Repeated Cocaine Exposure Decreases Dopamine D2-Like Receptor Modulation of Ca\textsuperscript{2+} Homeostasis in Rat Nucleus Accumbens Neurons

MARIELA F. PEREZ, KERSTIN A. FORD, IVAN GOUSSAKOV, GRACE E. STUTZMANN, AND XIU-TI HU

1IFEC, CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende s/n, Ciudad Universitaria, 5000 Córdoba, Argentina
2Department of Neuroscience, Rosalind Franklin University of Medicine and Science/The Chicago Medical School, North Chicago, Illinois 60064-3095
3Department of Pharmacology, Center for Compulsive Behaviors and Addiction, Rush University Medical Center, Chicago, Illinois 60612

KEY WORDS withdrawal; L-type Ca\textsuperscript{2+} channel; PKA; calcineurin; patch clamp

ABSTRACT The nucleus accumbens (NAc) is a limbic structure in the forebrain that plays a critical role in cognitive function and addiction. Dopamine modulates activity of medium spiny neurons (MSNs) in the NAc. Both dopamine D\textsubscript{1}-like and D\textsubscript{2}-like receptors (including D1R or D\textsubscript{1,5}R and D2R or D\textsubscript{2,3,4}R, respectively) are thought to play critical roles in cocaine addiction. Our previous studies demonstrated that repeated cocaine exposure (which alters dopamine transmission) decreases excitability of NAc MSNs in cocaine-sensitized, withdrawn rats. This decrease is characterized by a reduction in voltage-sensitive Na\textsuperscript{+} currents and high voltage-activated Ca\textsuperscript{2+} currents, along with increased voltage-gated K\textsuperscript{+} currents. These changes are associated with enhanced activity in the D1R/cAMP/PKA/protein phosphatase 1 pathway and diminished calcineurin function. Although D1R-mediated signaling is enhanced by repeated cocaine exposure, little is known whether and how the D2R is implicated in the cocaine-induced NAc dysfunction. Here, we performed a combined electrophysiological, biochemical, and neuroimaging study that reveals the cocaine-induced dysregulation of Ca\textsuperscript{2+} homeostasis with involvement of D2R. Our novel findings reveal that D2R stimulation reduced Ca\textsuperscript{2+} influx preferentially via the L-type Ca\textsuperscript{2+} channels and evoked intracellular Ca\textsuperscript{2+} release, likely via inhibiting the cAMP/PKA cascade, in the NAc MSNs of drug-free rats. However, repeated cocaine exposure abolished the D2R effects on modulating Ca\textsuperscript{2+} homeostasis with enhanced PKA activity and led to a decrease in whole-cell Ca\textsuperscript{2+} influx. These adaptations, which persisted for 21 days during cocaine abstinence, may contribute to the mechanism of cocaine withdrawal. Synapse 65:168–180, 2011.

INTRODUCTION

The nucleus accumbens (NAc) is a forebrain structure that regulates cognitive function and drug-motivated behaviors in humans and animals (Hyman et al., 2006; Robbins and Everitt, 2002). Dopamine innervation from the midbrain mediates function of medium spiny neurons (MSNs) in the NAc by activating the D1R (D\textsubscript{1,5}R) and D2R (D\textsubscript{2,3,4}R). D2R modulates cellular activity via numerous signaling pathways (Beaulieu et al., 2007; Beom et al., 2004; Pedrosa et al., 2004; Senogles, 2000; Zhang et al., 2004). In striatal MSNs, D2R stimulation facilitates G\textsubscript{b1,5}/phospholipase C\textsubscript{b1} coupling (Hernandez-Lopez et al., 2000) and phosphorylation of inositol-1,4,5-triphosphate receptors (IP\textsubscript{3}R) by PKA (Hu et al., 2005a). Such actions increase intracellular Ca\textsuperscript{2+} release and decrease activity of L-type Ca\textsuperscript{2+} channels, respectively (Bonci and Hopf, 2005; Greengard, 2001). Both the D2R effects activate calcineurin, which is a contractile protein phosphatase that mediates Ca\textsuperscript{2+} homeostasis and is involved in the regulation of synaptic plasticity and neuronal plasticity. The role of calcineurin in these processes is well documented, and its inhibition has been shown to produce similar effects as those induced by cocaine withdrawal [Bonci et al., 2005; Iwata et al., 2003].

Contract grant sponsor: USPHS; Contract grant numbers: DA 04093, DA 026746.
*Correspondence to: Xiu-Ti Hu, Department of Pharmacology, Center for Compulsive Behaviors and Addiction, Rush University Medical Center, 1735 W. Harrison Street, Cohn Research Building, Rm. 454, Chicago, IL 60612, USA. E-mail: xiu-ti_hu@rush.edu

Received 17 March 2010; Accepted 14 June 2010
DOI 10.1002/syn.20831
Published online 27 July 2010 in Wiley Online Library (wileyonlinelibrary.com).
dephosphorylates L-channels and IP_3Rs, thereby decreasing Ca^{2+} influx (Day et al., 2002; Hernandez-Lopez et al., 2000) but facilitating intracellular Ca^{2+} release (Bultynck et al., 2003; Groth et al., 2003).

Repeated cocaine exposure disturbs Ca^{2+} signaling in MSNs by enhancing and prolonging D1R/D2R stimulation. These changes result in part from reduced Ca^{2+} influx and calcineurin function (Hu et al., 2004, 2005b; Zhang et al., 2002), leading to a reduction in NAc excitability (see Hu, 2007 for review). Alterations in Ca^{2+} channel activity vary, depending upon subtypes of the channel. For instance, D1R stimulation increases L-channel activity with reinstatement of cocaine-seeking behavior in rats (Anderson et al., 2008; Self et al., 1998), whereas repeated cocaine exposure reduces Ca^{2+} currents (I_{Ca}) via N- and R-type Ca^{2+} channels in NAc MSNs (Zhang et al., 2002). Such differences can be attributed in part to enhanced phosphorylation of L-channels by PKA (Hernandez-Lopez et al., 1997) and dephosphorylation of N-/R-channels by protein phosphatase 1, respectively (Surmeier et al., 1995; Zhang et al., 2002). However, it is unknown whether and how D2R-mediated Ca^{2+} homeostasis in NAc MSNs is altered after repeated cocaine exposure. Repeated D2R stimulation reduces D2R-coupled G_i/G_o protein levels in the NAc (Nestler, 2004; Nestler et al., 1990) and induces desensitization and internalization of D2R after phosphorylation (Gainetdinov et al., 2004). These findings strongly suggest that dysregulated Ca^{2+} homeostasis and signaling are associated with D2R downregulation in striatal MSNs.

Cocaine-induced D2R dysfunction plays a critical role in neuroadaptation of Ca^{2+} influx and related signaling. Thus, chronic cocaine exposure reduces the α-subunits of D2R-coupled G_i/o protein (Nestler et al., 1990), Ca^{2+} influx (Hu et al., 2004; Zhang et al., 2002), and activity of the G_i/o-adenylyl cyclase/cAMP/PKA/Ca^{2+}/ calcineurin pathway in NAc MSNs (Hu et al., 2005b). Cocaine abuse also decreases D2R availability in the striatum of abstinent humans (Volkow et al., 1999). On the basis of these findings, we hypothesized that D2R modulation of Ca^{2+} homeostasis and signaling is decreased in NAc MSNs in cocaine-sensitized, withdrawn rats. This study was performed to determine whether (1) D2R modulates Ca^{2+} channel function and intracellular Ca^{2+} release in NAc MSNs of drug-free rats, and (2) repeated cocaine exposure decreases D2R modulation of Ca^{2+} channel activity and Ca^{2+} release via the D2R-coupled Ca^{2+}/ calcineurin pathways.

**MATERIALS AND METHODS**

**Animals and pretreatments**

Adolescent male Sprague-Dawley rats at 4–5 weeks of age (Spear, 2000), which were more vulnerable to the development of drug addiction (Badanich et al., 2006, 2008; Schramm-Sapyta et al., 2006), were used in this study. They were group housed in a temperature and humidity-controlled vivarium under a 12-h light/dark cycle. Food and water were freely available. After 3 days acclimation to the vivarium, rats 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 rats used in this study received repeated injections of saline or cocaine in their home cage. All experiments were performed after a short (3-day) or long-term (21-day) withdrawal from pretreatments.

**Whole-cell recordings in brain slices**

All procedures were in strict accordance with the National Research Council Guide for the Care and use of Laboratory Animals (NIH Publication No. 85-23, 1996) and were approved by our Institutional Animal Care and Use Committee. Rats were decapitated under halothane anesthesia. Brain was immediately excised and immersed in ice-cold artificial cerebrospinal fluid containing (in mM): NaCl 124, KCl 2.5, NaHCO3 26, MgCl2 2, CaCl2 2, and glucose 10; pH 7.4; 310 mosM/L. Coronal slices (300 μm) containing the NAc were cut with a vibratome (Leica VT1000S, Bannockburn, IL) and incubated in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid for 1 h at room temperature before recording. Slices were anchored in a recording chamber and perfused with gravity-fed oxygenated artificial cerebrospinal fluid (34°C) at a flow rate of 2–3 ml/min. Patch recording pipettes (3–5 Ω) were pulled from Corning 7056 glass capillaries (Corning, NY) with a horizontal pipette puller (Flaming/Brown P-97, Sutter Instruments, Novato, CA) and filled with internal recording solution (in mM): CsOH 130, HEPES 10, MgCl2 2, CaCl2 2, and glucose 10; pH 7.4; 310 mosM/L. To avoid influence of Ca^{2+} chelation that alters dynamic D2R modulation of ion channel activity, Ca^{2+} chelators were not included in the pipette solution (Hu et al., 2005a,b). Recordings were initiated in visually identified MSNs within the core region of the NAc using differential interference contrast microscopy and an amplifier (Axopatch 200B, Axon Instruments, Union City, CA).

---

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| cAMP         | cyclic adenosine monophosphate |
| D1R          | dopamine D_1-like receptors |
| D2R          | dopamine D_2-like receptors |
| IP_3R        | inositol-1,4,5-trisphosphate receptor |
| MSNs         | medium spiny neurons |
| NAc          | nucleus accumbens |
| PKA          | protein kinase A |

---

**Synapse**
After whole-cell configuration was formed, voltage-clamp mode was converted to current-clamp mode. Voltage signals were recorded, amplified in a bridge mode, and digitized by an interface (DigiData 1322A Series) into a computer running analysis software (pCLAMP 9) (Axon Instruments). To prevent influences of synaptic activities on the membrane potential ($V_m$), glutamate receptors and GABA$_A$ receptors were blocked during recording. Na$^+$ and K$^+$ channel were also blocked to separate the voltage-gated Ca$^{2+}$ channels (see Drug Application below for detail). High voltage-activated Ca$^{2+}$ plateau potentials were generated by injecting step depolarizing current pulses starting from 0 nA with 0.05 nA increments and 40 ms duration, which were delivered at 10 s intervals. The recording period in each episode was 3 s. Stabilized Ca$^{2+}$ potentials were recorded before application of agonists, antagonists, or blockers as control. Characteristics of Ca$^{2+}$ potentials were obtained from the initial Ca$^{2+}$ spike evoked by the minimal depolarizing current (rheobase). MSNs with stable resting membrane potential were recorded and used for analysis. Resting membrane potential was held at $-80 \text{ mV}$ (near the mean of $-78 \text{ mV}$) during drug application and recording. This gave each NAc MSN the same basal potential level; thus, the results obtained from different cells would be comparable (Hu et al., 2004). The amplitude of Ca$^{2+}$ potentials was measured from the spike threshold to its peak. The half-amplitude duration of Ca$^{2+}$ potentials was measured at the amplitude level at which one-half of the spike peak was reached. The integrated area (size) of Ca$^{2+}$ potentials was defined and measured under the curve, which initiated at the rising part of the spike from resting membrane potential and ended with the recording period.

Separate subgroups of NAc MSNs were recorded with application of different drugs and ion channel blockers that were added in artificial cerebrospinal fluid immediately before use. Selective blockers/inhibitors for Na$^+$ channels (tetrodotoxin, TTX, 1 $\mu$M), K$^+$ channels (tetraethylammonium, TEA, 20 $\mu$M), glutamate receptors (kynurenic acid, 2.5 $\mu$M), and GABA$_A$ receptors (SR-95531 or 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)-pyridazinium bromide, 4 $\mu$M) were applied in bath in all experiments. Quinpirole, a selective D2R agonist (1–10 $\mu$M), was used to assess D2R modulation of Ca$^{2+}$ spikes and release. The selective D2R antagonist eticlopride (10 $\mu$M) was used to block the effects of quinpirole. Selective Ca$^{2+}$ channel blockers for L-type (nifedipine, 5 $\mu$M), N- (o-conotoxin GVIA, 1 $\mu$M), and P/Q-type (o-conotoxin MVIIIC, 2 $\mu$M) channels were also applied in the bath to assess the role of non-L-type Ca$^{2+}$ channels. Active calcineurin (100 U) was added in the internal solution and applied directly in cytosol. Cyclosporin A (20 $\mu$M), a selective inhibitor for calcineurin, was applied externally.

**PKA assays**

The NAc and motor cortex from saline- or cocaine-pretreated rats, with a short- or long-term withdrawal, were dissected and lysed in hypotonic buffer [10 mM HEPES, pH 7.9, 1.5 mM MgCl$_2$, 10 mM KCl, 1 mM DTT, 25 $\mu$M (-)-$p$-bromotetramisole oxalate, 5 $\mu$M cantharidin, 5 $\mu$M microcystin-LF, and 5 $\mu$M cyclosporin A] and were supplemented with Complete Protease Inhibitor tablets (Roche Diagnostics, Indianapolis, IN). PKA activity in 2 $\mu$g of each sample was determined by the PepTag PKA assay (Promega, Madison, WI) (Dong et al., 2005). Positive controls contained 10 ng of purified PKA catalytic subunit (Promega), whereas the negative controls contained no PKA. The PepTag assay used the Leu-Arg-Arg-Ala-Ser-Leu-Gly (Kemptide) peptide substrate tagged with a UV-fluorescent dye. The PKA activity was detected by the amount of phosphorylated substrate migrating toward the anode. Quantification was performed by calculating luminescence intensity using TotalLab software (Nonlinear Dynamics, Durham, NC).

**Two-photon calcium imaging**

Live-cell Ca$^{2+}$ imaging of individual neurons in thick brain slice preparations was performed using a custom-made video-rate two-photon imaging system based on an Olympus BX51 microscope frame (Nguyen et al., 2001), which is the gold standard technique currently available. Relative changes in [Ca$^{2+}$]$_{in}$ were readily measured and compared within and between samples. Although the absolute [Ca$^{2+}$]$_{in}$ could not be measured, a relative change from this baseline and estimated concentrations based on established baseline values were made. The resting Ca$^{2+}$ levels were interpreted to be similar based on similar fluorescence intensity values at the same laser intensity and power levels, and similar depths within the tissue slice. In addition, other Ca$^{2+}$-dependent effects that would affect passive and active membrane properties were also not different between groups, which were consistent with the observed fluorescent levels. Individual MSNs were filled with the Ca$^{2+}$ indicator fura-2 (50 $\mu$M) via a patch pipette as described (Stutzmann et al., 2003). Excitation was provided by trains (80 MHz) of $\sim$100 fs pulses at 780 nm from a Ti:sapphire laser (Mai Tai Broadband, Spectra-Physics, Mountain View, CA). The laser beam was scanned at 30 fps using a custom-built scanner and focused through a 40× water-immersion objective (NA = 0.8). Emitted fluorescence light was detected by a wide-field photomultiplier (Electron Tubes, Rockaway, NJ) to derive a video signal that was captured and analyzed by Video Savant 5.0 software (IO Industries, ON, Canada). Further analysis of background-corrected images was performed using Metamorph software. For clarity, images and traces of fura-2 fluorescence are
expressed as inverse pseudo-ratios: \( F_0/\Delta F \) (\( F_0 \) is the average resting fluorescence before stimulation, and \( \Delta F \) is the decrease of fura-2 fluorescence resulting from increased [Ca\(^{2+}\)] when excited at 780 nM), so that increases in [Ca\(^{2+}\)] correspond to increasing ratios.

Statistical analysis

Student’s \( t \)-test was used for comparison of drug effects on the membrane properties, characteristics of Ca\(^{2+}\) plateau potentials, PKA activity, and intracellular Ca\(^{2+}\) release between control and drug-treated NAc cells in saline- or cocaine-pretreated rats. Repeated-measures ANOVA was used for comparison of the quinpirole-induced changes in the dose-response curves between saline- and cocaine-withdrawn groups. Newman-Keuls test was carried out for post hoc comparisons.

RESULTS

D2R stimulation reduces the size of evoked Ca\(^{2+}\) plateau potentials in NAc MSNs

All medium spiny NAc cells were recorded in the core region. Blockade of K\(^+\) channels (TEA in the bath and cesium in cytosol) depolarized resting membrane potential in NAc MSNs (SAL group = −61.1 ± 2.2, COC group = −60.7 ± 2.0 mV) when compared with that without such blockade (≈−79 mV) (Zhang et al., 1998), which effectively stabilized generation of Ca\(^{2+}\) potentials (Hu et al., 2004; Nasif et al., 2005a). This membrane depolarization was attributed mainly to blockade of the outflowing K\(^+\) currents that were activated at the resting status of cells to maintain resting membrane potential at hyperpolarized levels. This change in resting membrane potential was not significantly affected by D2R stimulation (data not shown). Ca\(^{2+}\) plateau potentials were evoked by depolarizing current pulses with blockade of Na\(^+\) channels and K\(^+\) channels (Fig. 1A). Bath-applied quinpirole (1–10 μM) decreased Ca\(^{2+}\) channel activity in a dose-dependent manner (Figs. 1B and 1C). Because 10 μM of quinpirole was effective in producing a significant reduction in the size of evoked Ca\(^{2+}\) plateau potentials (n = 40 cells, one-way ANOVA, \( F_{(4,39)} = 5.07, *P < 0.03 \)), it was used in the rest of experiments of this study. D2R-mediated decrease in Ca\(^{2+}\) influx was reflected by a reduction in the size of evoked Ca\(^{2+}\) spikes (control vs. quinpirole: 57,196 ± 8353 vs. 39,055 ± 5629 mV × ms; n = 15 cells, paired \( t \)-test, \( t_{(14)} = 5.61, *P < 0.05 \)), and the duration of Ca\(^{2+}\) potentials (measured at the half-amplitude level (control vs. quinpirole: 336.3 ± 23.8 vs. 213 ± 23.4 ms and 47.5 ± 2.7 vs. 38.5 ± 2.9 mV, respectively; n = 15 cells/group, paired \( t \)-test, \( t = 5.20 \) and \( t = 5.21 \), all \( *P < 0.05 \) (Fig. 2A–D). Rheobase and firing threshold of evoked Ca\(^{2+}\) spikes were not significantly affected by quinpirole (SAL vs. COC: 0.7 ± 0.05 vs. 0.7 ± 0.05 nA, and −13.6 ± 1.7 vs. −12.4 ± 1.5 mV, respectively). The effects of D2R stimulation on Ca\(^{2+}\) influx were washed out by fresh medium (n = 15, paired \( t \)-test, \( P > 0.05 \) (Figs. 2A–2D) or blocked by the selective D2R antagonist eticlopride (10 μM) (control vs. eticlopride + quinpirole: area, 34,479 ± 1969 vs. 35,450 ± 2709 mV × ms; amplitude: 38.3 ± 4.1 vs. 41.2 ± 2.3 mV; duration: 235.1 ± 15.8 vs. 255.3 ± 24.5 ms; n = 10 cells, paired \( t \)-test, \( P > 0.05 \) (Fig. 2E–H).

Selective blockade of L-type, but not N- and P/Q-type Ca\(^{2+}\) channel, mimics and occludes D2R-mediated reduction in Ca\(^{2+}\) spikes

Given the fact that generation of Ca\(^{2+}\) spikes in striatal cells of young rats depends primarily on activation of high voltage-activated Ca\(^{2+}\) channels (Hu et al., 2004; Surmeier et al., 1995; Zhang et al., 2002), we evaluated whether and how the activity and which subtype of high voltage-activated Ca\(^{2+}\) channels was modulated by the D2R. Bath application of nifedipine (5 μM), a specific L-channel blocker, significantly reduced the amplitude, duration, and size of evoked Ca\(^{2+}\) spikes (control vs. nifedipine: 51.0 ± 2.9 vs. 42.5 ± 4.4 mV; 319.6 ± 32.6 vs. 251.7 ± 35.7 ms, and...
50,816 ± 5800 vs. 39,811 ± 4425 mV × ms, respectively; n = 10 cells, paired t-test, t = 3.51, t = 4.22, and t = 3.07, all *P < 0.05) (Fig. 3A). With blockade of L-channels, quinpirole (10 µM) was no longer able to suppress Ca²⁺ potentials (nifedipine vs. nifedipine + quinpirole: 39,811 ± 4425 vs. 37,802 ± 4539 mV × ms, 42.5 ± 4.4 vs. 39.8 ± 4.7 mV, and 251.7 ± 35.7 vs. 248 ± 37.9 ms, respectively; n = 10 cells, paired t-test, both *P < 0.05). Short bars represent means ± S.E. E: Concurrent application of the selective D2R antagonist eticlopride (10 µM) blocked the quinpirole-induced reduction in the size of Ca²⁺ potentials. F–H: There was no significant difference in the area, amplitude, and duration of Ca²⁺ spikes between control and eticlopride + quinpirole-treated cells. Bath-applied eticlopride produced no significant change in Ca²⁺ potentials (means ± S.E., n = 10 cells; paired t-test, all P > 0.05).

Fig. 2. D2R-modulated inhibition in Ca²⁺ potentials was reversible and prevented by D2R antagonist. A: Representative recording traces showing that D2R-modulated inhibition of Ca²⁺ spikes was reversible. B–D: Bar graphs indicate that the significant reductions in the size, amplitude, and duration of Ca²⁺ spikes induced by quinpirole (10 µM) were reversed following washout (n = 10 NAc cells; paired t-test, *P < 0.05). Short bars represent means ± S.E. E: Concurrent application of the selective D2R antagonist eticlopride (10 µM) blocked the quinpirole-induced reduction in the size of Ca²⁺ potentials. F–H: There was no significant difference in the area, amplitude, and duration of Ca²⁺ spikes between control and eticlopride + quinpirole-treated cells. Bath-applied eticlopride produced no significant change in Ca²⁺ potentials (means ± S.E., n = 10 cells; paired t-test, all P > 0.05).

Fig. 3. Blockade of L-, but not N- or P/Q-type Ca²⁺ channels, mimicked and occluded D2R-modulated inhibition of Ca²⁺ spikes. A1: Representative traces showing that selective blockade of L-channel by nifedipine (5 µM) reduced the duration and amplitude of Ca²⁺ spikes, whereas the D2R-modulated inhibition of Ca²⁺ potentials was occluded by blockade of L-channel. A2: Bar graphs indicate a significant decrease in the Ca²⁺ spike area with L-channel blockade (n = 10 cells, respectively; means ± S.E., paired t-test, both *P < 0.05). There was no significant difference in the Ca²⁺ spike between nifedipine-treated and nifedipine plus quinpirole-treated cells (n = 10 cells; paired t-test, #P > 0.05). B1: Selective blockade of N-type Ca²⁺ channels with α-conotoxin GVIA (1 µM) reduced the duration of Ca²⁺ potentials, and this effect was further enhanced by concurrent application of quinpirole. B2: There was a significant decrease in the Ca²⁺ spike area between control cells and that with application of α-conotoxin GVIA or α-conotoxin GVIA plus quinpirole and between cells treated with α-conotoxin GVIA and α-conotoxin GVIA + quinpirole (n = 10 cells; paired t-test, all *P < 0.05). C1: Selective blockade of P/Q-type channels with α-conotoxin MVIIC (2 µM) reduced the Ca²⁺ spike area, which was also enhanced by quinpirole. C2: There was a significant difference in the spike area between control cells and that with α-conotoxin MVIIC or α-conotoxin MVIIC + quinpirole and between cells treated with α-conotoxin MVIIC and α-conotoxin MVIIC + quinpirole (n = 10 cells; paired t-test, all *P < 0.05).
t-test, all \( P > 0.05 \) (Fig. 3A). Because the size of \( \text{Ca}^{2+} \) spikes was affected by the duration and amplitude, it was measured and used as the primary result and was compared among all experimental groups throughout this study.

In contrast, blockade of other types of high voltage-activated \( \text{Ca}^{2+} \) channels did not eliminate D2R-mediated inhibition in \( \text{Ca}^{2+} \) spikes. Bath-applied \( \alpha \)-conotoxin GVIA (5 \( \mu \)M), a specific N-type \( \text{Ca}^{2+} \) channel blocker, significantly reduced the area of \( \text{Ca}^{2+} \) spikes (control vs. \( \alpha \)-conotoxin GVIA: 58,290 \( \pm \) 1117 vs. 48,208 \( \pm \) 10079 mV \( \times \) ms, \( n = 10 \) cells, paired \( t \)-test, \( t = 3.55, * P < 0.05 \)) (Fig. 3B). The N-channel blocker-induced reduction in \( \text{Ca}^{2+} \) spikes was enhanced by concurrent application of quinpirole, which produced an additive decrease in the size of \( \text{Ca}^{2+} \) spikes (\( \alpha \)-conotoxin GVIA vs. \( \alpha \)-conotoxin GVIA + quinpirole: 43,393 \( \pm \) 8605 vs. 27,773 \( \pm \) 5891 mV \( \times \) ms, \( n = 10 \) cells, paired \( t \)-test; \( t = 4.42, * P < 0.05 \)) (Fig. 3B). \( \alpha \)-Conotoxin MVIIC (2 \( \mu \)M), a specific blocker for P/Q-type \( \text{Ca}^{2+} \) channels (Brown and Randall, 2005; Phillips and Stampf, 2000), also significantly diminished the area of \( \text{Ca}^{2+} \) spikes (control vs. \( \alpha \)-conotoxin MVIIC: 43,245 \( \pm \) 4962 vs. 33,042 \( \pm \) 2309 mV \( \times \) ms, \( n = 10 \) cells, paired \( t \)-test, \( t = 2.77, * P < 0.05 \)) (Fig. 3C). Application of quinpirole with the P/Q-channel blocker also produced an additional reduction in the size of \( \text{Ca}^{2+} \) spike (\( \alpha \)-conotoxin MVIIC vs. \( \alpha \)-conotoxin MVIIC + quinpirole: 33,042 \( \pm \) 2309 vs. 21,383 \( \pm \) 5609 mV \( \times \) ms, \( n = 10 \) cells, paired \( t \)-test, \( t = 4.45, * P < 0.05 \)) (Fig. 3C).

Calcineurin suppresses \( \text{Ca}^{2+} \) plateau potentials and occludes D2R-mediated inhibition of \( \text{Ca}^{2+} \) spikes

To determine whether and how \( \text{Ca}^{2+} \) channel activity is modulated by the D2R-coupled \( \text{Ca}^{2+} \)/calcineurin pathway, we evaluated the interaction of calcineurin and D2R stimulation. We found that cytosolic application of active calcineurin (100 U) mimicked the D2R-mediated reduction in \( \text{Ca}^{2+} \) spikes (control vs. calcineurin: 54,460 \( \pm \) 3059 vs. 32,722 \( \pm \) 2141 mV \( \times \) ms, \( n = 10 \) cells, \( t \)-test, \( t = 5.84, * P < 0.05 \)). However, concurrent application of quinpirole with calcineurin produced no further reduction; and the calcineurin-induced decrease in the size of \( \text{Ca}^{2+} \) potentials was not significantly affected by quinpirole (calcineurin vs. calcineurin + quinpirole: 32,722 \( \pm \) 2141 vs. 33,823 \( \pm \) 2397 mV \( \times \) ms, \( n = 10 \) cells, paired \( t \)-test; \( P > 0.05 \); control vs. calcineurin + quinpirole: 54,460 \( \pm \) 3059 vs. 33,823 \( \pm \) 2397 mV \( \times \) ms; paired \( t \)-test, \( t = 5.06, * P < 0.05 \)) (Fig. 4A–B).

To further investigate the calcineurin effects on \( \text{Ca}^{2+} \) channel activity, we assessed if inhibition of calcineurin activity could induce an opposite responsiveness in \( \text{Ca}^{2+} \) spikes. In contrast to calcineurin, bath application of cyclosporin A (20 \( \mu \)M) (Hu et al., 2005a), a selective inhibitor for calcineurin, significantly increased the duration and size of \( \text{Ca}^{2+} \) potentials in NAc MSNs (control vs. cyclosporin A: 367.9 \( \pm \) 24 vs. 404.9 \( \pm \) 33.1 ms and 48,980 \( \pm \) 2686 vs. 55,988 \( \pm \) 3773 mV \( \times \) ms, \( n = 10 \) cells, respectively; paired \( t \)-test, \( t = 3.28 \) and \( t = 5.43 \), respectively, both \( * P < 0.05 \)) (Fig. 4C and D). This effect of cyclosporin A on \( \text{Ca}^{2+} \) potentials was not affected by concurrent application of quinpirole (cyclosporin A vs. cyclosporin A + quinpirole: 55,988 \( \pm \) 3773 vs. 54,596 \( \pm \) 3874 mV \( \times \) ms, \( n = 10 \) cells, paired \( t \)-test, \( P > 0.05 \); control vs. cyclosporin A + quinpirole: 48,980 \( \pm \) 2686 vs. 54,596 \( \pm \) 3874 mV \( \times \) ms, paired \( t \)-test, \( t = 3.55, * P < 0.05 \)) (Fig. 4D).

Repeated cocaine exposure and withdrawal decreases \( \text{Ca}^{2+} \) channel function and abolishes D2R-mediated inhibition of \( \text{Ca}^{2+} \) spikes

In this study, we extended our earlier research regarding the decreased activity of high voltage-activated \( \text{Ca}^{2+} \) channels in medium spiny NAc neurons of Synapse.
rats after a 3-day withdrawal (Hu et al., 2004) by investigating two additional questions: (1) does the decreased Ca$^{2+}$ channel activity persist after longer withdrawal? And (2) are the effects of D2R on modulating high voltage-activated Ca$^{2+}$ channel activity in NAc MSNs of cocaine-pretreated rats altered after cocaine withdrawal? To study the first question, we compared the integrated area of Ca$^{2+}$ spikes in NAc from saline- and cocaine-pretreated rats. The size of Ca$^{2+}$ potentials was significantly reduced in cocaine-pretreated NAc neurons either after a 3-day or a 21-day abstinence (3-day/withdrawl: SAL vs. COC: 174 vs. 262.4 ± 13.5 ms; 21-day/withdrawl: SAL vs. COC = 233.1 ± 16.6 vs. 174.6 ± 11.2 ms; t = 2.16 and t = 2.33; all *P < 0.05). Rheobase was also increased in cocaine-pretreated cells (3-day/withdrawl: SAL vs. COC = 0.66 ± 0.04 vs. 1.0 ± 0.06 nA; 21-day/withdrawl: SAL vs. COC = 0.7 ± 0.08 vs. 1.1 ± 0.11 nA; t-test, t = 3.5 and t = 2.4, respectively; all *P < 0.05) (Fig. 5E). These changes indicate that the intrinsic excitability of medium spiny NAc neurons was remarkably reduced by downregulating high voltage-activated Ca$^{2+}$ channel function in cocaine-sensitized, withdrawn rats.

The second question was studied by comparing the spike area of medium spiny NAc neurons between saline- and cocaine-pretreated rats. Associated with cocaine-induced decrease in Ca$^{2+}$ influx, the D2R effects on reducing the duration of evoked Ca$^{2+}$ spikes were diminished in cocaine-pretreated NAc neurons after withdrawal. Under this circumstance, quinpirole was no longer able to suppress Ca$^{2+}$ spikes in cocaine-withdrawn NAc neurons, even at a higher concentration (3-day/withdrawl group: SAL vs. COC: n = 14 vs. 13 cells, and 21-day/withdrawl group: SAL vs. COC, n = 12 vs. 11 cells; two-way ANOVA with repeated measures, F(2,40) = 4.16, *P < 0.05, and F(2,40) = 14.2, *P < 0.05, respectively, both compared to quinpirole 0 μM; control) (Fig. 5E–H).

Repeated cocaine administration persistently increases PKA activity in the NAc after a 3-day or a 21-day withdrawal

Previous findings indicated that repeated cocaine treatment increases PKA activity in various brain regions, including NAc (Edwards et al., 2007; Hope et al., 2005; Scheggi et al., 2007). To determine if the persistent dysregulation of Ca$^{2+}$ channels in NAc cells (see above) was associated with this change, we assessed PKA activity in the NAc using a highly specific fluorescent peptide substrate (kemptide) after a 3-
Fig. 6. Repeated cocaine exposure enhanced PKA activity in the NAc but not motor cortex. A: Examples of gels show phosphorylation of peptide substrates resulting from PKA activation in the tissue from the NAc obtained from SAL- and COC-pretreated rats. PKA activity was increased after the short- or long-term cocaine withdrawal (3-day/withdrawal or 21-day/withdrawal, respectively). There was a significant difference in PKA activity in the NAc between SAL- and COC-pretreated rats after both short- and long-term withdrawal (SAL 3-day/withdrawal vs. COC 3-day/withdrawal, n = 20 vs. 22 rats, unpaired t-test, *P < 0.05; SAL 21-day/withdrawal vs. COC 21-day/withdrawal, n = 14 vs. 14 rats, unpaired t-test, *P < 0.05). The bars and vertical lines represent the mean ± S.E. B: There was no significant change in PKA activity in the motor cortex of SAL-pretreated rats when compared with COC-pretreated rats.

Repeated exposure to cocaine decreases D2R modulation of cytosolic Ca^{2+} release in NAc MSNs after a 3-day or a 21-day withdrawal

It has been suggested that D2R stimulation increases Ca^{2+} release in striatal cells (Greengard, 2001). Stimulation of D2R also elevates [Ca^{2+}]_i in cortical astrocytes (Khan et al., 2001). However, little is known whether and how D2R could dynamically modulate this activity in medium spiny NAc neurons. Here, we used a two-photon laser scanning Ca^{2+} imaging technology to evaluate cytosolic Ca^{2+} release in medium spiny NAc neurons in slice preparations from saline- or cocaine-withdrawn rats. The basal levels of resting Ca^{2+} level, as indicated by relative fluorescent intensity of the calcium indicator fura-2, were imaged for 3 min in both saline- and cocaine-pretreated rats. There was no significant difference in the relative baseline levels of fluorescent intensity between NAc neurons in saline- and cocaine-pretreated rats after a 3-day or a 21-day withdrawal (both P > 0.05) (Fig. 7A). D2Rs localized on the cell membrane of medium spiny NAc neurons were stimulated with bath application of quinpirole (10 μM) for 5 min, and then the changes in [Ca^{2+}]_i after D2R stimulation were recorded and compared to the baseline. D2R stimulation by quinpirole evoked a marked relative increase in the somatic levels of free Ca^{2+} in NAc MSNs in saline-pretreated rats (F_0/ΔF = 0.62% ± 0.21% or 162% ± 20.8% of the predrug baseline measurement, n = 8 cells in four rats) (Fig. 7A and C). This D2R effect lasted ~5 min and was washed out completely with fresh artificial cerebrospinal fluid, as indicated in the raw two-photon images (Fig. 7A). The relative changes in Ca^{2+} release in saline- or cocaine-pretreated NAc cells in response to D2R stimulation are presented with pseudocolored images (Fig. 7B).

Repeated cocaine exposure abolished the D2R-mediated intracellular Ca^{2+} release in medium spiny NAc neurons. There was no detected Ca^{2+} release in the presence of quinpirole in cocaine-pretreated NAc cells when compared with that in saline-pretreated cells after either a 3-day withdrawal (F_0/ΔF = −0.02% ± 0.1% or 98.3% ± 7.1% of baseline predrug response; n = 9 cells; t_{11,16} = 3.02; **P < 0.001) (Fig. 7A and C) or a 21-day withdrawal (SAL- vs. COC-pretreated: n = 10/9 cells, four rats in each group; F_0/ΔF = 0.37 ± 0.16% vs. −0.02 ± 0.02, respectively; t_{11,17} = 2.29;
P < 0.05) (Fig. 7C, right panel). Given the fact that the quinpirole-induced increase in \([\text{Ca}^{2+}]_{\text{in}}\) was observed without activation of voltage-gated \(\text{Ca}^{2+}\) channels and with blockade of ionotropic glutamate receptors, these findings indicate that D2R-mediated increase in \([\text{Ca}^{2+}]_{\text{in}}\) resulted from intracellular stores.

Protein levels of L-type \(\text{Ca}^{2+}\) channels and IP\(_3\)R are not significantly altered in the rat NAc after chronic cocaine treatment and withdrawal

\(\text{Ca}^{2+}\) influx via high voltage-activated \(\text{Ca}^{2+}\) channels and \(\text{Ca}^{2+}\) release from intracellular stores depend not only on the activity but also on the number of these \(\text{Ca}^{2+}\) channels and IP\(_3\) receptors, respectively. Thus, we also evaluated if the decrease of \(\text{Ca}^{2+}\) influx and \(\text{Ca}^{2+}\) release in cocaine-pretreated NAc cells was affected by reduced protein levels (reflecting a decrease in the number) of the L-channels and/or IP\(_3\)Rs. Specific antibodies were used to measure the levels of L-channels and IP\(_3\)Rs in the rat NAc. The total protein levels of \(\alpha_1\) subunit (pore-forming and ligand-binding protein) of L-channel and IP\(_3\)R were not significantly affected in the rat NAc after repeated cocaine treatment and withdrawal \((n = 18–31/\text{group}, \text{all } P > 0.05; \text{data not shown})\). Whether the surface expression (a.k.a. trafficking) of the L-channels and IP\(_3\)Rs was altered by repeated cocaine exposure and withdrawal remains to be determined in future investigations.

**DISCUSSION**

This study determined that D2R (D\(_2\),D\(_3\),D\(_4\)) stimulation modulates \(\text{Ca}^{2+}\) homeostasis and related signaling in rat NAc MSNs in the core region by decreasing \(\text{Ca}^{2+}\) influx preferentially via the L-channels and increasing intracellular \(\text{Ca}^{2+}\) release. Repeated cocaine exposure increased PKA activity and abolished the D2R modulation. These changes are found after a 3-day or a 21-day cocaine abstinence, indicating enduring neuroadaptations of NAc function in cocaine-sensitized, withdrawn rats.
D2R stimulation decreases Ca\textsuperscript{2+} influx by preferentially reducing L-channel activity: implications of Ca\textsuperscript{2+} release and calcineurin activation

D2R-mediated decrease in Ca\textsuperscript{2+} influx was reflected by reduced “size” of Ca\textsuperscript{2+} potentials, which was reversible, receptor specific, and dose dependent. This decrease was mimicked and occluded by blockade of the L- but not N- and P/Q-type Ca\textsuperscript{2+} channels, suggesting that D2R modulation preferentially reduced L-channel activity in NAc MSNs. Intracellular Ca\textsuperscript{2+} release and downstream calcineurin activation were implicated in the mechanisms, which may underlie the D2R-modulated decrease of L-channel activity. We previously revealed that D2R stimulation facilitates Na\textsuperscript{+} channel activity, most likely via elevating intracellular [Ca\textsuperscript{2+}]\textsubscript{in} in rat NAc MSNs (Hu et al., 2005a). Nevertheless, such Ca\textsuperscript{2+} release has never been proven by real-time Ca\textsuperscript{2+} imaging study. This study demonstrated that selective D2R stimulation increased [Ca\textsuperscript{2+}]\textsubscript{in} in the absence of membrane depolarization with blockade of ionotropic glutamate receptors, indicating a dynamic intracellular Ca\textsuperscript{2+} release in rat NAc MSNs. Such increased Ca\textsuperscript{2+} release could activate calcineurin and dephosphorylate L-channels, thereby subsequently reducing Ca\textsuperscript{2+} influx via the L-channel (Day et al., 2002; Groth et al., 2003). Moreover, we also found that calcineurin mimicked and occluded D2R-mediated suppression of Ca\textsuperscript{2+} spikes, and inhibition of calcineurin activity by cyclosporin A not only prolonged the duration but also prevented D2R suppression of Ca\textsuperscript{2+} spikes. These findings suggest that via a D2R-coupled, cAMP/PKA/IP3R/Ca\textsuperscript{2+}-mediated pathway (Hu et al., 2005a), and likely the others (Hernandez-Lopez et al., 2000), D2R stimulation activates calcineurin in a converged common path that may facilitate dephosphorylation of L-channels and therefore reduce Ca\textsuperscript{2+} influx in NAc MSNs (Fig. 8A).

Repeated cocaine exposure decreases Ca\textsuperscript{2+} influx with enhanced PKA activity

Another major finding of this study is that the cocaine-induced decrease in Ca\textsuperscript{2+} influx was associated with enhanced PKA activity in the NAc. Given the...
fact that the MSNs constitute 95% cell population in this brain region (Pasik, 1979), we could reasonably assume that the cocaine-induced increase of PKA activity in the NAc occurred mainly in MSNs. The mechanism underlying the decreased Ca$^{2+}$ influx has been related to enhanced D1R signaling and reduced D2R function with increased phosphorylation and diminished dephosphorylation of Ca$^{2+}$ channels, respectively (see Hu, 2007; Nestler, 2004 for review). Compelling evidence shows that cocaine-induced neuroadaptation in ion channels is attributable, at least in part, to facilitation of the cAMP/PKA cascade. For instance, upregulated D1R signaling (e.g., increase of Gs-coupled cAMP formation and PKA activity) is instance, upregulated D1R signaling (e.g., increase of Gs-coupled cAMP formation and PKA activity) is found in NAc cells after repeated cocaine treatment (Hope et al., 2005; Self et al., 1995; Terwilliger et al., 1991). With enhanced PKA/DARPP-32 activity and reduced calcineurin function (Hu et al., 2005b), both the Ca$^{2+}$ influx via high voltage-activated Ca$^{2+}$ channels and the evoked Ca$^{2+}$ spikes are significantly diminished in cocaine-pretreated NAc MSNs (Hu et al., 2004; Zhang et al., 2002).

Cocaine-induced decrease in Ca$^{2+}$ influx in NAc MSNs could result from enhanced PKA activity via a direct and indirect manner. Although direct phosphorylation of L-channels by PKA can facilitate activity of the channel, whole-cell Ca$^{2+}$ influx is actually reduced because of decreased $I_{Ca}$ through N- and R-type Ca$^{2+}$ channels in cocaine-withdrawn NAc MSNs (Zhang et al., 2002). This decrease in $I_{Ca}$ is most likely related to an indirect PKA action by which protein phosphatase 1 is activated and non-L-type high voltage-activated Ca$^{2+}$ channels are dephosphorylated (Surmeier et al., 1995; Zhang et al., 2002). It is worth noting that $I_{Ca}$ via the L-channels contribute to only ~30% of whole-cell Ca$^{2+}$ conductance, whereas the combined N- and R-type Ca$^{2+}$ currents consist of about 50% of Ca$^{2+}$ influx in control, drug-free NAc MSNs (Zhang et al., 2002). Even though repeated cocaine exposure tended to increase Ca$^{2+}$ influx via L-channels, such change was not statistically significant (at least not in the soma). Thus, we suggest that the reduced amount of $I_{Ca}$ across N- and R-type Ca$^{2+}$ channels was greater than the probably increased Ca$^{2+}$ currents via the L-channels and therefore led to suppression of Ca$^{2+}$ potentials in cocaine-preexposed NAc MSNs (Fig. 8B).

Repeted cocaine treatment abolishes D2R-modulated Ca$^{2+}$ release and inhibition of L-channel activity

This study also reveals an intracellular Ca$^{2+}$ release induced by D2R stimulation. This novel finding provides the first evidence for dynamic D2R modulation of Ca$^{2+}$ mobilization in rat NAc MSNs. However, repeated cocaine exposure decreased the D2R effect. This decrease could be attributed in part to a reduction in D2R/Gi6 coupling and IP$_3$R activity, but not to a decrease in IP$_3$R levels. Such conclusion results from the following facts: First, PKA phosphorylation inhibits IP$_3$R activity and diminishes Ca$^{2+}$ release from endoplasmic reticulum (Cameron et al., 1995; Ferris et al., 1991; Quinton and Dean, 1992; Tertyshnikova and Fein, 1998). Second, D2R-coupled Ca$^{2+}$ modulation of ion channel activity involves inhibition of PKA activity and disinhibition of IP$_3$R (Hu et al., 2005b). Third, D2R-mediated suppression of Ca$^{2+}$ spikes relies on reduced L-channel activity via dephosphorylation of the channel by calcineurin (Day et al., 2002; Hernandez-Lopez et al., 2000). Fourth, repeated cocaine exposure increases D1R/Gi coupling (Terwilliger et al., 1991) and decreases D2R/Gi coupling (Nestler et al., 1990) as well as calcineurin levels and efficacy in NAc MSNs (Hu et al., 2005b). Fifth, the IP$_3$R protein levels are not changed by repeated cocaine exposure. Thus, reduced D2R modulation of intracellular Ca$^{2+}$ release by cocaine provides an informative knowledge for us to better understand the mechanism of cocaine withdrawal. Although the exact subtypes of D2R implicated in the cocaine-induced changes remain unknown (all D$_{2,3,4}$R are found in the NAc, but may or may not be in the same cells), these findings suggest that diminished D2R signaling plays an important role in dysregulating Ca$^{2+}$ homeostasis in NAc MSNs of cocaine-preexposed, abstinent rats.

Cocaine-induced adaptations in NAc MSNs persist during withdrawal

The reduced Ca$^{2+}$ influx, enhanced PKA activity, decreased intracellular Ca$^{2+}$ release, and abolished inhibition of L-channel activity mediated by D2R persisted for at least 21 days after cocaine withdrawal. These findings are consistent with earlier in vivo studies, showing that repeated cocaine exposure endurably increases D1R-modulated inhibition of NAc activity in cocaine-withdrawn rats (Henry and White, 1991; Henry et al., 1989). They are also correlated with cocaine-induced behavioral sensitization and neuroadaptations in synaptic activity of NAc cells, which may increase cocaine craving and/or relapse in response to cocaine and cocaine-related cues (Conrad et al., 2008; Kourrich et al., 2007). These findings are in agreement with the perspective that cocaine-induced neuroadaptations in dopamine signaling pathways and ion channel function decrease the intrinsic excitability (Dong et al., 2006; Zhang et al., 1998) and activity of NAc MSNs (Hu, 2007; Kalivas and Hu, 2006). Such decrease in NAc activity provides a support for the reduced basal activity in the reward circuitry revealed by brain imaging study in cocaine-abstinent human (Kufahl et al., 2005;
Volkow et al., 2003b), which may contribute to the neuropathophysiology of cocaine addiction.

It is worth noting that cocaine-induced changes in the L-channels, voltage-gated K⁺ channels, and PKA activity also occur in the medial prefrontal cortex (mPFC) (Dong et al., 2005; Ford et al., 2009; Nasif et al., 2005a,b), suggesting that cocaine-induced neuroadaptation preferentially affects function of the mesocorticolimbic dopamine system (a.k.a. the reward pathway). Given that both NAc and mPFC play critical roles in regulating cognitive function and addiction, these findings suggest that cocaine-induced neuroadaptation in neuronal activity may primarily or initially occur in the two brain regions.

**Functional implications**

Reduced D2R modulation of Ca\(^{2+}\) homeostasis in MSNs reveals dysregulation of the NAc after withdrawal from repeated cocaine exposure. These results are in agreement with previous findings, showing involvement of D2R dysfunction in the mechanisms of cocaine withdrawal. For instance, brain imaging studies in cocaine-abstinent humans indicate decreased D2R availability in striatal cells (Volkow et al., 2003b). This decrease (likely via phosphorylation-induced internalization) is correlated with reduced glucose metabolism and oxygen consumption in the orbital and medial PFC during cocaine abstinence (known as PFC hypoactivity) (Volkow et al., 2003a, 2007). Such changes reflect a decreased basal activity in the orbital-mPFC and reduced excitatory outputs from these cortical regions to the NAc (Kalivas and Hu, 2006) and therefore may contribute to the neuro-pathogenesis of cocaine withdrawal symptoms, including but not limited to depression, apathy, anhedonia, and drug seeking (Hu, 2007). Our novel findings provide support for the perspective that cocaine-induced behavioral changes are fundamentally based upon neuroadaptations in ion channel function and dopamine/Ca\(^{2+}\) signaling in the NAc and mPFC, in which D2R dysregulation, along with D1R dysfunction, plays a crucial role. Determining the altered D2R function in the NAc of cocaine-preexposed rats extends our knowledge to better understand the mechanisms of cocaine addiction, which may eventually help us to develop more effective therapeutic treatments for drug addiction.

**ACKNOWLEDGMENT**

The authors thank Dr. Francis J. White for his comments to this study.

**REFERENCES**

Anderson SM, Famous KR, Sadri-Vakili G, Kumaresan V, Schmidt HD, Bass CE, Termanliger EF, Cha JH, Pierce RC. 2008. CaMKII: A biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. Nat Neurosci 11:344–353.
Hu X-T. 2007. Cocaine withdrawal and neuro-adaptations in ion channel function. J Pharmacol Exp Ther 323:1505–1512.

Hu X-T, Basu S, White FJ. 2004. Repeated cocaine administration suppresses HVA-Ca\(^{2+}\) potentials and enhances activity of K\(^{-}\) channels in rat nucleus accumbens neurons. J Neurophysiol 92:1507–1516.

Hu X-T, Dong Y, Zhang XF, White FJ. 2005a. Dopamine D2 receptor-activated Ca\(^{2+}\) signaling modulates voltage-sensitive sodium currents in rat nucleus accumbens neurons. J Neurophysiol 93:1406–1417.

Hu X-T, Ford K, White FJ. 2005b. Repeated cocaine administration decreases calcineurin (PP2B) but enhances DARPP-32 modulation of sodium currents in rat nucleus accumbens neurons. Neuropharmacology 30:916–926.

Hyman SE, Malenka RC, Nestler EJ. 2006. Neural mechanisms of addiction: The role of reward-related learning and memory. Annu Rev Neurosci 29:665–698.

Kalivas PW, Hu X-T. 2006. Exciting inhibition in psychostimulant addiction. Trends Neurosci 29:610–616.

Khan ZL, Koulen P, Rubinstein M, Grandy DK, Goldman-Rakic PS. 2001. An astroglia-linked dopamine D2-receptor action in prefrontal cortex. Proc Natl Acad Sci USA 98:1964–1969.

Kourrich S, Rothwell PE, Klug JR, Thomas MJ. 2007. Cocaine exposure controls bidirectional synaptic plasticity in the nucleus accumbens. J Neurosci 27:7921–7928.

Kufahl PR, Li Z, Risinger RC, Rainen CJ, Wu G, Bloom AS, Li SJ. 2005. Neural responses to acute cocaine administration in the human brain detected by fMRI. Neuroimage 28:904–914.

Nasif FJ, Hu X-T, White FJ. 2005a. Repeated cocaine administration increases voltage-sensitive calcium currents in response to membrane depolarization in medial prefrontal cortex pyramidal neurons. J Neurosci 25:5674–5679.

Nasif FJ, Sidiropoulou K, Hu X-T, White FJ. 2005b. Repeated cocaine administration increases membrane excitability of pyramidal neurons in the rat medial prefrontal cortex. J Pharmacol Exp Ther 315:1305–1313.

Nestler EJ. 2004. Historical review: Molecular and cellular mechanisms of opiate and cocaine addiction. Trends Pharmacol Sci 25:210–218.

Nestler EJ, Terviliger RZ, Walker JR, Sevarino KA, Duman RS. 1990. Chronic cocaine treatment decreases levels of the G protein subunits Gi alpha and Go alpha in discrete regions of rat brain. J Neurochem 55:1073–1082.

Nguyen QT, Callamaras N, Hsieh C, Parker I. 2001. Construction of a two-photon microscope for video-rate Ca\(^{2+}\) imaging. Cell Calcium 30:383–393.

Pasik P. 1979. The internal organization of the neostriatum mamals. In: Divac I, Oberg RGE, editors. The neostriatum. Oxford: Pergamon Press. p 5–36.

Pedrosa R, Gomes P, Zeng C, Hopfer U, Jose PA, Soares-da-Silva P. 2004. Dopamine D3 receptor-mediated inhibition of Na\(^{+}/H\) exchanger activity in normotensive and spontaneously hypertensive rat proximal tubular epithelial cells. Br J Pharmacol 142:1345–1353.

Phillips PE, Stamford JA. 2000. Differential recruitment of N-, P- and Q-type voltage-operated calcium channels in striatal dopamine release evoked by ‘regular’ and ‘burst’ firing. Brain Res 884:139–146.

Quinton TM, Dean WL. 1992. Cyclic AMP-dependent phosphorylation of the inositol-1,4,5-trisphosphate receptor inhibits Ca\(^{2+}\) release from platelet membranes. Biochem Biophys Res Commun 184:883–890.

Robbins TW, Everitt BJ. 2002. Limbic-striatal memory systems and drug addiction. Neurobiol Learn Mem 76:625–636.

Scheffgi S, Raone A, De Montis MG, Tagliamonte A, Gambarara C. 2007. Behavioral expression of cocaine sensitization in rats is accompanied by a distinct pattern of modifications in the PKA DARPP-32 signaling pathway. J Neurochem 103:1185–1183.

Schramm-Saptya NL, Morris RW, Kuhn CM. 2006. Adolescent rats are protected from the conditioned aversive properties of cocaine and lithium chloride. Pharmacol Biochem Behav 84:344–352.

Self DW, Mclenahan AW, Beitner-Johnson D, Terviliger RZ, Nestler EJ. 1995. Biochemical adaptations in the mesolimbic dopamine system in response to heroin self-administration. Synapse 21:312–318.

Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ. 1998. Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J Neurosci 18:1848–1859.

Senogles SE. 2000. The D2s dopamine receptor stimulates phospholipase D activity: A novel signaling pathway for dopamine. Mol Pharmacol 58:455–462.

Spear L. 2000. Modeling adolescent development and alcohol use in animals. Alcohol Res Health 24:115–123.

Stutzmann GE, Laferla FM, Parker I. 2003. Ca\(^{2+}\) signaling in mouse cortical neurons studied by two-photon imaging and photo-released inositol triphosphate. J Neurosci 23:756–765.

Surmeier DJ, Bargas J, Hemmings HC Jr, Nairn AC, Greengard P. 1995. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. Neuron 14:385–397.

Tertsysnikova S, Fein A. 1998. Inhibition of inositol 1,4,5-trisphosphate-induced Ca\(^{2+}\) release by cAMP-dependent protein kinase in a living cell. Proc Natl Acad Sci USA 95:1613–1617.

Terviliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM, Nestler EJ. 1991. A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. Brain Res 548:100–110.

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Gifford A, Hitzemann R, Ding YS, Pappas N. 1999. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. Am J Psychiatry 156:1440–1443.

Volkow ND, Fowler JS, Wang GJ, Zhang H, Jenks BG, Ciccarelli A, Roubos EW, Scheenen WJ. 2004. Dopamine D2-receptor activation differentially inhibits N- and R-type Ca\(^{2+}\) channels in Xenopus melanotrope cells. Neuroendocrinology 80:368–378.