such changes have yet to be proven effective in cocaine-dependent individuals (Gawin, 1991; Kleber, 1995; Cornish and O'Brien, 1996). This likely reflects an incomplete understanding of neuroadaptations that result from repeated cocaine use (Self and Nestler, 1995; Hyman, 1996).

Over the past several years, we have used *in vivo* electrophysiological recordings of single NAc neurons to identify time-dependent alterations in responsiveness to cocaine, DA, and other neurotransmitters after repeated cocaine administration (Henry and White, 1991,1995; White et al., 1992,1995a,b). We have demonstrated a close temporal relationship between the persistence of one well established behavioral consequence of repeated cocaine administration, sensitization, and enhanced responsiveness of NAc neurons to stimulation of the D1 class of DA receptors (Henry and White, 1991,1995). Additional NAc neuronal alterations are evident at early withdrawal times, including enhanced inhibitory effects of GABA and serotonin (White et al.,

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1992; Henry and White, 1995) and markedly attenuated excitatory responses to glutamate (White et al., 1995b). Some of these changes were tested after longer withdrawal times (GABA, serotonin) and found to be transient, lasting <7 d. Because there is no evidence to suggest that cocaine dependence alters receptor systems for all of these neurotransmitters within the NAc, we considered the possibility that modifications had occurred in the intrinsic membrane properties of NAc neurons. Here we demonstrate that during the early period of withdrawal from repeated administration of cocaine, there is a marked decrease in the excitability of NAc neurons resulting from reduced conductance through VSSCs.

MATERIALS AND METHODS

Animals and treatments. Adult male Sprague Dawley rats initially weighing 150–175 gm were housed in groups of two to four in a temperature- and humidity-controlled vivarium under a 12 hr light/dark cycle. Food and water were freely available. After a 1 week acclimation to the vivarium, rats either were used for acute experiments regarding sodium current modulation or randomly assigned to one of three groups that received once daily intraperitoneal injections of saline (1.0 ml/kg), (−) cocaine HCl (15.0 mg/kg), or lidocaine HCl (50.0 mg/kg) for 5 consecutive days. Drug doses are given as the HCl salts. All experiments were conducted on the third day after cessation of injections.

Brain slice recordings. 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 our Institutional Animal Care and Use Committee. Rats were decapitated under halothane anesthesia, and brains were rapidly excised and dissected into blocks (3–4 mm) containing the NAc. The blocks of tissue were immersed in ice-cold artificial cerebrospinal fluid (ACSF) [(in mM): NaCl 124, KCl 5, $MgSO_4$ 2, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2.4, and D-glucose 10, pH 7.4 ± 0.05] and sectioned in the coronal plane with a motorized vibrating microtome. Brain slices were transferred to a holding chamber and incubated in oxygenated (95% O_2:5% CO_2) ACSF for at least 60 min before experimentation. Slices were then transferred to an interface-type recording chamber where they rested on nylon mesh and were superfused continuously with warm (35–36°C) oxygenated ACSF (flow rates of 1–2 ml/min). Borosilicate glass capillaries were pulled (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 (100 msec duration, −0.8 to +1.5 nA) through the recording electrode using the active 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 amplified and distributed to an oscilloscope and a 486-based computer running pCLAMP software (Axon Instruments). Recordings were made primarily from the core region of the NAc, although shell neurons were also included. As detailed previously (O'Donnell and Grace, 1993), we observed no difference in the membrane properties of shell and core neurons.

Whole-cell recordings. NAc neurons were freshly dissociated from slices obtained from rats (100–150 gm) as described above. Slices were cut while bathed in a low Ca^{2+} (100 μM), HEPES-buffered salt solution (in mM): 140 sodium isethionate, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 mM HEPES, pH 7.4, 300–305 mOsm/l). Slices were then incubated for 1–6 hr at room temperature in a $NaHCO_3$-buffered saline (Earle's Balanced Salts solution), bubbled with 95% O_2, 5% CO_2. Slices were then removed into the low Ca^{2+} buffer, and with the aid of a dissecting microscope, the NAc (including both core and shell regions) was micropunched and placed in an oxygenated stir chamber containing pronase (1–1.5 mg/ml) in HEPES-buffered HBSS at 35°C. After 20–30 min of enzyme digestion, tissue was rinsed three times in the low Ca^{2+}, HEPES-buffered saline and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was then plated into a petri dish mounted on the stage of an inverted microscope containing 1 ml of HEPES-buffered HBSS. After the cells were allowed to settle, the solution bathing the cells was changed to our normal recording external solution.

Whole-cell recordings used standard techniques. Electrodes were pulled from Corning 7052 glass capillaries and fire-polished before use. Sodium current was isolated by using the following solutions: internal (in mM) 120 CsF, 10 NaCl, 10 EGTA, 2 Na_2ATP, 10 HEPES, pH 7.3, 265–270 mOsm/l; external (in mM) 100 NaCl, 30 choline chloride, 5 CsCl, 1 $MgCl_2$, 1 $CaCl_2$, 0.4 $CdCl_2$, 10 glucose, 10 HEPES, pH 7.4, 300–305 mOsm/l. Electrodes filled with this solution had a resistance of 2–4 MΩ when tested in the bath solution. The junction potential of −5 mV was measured between the electrode and bath solution and was not compensated. Recordings were obtained with an Axon Instruments 200A patch-clamp amplifier and controlled and monitored with a PC 486 running pCLAMP6 with a 2 kHz filter. Step depolarizing pulses were applied at intervals of 5–10 sec to allow enough time for Na^+ channels to recover from inactivation. After seal rupture, series resistance (<10 MΩ) was compensated (70–80%) and periodically monitored. All currents were leak-subtracted. Adequate voltage control was determined by standard methods (Colatsky and Tsien, 1979). Recordings were made only from medium-sized neurons (6–12 μm somal diameter) that had only a few short proximal dendrites. Drugs were applied with a DAD-12 superfusion system that allowed rapid application and removal of drugs (ALA Scientific Instruments, Westbury, NY). All experiments were performed at room temperature (20–22°C). To record from freshly dissociated cortical neurons, we dissected the superficial layers (1–3) of the fronto-parietal motor cortex. All procedures were as described for NAc neurons, except that the external Na^+ concentration was decreased to 30 mM because of larger currents evoked in the cortical neurons. Choline chloride was increased to compensate the reduced osmolarity.

RESULTS

Cocaine pretreatment alters the membrane properties of NAc neurons

To examine the membrane properties of NAc neurons in cocaine-pretreated rats, we first made intracellular recordings from rat brain slices *in vitro*. Cocaine-pretreated NAc neurons exhibited a number of significant alterations in passive and active membrane properties, as compared with saline-pretreated controls (Table 1). Resting membrane potentials were hyperpolarized, action potential generation by depolarizing current injection required higher current intensities (rheobase), Na^+ spike thresholds were increased, and spike amplitudes were reduced. In many neurons, the afterhyperpolarization amplitudes were also increased, but variability in this measure precluded it from reaching statistical significance (Table 1, Fig. 1). Figure 1 shows examples of action potentials obtained from control and cocaine-pretreated neurons such that the decreased spike amplitudes of cocaine-treated neurons can be appreciated. Taken together, these findings indicate that the excitability of NAc neurons is significantly reduced by repeated cocaine administration.

Although the absolute magnitude (<20%) of certain changes [resting membrane potential (RMP), action potential threshold and amplitude] may appear small (Table 1), they are not only statistically reliable but also likely to have considerable functional impact. These alterations are likely to diminish markedly the responsiveness of NAc neurons to excitatory inputs and thus the normal output of the NAc. Alterations in RMP and threshold would result in the need for greater excitatory barrage, because under normal circumstances, maintained coordinated synaptic activation is required to depolarize medium spiny neurons to the point of spike activity (O'Donnell and Grace, 1995; Wilson and Kawaguchi, 1996). Moreover, the more hyperpolarized resting state of the neurons will decrease the likelihood of firing by maintaining the slowly inactivating potassium channel in a non-inactivated state (Nisenbaum et al., 1994). Even when firing, the reductions in spike amplitude will diminish transmitter release substantially (Charlton and Bittner, 1978; Baxter and Bittner, 1981). To appreciate the magnitude of the changes in such membrane properties observed in our experiments, they should be compared with those produced by other *in vivo* manipulations on medium spiny neurons as well as with effects of repeated cocaine

Table 1. Repeated cocaine administration *in vivo* altered the membrane properties of NAc neurons recorded *in vitro*

	Saline	Cocaine	Lidocaine
Number of neurons	36	29	14
Number of rats	25	19	4
Passive membrane properties			
RMP (mV)	−79.1 ± 0.8	−83.8 ± 0.6**	−76.9 ± 1.5
R_{in} (mΩ)	38.3 ± 3.1	42.4 ± 3.1	32.1 ± 2.9
Time constant (τ, msec)	5.6 ± 0.5	4.7 ± 0.8	5.9 ± 1.3
Active membrane properties			
Current to generate AP (nA)	0.65 ± 0.03	0.96 ± 0.06**	0.69 ± 0.09
AP threshold (mV)	−46.7 ± 1.5	−40.9 ± 1.7*	−46.1 ± 2.1
AP amplitude (mV)	61.1 ± 1.1	52.5 ± 1.7**	59.2 ± 1.2
AP duration (msec)	2.1 ± 0.1	2.2 ± 0.1	1.5 ± 0.1*
AHP amplitude (mV)	10.3 ± 0.6	12.8 ± 1.0	11.7 ± 1.0

Intracellular recording was used to measure the membrane properties of nucleus accumbens neurons from brain slices obtained from the three groups of rats. Values represent the mean ± SEM for the number of neurons indicated (*$p < 0.05$; **$p < 0.01$; with Student's t test used to compare with control). Resting membrane potential (RMP) was measured in the absence of injected current before the initiation of other manipulations. Apparent input resistance (R_{in}) and time constants were determined by the membrane potential changes induced by hyperpolarizing current pulses, with the aid of algorithms within the pCLAMP software. Action potentials (APs) were generated by injecting step depolarizing current pulses of 0.1 nA increments. AP characteristics were obtained from the initial spike evoked by the minimal depolarizing current pulse in every NAc neuron recorded; amplitudes were measured from the spike threshold. After hyperpolarization (AHP) amplitudes were measured from the equipotential point of the spike threshold to the maximum deflection of the hyperpolarization after the end of the AP. AP duration was measured at the broadest point of the AP.

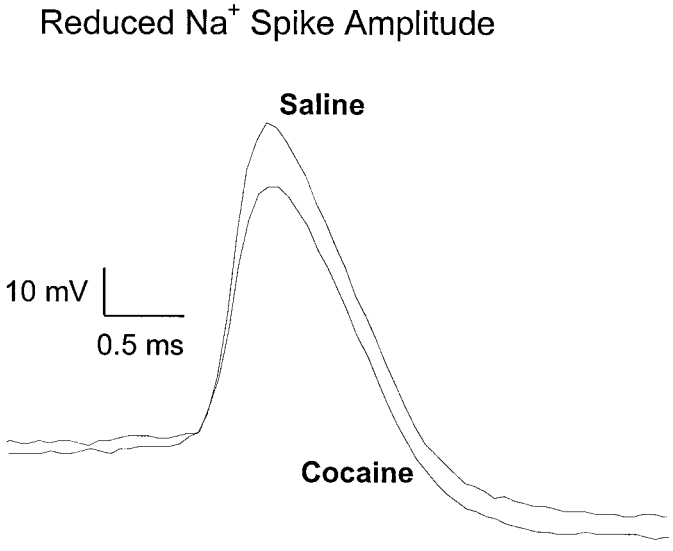

Figure 1. Repeated cocaine administration reduces action potential amplitudes of NAc neurons. Representative traces showing action potentials recorded from NAc neurons in saline-pretreated and cocaine-pretreated rats on the third day of withdrawal. Note the reduced amplitude of the action potential in the cocaine-pretreated neuron. The *traces* are normalized for action potential thresholds, which were −40.2 mV in the control neuron and −45.0 mV in the cocaine neuron. Resting membrane potentials were −79 mV for control and −86 mV for cocaine. Action potentials were evoked with intracellular depolarizing current injection (0.7 and 0.9 nA for saline and cocaine, respectively).

on other brain neurons. The changes that we observed after repeated cocaine administration are similar in magnitude to those observed in medium spiny striatal neurons 1–5 hr after transient forebrain ischemia (Xu, 1995) and are larger than those produced by repeated cocaine administration on the membrane properties of dorsolateral septal nucleus neurons (Simms and Gallagher, 1996; Shoji et al., 1997).

The reduction in excitability of NAc neurons by repeated cocaine administration cannot be attributed to the local anesthetic properties of cocaine because it is unlikely that cocaine or its metabolites were present in the slice after a 3 d withdrawal period and extensive superfusion of the slice. Moreover, repeated administration of the local anesthetic lidocaine (50 mg/kg for 5 d), which lacks significant activity at monoamine transporters (Ritz et al., 1987), failed to mimic any of the effects observed with repeated cocaine (Table 1).

Because D1 receptor-mediated responses are significantly enhanced by this cocaine pretreatment regimen (Henry and White, 1991) and because D1 receptor stimulation can hyperpolarize medium spiny NAc neurons (Uchimura et al., 1986; Uchimura and North, 1990), increase rheobase (O'Donnell and Grace, 1996), and reduce action potential amplitude (Schiffmann et al., 1995), we next determined whether endogenous DA within the slice had rendered NAc neurons less excitable by stimulating supersensitive D1 receptors. Bath perfusion with the selective D1 receptor antagonist SCH 23390 (1–2 μM) did not alter the membrane properties of NAc neurons recorded from cocaine-pretreated rats ($n = 5$; data not shown), indicating that there was no basal activation of D1 receptors in our slices.

Na^+ currents in NAc neurons are decreased by D1 receptor stimulation

Computer modeling coupled to direct observations of motoneurons indicates that alterations in rheobase, spike threshold, and spike amplitude can occur with reductions in Na^+ conductance (Halter et al., 1995). In medium spiny neurons of the dorsal striatum, both fast and slowly inactivating Na^+ conductances are modulated by DA receptors (Surmeier et al., 1992; Cepeda et al., 1995; Schiffmann et al., 1995). Given the marked alterations in synaptic DA levels (and thus DA receptor activation) that occur

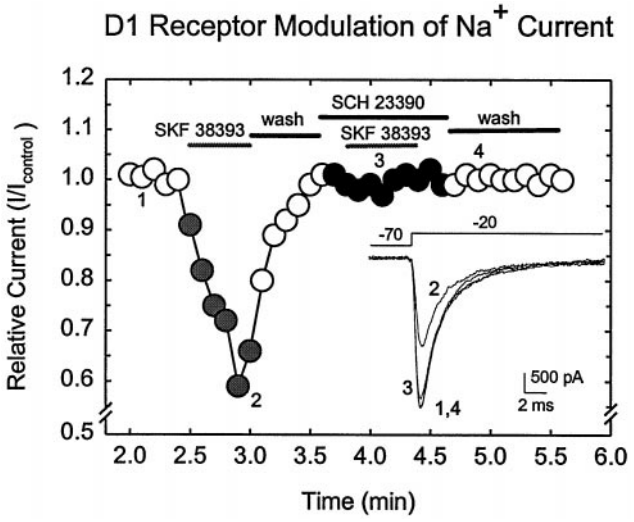

Figure 2. Stimulation of dopamine D1 receptors suppresses whole-cell Na^+ currents in NAc neurons. Current traces (*inset*) and time course showing that the D1 receptor selective agonist SKF 38393 (1 μM) reversibly suppressed whole-cell Na^+ current in an untreated NAc neuron. The effect was observed in 33/40 neurons tested (mean ± SEM = 24.5 ± 2.1% suppression; see Fig. 8 for concentration–effect curves). The dopamine D1 receptor-selective antagonist SCH 23390 (1 μM) completely prevented the suppression of current by SKF 38393 (n = 6). Currents were evoked by stepping the membrane from the holding potential of −70 mV to the test potential of −20 mV (see *protocol above traces*) where Na^+ current was near-maximal (*I–V* tests not shown). Na^+ currents were completely prevented by tetrodotoxin (1–2 μM; not shown), exhibited a reversal potential of ~50 mV, and inactivated rapidly. The *number* associated with each trace indicates the point during the time course at which the trace was obtained.

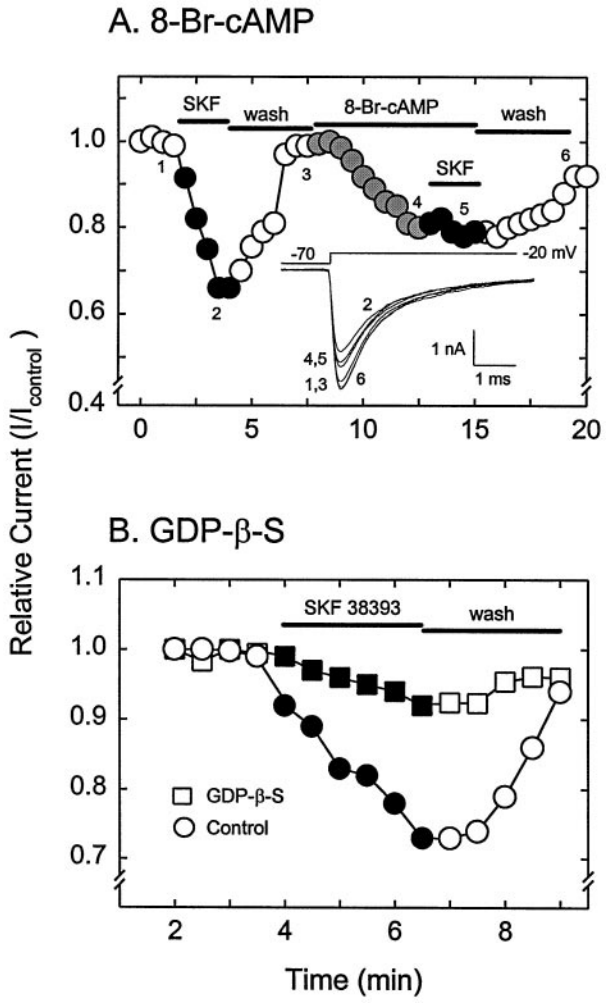

Figure 3. The D1 agonist-induced suppression of Na^+ current involves G_s protein activation of cAMP. *A*, This combination of time plot and current traces (*inset*) shows that the suppression produced by SKF 38393 was mimicked by the membrane-permeable cAMP analog *8-Br-cAMP* (50 μM; n = 6; 37.3 ± 10.6% suppression), which also occluded the effect of SKF 38393. *B*, These time–response curves compare the suppression of Na^+ currents by SKF 38393 in a control neuron and a neuron that had been dialyzed with the nonhydrolyzable GDP analog *GDP-β-S*, which prevents activation of G_s proteins. Note the marked reduction in the efficacy of SKF 38393 (2.8 ± 1.4% suppression; n = 7).

with repeated cocaine administration (Weiss et al., 1992; Kalivas and Duffy, 1993), we reasoned that alterations may have occurred in the steady state of VSSCs modulated by DA receptors. The remainder of our study focused on this possibility. First, we determined whether Na^+ currents in medium spiny neurons of the NAc, like those in the dorsal striatum, are modulated by D1 receptors.

We used the whole-cell configuration of the patch-clamp technique to record Na^+ currents from freshly dissociated NAc neurons. Medium spiny NAc neurons (those with capacitance <10 pF) (Surmeier et al., 1992) exhibited DA receptor modulation of VSSCs that was identical to that reported for the dorsal striatum (Surmeier et al., 1992; Cepeda et al., 1995; Schiffmann et al., 1995). Stimulation of D2 class receptors with the selective agonist quinpirole exerted mixed effects on whole-cell Na^+ currents, increasing conductance in some neurons but decreasing it in others (White et al., 1997). In contrast, D1 receptor stimulation with SKF 38393 (0.1–10 μM) consistently suppressed whole-cell, tetrodotoxin-sensitive Na^+ current in ~80% of the neurons we tested (Figs. 2, 8). This effect was completely prevented by the D1 receptor antagonist SCH 23390 (1 μM) (Fig. 2) but not by the D2 receptor-selective antagonist eticlopride (1 μM; n = 3; not shown). The inhibitory modulation was produced through the classic D1 receptor transduction pathway involving activation of G_s protein, stimulation of adenylyl cyclase activity, formation of cAMP, and dissociation of regulatory and catalytic subunits of cAMP-dependent protein kinase (PKA), leading to phosphorylation of VSSCs. Thus, the SKF 38393-induced suppression of whole-cell Na^+ conductance was mimicked by bath application of the membrane-permeable cAMP analog 8-bromo-cAMP (50 μM), which also occluded the inhibition produced by SKF 38393, demonstrating that the agonist effect was mediated by increased cAMP formation (Fig. 3*A*). Similar suppression of Na^+ currents was observed after intracellular dialysis (via the patch-clamp electrode) with the catalytic subunit of PKA (Fig. 4*A*), which also precluded SKF 38393-induced inhibition. In addition, the modulation observed with the D1 receptor-selective agonist was prevented by intracellular dialysis with either an inhibitor of PKA, PKI [5–24] (Fig. 4*B*), or an inhibitor of G_s activation, the nonhydrolyzable GDP analog guanosine 5′-*0*-(2-thiodiphosphate) (*GDP-β-S*, Fig. 3*B*). The voltage dependence of both activation and inactivation were unchanged (Fig. 5). This profile of effects is consistent with PKA phosphorylation of brain type IIA sodium channel α-subunits (Gershon et al., 1992; Li et al., 1992).

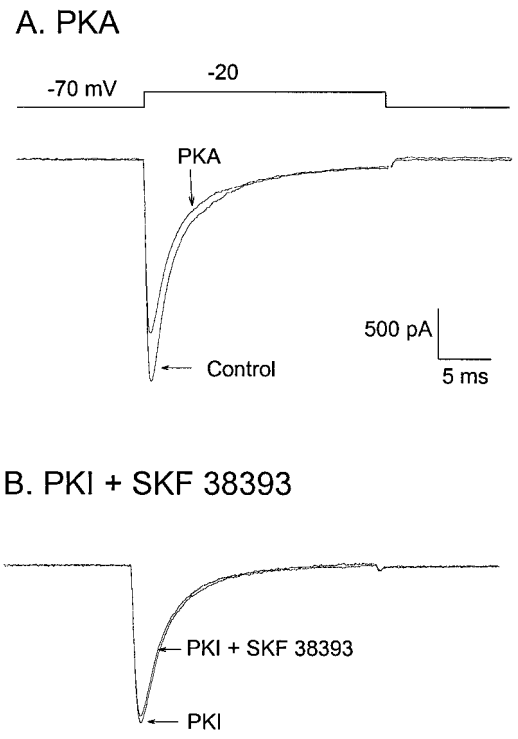

Figure 4. The D1 agonist-induced suppression of Na^+ currents involves cyclic-AMP-dependent protein kinase (*PKA*). *A*, The suppression of Na^+ current by D1 agonists was mimicked by the catalytic subunit of PKA (50–100 U/ml; $n = 8$), which was dialyzed into the neurons through the patch pipette. Na^+ current reduction was observed 4 min after membrane seal rupture. *B*, The PKA inhibitor *PKI* [5–24] (10 μM) prevented the suppression of Na^+ current by SKF 38393 after 4 min of dialysis into the neuron ($n = 9$).

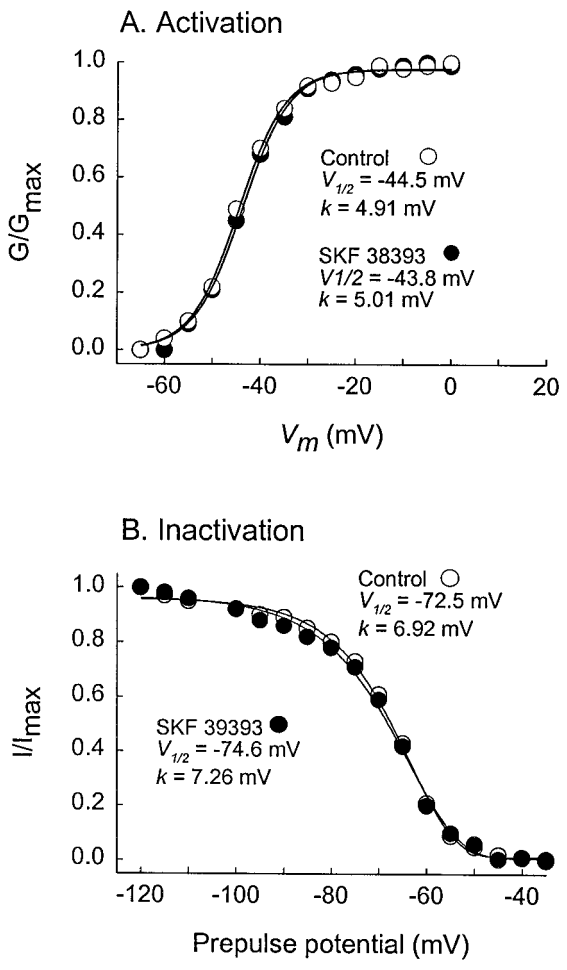

Figure 5. D1 agonist-induced suppression of Na^+ current occurred without alterations in the voltage dependence of activation or inactivation. *A*, The voltage-dependence of activation of whole-cell Na^+ current was not altered by SKF 38393. Relative conductance is plotted as a function of test potential. Activation curves were determined by voltage-clamping cells at −70 mV and applying voltage steps to potentials of −70 to +60 mV. To ensure that channel inactivation was removed completely, a lower frequency (0.1–0.2 Hz) was used. Peak currents were obtained at every test potential. Conductance was derived according to the equation $G_{Na} = I_{Na,peak}/(V - V_{Na})$, where G_{Na} is conductance, $I_{Na,peak}$ is peak Na^+ current, V is test potential, and V_{Na} is the Na^+ reversal potential. The steady-state activation curves (*m*-curve) were determined by plotting $G_{Na}/G_{Na,max}$ versus membrane potential as the Boltzmann equation $m = [1 + \exp(V - V_{½})/k] - 1$ where $V_{½}$ is the midpoint of the *m*-curves and k is the curve slope factor. *B*, The voltage dependence of inactivation was not altered by SKF 38393. Relative current is plotted as a function of prepulse potential. Steady-state inactivation curves were determined with a conditioning prepulse protocol. Cells were voltage-clamped at −70 mV. Membrane potential was first stepped (prepulse) to potentials from −120 to −40 mV (200 msec duration) before evoking Na^+ currents with a step to −20 mV. The steady-state inactivation curves (*h*-curves; $h = I_{Na,peak}/I_{Na,max}$ vs conditioning potential) were fitted to a Boltzmann equation $h = [1 + \exp(V - V_{½})/k] - 1$, where $V_{½}$ is the half-point of the inactivation curve and k is the slope factor. Time constants for inactivation were obtained by fitting traces to a single exponential.

Na^+ current density is reduced in cocaine-pretreated NAc neurons

Having characterized the DA receptor modulation of VSSCs in NAc neurons, we next examined whether Na^+ conductance was altered by repeated cocaine administration. In NAc neurons obtained from cocaine-pretreated rats, peak whole-cell Na^+ current density (current/capacitance) was greatly reduced (37%) compared with that of saline-pretreated control neurons (Fig. 6*A*). This effect was clearly caused by a reduction in current, because cell capacitance was not significantly different (*t* test) in the two groups of neurons: saline, 6.1 ± 0.34; cocaine, 6.3 ± 0.37. Reductions in peak Na^+ currents can result in reduced action potential amplitudes in medium spiny neurons (Fraser et al., 1993), as we observed in our current-clamp recordings. *I-V* recordings (Fig. 6*B,C*) indicated that the reduction in Na^+ current was accompanied by a significant depolarizing shift of 5.2 mV in the voltage-dependence of activation and a reduction in the slope of steady-state activation (Fig. 6*D*). The +5.2 mV shift in the voltage dependence of activation may be directly responsible for the +5.8 mV increase in firing threshold observed in brain slices. Indeed, mathematical modeling of Na^+ channel function indicates that a +2.2 mV alteration in activation voltage produces a +2.6 mV shift in threshold (Halter et al., 1995). We found no significant alteration in the voltage-dependence of inactivation (Fig. 6*E*). As in our current-clamp recordings, repeated administration of the Na^+ channel blocker lidocaine failed to reproduce the alterations in whole-cell Na^+ currents observed after repeated cocaine (see Fig. 6 legend). Thus, the effects cannot be attributed to the local anesthetic properties of cocaine. In addition, recordings from acutely dissociated cortical neurons failed to reveal changes in VSSCs such as those seen in the NAc (Table 2), indicating that the effects do not reveal a general action on brain neurons but rather reflect the intrinsic properties of the neurons

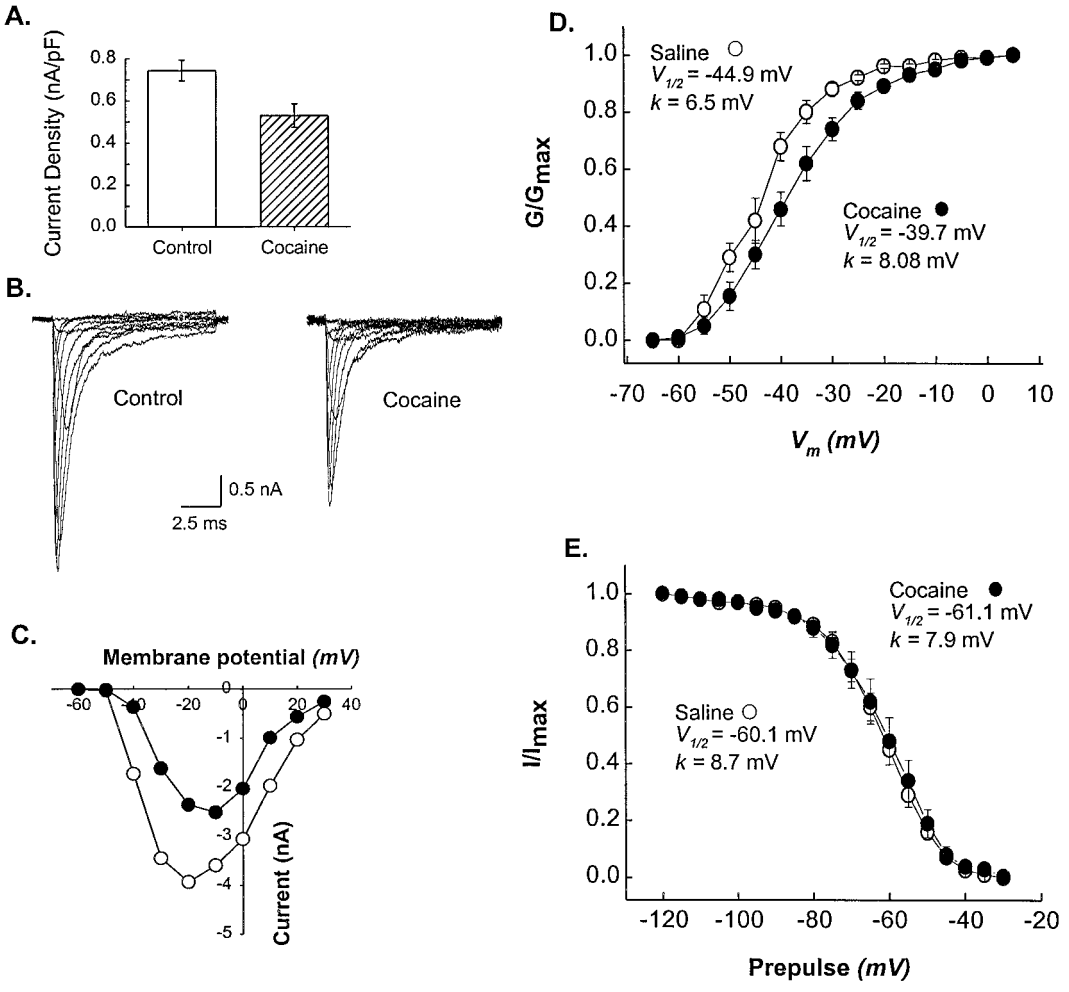

Figure 6. Repeated cocaine treatment reduced NAc Na^+ currents and caused a depolarizing shift in the voltage dependence of activation. *A*, NAc neurons ($n = 33$) from cocaine-pretreated rats exhibited significantly ($t_{68} = 2.75$; $p = 0.0075$) reduced peak whole-cell Na^+ currents (measured at −20 mV) as compared with neurons ($n = 37$) obtained from saline-pretreated rats. Results are presented as current density to normalize for capacitance (see Results). *B*, Recordings showing *I–V* relationship for Na^+ currents in a control and a cocaine-pretreated NAc neuron. *C*, Plots of the *I–V* curves for the two neurons shown in *B*. *D*, Cocaine pretreatment caused a depolarizing shift in the voltage dependence of activation and reduced the slope factor (k). Values were obtained for each neuron at each membrane voltage, and the mean ± SEM were plotted. Half-maximal activation ($V_{1/2}$) and slope (k) values were obtained individually for each neuron and compared with t tests. Half-maximal activation occurred at significantly ($t_{28} = 2.26$; $p = 0.031$) more depolarized potentials in cocaine-pretreated neurons (−40.3 ± 1.8 mV; $n = 16$) as compared with saline-pretreated neurons (46.1 ± 1.2; $n = 14$). The slope factor was also significantly decreased ($t_{28} = 3.13$; $p = 0.004$) in the cocaine group (8.85 ± 0.8) as compared with the saline group (4.01 ± 0.8). Note that the $V_{1/2}$ and k values shown in the figure were derived from the curves fitted to the mean values depicted in the figure, not from the mean values (given here) obtained by averaging all of the neurons in the sample. *E*, The voltage dependence of inactivation was not altered by repeated cocaine treatment. Values were obtained from the mean ± SEM as in *B* ($n = 16$ for cocaine; $n = 13$ for saline). Note that repeated administration of lidocaine failed to produce effects similar to those of cocaine: Na^+ current density = 0.81 ± 0.058, $n = 9$; activation $V_{1/2} = -42.4 \pm 1.8$, $k = 5.2 \pm 0.8$, $n = 8$; and inactivation $V_{1/2} = -60.6 \pm 2.5$, $k = 7.0 \pm 0.4$, $n = 9$. None of these values was significantly different from control (t tests). See Figure 5 legend for details regarding activation and inactivation curves.

and the particular set of neurotransmitter inputs and receptors that are impacted by cocaine.

A possible mechanism for the reduction in peak Na^+ current density observed in cocaine-pretreated NAc neurons is increased phosphorylation. VSSCs contain several consensus sites for PKA phosphorylation and at least two for PKC phosphorylation. In both cases, phosphorylation leads to a decrease in Na^+ conductance (for review, see Catterall, 1992). However, the reduction in current produced by PKA phosphorylation is not accompanied by a depolarizing shift and a reduced slope of the voltage dependence of activation, as observed in our neurons. There are reports that PKC phosphorylation of rat brain IIA Na^+ channel α-subunits expressed in *Xenopus* oocytes reduced peak Na^+ current and caused a depolarizing shift in the voltage dependence of activation similar to that reported here (Dascal and Lotan, 1991). However, this effect was not seen by Catterall and colleagues, who reported reductions in peak current accompanied by a slowing of inactivation in both cultured rat brain neurons and Chinese hamster ovary cells expressing the IIA α-subunit (Numann et al., 1991). To determine how PKC phosphorylation of VSSCs alters conductance in rat NAc neurons, we tested the effects of the PKC activator phorbol 12-myristate 13-acetate (PMA). Bath application of PMA (1 μM) produced variable reductions in Na^+ current (18.7 ± 2.0%), with no alterations in the voltage dependence of activation or inactivation (Fig. 7). In approximately half of the neurons tested, we observed a slight

Table 2. Repeated cocaine administration did not alter whole-cell Na^+ current in freshly dissociated cortical neurons

	Control (n = 9)	Cocaine (n = 8)
Na^+ current density (nA/pF)	0.35 ± 0.03	0.34 ± 0.03
Half-maximal activation (mV)	−34.8 ± 1.0	−36.9 ± 1.0
Activation slope (mV)	5.2 ± 0.6	4.9 ± 0.7
Half-maximal inactivation (mV)	−61.8 ± 1.7	−62.8 ± 2.4
Inactivation slope (mV)	6.5 ± 0.5	6.4 ± 0.6

slowing of inactivation (Fig. 7). These results suggest that the depolarizing shift in the voltage dependence of activation is unlikely to have resulted from enhanced PKC phosphorylation.

D1 receptor suppression of Na^+ currents in NAc neurons is not altered by cocaine pretreatment

Our previous *in vivo* work has suggested that D1 receptor-mediated suppression of NAc neuronal activity was enhanced in cocaine-pretreated rats (see introductory remarks). Therefore, we next compared the ability of SKF 38393 (0.1–10 μM) to suppress Na^+ conductance in NAc neurons dissociated from cocaine- and saline-pretreated rats. To our surprise, the suppression of Na^+ current by SKF 38393 was nearly identical in the two sets of neurons (Fig. 8), indicating that there was no D1 receptor supersensitivity in the cocaine-pretreated NAc neurons using this measure. This finding suggests that PKA phosphorylation of VSSCs is not maximal after repeated cocaine administration, given that D1 receptor stimulation can further suppress Na^+ conductance. Despite the similar suppression of Na^+ conductance produced by D1 receptor stimulation in the two groups of neurons, the *in vivo* effect in cocaine-pretreated rats would be a further reduction in Na^+ conductance caused by the decreased basal state of conductance produced by cocaine pretreatment.

DISCUSSION

Repeated administration of cocaine causes many adaptations within neural systems, from alterations in transporters, receptors, and transduction molecules to changes in gene expression (for review, see Hammer, 1995). The present results demonstrate a new adaptation: reductions in the basal functioning of VSSCs in NAc neurons. Our previous *in vivo* recordings demonstrated that NAc neurons were less responsive to the excitatory effects of locally applied glutamate (White et al., 1995b), but the present results indicate a more global reduction in excitability because VSSCs are responsible for action potential

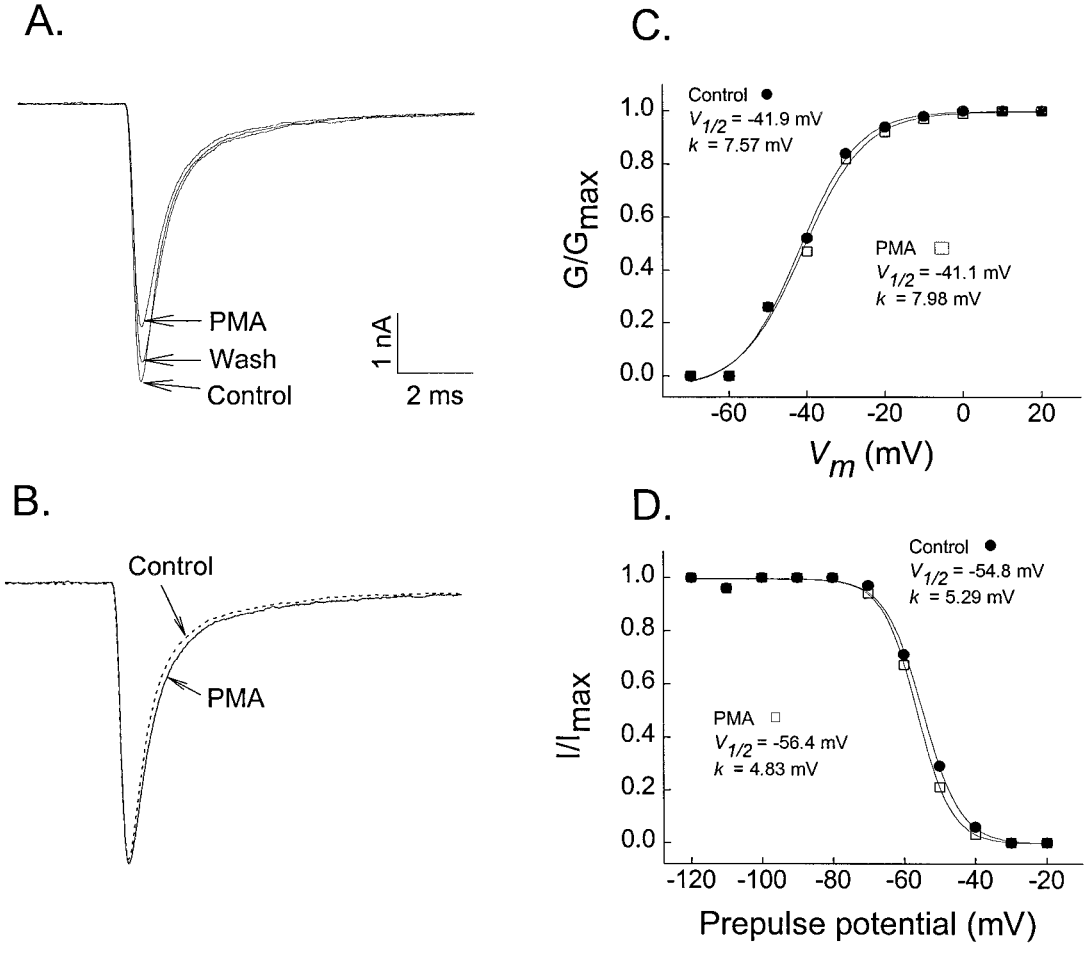

Figure 7. Phosphorylation of sodium channels by PKC decreased whole-cell Na^+ current and slightly delayed inactivation. *A*, Administration of the PKC activator PMA (1 μM) decreased peak Na^+ current. *B*, The traces in *A* have been rescaled such that current amplitude is normalized in the two conditions. This allows better detection of the slower inactivation in the presence of PMA. However, this effect was observed in only four of nine neurons. *C*, The voltage dependence of activation was not altered by PMA treatment (n = 3). *D*, The voltage dependence of inactivation was not altered by PMA treatment (n = 3). See Figure 5 legend for details of activation and inactivation curves.

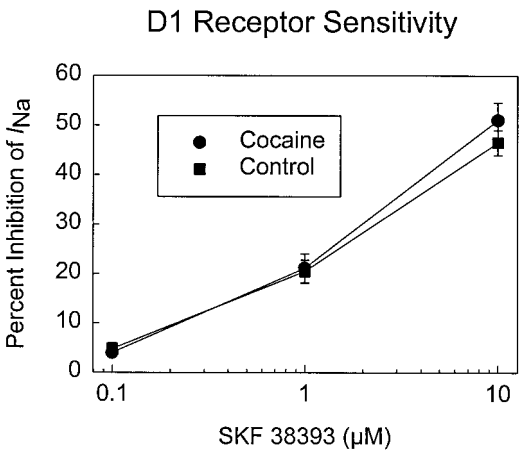

Figure 8. D1 agonist-induced suppression of whole-cell Na^+ current in NAc neurons is not altered by repeated cocaine administration. The ability of the DA D1 receptor-selective agonist SKF 38393 (0.1–10 μM) to suppress whole-cell Na^+ current was not significantly different (ANOVA) in NAc neurons recorded from cocaine-treated rats and saline-treated control rats. Sample sizes for the 0.1, 1.0, and 10.0 μM concentrations of SKF 38393 are 6, 6, and 5 for both control and cocaine groups. In addition, another group of 30 cocaine pretreated neurons were tested with 1.0 μM SKF 38393 and exhibited average inhibition of 27.2 ± 1.7%, a value that is not significantly different from that obtained in 33 untreated neurons (24.5 ± 2.1%), as described in Figure 2 legend (*t* test).

generation. The effect of chronic cocaine cannot be attributed to direct effects on VSSCs because during *in vivo* administration our dose regimen produces brain concentrations of cocaine (<3 μM) (Pettit et al., 1990) that are considerably less than those (25–50 μM) needed to interact with VSSCs and to alter membrane potential (Reith et al., 1986; Zimanyi et al., 1989; Wheeler et al., 1993). In addition, repeated administration of the local anesthetic lidocaine, which blocks VSSCs but does not exert actions on monoamine transporters, failed to mimic the effects of cocaine both in our brain slice and in dissociated neuron preparations. Moreover, recordings from acutely dissociated cortical neurons failed to reveal changes in VSSCs such as those seen in the NAc, indicating that the effects do not reveal a general action on brain neurons but reflect instead the specific set of monoamine and other receptors and signaling pathways that are expressed by NAc neurons and impacted by the ability of cocaine to increase synaptic concentrations of dopamine and other neurotransmitters. We propose that by increasing synaptic levels of DA and other neurotransmitters within the NAc, cocaine causes compensatory alterations in signaling pathways that regulate excitability of NAc neurons and thereby renders the NAc considerably less responsive to excitatory commands.

Repeated cocaine administration alters membrane properties of NAc neurons

During cocaine withdrawal, NAc neurons exhibited a number of alterations in membrane properties, including hyperpolarized membrane potentials, increased action potential thresholds, increased rheobase, and decreased action potential amplitude, effects that were not caused by cocaine during the recordings because our experiments were conducted 3 d after the last cocaine injection. Although these changes are not related to direct effects of cocaine on VSSCs, they do indicate that repeated cocaine causes adaptations in voltage-dependent conductances that control neuronal excitability. The resting membrane potential of NAc neurons is primarily determined by an inward rectifying K^+ (K_{IR}) channel (Uchimura et al., 1989). Within medium spiny neurons of the NAc, K_{IR} is known to be modulated by DA receptors (Uchimura and North, 1990). Given that D1 receptors hyperpolarize NAc neurons by opening K_{IR} (Uchimura et al., 1989; Uchimura and North, 1990) and that the D1 receptor signaling pathway is upregulated by repeated cocaine administration (Self and Nestler, 1995), it seems likely that the more hyperpolarized resting membrane potentials resulted from enhanced K_{IR} conductance in the cocaine-pretreated NAc neurons. Possible changes in this and other K^+ channels are being pursued in additional experiments. The remainder of the present study focused on VSSC modulation because the set of additional alterations in Na^+ spike characteristics (rheobase, threshold, amplitude) is most suggestive of Na^+ current changes (Halter et al., 1995).

Reduced excitability of NAc neurons by repeated cocaine treatment results from decrements in whole-cell Na^+ currents

Our whole-cell recordings indicate that, as in the neighboring dorsal striatum (Surmeier et al., 1992; Cepeda et al., 1995; Schiffmann et al., 1995), Na^+ conductance in medium spiny NAc neurons is consistently reduced by DA D1 receptor stimulation through activation of the cAMP–PKA pathway and phosphorylation of VSSCs. In addition, our findings indicate that repeated cocaine treatment markedly reduced (37%) the basal peak Na^+ current while it shifted the voltage dependence of activation in the depolarizing direction. These changes would explain the increased rheobase, increased spike threshold, and reduced spike amplitude observed in our slice recordings. The reduction in whole-cell Na^+ conductance could be attributed to reduction in single-channel current, the number of active Na^+ channels, or the open probability of the channels during depolarization. Although single-channel recordings will be required to distinguish between these possibilities, there are several findings that lead us to believe that the responsible mechanism may be a reduction in open probability. Enhanced phosphorylation of VSSCs by cAMP-dependent protein kinase reduces whole-cell Na^+ current by decreasing channel open probability (Gershon et al., 1992; Li et al., 1992). The cAMP–PKA pathway in the NAc is clearly upregulated by repeated cocaine treatment, with increases in levels of both cAMP and PKA (Terwilliger et al., 1991). Because the activity of VSSCs is dynamically regulated by fluctuations in cAMP and PKA concentrations (Catterall, 1992), the basal state of VSSC phosphorylation may be enhanced in NAc neurons recorded from rats after repeated cocaine treatment. Prolonged substrate phosphorylation produced by repeated psychomotor stimulant administration has several precedents, including tyrosine hydroxylase and extracellular signal-related kinase (ERK) in the VTA (Beitner-Johnson and Nestler, 1991; Berhow et al., 1996) and cAMP response element-binding protein (CREB) in the NAc (Cole et al., 1995; Fitzgerald and Nestler, 1995). How does the upregulation of the cAMP–PKA pathway come about? A likely candidate is the relatively persistent decrease in NAc levels of G_i and G_o proteins produced by repeated cocaine administration (Nestler et al., 1990; Terwilliger et al., 1991; Striplin and Kalivas, 1993). Because these proteins couple many receptors to inhibition of adenylyl cyclase, the decreased functional activity in this opposing system would lead to increased cAMP and PKA concentrations.

The second alteration in Na^+ conductance that we observed in

cocaine-pretreated NAc neurons was a depolarizing shift and a reduced slope of the voltage dependence of activation. These effects are not associated with enhanced PKA phosphorylation of VSSCs (Catterall, 1992). They also could not be attributed to PKC phosphorylation, as suggested by experiments in which PKC was used to phosphorylate Na^+ channels expressed in *Xenopus* oocytes (Dascal and Lotan, 1991), because phorbol ester administration to our neurons reduced whole-cell Na^+ current without modifying the voltage dependence of activation or inactivation. So, what caused the depolarizing shift in the voltage dependence of activation in our cocaine-pretreated neurons? One possible mechanism would be a reduction in direct G-protein modulation of VSSCs (Ma et al., 1994), which may also result from the reduction in levels of these proteins caused by repeated cocaine treatment (Nestler et al., 1990; Terwilliger et al., 1991; Striplin and Kalivas, 1993).

D1 receptor modulation of Na^+ conductance is normal in cocaine-withdrawn NAc neurons

Although our earlier *in vivo* recordings have consistently indicated that repeated cocaine administration causes a persistent enhancement in the ability of D1 receptor stimulation to suppress the firing of NAc neurons (Henry et al., 1989; Henry and White, 1991,1995), the suppression of whole-cell Na^+ current by D1 receptor stimulation was normal in cocaine-pretreated neurons. The lack of an enhanced D1 receptor-mediated response is also consistent with many reports that D1 receptors are unaltered by repeated cocaine treatment using standard intermittent injection procedures (for review, see Self and Nestler, 1995). Thus, the apparent supersensitivity of D1 receptor-mediated inhibitory responses has been attributed to alterations in the cAMP transduction cascade (Self and Nestler, 1995; White et al., 1995a). Our new findings implicate the decreased excitability of NAc neurons in the apparent D1 receptor supersensitivity.

In the anesthetized preparation used in our earlier *in vivo* recordings, most NAc neurons were quiescent and thus were induced to fire by iontophoretic application of glutamate. A reduction in excitability by downregulation of Na^+ conductance would explain the reduced ability of glutamate to drive the NAc neurons in cocaine-pretreated rats (White et al., 1995b), an effect that cannot be explained by alterations in NAc glutamate receptor subunit expression during the early days of withdrawal from repeated cocaine administration (Fitzgerald et al., 1996). The reduction in Na^+ conductance would make D1 receptor stimulation appear more effective at producing inhibition because the effect has to be measured against glutamate-induced activity. Because the efficacy of glutamate is reduced, there exists a bias toward inhibitory efficacy, which would explain why NAc neurons also appear more sensitive to other inhibitory agents such as GABA and serotonin (Henry and White, 1991; White et al., 1992). Time course studies indicate that the enhanced inhibitory efficacies of GABA and serotonin, unlike that of D1 receptor agonists, are quite transient (White et al., 1995a). If these transient alterations are a reflection of reduced Na^+ conductance, then it may be that the latter effect is also transient and that the apparent *in vivo* D1 receptor supersensitivity observed at later withdrawal times (weeks) reflects alterations that are more specific to that receptor system.

Functional implications of whole-cell plasticity for cocaine withdrawal

Our studies have demonstrated a novel form of whole-cell (nonsynaptic) plasticity produced by repeated cocaine administration: reduced Na^+ currents in NAc neurons. Although it is certain that cocaine-induced alterations in transmission at specific synapses will modify the responsiveness of NAc neurons to selected inputs (synaptic plasticity), as demonstrated previously in the VTA (Bonci and Williams, 1996) and dorsal lateral septal nucleus (Shoji et al., 1997), the reduction of VSSC function will produce an indiscriminate decrease in the responsiveness of the NAc to excitatory commands because these channels govern the initiation of action potentials. In addition, reduced Na^+ conductance could also impact associative synaptic plasticity because VSSCs expressed within neuronal soma and dendrites carry backpropagating action potentials that modulate dendritic Ca^{2+} influx (Johnston et al., 1996; Stuart et al., 1997).

The NAc is a structure in which functionally distinct ensembles of neurons are recruited by convergent excitatory inputs to coordinate patterns of movement and affect (for review, see Pennartz et al., 1994). Given that major excitatory inputs to NAc originate within structures known to be involved in motivated behavior and the learned associations between such behavior and both specific environmental settings and emotional states (prefrontal cortex, hippocampus, and basolateral amygdala), a reduction in the excitability of NAc neurons would decrease processing of such information and could thereby lead to cocaine withdrawal effects such as anergia, anhedonia, and depression. In rat models, such deficits are observed during the early days of withdrawal from repeated cocaine administration (Markou and Koob, 1991; Koeltzow et al., 1996), when Na^+ currents are depressed. Accordingly, the further reduction in Na^+ currents after D1 receptor agonist administration suggests that the proposed use of such drugs as a replacement therapy for cocaine-dependent individuals (Self et al., 1996) may be contraindicated during the early period of cocaine withdrawal.

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