Repeated administration of cocaine leads to numerous behavioral alterations in both humans and laboratory animals. In humans, cocaine dependence is accompanied by withdrawal symptoms, including anhedonia, anergia, anxiety, depression, and cocaine craving (Gawin, 1991). Many of these symptoms have been successfully modeled in animals (Carroll and Lac, 1987; Kokkinidis and McCarter, 1990; Markou and Koob, 1991; Harris and Aston-Jones, 1993; Mutschler and Pierce and Kalivas, 1997; Kalivas et al., 1998; White and Kalivas, 1998). We have focused our attention on alterations in dopamine D1 receptors within the nucleus accumbens (NAc) and in withdrawal symptoms after cessation of repeated cocaine administration. These receptors couple to a variety of ion channels to modulate neuronal excitability. Using whole-cell recordings from dissociated adult rat NAc medium spiny neurons (MSNs), we show that, as in dorsal striatal MSNs, D1 receptor stimulation suppresses N- and P/Q-type Ca2+ currents (I_{Ca}) by activating a cAMP/protein kinase A/protein phosphatase (PP) signaling system, presumably leading to channel dephosphorylation. We also report that during withdrawal from repeated cocaine administration, basal I_{Ca} density is decreased by 30%. Pharmacological isolation of specific I_{Ca} components indicates that N- and R-type, but not P/Q- or L-type, currents are significantly reduced by repeated cocaine treatment. Inhibiting PP activity with okadaic acid enhances I_{Ca} in cocaine withdrawn, but not control, NAc neurons, suggesting an increase in constitutive PP activity. This suggestion was supported by a significant decrease in the ability of D1 receptor stimulation and direct activation of cAMP signaling to suppress I_{Ca} in cocaine-withdrawn NAc neurons. Chronic cocaine-induced reduction of I_{Ca} in NAc MSNs will globally impact Caz+-dependent processes, including synaptic plasticity, transmitter release, and intracellular signaling cascades that regulate membrane excitability. Along with our previously reported reduction in whole-cell Na+ currents during cocaine withdrawal, these findings further emphasize the important role of whole-cell plasticity in reducing information processing during cocaine withdrawal.

**ABSTRACT**

Dopamine D1 receptors within the nucleus accumbens (NAc) are intricately involved in the rewarding effects of cocaine and in withdrawal symptoms after cessation of repeated cocaine administration. These receptors couple to a variety of ion channels to modulate neuronal excitability. Using whole-cell recordings from dissociated adult rat NAc medium spiny neurons (MSNs), we show that, as in dorsal striatal MSNs, D1 receptor stimulation suppresses N- and P/Q-type Ca2+ currents (I_{Ca}) by activating a cAMP/protein kinase A/protein phosphatase (PP) signaling system, presumably leading to channel dephosphorylation. We also report that during withdrawal from repeated cocaine administration, basal I_{Ca} density is decreased by 30%. Pharmacological isolation of specific I_{Ca} components indicates that N- and R-type, but not P/Q- or L-type, currents are significantly reduced by repeated cocaine treatment. Inhibiting PP activity with okadaic acid enhances I_{Ca} in cocaine withdrawn, but not control, NAc neurons, suggesting an increase in constitutive PP activity. This suggestion was supported by a significant decrease in the ability of D1 receptor stimulation and direct activation of cAMP signaling to suppress I_{Ca} in cocaine-withdrawn NAc neurons. Chronic cocaine-induced reduction of I_{Ca} in NAc MSNs will globally impact Caz+-dependent processes, including synaptic plasticity, transmitter release, and intracellular signaling cascades that regulate membrane excitability. Along with our previously reported reduction in whole-cell Na+ currents during cocaine withdrawal, these findings further emphasize the important role of whole-cell plasticity in reducing information processing during cocaine withdrawal.

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system (for review, see Nestler, 1997), resulting in an enhanced basal phosphorylation state of voltage-sensitive Na⁺ channels, an effect known to reduce Na⁺ currents (for review, see Catterall, 2000).

Herein, we have focused on voltage-sensitive calcium current (I_{Ca}) as a potential target of cocaine-induced neuroadaptations in NAc MSNs. Previous work within the dorsal striatum has demonstrated that DA D1 receptors suppress N- and P/Q-type Ca²⁺ channel conductances, through a cAMP/PKA/protein phosphatase-1 signaling system (Surmeier et al., 1995). Accordingly, we hypothesized that repeated DA D1 receptor stimulation produced by cocaine-induced inhibition of DA reuptake and enhancement of extracellular DA levels would also lead to alterations in the basal state of Ca²⁺ channel phosphorylation and I_{Ca}. We now report that during withdrawal from repeated cocaine administration, NAc MSNs exhibit a marked reduction in I_{Ca} through N- and R-type Ca²⁺ channels.

Materials and Methods

Animals and Treatments. Adult male Sprague-Dawley rats initially weighing 150 to 175 g were housed in groups of two to four in a temperature- and humidity-controlled vivarium under a 12-h light/dark cycle. Food and water were freely available. After 1 week of acclimation, rats were designated for use in acute experiments regarding I_{Ca} modulation or were randomly assigned to groups that received once daily i.p. injections of saline (1.0 ml/kg) or (–)-cocaine HCl (15.0 mg/kg as the salt) for five consecutive days. Experiments using the treated rats were conducted on the 3rd day after cessation of injections (i.e., day 8).

Acute Dissociation Procedure. On the day of the experiment, rats were anesthetized with halothane and decapitated. Brains were quickly removed and dissected into blocks containing the NAc. Slices of 400 µm were cut with a motorized vibrating microtome while bathed in a low-Ca²⁺ (100 µM), HEPES-buffered salt solution containing 140 mM sodium isethionate, 2 mM KCl, 4 mM MgCl₂, 0.1 mM CaCl₂, 23 mM glucose, and 15 mM HEPES, pH 7.4 (300–350 mOsM/l). Slices were then incubated for 1 to 6 h at room temperature in a NaHCO₃-buffered saline (Eagle’s balanced salts solution) bubbled with 95% O₂, 5% CO₂. Slices were then removed into the low-Ca²⁺ buffer, and with the aid of a dissecting microscope, the NAc, including both core and shell, was micropunched and placed in a NaHCO₃-buffered saline (Eagle’s balanced salts solution) bubbled with 95% O₂, 5% CO₂. Slices were then transferred into the low-Ca²⁺ buffer and, with the aid of a dissecting microscope, the NAc, including both core and shell, was micropunched and placed in an oxygenated Cell-Stir chamber (Wheaton, Millville, NJ) containing pronase (1–1.5 mg/ml) in HEPES-buffered Hanks’ balanced salt solution at 35°C. After 20 to 30 min of enzyme digestion, tissue was rinsed three times in the low-Ca²⁺ solution, HEPES-buffered saline and mechanically dissociated with a gridded series of fire-polished Pasteur pipettes. The cell suspension was then plated into a Petri dish on a temperature- and humidity-controlled stage. Recordings were obtained with an Axon Instruments (Union City, CA) 200A patch-clamp amplifier and controlled and monitored with a PC 486 running pCLAMP6 (version 6.01) with a 2-kHz filter and a DigiData 1200 interface (333 kHz; Axon Instruments). After seal rupture, series resistance (<10 MΩ) was compensated (70–80%) and periodically monitored. All currents were leak subtracted using the P/6 procedure in pCLAMP6. Recordings were made only from medium-sized neurons (<14 µm in diameter) that had only a few short proximal dendrites. Drugs were applied with a DAD-12 superfusion drug application system controlled by a 386 computer (ALA Scientific Instruments, Westbury, NY). All experiments were performed at room temperature (20–22°C). SKF 38393, SCH 23390, nifedipine, and okadaic acid were purchased from Sigma/RBI (Natick, MA); ω-conotoxin GVIA (CgTx) and PKI[4-25] were purchased from Peninsula Laboratories (Belmont, CA); ω-agatoxin TK (AgTx) was purchased from Peptides International (Louisville, KY); and 8-bromo-cAMP (8-Br-cAMP), Hanks’ balanced salt solution, Earle’s balanced saline solution, and tetrodotoxin were purchased from Sigma-Aldrich (St. Louis, MO).

Statistics. Differences between drug effects during withdrawal from all treatments were evaluated with Student’s paired t tests. Differences between cocaine- and saline-pretreated NAc neurons were evaluated with Student’s nonpaired t tests.

Results

Whole-Cell I_{Ca} in NAc MSNs Is Comprised Primarily of High Voltage-Activated (HVA) Calcium Channels. Neurons used for recording were chosen according to their diameter and/or whole-cell capacitance. Only medium-sized neurons (7–14 µm in diameter) with short remaining processes were used. These neurons exhibited whole-cell capacitance of 3 to 9 pF as previously reported for MSNs in both dorsal striatum and NAc (Surmeier et al., 1995; Churchill and MacVicar, 1998; Zhang et al., 1998).

Whole-cell I_{Ca} recordings (using Ba²⁺ as the charge carrier) were elicited by depolarizing pulses from a holding potential of −80 mV. The current-voltage relationship observed in a typical NAc neuron is shown in Fig. 1. Current was activated at about −40 mV, peaked at 0 mV, and reversed at +55 mV. Similar to results obtained from acutely dissociated MSNs of the dorsal striatum (Bargas et al., 1994), but in contrast to a previous report on the NAc (Churchill and MacVicar, 1998), we seldom observed evidence of low-voltage-activated (LVA, T-type) currents (those activated at relatively hyperpolarized potentials) in MSNs of the NAc (<10% of neurons, n > 100).

Because HVA Ca²⁺ currents (those activated at relatively depolarized membrane potentials) are comprised of a heterogeneous group of channel types (for review, see Dunlap et al., 1995), we first sought to characterize the Ca²⁺ channel subtypes in NAc neurons. Bath application of the N-type Ca²⁺ channel blocker ω-CgTx GVIA (2 µM) reduced I_{Ca} by 25.3 ± 1.4% (n = 14), the P/Q channel blocker ω-AgTX TK (100–200 nM) reduced I_{Ca} by 22.8 ± 2.5% (n = 15), and the L-type Ca²⁺ channel antagonist nifedipine (5 µM) reduced current by 31.0 ± 2.2% (n = 13). The nonselective channel blocker CdCl₂ (0.4 mM) completely suppressed I_{Ca} (Fig. 2), suggesting that approximately 20% of the I_{Ca} is carried through R-type channels, so named because of their resistance to antagonists and toxins (Randall and Tsien, 1997).

DA D1 Receptor Stimulation Reduces N-Type and P/Q-Type I_{Ca} in NAc MSNs through a cAMP/PKA Enhancement of Protein Phosphatase Activity. During a thorough characterization of the effects of DA D1 receptor...
stimulation on $I_{Ca}$ in MSNs of the dorsal striatum, Surmeier et al. (1995) proposed that D1 receptor stimulation suppresses N- and P/Q-type currents by enhancing a protein phosphatase-1-mediated dephosphorylation of the channels through the cAMP/PKA signaling system. To determine whether similar mechanisms exist for MSNs of the NAc, we next conducted a series of pharmacological studies. We observed that bath application of the DA D1 receptor-selective agonist SKF 38393 at a subsaturating concentration (1 μM) reversibly reduced $I_{Ca}$ (Fig. 3), with a slow onset and recovery time course; the extent of suppression averaged 21.2 ± 1.6% ($n = 21$). At lower concentrations (0.1 μM), the modulation was weak and variable (8.9 ± 3.2%, $n = 6$; Fig. 3). Higher concentrations (100 μM) produced greater inhibition, but the effect was difficult to washout (29.3 ± 2.2%, $n = 5$; Fig. 3). Accordingly, all subsequent studies used the 1 μM concentration of SKF 38393. The DA D1 receptor-selective antagonist SCH 23390 significantly attenuated the effect of 1 μM SKF 38393 ($n = 6$; Fig. 3). The modulation produced by D1 receptor activation could be mimicked by 8-Br-cAMP (50 μM), a membrane-permeable cAMP analog, indicating in-
volvement of the adenylyl cyclase-cAMP system coupled to D1 receptors (Fig. 3). The mean reduction was 23.7 ± 1.6% \((n = 7)\). Furthermore, the suppression of \(I_{\text{Ca}}\) by 8-Br-cAMP was prevented by intracellular dialysis with the protein kinase inhibitor PKI [4-25] \((10 \mu M, n = 5; \text{Fig. 4A})\); similar results were obtained using SKF 38393 rather than 8-Br-cAMP \((n = 4, 2.1 \pm 0.3\% ; \text{Fig. 4A})\). Finally, bath application of okadaic acid \((1 \mu M, n = 5)\), a nonselective inhibitor of protein phosphatases (PPs) type 1 and type 2A, prevented the inhibitory effect of SKF 38393 \((\text{Fig. 4B})\). To identify the \(Ca^{2+}\) channel subtypes subject to D1 receptor modulation, we again used the selective N-type and P/Q-type channel toxins \(\omega\)-CgTx \((2 \mu M\) and \(\omega\)-AgTx \((100–200 \text{nM})\) to block these channels and reexamined the effect of SKF 38393 on \(I_{\text{Ca}}\). During application of the two toxins, \(1 \mu M\) SKF 38393 no longer suppressed \(I_{\text{Ca}}\); in fact, a slight, but not statistically significant, enhancement was usually observed \((\text{mean enhancement, 5.2 ± 2.1, n = 5; Fig. 5})\), indicating that D1 receptor activation specifically acted on N- and P/Q-type channels to suppress current. Taken together, these findings suggest that, as in dorsal striatal MSNs, DA D1 receptor stimulation reduces N- and P-type \(Ca^{2+}\) channels in NAc neurons by producing a cAMP/PKA enhancement of PP activity, leading to dephosphorylation of \(Ca^{2+}\) channels.

Repeated Cocaine Treatment Decreases \(I_{\text{Ca}}\) Primarily through N- and R-Type Channels. We measured the \(I_{\text{Ca}}\) density to compare the basal state of \(I_{\text{Ca}}\) in animals treated repeatedly with cocaine or saline. Current density was estimated by using total cell capacitance as an index of membrane area \((\text{pA/pF})\). Repeated cocaine treatment produced a significant 30% reduction in basal \(I_{\text{Ca}}\) density \((\text{Fig. 6A})\), which was solely attributable to peak currents because there was no significant difference between the two groups of cells with respect to cell capacitance. There were no differences in the voltage dependence of activation or inactivation when cocaine-pretreated neurons were compared with control neurons \((\text{data not shown})\). The reduction in basal \(I_{\text{Ca}}\) was region specific because we failed to observe a similar effect when we recorded from motor cortex neurons after repeated cocaine treatment \((\text{Fig. 6B})\).

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### Figures

**Fig. 4.** Dopamine D1 receptor modulation of whole-cell \(I_{\text{Ca}}\) in NAc neurons involves PKA and a PP. A, when the PKA inhibitor PKI [4-25] \((10 \mu M, n = 5)\) was included in the recording solution to allow it to dialyze into the neuron, suppression of current by 8-Br-cAMP \((\text{Br})\) was significantly prevented \((t_{10} = 9.9, p < 0.0001)\). The left two columns in the graph inset show the results of 8-Br-cAMP alone and in the presence of PKI (means ± S.E.M.). Similar results were obtained with SKF 38393 \((\text{SKF})\), as shown in the right two columns of inset graph \((\text{statistics were not performed due to the small sample size in the PKI group; n = 4})\). B, bath application of the PPI/PP2A inhibitor okadaic acid \((\text{OA})\) \((1 \mu M)\) also significantly \((t_{4} = 10.7, p = 0.001, \text{paired test})\) prevented the suppression of current by SKF 38393 \((\text{1 \mu M})\), as shown in the inset graph.

**Fig. 5.** DA D1 receptor stimulation suppresses N- and P/Q-type \(I_{\text{Ca}}\) components. The ability of SKF 38393 \((\text{SKF})\) \((1 \mu M)\) to suppress \(I_{\text{Ca}}\) is abolished after administration of the N-type antagonist CgTx and the P/Q-type antagonist AgTx. Toxin concentrations are as in Fig. 2. Note the slight increase in \(I_{\text{Ca}}\) that occurred during D1 receptor stimulation when N- and P/Q-type channels were blocked. Inset, traces shown were taken at the times indicated by the corresponding numbers.
both N- and R-type currents were significantly reduced by repeated cocaine treatment (Fig. 6B).

Application of the PP1/PP2A inhibitor okadaic acid (1 μM) enhanced peak \( I_{\text{Ca}} \) in neurons obtained from cocaine-pretreated rats, in striking contrast to its lack of effect in saline-pretreated neurons (Fig. 6C). This finding is consistent with the possibility that the decrease in \( I_{\text{Ca}} \) caused by cocaine pretreatment is due to an increase in constitutive phosphatase activity.

**D1 Receptor Modulation of \( I_{\text{Ca}} \) Is Decreased in Cocaine-Pretreated NAc Neurons.** Previous in vivo electrophysiological evidence suggested that repeated cocaine administration increases the functional sensitivity of DA D1 receptors in the NAc (Henry and White, 1991, 1995). However, D1 receptor modulation of whole-cell \( \text{Na}^+ \) current was not altered after repeated cocaine administration (Zhang et al., 1998). To examine whether DA D1 receptor modulation of \( I_{\text{Ca}} \) was altered by repeated cocaine administration, we compared the ability of SKF 38393 to suppress peak \( I_{\text{Ca}} \) in NAc neurons dissociated from cocaine- and saline-pretreated rats. We found that the response to 1 μM SKF 38393 was significantly reduced compared with the saline-pretreated group (Fig. 6D). To determine whether this was due to a change in D1 receptors or to signaling events downstream of the receptors, we also evaluated the suppression of current produced by 8-Br-cAMP. As with the D1 agonist, the response to 8-Br-cAMP was significantly reduced in the cocaine-pretreated neurons compared with saline-pretreated neurons (Fig. 6D).

**Discussion**

Our findings indicate that during withdrawal from repeated cocaine administration, there is a marked reduction in \( I_{\text{Ca}} \) carried through N- and R-type channels in MSNs of the NAc. As with the reductions in whole-cell \( \text{Na}^+ \) currents that we reported previously (Zhang et al., 1998), the reductions in \( I_{\text{Ca}} \) were region-specific in that we did not observe similar changes in neurons dissociated from motor cortex, an area that lacks appreciable dopaminergic innervation. Our findings suggest that down-regulation of \( I_{\text{Ca}} \) results from an alteration in the basal state of phosphorylation of N-type channels as well as additional alterations in R-type channels.

**HVA Ca\(^{2+}\) Channels Are Primarily Responsible for \( I_{\text{Ca}} \) in Most Acutely Isolated NAc MSNs.** We observed that over 90% of adult NAc MSNs exhibit only HVA \( I_{\text{Ca}} \), with only sporadic neurons exhibiting activation at relatively hyperpolarized potentials. In addition, micromolar concentrations of \( \text{Cd}^{2+} \), which only weakly (ED\(_{50} > 50 \) μM) block LVA (or T-type) \( \text{Ca}^{2+} \) channels (for review, see Randall, 1998), completely eliminated \( I_{\text{Ca}} \) in every neuron tested. Our findings are in close agreement with those of Surmeier and colleagues who demonstrated that neonatal MSNs express LVA \( I_{\text{Ca}} \) when cultured from embryonic rat brain or dissociated during early postnatal development (P2–P7), but not when dissociated from later postnatal animals, leading these authors to suggest a developmental loss of LVA \( \text{Ca}^{2+} \) currents (Bargus et al., 1991, 1994). In contrast to our findings, another recent report (Churchill and MacVicar, 1998) indicated that 55% of NAc neurons acutely isolated from rats between P24 and P32 exhibited LVA currents. These authors also reported that 77% of cultured and only 42% of acutely isolated P5 to P11 neurons exhibited prominent LVA currents. These findings suggest that more extensive dendritic arbors in the cultured neurons may contribute to the increased probability of detecting LVA currents. Our findings from neurons isolated from rats between P47 and P52 contained very short (<40 μm) dendritic processes, which may have reduced the likelihood of observing LVA currents. Recent autoradiographic analyses indicate abundant expression of the LVA T-type α\(_{1G}\), α\(_{1H}\), and α\(_{1I}\) subunits within the juvenile and adult striatum (McRory et al., 2001) and intracellular recordings of NAc MSNs in brain slices indicate the presence of a presumed LVA current (O'Donnell and Grace, 1993). However, it is possible that the current observed in brain slices is due to L-type channels with a more negative activation threshold (Avery and Johnston, 1996). As for HVA currents, we used selective blockers of various \( \text{Ca}^{2+} \) channels to demonstrate that the whole-cell current was comprised of N-type (~25%), P/Q-type (~25%), and L-type (~30%), with a toxin/antagonist resistant (or R-type) component of about 20%. These values are similar to those reported by Churchill and MacVicar (1998) who further established that Q-type channels might primarily carry the P/Q current.

**DA D1 Receptors Suppress N- and P/Q-Type Currents in NAc MSNs by a cAMP/PKA/PP Signaling Cascade.** Surmeier et al. (1995) proposed a model for DA D1 receptor suppression of N- and P/Q-type \( I_{\text{Ca}} \) in striatal MSNs in which N- and P/Q-type \( \text{Ca}^{2+} \) channels are fully phosphor-
yalated at rest, presumably by cGMP-dependent protein kinase. Because cGMP-dependent protein kinase and PKA phosphorylate similar substrates, an enhancement of PKA activity exerts no direct effects on N- and P/Q-type channels. Instead, PKA activation phosphorylates targeting proteins for PP1, releasing the catalytic subunit of PP1 into the cytosol to dephosphorylate N- and P/Q-type Ca\(^{2+}\) channels. Our results are consistent with this model. We found that DA D1 receptor signaling suppressed \(I_{\text{Ca}}\) in NAc MSNs. Stimulation of DA D1 receptors with the selective agonist SKF 38393 decreased \(I_{\text{Ca}}\) by approximately 25%, an effect that was prevented by the DA D1 receptor antagonist SCH 23390 and mimicked by the membrane-permeable cAMP analog 8-Br-cAMP. When N- and P/Q-type currents were blocked with \(\omega\)-CgTx and \(\omega\)-AgTx, respectively, SKF 38393 no longer suppressed \(I_{\text{Ca}}\). In fact, some neurons exhibited a slight enhancement of \(I_{\text{Ca}}\), a finding that is consistent with reports that DA D1 receptors can facilitate L-type \(I_{\text{Ca}}\) in striatal MSNs because L-type channels are not subject to the PKA-activated PP1 dephosphorylation (Surmeier et al., 1995; Hernández-López et al., 1997).

To test further the involvement of the cAMP/PKA cascade, we showed that 8-Br-cAMP-induced and SKF 38393-induced suppression of \(I_{\text{Ca}}\) was abolished by inclusion of the PKA inhibitor PKI [4–35] in the recording pipette. Finally, we found that the nonselective PP1/PP2A inhibitor okadaic acid prevented the suppression of currents normally observed during activation of the cAMP/PKA signaling pathway. Given that the selective peptide inhibitor of PP1 (phospho-DARPP-32) also eliminated D1 agonist-induced suppression of \(I_{\text{Ca}}\) in striatal MSNs (Surmeier et al., 1995), PP1 is probably the dominant form of PP involved in the modulation. Therefore, DA D1 receptors seem to modulate N- and P/Q-type Ca\(^{2+}\) currents in NAc MSNs via the same cAMP/PKA/PP1 pathway proposed for MSNs within the dorsal striatum.

**Repeated Cocaine Administration Reduces Basal \(I_{\text{Ca}}\) Primarily through N- and R-Type Channels.** On the 3rd day of withdrawal from five daily cocaine injections, basal \(I_{\text{Ca}}\) in NAc MSNs was reduced by 30%. Use of selective antagonists for the various HVA channels indicated that both N- and R-type \(I_{\text{Ca}}\) components were significantly reduced. Experiments with okadaic acid support the likelihood that the reduction in basal \(I_{\text{Ca}}\) is due to enhanced signaling through the cAMP/PKA/PP1 cascade proposed above because this PP inhibitor increased \(I_{\text{Ca}}\) only in NAc neurons dissociated from cocaine-pretreated rats. This finding suggests greater constitutive PP1 activity in the cocaine-pretreated neurons. But because the okadaic acid effect was only about one-half (17%) of what would be expected if greater PP1 activity was fully responsible for the reduction in basal whole-cell \(I_{\text{Ca}}\) (30%), it is possible that repeated cocaine reduced basal \(I_{\text{Ca}}\) density by additional mechanisms, particularly with respect to decreased R-type current. One possibility would be an alteration in the expression of Ca\(^{2+}\) channel subunits as has been reported after chronic DA receptor stimulation in other systems (Fass et al., 1999). However, we can only speculate given that nothing is known regarding neuromodulation of R-type Ca\(^{2+}\) channels in MSNs (Foehring et al., 2000).

The failure of repeated cocaine administration to alter P/Q-type channels is surprising given that DA D1 receptor modulation of these channels seems to use the same signaling system as N-type channels. If the down-regulation of N-type currents during cocaine withdrawal occurs because of enhanced signaling through the cAMP/PKA/PP1 pathway, shouldn't we expect a similar down-regulation of the P/Q-type current? It is intriguing to consider the possibility that compensatory mechanisms may protect these channels from similar neuroadaptations produced by cocaine withdrawal. Such mechanisms might involve subcellular variations in kinase/phosphatase-anchoring proteins, alterations in gene expression, or in modulation of Ca\(^{2+}\) channels by G protein \(\beta\gamma\)-subunits.

We also observed a reduction in the ability of DA D1 receptor stimulation to suppress \(I_{\text{Ca}}\) in neurons obtained from cocaine-pretreated rats. Although there are reports of reduced D1 receptors after self-administration of high daily doses of cocaine (Laurier et al., 1994; De Montis et al., 1998), this has not been typically observed with the type of lower dose regimen used herein (for review, see White et al., 1997). Indeed, the similar reduction in the ability of 8-Br-cAMP to suppress \(I_{\text{Ca}}\) indicates that the mechanism responsible must be downstream from D1 receptors. The ability of okadaic acid to increase \(I_{\text{Ca}}\) only in cocaine-pretreated neurons suggests that an increase in constitutive phosphatase activity may reduce the efficacy of D1 receptor stimulation.

**Functional Implications.** We previously reported a whole-cell depression of Na\(^{+}\) currents in NAc MSNs during withdrawal from repeated cocaine treatment. We proposed that this neuroadaptation would render the NAc much less responsive to excitatory inputs from glutamatergic neurons in the prefrontal cortex, ventral subiculum, and basolateral amygdala. Given that convergent excitatory drive from these structures is required for NAc neurons to fluctuate between their hyperpolarized “down” state to a more depolarized “up” state necessary for spike activity (O’Donnell and Grace, 1995; Groenewegen et al., 1999; O’Donnell et al., 1999), the reduced excitability caused by down-regulated Na\(^{+}\) current would reduce the ability of NAc neurons to relay essential cognitive and motivational commands from the limbic system to the motor system, perhaps accounting for cocaine withdrawal symptoms of anhedonia, anergia, and depression.

Our present findings indicate that even if the NAc received sufficient excitatory drive to overcome the state of whole-cell depression, relay of information through this structure might still be compromised. Entry of Ca\(^{2+}\) through N-type and R-type channels is pivotal for depolarization-induced Ca\(^{2+}\) entry into nerve terminals, an event that is necessary for exocytosis (Dunlap et al., 1995; Wu et al., 1998). Assuming that the reduction in \(I_{\text{Ca}}\) observed with our somatic recordings would also be expressed at nerve terminals where even small decreases in Ca\(^{2+}\) flux can have profound effects on transmitter release (Heidelberg et al., 1994; Mintz et al., 1995), a 30% reduction in basal \(I_{\text{Ca}}\) during cocaine withdrawal could dramatically reduce release of \(\gamma\)-aminobutyric acid and colocalized neuropeptides from MSNs, furthering the loss of information processing between the NAc and its targets. In addition, both somatic and dendritic Na\(^{+}\) and Ca\(^{2+}\) currents play important roles in synaptic plasticity (Häußer et al., 2000). Accordingly, this global depression in neuronal excitability is likely to disrupt the ability of the NAc to integrate motivational and reward-associated learning processes in ways that may contribute to the pathology of drug addiction.
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Address correspondence to: Dr. Francis J. White, Department of Cellular and Molecular Pharmacology, Finch University of Health Sciences, The Chicago Medical School, 3333 Green Bay Rd., North Chicago, IL 60048. E-mail: francis.white@finchcms.edu