Title: Dopamine D₂ Receptor Modulation of K⁺ Channel Activity Regulates Excitability of Nucleus Accumbens Neurons at Different Membrane Potentials

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Running head: D₂R modulation of K⁺ channel function

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Abstract

The nucleus accumbens (NAc) is a forebrain area in the mesocorticolimbic dopamine (DA) system that regulates many aspects of drug addiction. Neuronal activity in the NAc is modulated by different subtypes of DA receptors. Although DA signaling has received considerable attention, the mechanisms underlying D2-class receptor (D2R) modulation of firing in medium spiny neurons (MSNs) localized within the NAc remain ambiguous. In the present study, we performed whole-cell current-clamp recordings in rat brain slices to determine whether and how D2R modulation of K⁺ channel activity regulates the intrinsic excitability of NAc neurons in the core region. D2R stimulation by quinpirole or DA significantly and dose-dependently decreased evoked Na⁺ spikes. This D2R effect on inhibiting evoked firing was abolished by antagonism of D2Rs, reversed by blockade of voltage-sensitive, slowly-inactivating A-type K⁺ currents (I_As), or eliminated by holding membrane potentials at levels in which I_As was inactivated. It was also mimicked by inhibition of cAMP-dependent protein kinase (PKA) activity, but not phosphatidylinositol-specific phospholipase C (PI-PLC) activity. Moreover, D2R stimulation also reduced the inward rectification and depolarized the resting membrane potentials (RMP) by decreasing “leak” K⁺ currents. However, the D2R effects on inward rectification and RMP were blocked by inhibition of PI-PLC, but not PKA activity. These findings indicate that, with facilitated intracellular Ca²⁺ release and activation of the D2R/Gq/PLC/PIP₂ pathway, the D2R-modulated changes in the NAc excitability are dynamically regulated and integrated by multiple K⁺ currents, including but are not limited to I_As, inwardly rectifying K⁺ currents (I_Kir), and “leak” currents (I_K-2p).
Introduction

The NAc is a limbic structure innervated by the DAergic input from the ventral tegmental area of the midbrain and the glutamatergic input from the mPFC. This brain region is involved in control of cognitive tasks (Pennartz et al. 1994; Nicola et al. 2000; Kalivas et al. 2005). Activity of NAc neurons is regulated by both DA D1 and D2-class receptors (White and Wang 1986; Hu and White 1997; Hu et al. 2005). Dysfunction in the mesocorticolimbic DA system has been implicated in certain neuropsychiatric disorders, including drug addiction (White and Kalivas 1998; Wise 1998; Hyman and Malenka 2001; Hyman 2005; Kalivas et al. 2005; Kalivas and Hu 2006). Previous findings have indicated that D2R activation modulates the intrinsic excitability of DA-innervated neurons, usually causing a decrease in evoked action potentials (Hu and Wang 1988; Gulledge and Jaffe 1998; Cepeda et al. 2001; West and Grace 2002; Tseng and O'Donnell 2004). This inhibitory effect of D2R modulation on evoked Na+ spike firing has been related to activation of a variety of K+ currents (Greif et al. 1995; Congar et al. 2002; Ljungstrom et al. 2003). However, the mechanisms underlying activation of these K+ currents remain unknown.

However, previous findings have also revealed some excitatory effects of D2R stimulation on evoked neuronal activity. For instance, co-activation of the D2R and the D1R can depolarize cell membrane in dorsal striatal cells and increase evoked Na+ spike firing in NAc shell cells by inhibition of Na+/K+ ATPase and combination of G_{i/o} subunits released from D2R/G_{i/o} coupling and G_{as}-like subunits from D1R/G_{s} coupling, respectively (Bertorello et al. 1990; Hopf et al. 2003). Moreover, D2R stimulation also facilitates Ca2+ mobilization (Parikh et al. 1996; Greengard et al. 1999), leading to an enhancement in voltage-sensitive sodium currents via facilitating dephosphorylation of the Na+ channel by calcineurin (Hu et al. 2005). In addition,
stimulation of D₂Rs also reduces the inward rectification in freshly-dissociated mPFC pyramidal neurons by inactivating the cAMP/PKA cascade (Dong et al. 2004), which may induce membrane depolarization from the resting levels if the “leak” K⁺ currents are suppressed. In spite of the apparent discrepancies in the D₂R regulation of ion channel/pump activity that have caused many controversies in the past with respect to the inhibitory or excitatory effects of D₂R stimulation, these findings actually indicate that the D₂R is functionally involved in modulation of the intrinsic excitability of DA-innervated neurons by dynamically integrating activity of voltage-gated ion channels, including various types of K⁺ channels at different membrane potentials.

Given the above, we hypothesized that D₂R stimulation would significantly change the excitability of medium spiny NAc neurons by modulating the function of a variety of K⁺ channel subtypes. The present study was performed to determine whether and how D₂R stimulation changes the evoked Na⁺ spike firing, the inward rectification, and the RMP by modulating the activity of VGKCs (mainly IA), IKir, and the “leak” K⁺ currents (IK-2P) in medium spiny neurons located within the core NAc of rats.
Material and methods

Animals

Adolescent male Sprague-Dawley rats (Harlan Indianapolis, IN) at age of 4-5 week-old (Spear 2000) were group housed in a temperature- and humidity-controlled vivarium under a 12 hr light/dark cycle. Food and water were freely available. After ≥ 3 days acclimation to the vivarium, rats were used for acute experiments examining the D2R modulation of MSNs membrane activity.

Preparation of Brain Slices

All procedures were in strict accordance with the Guide for the Care and use of Laboratory Animals (1996) and were approved by our Institutional Animal Care and Use Committee. Rats were decapitated under halothane anesthesia and the brain was immediately excised and immersed in ice-cold artificial cerebrospinal fluid (aCSF) 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) and incubated in oxygenated (95% O2/5% CO2) aCSF for 1 hr at room temperature before recording.

Whole-cell Current Clamp Recordings in Brain Slices

Brain slices were anchored in a recording chamber and perfused by gravity-fed oxygenated aCSF (34°C) with the GABA_A receptor blocker SR-95531 (4 mM) and the glutamatergic receptor blocker kynurenic acid (2.5 mM) at a flow rate of 2-3 ml/min. Patch recording pipettes (3-5 MΩ) were pulled from Corning 7056 (Corning, NY) glass capillaries with horizontal pipette puller (Flaming/Brawn P-97, Sutter Instruments, CA) and filled with
internal recording solution (in mM): K⁺-gluconate 120, HEPES 10, KCl 20, MgCl₂ 2, Na₂ATP 3, Na₂GTP 0.3, biocytin 0.1 %. Recordings were initiated in visually identified MSNs within the core of the NAc using differential interference contrast (DIC) microscopy (Stuart et al. 1993) and an Axopatch 200B amplifier (Axon Instruments, Union City, CA). After a whole-cell configuration was formed, voltage-clamp mode was converted to current-clamp recording. Voltage signals were amplified in bridge mode and digitized by a DigiData 1200 Series (Axon Instruments) and distributed to a computer running pCLAMP 9 software (Axon Instruments).

Na⁺-dependent action potentials were generated by injection of step depolarizing current pulses with 0.05 nA increments, ranging from 0 to 0.5 nA. Characteristics of the action potentials were obtained from the initial spike evoked by the minimal depolarizing current pulse (rheobase) in each MSN recorded. In all cases Na⁺ spikes were evoked from RMP. The amplitude of action potential (mV) was measured from spike threshold to peak level. The deepness of afterhyperpolarization (AHP amplitude, mV) was measured from the equipotential point of the spike threshold to the maximum deflection of the membrane hyperpolarization after the end of the action potential. The half-action potential duration was measured at half-amplitude levels.

To determine the effects of applied drugs on evoked Na⁺ spikes as well as on hyperpolarized membrane potentials, the majority of NAc neurons were recorded under a condition in which RMP was held at -80 mV. This approach gave each individual NAc neuron the same basal (control) potential level and therefore made the results obtained from different cells comparable (Hu et al. 2004). In MSN, it has been shown that secondary dendrites are a crucial cellular compartment for postsynaptic D2 modulation of AMPA currents (Hernandez-
Echeagaray et al. 2004). To avoid influences from depolarization/hyperpolarization of dendritic membrane in the soma, AMPA and NMDA currents were blocked by kynurenic acid applied in the bath medium (see above). In another group of cells, the drug effects on evoked Na⁺ spikes were studied by holding resting membrane potential at -50 mV. In these cells, Na⁺-dependent action potentials were generated by injection of step depolarizing current pulses with 0.04 nA increments ranging from 0 to 0.2 nA. Injection of higher currents caused artificial distortions in the form of Na⁺ spikes, which made analysis of evoked action potentials very difficult. Under this depolarized RMP, certain voltage-gated K⁺ channels were inactivated and their effects on action potentials were eliminated. In addition, to determine the effects of applied drugs on RMP, a group of NAc neurons was recorded at their resting status without any membrane potential holding. To calculate percent change in spiking for the time-course experiments, a current pulse was selected to evoke six or seven spikes as basal activity. This current pulse was then used at different time points in all cells. Firing rates of evoked spikes were averaged from a two-minute time period before drug application in each cell. Such values were then normalized to 100% as control and used to compare with that during drug application. To calculate the % of change in the dose-response curve, the firing rate of evoked spikes was averaged from the last two minutes of quinpirole perfusion (the 3rd to 5th min) in which quinpirole achieved its maximal effect, and then compared to the normalized control (100 %).

The current-voltage relationship (I-V curve) was studied with perfusion of the specific Na⁺ channel blocker tetrodotoxin (TTX, 1 μM) and the calcium channel blocker cadmium (Cd²⁺, 200 μM). Five minutes after TTX and Cd²⁺ application, the cell membrane was hyperpolarized by injecting negative current pulses (200 msec duration, -0.8 to 0 nA). Under these conditions,
recorded changes in the I-V curve would reflect activation or inactivation of $I_{Kir}$ (Nisenbaum and Wilson 1995). Membrane properties were studied in the following manners: RMP was measured in the absence of injected current, the input resistance ($R_{in}$, MΩ) was determined from linear regression in the linear range (± 0.1 nA range) of the I-V curve established by plotting the steady-state potential change in response to hyperpolarizing current pulses. Time constants were determined by the fit function of pCLAMP software. The whole-cell pipette series resistance was less than 20 MΩ and bridge was compensated. Only NAc cells that had a stable RMP at or more negative than -75 mV with evoked spikes that overshot across 0 mV membrane potentials were used for analysis of membrane properties and further drug treatment.

**Drug Application**

Separate subgroups of NAc neurons were recorded with application of different drugs and ion channel blockers. Selective agonist and antagonist for D2Rs (quinpirole, 10 µM and eticlopride, 10 µM, respectively) were used to determine whether D2R stimulation affects the evoked action potentials, the inward rectification during membrane hyperpolarization, and RMP. Moreover, the effects of D2R agonists on evoked Na+ spikes were also studied when the membrane potentials were held at a more depolarized membrane potential level (approximately -50 mV). In addition, the effects of DA on evoked firing were studied with different concentrations (0, 20, 40 and 80 µM). DA at a concentration of 40 µM was used in the time-course experiment. To minimize oxidation, all experiments with DA were conducted in the dark. To confirm the effects of quinpirole on D2R-mediated inhibition of firing, the D1R antagonist SCH-23390 (10 µM) was concurrently applied with DA in the time-course experiments. The selective PKA inhibitor H-89 (10 µM) was applied in bath solution, whereas Rp-cAMPs (500
μM), another selective PKA inhibitor, was dialyzed to the cytosol via the recording pipette. Since internally applied Rp-cAMPs might affect RMP during formation and stabilization of whole-cell configuration and thereby causing inaccurate measurement of control RMP, data from other experimental groups were pooled to form the control group. Measurements of RMP from this group were then compared with that affected by Rp-cAMPs and quinpirole plus Rp-cAMPs, respectively. The selective phosphatidylinositol-specific PLC (PI-PLC) inhibitor (ET-18-OCH₃, 1-O-Octadecyl-2-O-methyl-sn-glycero-3-phosphorylcholine, 500 μM) was externally applied in the aCSF medium. These inhibitors were used to determine whether the D₂R-mediated changes in NAc neurons were modulated via the cAMP/PKA cascade or through other signaling pathway(s) associated with activation of PLC.

The relatively selective A-type K⁺ channel blocker 4-aminopyridine (4-AP, at a low concentration of 10 μM) was used to determine whether and how IA was involved in the D₂R-mediated changes in evoked action potentials. All drugs were prepared according to manufacturer’s specifications (SIGMA Chemicals, St. Louis, MO). Bath solutions with such drugs and ion channel blockers were made with aCSF immediately before use.

Statistical Analysis

Unpaired Student’s t-tests were used to estimate the significance of the difference (*p<0.05 and **p<0.01) in the membrane properties between control and drug-treated groups of NAc neurons. Repeated measures analysis of variance (ANOVA) was used for comparison of the drug-induced changes in the current- response (evoked spikes) curves as well as in the inward rectification curves between control and drug-treated groups. Comparisons of the drug-induced
alterations in evoked action potentials in the time-course experiments were carried out with
ANOVA. In addition, post-hoc comparisons were carried out using the Newman-Keuls test.
Results

*D2R stimulation decreased evoked Na⁺ spikes in NAc neurons*

All NAc neurons were MSNs as evidenced by DIC microscopy and recorded within the core region (O'Donnell and Grace 1993; Hu et al. 2004). The majority of neurons has shown a slow, repetitive spike-firing pattern reported for typical MSNs (Nisenbaum et al. 1994; Mahon et al. 2000). To study the effects of D2R stimulation on the properties of evoked Na⁺ spikes, a series of current pulses (200 msec) was delivered to NAc neurons, at an interval of 20 sec between each pulse. These current pulses ranged from 0 to +0.5 nA, in 0.05 nA steps (Fig. 1A). Current-evoked spike response curves showed that bath application of quinpirole (10 µM) significantly and reversibly decreased evoked action potentials in the majority of MSNs (13 of 20 cells recorded, 65%) (control vs. quinpirole, n=20 cells, repeated measures ANOVA, $F_{1, 38} = 6.89$, $p<0.05$; post-hoc Newman-Keuls test, *$p<0.05$) (Fig. 1B). Quinpirole dose-dependently decreased evoked Na⁺ spikes at all doses tested (2, 4, 6, and 10 µM) (n= 10 cells for each concentration, unpaired *t*-test, *$p<0.05$) (Fig. 1C). Because greater reduction in firing was observed with 10 µM of the D2R agonist (30.6 ± 3.1 %), this concentration was used for later experiments in this study. In time-course experiments, quinpirole-induced reduction in firing occurred quickly and achieved its maximal levels 3-5 min with application of the agonist (n=13 cells; ANOVA, $F_{77, 803} = 4.75$, $p<0.05$; post-hoc Newman-Keuls test, $p<0.05$) (Fig. 1D-1F).

The effects of DA (10, 20, 40, and 80 µM) on evoked firing were also studied in NAc cells. Bath application of DA significantly and reversibly decreased evoked action potentials at 40 and 80 µM (control vs. DA 40 µM: 100.3 ± 0.29 % vs. 87.2 ± 0.5 %; control vs. DA 80 µM: 100.3 ± 0.29 % vs. 86.6 ± 0.7 %, respectively; n=10 cells for each concentration, unpaired *t*-test,
* p<0.05) (Fig. 2A and B). To further determine whether the quinpirole-induced decrease in evoked firing was mediated by D2Rs, DA (40 µM) was concurrently applied with SCH-23390 (10 µM), a selective D1R antagonist. Similar to quinpirole, combined application of DA and SCH-23390 induced a significant and reversible decrease in evoked Na\(^+\) spikes (n=11 cells, ANOVA, \(F_{45, 460} = 3.48, p<0.05\); post-hoc Newman-Keuls test, \(p<0.05\)) (Fig. 2C and D). Furthermore, this DA-mediated reduction in evoked Na\(^+\) spikes was completely abolished when the D2R antagonist eticlopride (10 µM) was applied concurrently with DA (40 µM) and SCH-23390 (10 µM). Under these experimental conditions there was no significant difference in evoked firing along the time course (n=8 cells, control vs. DA plus SCH-23390 and eticlopride, ANOVA, \(F_{45, 322}=0.49, p>0.05\)) (Fig. 2E and F). This result confirms that the DA- or quinpirole-induced reduction in evoked Na\(^+\) spikes is selectively mediated by activation of the D2R.

The D2R-mediated reduction in evoked firing was accompanied by significant alterations in certain membrane properties, including increased rheobase (control vs. quinpirole: 0.23 ± 0.01 vs. 0.27 ± 0.02 nA, n=20 cells, paired \(t\)-test, \(p<0.05\)) and reduced threshold of action potential (control vs. quinpirole: -40.25 ± 0.68 vs. -42.06 ± 0.92 mV, n=20 cells; paired \(t\)-test, \(p<0.05\)). There were no significant changes in the input resistance, spike amplitude, duration of action potential measured at the half-amplitude level (half AP duration), and amplitude of AHP (control vs. quinpirole: 113.34 ± 10.68 vs. 109.41 ± 9; 83.58 ± 1.62 vs. 82.55 ± 1.71; 0.95 ± 0.03 vs. 0.97 ± 0.04; 13.92 ± 0.59 vs. 13.15 ± 0.61 mV, respectively, n=20 cells, paired \(t\)-test, \(p>0.05\)).

D2R-mediated inhibition in evoked firing was blocked by concurrent application of eticlopride, a selective D2R antagonist. Nevertheless, since some MSNs did not show reduction
in evoked spikes in response to D₂R stimulation, this antagonist experiment was performed only in spiny cells that showed agonist effect. The D₂R-mediated inhibition in spike firing was identified first and washed out. Then these cells were recorded with concurrent application of quinpirole and eticlopride with the same concentration (10 µM). Under these conditions, quinpirole-induced reduction in evoked firing was completely blocked by eticlopride (n=6 cells, ANOVA, $F_{52, 340} = 3.56, p< 0.05$, post-hoc Newman-Keuls test, *$p<0.05$) (Fig. 3A and B).

*Inhibition of PKA, but not PLC, mimicked the D₂R-mediated decrease of evoked Na⁺ spikes*

It is well-established that activation of D₂Rs inhibits adenylyl cyclase (AC) activity, thereby reducing cytosolic cAMP levels and PKA activity (Stoof and Kebabian 1981; 1982; Sibley 1995). Therefore, if the D₂R-mediated decrease in evoked Na⁺ spikes was regulated by the cAMP/PKA cascade, inhibition of PKA activity should resemble the effects of quinpirole on suppressing evoked action potentials. Indeed, direct inhibition of PKA activity by internally dialyzed Rp-cAMPs (500 µM) mimicked this effect of D₂R stimulation on decreasing Na⁺ spikes with greater potency (control vs. Rp-cAMPs: n=13 cells, repeated measures ANOVA, $F_{1, 22} = 60.41, p<0.01$; post-hoc Newman-Keuls test, *$p<0.05$) (Fig. 4A and B). Under this condition, the inhibitory effect of D₂R stimulation on firing was occluded and quinpirole was no longer able to produce further reduction in the evoked spikes. Thus, there was no significant difference in the current-spike response curves between NAc neurons recorded with application of Rp-cAMPs alone and those treated with the PKA inhibitor plus quinpirole (Rp-cAMPs vs. Rp-cAMPs + quinpirole: n=11 cells, repeated measures ANOVA, $F_{1, 20} = 0.2, p>0.05$) (Fig. 4B). Increasing the depolarizing currents to the levels greater than 0.5 nA did not induce further increase in evoked Na⁺ spikes in either Rp-cAMPs-treated or Rp-cAMPs plus quinpirole-treated cells,
indicating that a maximal effect on blocking PKA activity to suppress action potential had been achieved (data not shown). Moreover, bath-applied H-89 (10 μM), a different PKA inhibitor, also decreased evoked Na⁺ spikes and occluded the inhibitory effects of quinpirole on evoked firing (control vs. H-89: n=6 cells, repeated measures ANOVA, $F_{1, 10} = 7.9, p<0.05$, post-hoc Newman-Keuls test, *$p<0.05$; H-89 vs. H-89 + quinpirole: n=6 cells, repeated measures ANOVA, $F_{1, 10} = 0.2, p>0.05$) (Fig. 4C and D).

In contrast, inhibition of PI-specific PLC by externally applied ET-18-OCH₃ (500 μM) failed to affect the ability of D₂R stimulation in decreasing evoked Na⁺ spikes. Under this condition, quinpirole-induced reduction in evoked firing was not affected by inhibition of PI-PLC (control vs. ET-18-OCH₃ + quinpirole: n=12 cells, repeated measures ANOVA, $F_{1, 22} = 5.06, p<0.05$, post-hoc Newman-Keuls test, *$p<0.05$) (Fig. 5). Nevertheless, there was still a significant difference in evoked spikes between MSNs treated with ET-18-OCH₃ alone and ET-18-OCH₃ plus quinpirole (ET-18-OCH₃ vs. ET-18-OCH₃ + quinpirole: n=12 cells, repeated measures ANOVA, $F_{1, 22} = 4.93, p<0.05$, post-hoc Newman-Keuls test, *$p<0.05$) (Fig. 5). In addition, ET-18-OCH₃ by itself did not cause any significantly changes in evoked Na⁺ spikes (control vs. ET-18-OCH₃, n=12 cells; repeated measures ANOVA, $F_{1, 22} = 0.0035, p>0.05$) (Fig. 5).

*Participation of slow-inactivating A-type K⁺ current in D₂R-mediated reduction of Na⁺ spikes*

Previous studies indicate that one of the major determinants of neuronal firing are VGKCs (or delayed rectifiers) (Hille 2001). Neostriatal MSNs possess at least three types of VGKCs, including two types of A-currents (e.g., slow-inactivating A-type K⁺ current, $I_{A\text{ss}}$, and
fast-inactivating A-type K⁺ current, \( I_{\text{Af}} \) and a non-inactivating K⁺ current (Surmeier et al. 1991; 1992). Although \( I_{\text{As}} \) makes a relatively minor contribution to the total amount of K⁺ currents generated by VGKCs (Surmeier and Kitai 1993; Hopf et al. 2003), it plays an important role in controlling spike firing, because inhibition of \( I_{\text{As}} \) increases evoked Na⁺ spike firing (Nisenbaum et al. 1994; Wickens and Wilson 1998; Mahon et al. 2000). In addition, most striatal MSNs show \( I_{\text{As}} \) with absence of \( I_{\text{Af}} \) (Surmeier and Kitai 1993), while D₂R stimulation increases \( I_{\text{As}} \) in these cells (Surmeier and Kitai 1993).

To determine whether \( I_{\text{As}} \) was functionally involved in the D₂R-mediated reduction in evoked Na⁺ spikes in NAc MSNs, 4-AP (10 µM), a relatively selective inhibitor for \( I_{\text{As}} \) at a concentration range of 5-60 µM (Surmeier et al. 1991), was used in our experiments. Bath application of 4-AP markedly increased evoked action potentials in all MSNs recorded (n=13 cells). Under this condition, quinpirole failed to inhibit evoked Na⁺ spikes (Fig. 6A). Current-spike response curves indicate that there was a significant increase in the number of evoked action potentials in 4-AP-treated cells as compared to that in control group (control vs. 4-AP: n=13 cells, repeated measures ANOVA, \( F_{1, 24} = 4.5, p<0.05 \); post-hoc Newman-Keuls test, \( *p<0.05 \) (Fig. 6B). There also was a significant difference in evoked spikes between NAc neurons treated with 4-AP plus quinpirole and control cells without drug treatment (control vs. 4-AP + quinpirole: n=13 cells, repeated measures ANOVA, \( F_{1, 24} = 4.42, p<0.05 \); post-hoc Newman-Keuls test, \( *p<0.05 \)). In contrast, there was no significant difference in the number of evoked spikes between NAc neurons treated with 4-AP alone and 4-AP plus quinpirole (4-AP vs. 4-AP + quinpirole: n=13 cells, repeated measures ANOVA, \( F_{1, 24} = 0.02, p>0.05 \) (Fig. 6B).
To confirm the involvement of $I_{As}$ in inhibiting evoked spikes mediated by D2Rs, we took advantage of the different response characteristics of $I_{As}$ and $I_{Af}$ to voltage-dependent inactivation. In a group of cells, RMP was held at –50 mV that resulted in inactivation of $I_{As}$ (Gabel and Nisenbaum 1998; Surmeier et al. 1991; 1994). To study the properties of evoked Na$^+$ spikes either with or without D2R stimulation, current pulses were applied from 0 to +0.2 nA with 0.04 nA increments. Under this circumstance, D2R stimulation by quinpirole did not cause any significant changes in evoked firing with application of different current intensities (control vs. quinpirole: n=10 cells, repeated measures ANOVA, $F_{1, 6} = 5.93, p>0.05$) (Fig. 7A and B).

**D2R stimulation reduced inward rectification during membrane hyperpolarization**

One of the defining electrophysiological properties of striatal MSNs is a pronounced inward rectification, which mainly represents activation of inwardly rectifying K$^+$ currents ($I_{Kir}$) evoked by membrane hyperpolarization (Nisenbaum and Wilson 1995). To determine whether D2R stimulation affected the activity of $I_{Kir}$, the inward rectification was studied by comparing the I-V curves across cells during membrane hyperpolarization between NAc neurons with or without D2R stimulation. Furthermore, since D2R stimulation can not only decrease activity of the cAMP/PKA cascade (Sibley 1995; Stoof and Kebabian 1981; 1982), but also activate the PLC$\beta$1-IP$_3$-calcineurin-signaling cascade in striatal MSNs (Hernandez-Lopez et al. 2000), we also investigated whether the potential changes in $I_{Kir}$ mediated by D2Rs were related to the two distinct signaling pathways.

With injection of negative current pulses (0 to -0.8 nA), membrane potentials of control neurons were hyperpolarized from the initial level of -80 mV to approximately -125 mV. An
apparent inward rectification was induced, indicating activation of $I_{\text{Kir}}$ (and other hyperpolarization-activated currents). Quinpirole significantly attenuated the inward rectification, leading the membrane potential to a more hyperpolarized level (shifting the I-V plot downward and inducing greater linearity) (control vs. quinpirole: n=13 cells, repeated measures ANOVA, $F_{1,21} = 7.4, p<0.02$; post-hoc Newman-Keuls test, $*p<0.05$) (Fig. 8A and B).

However, this D$_2$R-action on reducing the inward rectification was completely blocked by concurrent bath application of the PI-PLC inhibitor ET-18-OCH$_3$ (500 µM) (Fig. 8A). There was a significant difference in the I-V curves between NAc neurons recorded with quinpirole alone and cells recorded with ET-18-OCH$_3$ plus quinpirole (quinpirole vs. ET-18-OCH$_3$ + quinpirole: n=10 cells, repeated measures ANOVA, $F_{1,18} = 10.07, p<0.01$; post-hoc Newman-Keuls test, $*p<0.05$) (Fig. 8B). There was no significant difference between control NAc cells and neurons treated with ET-18-OCH$_3$ plus quinpirole (control vs. ET-18-OCH$_3$ + quinpirole: n=10 cells, repeated measures ANOVA, $F_{1,18} = 0.15, p>0.05$) (Fig. 8B). In addition, inhibition of PI-PLC by ET-18-OCH$_3$ alone caused no significant change in the inward rectification (control vs. ET-18-OCH$_3$: n=10 cells, repeated measures ANOVA, $F_{1,17} = 0.13, p>0.05$) (Fig. 8A and B).

Unlike inhibition of PI-PLC, inhibition of PKA activity by internally dialyzed Rp-cAMPS (500 µM) did not block the D$_2$R-mediated reduction in the inward rectification (Rp-cAMPS vs. Rp-cAMPS + quinpirole: n=13 cells, repeated measures ANOVA, $F_{1,24} = 8.62, p<0.01$; post-hoc Newman-Keuls test, $*p<0.05$). In addition, cytosolic application of Rp-cAMPS alone produced no significant change in the inward rectification of NAc neurons (control vs. Rp-cAMPS: n=13 cells, repeated measures ANOVA, $F_{1,24} = 1.61, p>0.05$) (Fig. 8A and C).
D<sub>2</sub>R stimulation depolarized RMP

It is well-known that, despite their name, certain inward rectifiers carry outward K<sup>+</sup> currents to maintain RMP of neurons (Hille 2001). Blockade of these out-flowing K<sup>+</sup> currents leads to depolarization from RMP (Nasif et al. 2005). Because these K<sup>+</sup> channels belong to the superfamily of the inward rectifier and activity of inwardly rectifying K<sup>+</sup> channels is reduced with D<sub>2</sub>R stimulation (see above), we also studied whether RMP of NAc neurons was modulated by D<sub>2</sub>Rs. In this experiment, RMP was not clamped during recording. Quinpirole induced a small but significant membrane depolarization in all NAc neurons recorded (control vs. quinpirole: -82.92 ± 0.63 vs. -80.73 ± 0.85 mV, n=14 cells, paired t-test, *p<0.05). This effect of quinpirole on RMP was washed out and returned to more hyperpolarized levels (Fig. 9A). There was no significant difference in RMP recorded from cells in control group vs. washout group (-82.92 ± 0.63 vs. -82.47 ± 1.27 mV, n=14 cells, paired t-test, p>0.05) (Fig. 9B). However, concurrent bath application of the selective PI-PLC inhibitor ET-18-OCH<sub>3</sub> blocked this effect of D<sub>2</sub>R stimulation on RMP (control vs. quinpirole + ET-18-OCH<sub>3</sub>: -80.73 ± 0.87 vs. -80.15 ± 0.77 mV, n=12 cells; paired t-test; p>0.05), without inducing any significant changes in RMP (control vs. ET-18-OCH<sub>3</sub>: -80.73 ± 0.87 vs. -80.66 ± 1.08 mV, n=12 cells, paired t-test, p>0.05) (Fig. 9C). In contrast, co-application of Rp-cAMPs failed to block quinpirole-induced depolarization in RMP (Rp-cAMPs vs. Rp-cAMPs + quinpirole: -80.01 ± 0.7 vs. -78.01 ± 0.74 mV; n=11 cells, paired t-test, *p<0.05; and control vs. Rp-cAMPs + quinpirole: -80.6 ± 0.6 vs. -78.01 ± 0.74 mV, n=11 cells, unpaired t-test; **p<0.01) (Fig. 9D). Rp-cAMPs alone did not produce significant change in RMP as compared to control (control vs. Rp-AMPs: -80.6 ± 0.6 vs. -80.01 ± 0.7 mV, n=11 cells, unpaired t-test, p>0.05).
Discussion

The present study has demonstrated that the D₂R-mediated reduction in evoked action potentials was receptor-specific which involves inhibition of PKA activity and activation of $I_{As}$. We have also determined that D₂R stimulation attenuated the inward rectification in response to membrane hyperpolarization, indicating a decreased $I_{Kir}$. In addition, D₂R stimulation induced a small but significant RMP depolarization, revealing a reduction in “leak” K⁺ currents. These findings indicate that D₂Rs modulate the intrinsic excitability of NAc cells with integrated regulation of different K⁺ channel types via multiple signaling pathways.

$D₂R$-mediated $I_{As}$ activation decreases evoked Na⁺ spikes

The major finding of this study is that D₂R stimulation, either by DA or the selective D₂R agonist quinpirole, suppressed evoked Na⁺ spikes. The predominant mechanism underlying the D₂R-modulated suppression of evoked Na⁺ spikes should be attributed to activation of $I_{As}$ in the core NAc cells. K⁺ channels are one of the key regulators of the intrinsic excitability in both dorsal and ventral striatal MSNs (Surmeier and Kitai 1993; 1997; Wickens and Wilson 1998; Hu et al. 2004). There are at least three major K⁺ currents activated by membrane depolarization in striatal MSNs (Surmeier et al. 1991; Nisenbaum and Wilson 1995). Among them, $I_{As}$ plays an important role in regulating firing (Nisenbaum et al. 1994; Wickens and Wilson 1998; Mahon et al. 2000). D₂R stimulation usually enhances $I_{As}$ though D₁R stimulation decreases the current in striatal MSNs (Surmeier and Kitai 1993). Associated with D₂R stimulation, evoked action potentials are decreased in dorsal striatal cells, not only with increased $I_{As}$ but also with decreased Ca²⁺ influx through L-type Ca²⁺ channels which is mediated by a novel $G_{βγ/PLC_β1/IP_3/Ca^{2+}}$/calcineurin pathway (Hernandez-Lopez et al. 2000).
Accordingly, findings from the present study have indicated further that D2R-modulated reduction in evoked firing recorded in MSNs of the core NAc should be attributed primarily to activation of A-type K\(^+\) channels because either blockade or inactivation of A-type K\(^+\) channels abolishes the D2R-mediated inhibition in evoked Na\(^+\) spikes. These results are consistent with and supportive of previous findings regarding the inhibitory effects of A-type K\(^+\) channels on evoked action potentials in striatal MSNs. Interestingly, \(I_{As}\) is also found to be inhibited by co-activation of D1Rs and D2Rs in NAc cells located within the shell region, leading to an increase in evoked firing (Hopf et al. 2003). However, unlike activation of A-type K\(^+\) channels in the core NAc cells which is regulated most likely by the D2R and neuronal Ca\(^{2+}\) sensor proteins (see below), increased firing in the shell NAc cells is mediated by combined G\(_{\gamma}\) subunits released from D2R/Gi/o coupling and G\(_{\alpha}\)-like subunits from D1R/Gs coupling (Hopf et al. 2003). In addition, increased firing in striatal cells can also be induced by co-activation of the D1R and D2R via a synergistic inhibition of Na\(^+\)-K\(^+\) ATPase (Bertorello et al. 1990). Given the above, we propose that (1) activation of the D2R in different NAc circuitries modulates activity of various types of ion channels by regulating multiple signaling pathways which leads to an integrated change in neuronal intrinsic excitability, and (2) MSNs located in the core and shell region of the NAc have distinct characters that may be related to their unique functions in the reward pathway.

Interestingly, some differences were observed between DA-induced inhibition in evoked firing (~80% of baseline) and that induced by co-application of DA and SCH23390 (~60% of baseline). The mechanism of this phenomenon should be attributed to involvement of D1R modulation of ion channel activity. It is established that stimulation of D1Rs leads to activation of L-type Ca\(^{2+}\) channels in medium spiny striatal cells (Hernandez-Lopez et al. 1997). This
specific effect of D₁R modulation on the L-channel activity ought actually to increase the intrinsic excitability of these neurons in response to membrane depolarization. Thus, blockade of D₁Rs with enhanced stimulation of D₂Rs could induce an integrated regulation that leads to a greater suppression in evoked action potentials than DA does. It should be particularly true when the effects of D₂R-coupled A-type K⁺ channels is predominant.

**The mechanism underlying activation of Iₘs**

Despite numerous previous findings demonstrating a D₂R-modulated reduction in Na⁺ spikes in striatal MSNs, the exact mechanism underlying this D₂R action is unknown. The present study reveals that the D₂R-modulated increase in Iₘs is regulated via inhibition of PKA activity, suggesting that at least two PKA-related mechanisms may be involved. The first one seems to be associated with decreased phosphorylation of Iₘ channels by PKA since this kinase is inhibited by quinpirole, Rp-cAMPs or H-89. However, because PKA-induced phosphorylation usually increases activity of the delayed rectifier (Iₖ), including Iₘ channels (Koh et al. 1996; Hille 2001), this scenario is unlikely. The other one could be related to a D₂R-facilitated increase in Ca²⁺ mobilization, which elevates cytosolic free Ca²⁺ levels ([Ca²⁺]ᵢ) in striatal MSNs (Nishi et al. 1997; 1999). We have previously determined that D₂R-mediated increase in intracellular Ca²⁺ release is regulated by disinhibition of IP₃ receptors following inhibition of PKA activity in NAc neurons (Hu et al. 2005). Based upon these findings, we propose that D₂R-mediated activation of Iₘs in core NAc neurons is regulated by a signaling pathway involving inhibition of PKA activity and facilitation of Ca²⁺ mobilization.
Recent findings reveal that the neuronal Ca\textsuperscript{2+}-sensor (NCS) proteins (e.g., NCS-1 and K\textsuperscript{+} channel-interacting proteins, KChIPs) modulate $I_A$, leading to an increase in $I_A$ density and prolonged $I_A$ deactivation (An et al. 2000). More importantly, because some of these NCS proteins are functionally and conformationally coupled to D\textsubscript{2}Rs (see Bergson et al. 2003; Burgoyne et al. 2004 for review), while D\textsubscript{2}R-mediated increase in free [Ca\textsuperscript{2+}]\textsubscript{in} effectively activates them (Kabbani et al. 2002), these NCS proteins may play a critical role in D\textsubscript{2}R-modulation of $I_A$ and inhibition of firing. Thus, it is most likely that the D\textsubscript{2}R-mediated reduction of evoked Na\textsuperscript{+} spikes in the core NAc cells results from a consequence of inhibition of PKA activity, disinhibition of IP\textsubscript{3} receptors, facilitation of Ca\textsuperscript{2+} release, and activation of the NCS proteins, which eventually increases $I_{As}$.

D\textsubscript{2}R-mediated attenuation of inward rectification is modulated by activated PLC

Another important finding in this study is that D\textsubscript{2}R stimulation decreases the inward rectification in the core NAc cells. Previous investigations have found that several subtypes of K\textsubscript{ir} channels, including the classic inwardly rectifying K\textsuperscript{+} channels (IRK1-3 or K\textsubscript{ir} 2.1-3) and G protein–activated inward rectifiers (GIRK1 and 3, or K\textsubscript{ir} 3.1 and K\textsubscript{ir} 3.3) are located in NAc neurons (Karschin et al. 1996). It is also well-established that K\textsubscript{ir} channels, which are activated in response to membrane hyperpolarization and inactivated during depolarization, exert their role in diverse cellular functions, especially in regulating K\textsuperscript{+} homeostasis, synaptic inhibition, neuronal firing, and resting conductance (Wang 1995; Hille 2001; D'Ambrosio et al. 2002).

Findings from the present study indicate that the D\textsubscript{2}R-modulated decrease in the inward rectification was regulated by a D\textsubscript{2}R-coupled PI-PLC pathway because D\textsubscript{2}R-mediated reduction
in the inward rectification was abolished by inhibition of PI-PLC activity, but not of PKA. Although this finding is supported by considerable evidence which indicates that activation of PLC is associated with reduced activity of $K_{ir}$ channels (e.g., both IRKs and GIRKs) (Takano et al. 1995; Sharon et al. 1997; Leaney et al. 2001), the mechanism underlying D$_2$R-modulated decrease in the inward rectification during membrane hyperpolarization in NAc cells remains unknown. However, it is possible that the reduced inward rectification is related to a decrease in the cytosolic levels of phosphatidylinositol-4,5-bisphosphate (PIP$_2$). Upon D$_2$R-coupled activation, PLC hydrolyses PIP$_2$ and decreases local levels of PIP$_2$ in cells (Stauffer et al. 1998; van der Wal et al. 2001). This decrease in cellular PIP$_2$ levels could reduce $K_{ir}$ activity via two pathways. First, because binding of PIP$_2$ to certain sites of $K_{ir}$ channels increases activity of the channel (Huang et al. 1998; Kobrinisky et al. 2000; Du et al. 2004), reduced PIP$_2$ availability would diminish activity of IRKs and GIRKs (Meyer et al. 2001), thereby decreasing the inward rectification. Second, PIP$_2$ is hydrolyzed by PLC to form IP$_3$ and diacylglycerol (DAG). DAG activates protein kinase C (PKC), which can also inhibit $K_{ir}$ channel activity (Stevens et al. 1999; Mao et al. 2004) that should also decrease the inward rectification. Given the above, we propose that the D$_2$R-mediated reduction in the inward rectification (representing decreased $I_{Kir}$) should be attributed to activation of PI-PLC and a consequent reduction of intracellular PIP$_2$ levels.

_D$_2$R-mediated depolarization of RMP is also modulated by activation of PLC_

Despite their name, certain inward rectifiers carry outward (“background” or “leak”) K$^+$ currents which are activated at the RMP (Hille 2001). This type of K$^+$ channel consists of the two-pore domain ($K_{2p}$) and serves as a molecular determinant of several “leak” K$^+$ currents (Lesage and Lazdunski 2000; Goldstein et al. 2001; Kang and Kim 2006). These $K_{2p}$ channels
allow K+ currents ($I_{K-2P}$) outflow, helping to set and stabilize RMP at levels slightly above the K+ equilibrium potential. These K+ channels are highly expressed in the NAc (Karschin et al. 1996; Talley et al. 2001). Blockade of this K+ efflux depolarizes RMP in neurons (Nasif et al. 2005). Our findings not only show a D2R-modulated membrane depolarization from RMP, but also unmask its mechanism in which activation of PI-PLC was responsible for this D2R action on decreasing “leak” K+ currents. This result from our study is in agreement with a recent finding which demonstrates that activity of K2P channels is decreased by agonist-activated PLC and hydrolysis of PIP2 (Lopes et al. 2005). Taken together, these findings suggest that the D2R-mediated RMP depolarization is regulated by activation of PLC and depletion of PIP2, which lead to a decreased K2P channel activity.

Conclusions

In this study we have determined that D2R modulation inhibits evoked Na+ spike firing by activating $I_A$, reduces the inward rectification by diminishing $I_{Kir}$, and depolarizes RMP likely by decreasing the “leak” currents ($I_{K-2P}$) in MSNs located in the core NAc. We also reveal that the integrated D2R actions are regulated by multiple signaling pathways, including but are not limited to the cAMP/PKA cascade, D2R-coupled intracellular Ca$^{2+}$ release, and D2R-associated Gq/PI-PLC/PIP2 pathway. By modulating $I_{As}$, $I_{Kir}$, and $I_{K-2P}$ along with voltage-sensitive $I_{Na}$ and $I_{Ca}$ (see above) at different membrane potential levels, the D2R dynamically and integratively regulates the intrinsic excitability of NAc spiny neurons. Given that D2Rs play an important role in pathophysiology and treatment of neurodegenerative diseases, attention-deficit-hyperactivity disorder, schizophrenia, and drug addiction, novel findings from the present study should assist future investigations focusing on elucidation of the mechanisms underlying these diseases.
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Figure legends

Figure 1. D₂R stimulation decreased action potentials evoked in NAc neurons. **A₁:** A MSN in the core of NAc labeled by biocytin staining. **A₂:** Representative traces showing that evoked action potentials in a NAc neuron were reduced by stimulation of D₂Rs with quinpirole (10 µM). The depolarizing currents required for generation of action potentials were also increased with application of quinpirole (Left: control vs. Right: quinpirole). **B:** Current-evoked spike response curves showing that evoked Na⁺ spikes were significantly reduced in NAc neurons with quinpirole (n=20 cells, with post-hoc test, *p<0.05). **C:** Dose-response graphs showing that quinpirole-induced decrease in firing was significant at all concentrations studied (2-10 µM). Grater effect (≥ 30 %) was achieved with 10 µM. Bars represent means ± S.M.E. **D:** Quinpirole-induced decrease in firing was reversible and washed out by fresh bath solution. **E** and **F:** Time-response curves indicate that the D₂R-mediated inhibition in evoked Na⁺ spikes achieved its maximal levels approximately 5 min after bath application of quinpirole (10 µM). It returned to control levels after 3-5 minutes of washout (**E:** single cell; **F:** n=12 cells, with post-hoc test, *p<0.05).

Figure 2. DA decreased evoked firing in NAc spiny cells in a dose-dependent manner. **A:** Representative traces showing that application of DA (40 µM) reduced evoked action potentials. **B:** Dose-response (10, 20, 40, 80 µM) graphs showing that DA at concentrations of 40 and 80 µM induced a significant decrease in evoked firing (n=10 cells/concentration group, unpaired *t*-test, *p<0.05). Bars represent means ± S.M.E. **C:** Representative traces showing that, when DA (40 µM) was applied with the selective D₁R antagonist SCH23390 (10 µM), it produced a grater reduction in evoked firing as compared to its action along. **D:** The time-response curve shows
that evoked firing was significantly and reversibly decreased following concurrent application of 
DA and SCH23390 (n=11 cells, with post-hoc test, $p<0.05$). E: Representative traces showing 
that concurrent application of DA (40 µM) with the selective D₁R antagonist SCH23390 (10 µM) 
and the selective D₂R antagonist eticlopride (10 µM) did not produce significant change in 
evoked firing. F: The time-response curve shows that evoked firing was not significantly altered 
following concurrent application of DA, SCH23390 and eticlopride (n=8 cells, $p>0.05$).

Figure 3. D₂R-mediated inhibition of evoked firing was prevented by blockade of D₂Rs. A: 
Representative traces showing that concurrently applied eticlopride (10 µM) blocked quinpirole-
induced suppression in evoked Na⁺ spikes. B: The time-response curve shows that evoked firing 
was decreased following application of quinpirole. This inhibitory effect of D₂R agonist on Na⁺ 
spikes was washed out and blocked by concurrent application of eticlopride (n= 6 cells, with 
post-hoc test, $p<0.05$).

Figure 4. Inhibition of PKA mimicked D₂R-mediated reduction in evoked Na⁺ spikes. A: 
Representative traces showing that cytosolic application of Rp-cAMPs (500 µM) produced a 
similar but more profound inhibition of evoked Na⁺ spikes than the D₂R agonist (quinpirole, 10 
µM), in response to the same intensity of depolarizing current pulse (0.35 nA). B: Current-
evoked spike response curves showing a significant decrease in evoked spikes when Rp-cAMPs 
was dialyzed (control vs. Rp-cAMPs: n=13, $p<0.01$; with post-hoc test, *$p<0.05$) and with Rp-
cAMPs + quinpirole perfusion (control vs. Rp-cAMPs + quinpirole: n=13 cells, *$p<0.05$) as 
compared to control. In addition, no significant difference in evoked firing was found between 
cells treated with Rp-cAMPs and with Rp-cAMPs plus quinpirole (n=11 cells, $p>0.05$). Note
that plots from Fig. 1B (control vs. quinpirole) were included to compare the effects of Rp-cAMPs and quinpirole on evoked spikes. 

C: Representative traces show that perfusion of H-89 (10 µM), another selective PKA inhibitor, not mimicked but also occluded the inhibitory effect of quinpirole on evoked Na\(^+\) spikes. 

D: Current-evoked spike response curves indicate a significant decrease in evoked Na\(^+\) spikes following application of either H-89 alone (control vs. H-89: n= 6 cells, \(p<0.05\); with post-hoc test, \(*p<0.05\)) or H-89 plus quinpirole (control vs. H-89 + quinpirole: n=6 cells, \(*p<0.05\)), as compared to control. There was no significant difference in evoked firing between cells recorded with H-89 alone and H-89 plus quinpirole (n= 6 cells, \(p>0.05\)).

**Figure 5.** Inhibition of PI-PLC failed to affect D\(_2\)R-mediated reduction in evoked Na\(^+\) spikes. 

A: Representative traces showing that application of the selective PI-PLC inhibitor ET-18-OCH\(_3\) (500 µM) did not induce marked changes in evoked Na\(^+\) spikes either in the presence or absence of quinpirole. 

B: Current-evoked spike response curves showing that the effects of quinpirole on suppressing evoked action potentials were not affected by ET-18-OCH\(_3\) (n=12 cells, \(p>0.05\)). Therefore, there was a significant difference in evoked spikes between neurons recorded from control group and those treated with ET-18-OCH\(_3\) plus quinpirole (n=12 cells, with post-hoc test, \(*p<0.05\)).

**Figure 6.** Blockade of \(I_A\) increased evoked Na\(^+\) spikes and abolished D\(_2\)R-mediated reduction of firing. 

A: Representative traces showing that the number of action potentials evoked by depolarizing current pulses was markedly increased in NAc neurons following blockade of \(A\)-type K\(^+\) channels with bath application of 4-AP at a relative low concentration (10 µM).
Moreover, 4-AP also reversed the effects of quinpirole on suppressing firing activity. B: Current-spike response curves indicating that there was a significant difference in the number of evoked action potentials between control NAc cells and those treated with 4-AP alone (n=13 cells, with post-hoc test, *p<0.05) or 4-AP plus quinpirole (n=13 cells, with post-hoc test, *p<0.05). In addition, there was no significant difference in evoked firing between NAc neurons treated with 4-AP alone and 4-AP plus quinpirole (n=13 cells, p>0.05).

**Figure 7.** D$_2$R-mediated reduction in evoked Na$^+$ spikes was eliminated at more depolarized membrane potential levels. A: Representative traces showing action potentials evoked from a holding potential (V$_h$) at approximately -50 mV, either with or without D$_2$R stimulation. At this depolarized membrane potential, quinpirole (10 µM) failed to suppress evoked Na$^+$ spikes in NAc neurons. B: Current-spike response curves showing that evoked action potentials were not significantly affected by D$_2$R stimulation when the membrane potentials were held at -50 mV level (n=10 cells, p>0.05).

**Figure 8.** D$_2$R stimulation attenuated inward rectification during membrane hyperpolarization. A: Representative traces show the potential responsiveness of NAc neurons to injection of hyperpolarizing current pulses (-0.8 to 0 nA, 200 msec duration) in the control group and other compound-treated groups (quinpirole, 10 µM; ET-18-OCH$_3$, 500 µM; ET-18-OCH$_3$ + quinpirole; Rp-cAMPs, 500 µM; or Rp-cAMPs + quinpirole, respectively). RMP was held at -80 mV (V$_h$). Arrows indicate the time points at which the hyperpolarized membrane potentials were measured. B: The I-V curves indicate the changes in inward rectification induced by quinpirole and/or ET-18-OCH$_3$. D$_2$R stimulation by quinpirole significantly attenuated inward rectification.
as compared to control (n=13 cells, with post-hoc test, *p<0.05). This D2R action on inward rectification was completely blocked by ET-18-OCH3 (n=10 cells, with post-hoc test, *p<0.05), whereas application of this PI-PLC inhibitor alone did not significantly change inward rectification (control vs. ET-18-OCH3: n=10 cells, p>0.05). C: The I-V curves indicate the changes in inward rectification induced by quinpirole and/or Rp-cAMPs. Quinpirole produced a significant reduction in the inward rectification, either with or without the presence of Rp-cAMPs (control vs. quinpirole: n=13 cells; control vs. quinpirole + Rp-cAMPs: n=13 cells, with post-hoc test, *p<0.05). The PKA inhibitor Rp-cAMPs alone did not induce any significant changes in inward rectification as compared to control (control vs. Rp-cAMPs: n=13 cells, p>0.05).

**Figure 9.** D2R stimulation depolarized RMP by activation of PLC. A: Representative traces showing that RMP of a single NAc neuron was slightly depolarized with application of quinpirole and returned to control levels after washout. B: Bar graph showing that the D2R-mediated small depolarization of RMP was significant as compared to control or washout (n=14 cells, p<0.05). Furthermore, there is no significant difference in the RMP between control cells and neurons with washout of quinpirole (n=14, p>0.05). C: Bar graph showing that, although the PI-PLC inhibitor ET-18-OCH3 (500 µM) alone did not induce significant changes in RMP as compared to control, it blocked the ability of quinpirole to depolarize RMP (control vs. ET-18-OCH3 and control vs. ET-18-OCH3 +quinpirole, n=12 cells, p>0.05). D: Bar graph showing that the PKA inhibitor Rp-cAMPs (500 µM) failed to block the quinpirole-induced depolarization of RMP. Even with concurrent application of Rp-cAMPs, quinpirole was still able to induce a small but significant depolarization in RMP as compared to control (control vs. Rp-cAMPs: n=11 cells,
$p>0.05$; control vs. Rp-cAMPs + quinpirole: $n=11$ cells, $**p<0.01$; and Rp-cAMPs vs. Rp-cAMPs + quinpirole, $n=11$ cells, $p<0.05$). Bars represent means ± S.M.E.
Figure 1. D2R stimulation decreased action potentials evoked in NAc neurons. A1: A MSN in the core of NAc labeled by biocytin staining. A2: Representative traces showing that evoked action potentials in a NAc neuron were reduced by stimulation of D2Rs with quinpirole (10 μM). The depolarizing currents required for generation of action potentials were also increased with application of quinpirole (Left: control vs. Right: quinpirole). B: Current-evoked spike response curves showing that evoked Na+ spikes were significantly reduced in NAc neurons with quinpirole (n=20 cells, with post-hoc test, *p<0.05). C: Dose-response graphs showing that quinpirole-induced decrease in firing was significant at all concentrations studied (2-10 μM). Greater effect (~30%) was achieved with 10 μM. Bars represent means ± S.M.E. D: Quinpirole-induced decrease in firing was reversible and washed out by fresh bath solution. E and F: Time-response curves indicate that the D2R-mediated inhibition in evoked Na+ spikes achieved its maximal levels approximately 5 min after bath application of quinpirole (10 μM).
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(10 μM) and the selective D2R antagonist eticlopride (10 μM) did not produce significant change in evoked firing. F: The time-response curve shows that evoked firing was not significantly altered following concurrent application of DA, SCH23390 and eticlopride (n=8 cells, p>0.05).
Figure 3. D2R-mediated inhibition of evoked firing was prevented by blockade of D2Rs. A: Representative traces showing that concurrently applied eticlopride (10 μM) blocked quinpirole-induced suppression in evoked Na+ spikes. B: The time-response curve shows that evoked firing was decreased following application of quinpirole. This inhibitory effect of D2R agonist on Na+ spikes was washed out and blocked by concurrent application of eticlopride (n= 6 cells, with post-hoc test, p<0.05).
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spikes. C: Representative traces show that perfusion of H-89 (10 μM), another selective PKA inhibitor, not mimicked but also occluded the inhibitory effect of quinpirole on evoked Na+ spikes. D: Current-evoked spike response curves indicate a significant decrease in evoked Na+ spikes following application of either H-89 alone (control vs. H-89: n=6 cells, p<0.05; with post-hoc test, *p<0.05), or H-89 plus quinpirole (control vs. H-89 + quinpirole: n=6 cells, *p<0.05), as compared to control. There was no significant difference in evoked firing between cells recorded with H-89 alone and H-89 plus quinpirole (n=6 cells, p>0.05).
Figure 5. Inhibition of PI-PLC failed to affect D2R-mediated reduction in evoked Na+ spikes. A: Representative traces showing that application of the selective PI-PLC inhibitor ET-18-OCH₃ (500 μM) did not induce marked changes in evoked Na+ spikes either in the presence or absence of quinpirole. B: Current-evoked spike response curves showing that the effects of quinpirole on suppressing evoked action potentials were not affected by ET-18-OCH₃ (n=12 cells, p>0.05). Therefore, there was a significant difference in evoked spikes between neurons recorded from control group and those treated with ET-18-OCH₃ plus quinpirole (n=12 cells, with post-hoc test, *p<0.05).
Figure 6. Blockade of IA increased evoked Na+ spikes and abolished D2R-mediated reduction of firing. A: Representative traces showing that the number of action potentials evoked by depolarizing current pulses was markedly increased in NAc neurons following blockade of A-type K+ channels with bath application of 4-AP at a relative low concentration (10 μM). Moreover, 4-AP also reversed the effects of quinpirole on suppressing firing activity. B: Current-spike response curves indicating that there was a significant difference in the number of evoked action potentials between control NAc cells and those treated with 4-AP alone (n=13 cells, with post-hoc test, *p<0.05) or 4-AP plus quinpirole (n=13 cells, with post-hoc test, *p<0.05). In addition, there was no significant difference in evoked firing between NAc neurons treated with 4-AP alone and 4-AP plus quinpirole (n=13 cells, p>0.05).
Figure 7. D2R-mediated reduction in evoked Na+ spikes was eliminated at more depolarized membrane potential levels. A: Representative traces showing action potentials evoked from a holding potential (Vh) at approximately -50 mV, either with or without D2R stimulation. At this depolarized membrane potential, quinpirole (10 µM) failed to suppress evoked Na+ spikes in NAc neurons. B: Current-spike response curves showing that evoked action potentials were not significantly affected by D2R stimulation when the membrane potentials were held at 50 mV level (n=10 cells, p>0.05).
Figure 8. D2R stimulation attenuated inward rectification during membrane hyperpolarization. A: Representative traces show the potential responsiveness of NAc neurons to injection of hyperpolarizing current pulses (-0.8 to 0 nA, 200 msec duration) in the control group and other compound-treated groups (quinpirole, 10 μM; ET-18-OCH3, 500 μM; ET-18-OCH3 + quinpirole; Rp-cAMPS, 500 μM; or Rp-cAMPS + quinpirole, respectively). RMP was held at -80 mV (Vh). Arrows indicate the time points at which the hyperpolarized membrane potentials were measured. B: The I-V curves indicate the changes in inward rectification induced by quinpirole and/or ET-18-OCH3. D2R stimulation by quinpirole significantly attenuated inward rectification as compared to control (n=13 cells, with post-hoc test, *p<0.05). This D2R action on inward rectification was completely blocked by ET-18-OCH3 (n=10 cells, with post-hoc test, *p<0.05), whereas application of this PI-PLC inhibitor alone did not significantly change inward
rectification (control vs. ET-18-OCH3: n=10 cells, p>0.05). C: The I-V curves indicate the changes in inward rectification induced by quinpirole and/or Rp-cAMPs. Quinpirole produced a significant reduction in the inward rectification, either with or without the presence of Rp-cAMPs (control vs. quinpirole: n=13 cells; control vs. quinpirole + Rp-cAMPs: n=13 cells, with post-hoc test, *p<0.05). The PKA inhibitor Rp-cAMPs alone did not induce any significant changes in inward rectification as compared to control (control vs. Rp-cAMPs: n=13 cells, p>0.05).
Figure 9. D2R stimulation depolarized RMP by activation of PLC. A: Representative traces showing that RMP of a single NAc neuron was slightly depolarized with application of quinpirole and returned to control levels after washout. B: Bar graph showing that the D2R-mediated small depolarization of RMP was significant as compared to control or washout (n=14 cells, p<0.05). Furthermore, there is no significant difference in the RMP between control cells and neurons with washout of quinpirole (n=14, p>0.05). C: Bar graph showing that, although the PI-PLC inhibitor ET-18-OCH3 (500 μM) alone did not induce significant changes in RMP as compared to control, it blocked the ability of quinpirole to depolarize RMP (control vs. ET-18-OCH3 and control vs. ET-18-OCH3 +quinpirole, n=12 cells, p>0.05). D: Bar graph showing that the PKA inhibitor Rp-cAMPS (500 μM) failed to block the quinpirole-induced depolarization of RMP. Even with concurrent application of Rp-cAMPS, quinpirole was still able to induce a small but
significant depolarization in RMP as compared to control (control vs. Rp-cAMPs: n=11 cells, p>0.05; control vs. Rp-cAMPs + quinpirole: n=11 cells, **p<0.01; and Rp-cAMPs vs. Rp-cAMPs + quinpirole, n=11 cells, *p<0.05). Bars represent means ± S.M.E