Cellular/Molecular

# Cooperative Activation of $D_1$ and $D_2$ Dopamine Receptors Enhances a Hyperpolarization-Activated Inward Current in Layer I Interneurons

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**Layer I of the neocortex comprises axonal processes from widespread regions of the brain and a unique population of GABAergic interneurons. Dopamine is known to directly depolarize layer I interneurons, but the underlying mechanism is unclear. Using whole-cell recording techniques in neocortical brain slices, we have examined how dopamine increases excitability of layer I interneurons in postnatal day 7–11 rats. Dopamine (30 $\mu$M) caused a 10 mV depolarization of layer I neurons. Paradoxically, neither the $D_1$-like receptor agonist 6-chloro-2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzazepine hydrobromide (SKF81297) (1–10 $\mu$M) nor the $D_2$-like agonist quinpirole (10 $\mu$M) produced a significant depolarization. Depolarization was observed when SKF81297 and quinpirole were coapplied. When G-protein $\beta\gamma$ subunits were included in the recording pipette, $D_1$ but not $D_2$ agonists depolarized layer I neurons. Bath application of 4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride, a specific blocker of inwardly rectifying hyperpolarization-activated current ($I_h$) channels, hyperpolarized the neurons and occluded the action of dopamine. Voltage-clamp analysis demonstrated that dopamine increased the amplitude and shifted the voltage dependence of activation of $I_h$. These results indicate that $I_h$ contributes to the resting potential of layer I interneurons and is subject to modulation by dopamine.**

*Key words:* **neocortex; dopamine; layer I; interneuron; modulation; $I_h$**

## Introduction

Layer I of the neocortex resides just under the pial surface and is ~150 $\mu$m thick in the adult rat. Neuronal density in layer I is very low, and this layer has been referred to as the molecular or cell-free layer. Virtually all cells in layer I are GABAergic interneurons (DelRio et al., 1994; Li and Schwark, 1994). Studies *in vivo* and *in vitro* have begun to explore the function and connectivity of layer I interneurons. Ascending sensory information is relayed to layer I neurons rapidly, arriving 5–7 ms after whisker stimulation. These cells have receptive field properties similar to layer V pyramidal neurons (Zhu and Zhu, 2004), suggesting that these cells play a role in sensory processing. Multiple morphologically distinct types of layer I interneurons have been described previously (Bradford et al., 1978; Marin-Padilla, 1990; Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996b; Christophe et al., 2002; Chu et al., 2003). The axonal arborizations of layer I cells fall into two categories: cells with extensive arbors confined to layer I and interneurons with descending axons reaching as far as layers IV–V (Zhou and Hablitz, 1996b; Christophe et al., 2002). Layer I cells thus appear to be part of a complex cortical circuit and are positioned to participate in information processing.

Layer I receives a dense innervation from dopaminergic fibers (Berger, 1992; Smiley et al., 1992; Sesack et al., 1995). Dopamine has a direct depolarizing effect on layer I interneurons and produces an action potential-dependent, robust enhancement of spontaneous IPSCs (Zhou and Hablitz, 1999). The mechanism underlying the depolarization is unclear. Layer II–IV fast-spiking interneurons are depolarized by dopamine via an effect on an inward-rectifier $K^+$ current and a resting leak $K^+$ current (Gorelova et al., 2002). Dopamine has been reported to modulate an inwardly rectifying hyperpolarization-activated current ($I_h$) in rat ventral tegmental neurons (Jiang et al., 1993). Identifying the currents and receptor mechanisms involved in depolarization of layer I interneurons is essential for understanding the functional role of these cells and their modulation by dopamine.

Depending on the cell type, $I_h$ in the brain contributes to generation of rhythmic activity (McCormick and Pape, 1990) and determination of the resting membrane potential (Robinson and Siegelbaum, 2003). Four mammalian genes, termed *HCN1–HCN4*, encode hyperpolarization-activated, nonselective cation channels. Each subunit exhibits distinct patterns of activation and inactivation and varying sensitivities to cyclic nucleotides (Santoro et al., 2000; Wainger et al., 2001). Layer I interneurons display a prominent "sag" response during hyperpolarization, indicative of the presence of HCN channels (Zhou and Hablitz, 1995; Radnikow et al., 2002). Cajal-Retzius cells, another cell type located in layer I, also display robust $I_h$ currents early in development (Kilb and Luhmann, 2000; Radnikow et al., 2002). HCN channels are regulated by intracellular cyclic nucleotide levels (Chen et al., 2001), and dopamine receptor activation is known to

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alter intracellular cAMP levels (Missale et al., 1998). We therefore examined whether dopamine depolarizes layer I interneurons via an effect on $I_h$. Our studies demonstrate that dopamine, via a synergistic activation of $D_1$- and $D_2$-like receptors, depolarizes layer I GABAergic interneurons by enhancing $I_h$.

## Materials and Methods

*Slice preparation.* Neocortical slices were prepared from Sprague Dawley rats (7–11 d old). Animals were handled and housed according to the National Institutes of Health Committee on Laboratory Animal Resources guidelines. All experimental protocols were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Every effort was made to minimize pain and discomfort. Rats were anesthetized with ketamine and decapitated. The brain was removed quickly and placed in ice-cold artificial CSF (ACSF), which contained the following (in mM): 125 NaCl, 3.5 KCl, 0.5 $CaCl_2$, 3.5 $MgCl_2$, 26 $NaHCO_3$, and 10 D-glucose. The solution was bubbled with 95%$O_2$/5%$CO_2$ to maintain pH at ~7.4. Coronal brain slices (300 $\mu$m thick) containing the anterior cingulate cortex and the shoulder or Fr2 region of the frontal cortex (Paxinos and Watson, 1997) were cut using a vibratome. Slices were stored for 45 min at 37°C and kept at room temperature until recording. The oxygenated storage solution contained the following (in mM): 125 NaCl, 3.5 KCl, 26 $NaHCO_3$, 10 D-glucose, 2.5 $CaCl_2$, and 1.3 $MgCl_2$. Individual slices were subsequently transferred to a recording chamber continuously perfused (3 ml/min) with oxygenated saline at 32°C. A Zeiss (Thornwood, NY) Axioskop FS microscope equipped with Nomarski optics, a 40× water-immersion lens, and infrared illumination was used to view neurons in the slices. Layer I interneurons were identified by their distance from the pial surface and their firing properties. In addition, cells were intracellularly labeled with biocytin to confirm identification. Labeled cells were processed as described previously (Zhou and Hablitz, 1996b).

*Whole-cell recording.* Whole-cell current- and voltage-clamp recordings were obtained as described previously (Zhou and Hablitz, 1996b). Tight seals (>2 GΩ before breaking into whole-cell mode) were obtained using patch electrodes having an open-tip resistance of ~3 MΩ. Series resistance during recording varied from 10 to 20 MΩ and was not compensated. Recordings were terminated whenever significant increases (>20%) in series resistance occurred. The intracellular solution for recording synaptic currents contained the following (in mM): 125 K-gluconate, 10 KCl, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 0.5 EGTA. The osmolarity and pH were adjusted to 285 mOsm and 7.3, respectively. Recordings were done at 32°C.

*Data collection and analysis.* Signals were acquired using a Warner PC505A amplifier (Warner Instruments, Hamden, CT) controlled by Clampex 8.0 software (Molecular Devices, Foster City, CA) via a Digidata 1200B interface ( Molecular Devices). Current–voltage relationships and repetitive firing properties were studied using computer-generated commands. Responses were filtered at 5 kHz, digitized at 10–20 kHz, and analyzed using Clampfit 8.0 software ( Molecular Devices). Data are expressed as mean ± SEM. Statistical analysis of response before, during, and after the addition of dopaminergic agents was performed using a two-tailed Student's *t* test. $p < 0.05$ was considered significant.

*Drug application.* Dopamine (30 $\mu$M) was used as the endogenous agonist for dopamine receptors and was bath applied. After establishing whole-cell recording and obtaining control responses, dopamine and dopaminergic agonists were bath applied. After 10 min of drug application, experimental data were collected. Drug-free solution was then applied for 10 min, and wash responses were recorded. Antagonists were applied 20 min before acquiring control records as well as during agonist application. 6-Chloro-2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzazepine hydrobromide (SKF81297) and 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390) were used as selective $D_1$-like agonists and antagonists, respectively. Quinpirole and *S*(−)eticlopride were used as selective $D_2$-like agonists and antagonists, respectively. Quinpirole and SCH23390 were purchased from Tocris Cookson (Ellisville, MO), whereas SKF81297 and eticlopride were obtained from Sigma-Aldrich (St. Louis, MO). The drugs were stored in frozen stock solution and dissolved in the ACSF before each experiment. Sodium metabisulfite (50 $\mu$M $Na_2S_2O_5$) was used as an anti-oxidant (Sutor and Ten Bruggencate, 1990). The protein kinase A (PKA) peptide inhibitor (PKI 5-24) and bovine brain G-protein $\beta\gamma$ (G$\beta\gamma$) were obtained from Calbiochem (La Jolla, CA). For application, the peptide inhibitor and $\beta\gamma$ subunits were included in the patch pipette at a concentration of 1 $\mu$M and 20 nM, respectively.

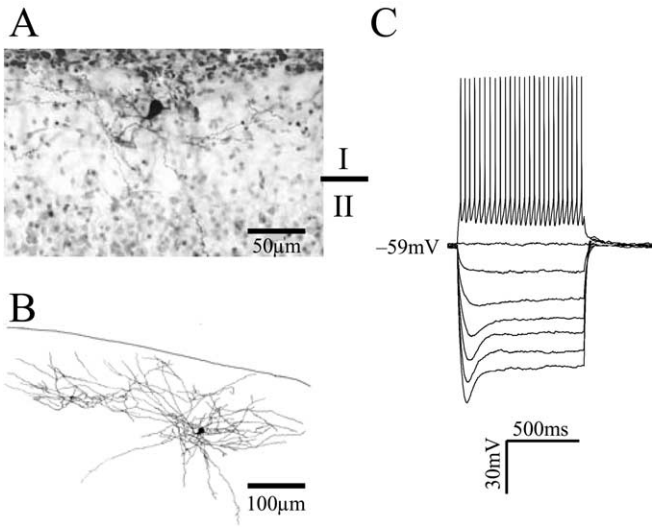


**Figure 1.** Identification of layer I interneurons in the rat prefrontal cortex. ***A***, Photomicrograph of a biocytin-labeled layer I interneuron. ***B***, Camera lucida reconstruction of the neuron from ***A***. This interneuron had an extensive dendritic tree restricted to the layer I and four axon collaterals that descended to layer II/III. ***C***, Current-clamp recording from the interneuron shown in ***A***. Injection of a depolarizing current elicited a train of action potentials that showed no accommodation. Action potential durations were brief (base duration, ~2 ms) and were followed by a fast afterhyperpolarization. Hyperpolarizing current pulses induced a hyperpolarization that reached a peak and sagged back toward rest. Such responses are a hallmark of hyperpolarization-activated, nonspecific cation channels.

## Results

### Properties of layer I interneurons

Under direct visualization, recordings were obtained from a total of 74 layer I interneurons from postnatal day 7 (P7) to P11 rats. In current-clamp recordings, the resting membrane potential was −55.8 ± 2 mV ($n = 40$), and the mean input resistance was 724 ± 73 MΩ ($n = 20$). A photomicrograph of a typical biocytin-labeled layer I interneuron is shown in Figure 1*A*. A camera lucida reconstruction of the cell is shown in Figure 1*B*. This cell was located near the pial surface and had an extensive axonal arbor extending up to 500 $\mu$m laterally within layer I. In addition, at least four axon collaterals were observed descending to layers II/III. Dendritic spines were not observed on these cells. Current-clamp recordings from this cell are shown in Figure 1*C*. A depolarizing current pulse elicited a train of action potentials that showed little or no accommodation. Action potential durations were brief (base duration, ~2 ms) and were followed by a fast afterhyperpolarization, as described previously (Zhou and Hablitz, 1996a). Hyperpolarizing current pulses evoked a voltage response that reached a peak and sagged back toward rest. Such responses are a hallmark of hyperpolarization-activated, nonspecific cation channels.

### Dopamine depolarizes interneurons and increases their excitability

Layer I receives a dense dopaminergic input from the ventral tegmental area (Lindvall et al., 1974). Previous studies from our

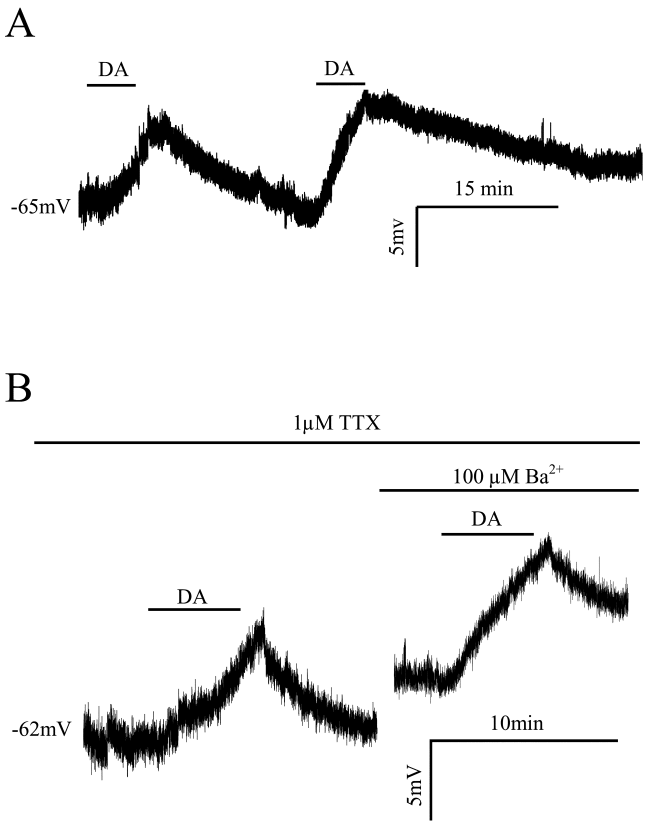


**Figure 2.** Dopamine depolarizes layer I interneurons in the prefrontal cortex. ***A***, Typical whole-cell current-clamp recording showing that dopamine (50 $\mu$M) reversibly depolarizes layer I interneurons. Depolarization response to dopamine recovered after a 10–20 min wash. Reapplication of dopamine induced a similar response in this cell. ***B***, In the presence of 1 $\mu$M TTX, dopamine (50 $\mu$M) depolarized layer I interneurons to a similar extent as seen in untreated conditions, indicating the depolarization was mediated by a direct effect of dopamine on the recorded cell and is independent of synaptic transmission. In the same interneuron after recovery from the first dopamine application, bath applying 100 $\mu$M $Ba^{2+}$ induced an ~5 mV depolarization because of the inhibition of a $Ba^{2+}$-sensitive inward rectifier potassium current. Application of dopamine in the presence of $Ba^{2+}$ elicited a membrane depolarization comparable with the control condition, suggesting dopamine depolarization of layer I interneurons does not involve modulation of a $Ba^{2+}$-sensitive inward rectifier current. DA, Dopamine.

laboratory have shown that dopamine depolarizes and increases the excitability of layer I interneurons (Zhou and Hablitz, 1999). In the present study, bath application of 30 $\mu$M dopamine produced a reversible depolarization in all of the tested cells ($n = 13$). Figure 2*A* shows an example of a typical membrane depolarization induced by dopamine. The maximum depolarization occurred 6 min after beginning the dopamine application. Responses recovered after a 10–20 min wash period. Reapplication of dopamine produced a similar response in this cell. The mean depolarization induced by dopamine was 10.8 ± 1 mV ($n = 5$; $p < 0.01$). Dopamine could potentially increase the release of several neurotransmitters that might indirectly affect the postsynaptic excitability. To determine whether the observed depolarization was mediated by a direct effect of dopamine on the recorded cell, action potential-dependent transmitter release was blocked by adding 1 $\mu$M TTX to the bathing medium. As shown in Figure 2*B*, dopamine depolarized the cell to a similar extent (9.8 ± 2.1 mV; $n = 8$) under these conditions. In subsequent experiments, 1 $\mu$M TTX was routinely added to the recording medium.

Previous studies of prefrontal cortex pyramidal cells (Dong et al., 2004) and interneurons (Gorelova et al., 2002) have shown that dopamine modulates inwardly rectifying potassium currents. We bath applied 0.1 mM $Ba^{2+}$ to determine whether inward rectifier potassium channels were involved in the dopamine-induced depolarization of layer I interneurons. $Ba^{2+}$ application depolarized interneurons by 6.4 ± 2 mV ($n = 8$), indicating the presence of $Ba^{2+}$-sensitive "leak" currents, as described for layer II/III pyramidal cells (Sutor and Hablitz, 1993). In the presence of 0.1 mM $Ba^{2+}$ medium, dopamine depolarized layer I cells to an extent comparable with control (11.5 ± 2 mV; $n = 3$). When $Ba^{2+}$ was applied in the presence of dopamine, an additional depolarization was observed. These results indicate that dopamine does not modulate a $Ba^{2+}$-sensitive inward rectifier current in layer I interneurons.

### Role of $D_1$- and $D_2$-like receptors in dopamine-mediated depolarizations

$D_1$- and $D_2$-like receptors are both expressed in superficial cortical layers (Vincent et al., 1993). Subtype-specific agonists and antagonists were used to determine the relative contributions of $D_1$ and $D_2$ receptors to the observed membrane depolarization. Paradoxically, as shown in Figure 3*A*, neither the $D_1$-like receptor agonist SKF81297 (1–10 $\mu$M) nor the $D_2$-like agonist quinpirole (10 $\mu$M) produced a significant depolarization (SKF81297: 0.2 ± 0.9 mV, $n = 5$, $p > 0.05$; quinpirole: 2.9 ± 1 mV, $n = 5$, $p > 0.05$). Behavioral (Ikemoto et al., 1997) and electrophysiological (Hopf et al., 2003) studies suggest that cooperative activation of $D_1$ and $D_2$ receptors is required for some dopamine-dependent behaviors. We therefore tested whether coapplying 1 $\mu$M SKF81297 and 10 $\mu$M quinpirole could mimic the action of dopamine. This treatment produced a significant depolarization of layer I interneurons (8.1 ± 2 mV; $n = 4$; $p < 0.01$) similar to that observed with dopamine. It was then hypothesized that if the dopamine-mediated depolarization required cooperative activation of $D_1$ and $D_2$ receptors, application of either a $D_1$ or a $D_2$ receptor antagonist should block the effect of dopamine. As shown in Figure 3*B*, it was found that in the presence of the $D_1$ antagonist SCH23390 (10 $\mu$M) or the $D_2$ antagonist eticlopride (1 $\mu$M), dopamine did not significantly depolarize layer I interneurons (SCH23390, 1.2 ± 0.7 mV; eticlopride, 1.2 ± 1.3 mV; both $n = 5$; $p > 0.05$). The pharmacological results are summarized in Figure 3*C*. These results strongly suggest that the dopamine-induced depolarization requires synergistic activation of both $D_1$- and $D_2$-like receptors.

### Dopamine-induced depolarization requires cAMP-dependent PKA activation

PKA has been shown to play a major role in dopamine signaling. If the dopamine-induced depolarization of layer I interneurons involved PKA, inhibition of PKA should block the depolarization. The addition of 1 $\mu$M PKA inhibitory peptide (PKI) in the recording pipette blocked the dopamine-induced depolarization [−1.1 ± 0.6 mV with PKI in the pipette ($n = 6$; $p < 0.01$) vs 10.8 ± 1.0 mV with dopamine without PKI], suggesting PKA signaling is involved. To test this further, 10 $\mu$M forskolin, a potent nonspecific adenylyl cyclase activator, was bath applied. Forskolin produced a significant depolarization (12.2 ± 3.4 mV; $n = 5$; $p < 0.01$ vs control), as shown in Figure 4*A*. The effect of forskolin was persistent and difficult to reverse after washing.

Typically, $D_1$ receptors are coupled to $G_s$ and activate adenylate cyclase (AC), whereas $D_2$ receptors are associated with $G_i$ and inhibit AC. Recently, a model was suggested that explains the cooperative interaction of $D_1$ and $D_2$ receptor activation (Hopf et

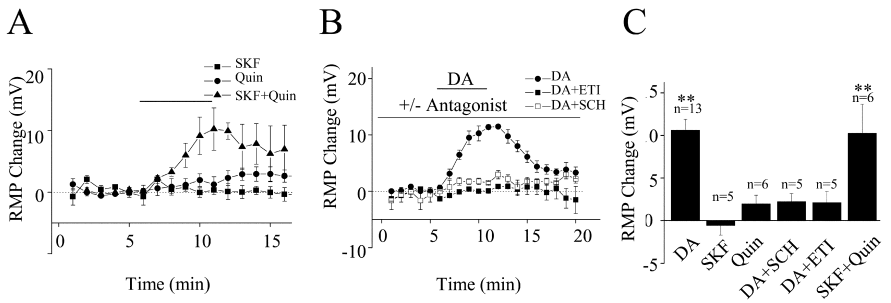


**Figure 3.** Synergistic activation of $D_1$- and $D_2$-like receptors is required for dopamine-induced membrane depolarization of layer I neurons. ***A***, Summary time course plot of the effect of the specific $D_1$ and $D_2$ agonists SKF81297 and quinpirole, respectively. Neither SKF81297 (1 $\mu$M) nor quinpirole (10 $\mu$M) depolarized layer I interneurons. Coapplication of SKF81297 (1 $\mu$M) and quinpirole (10 $\mu$M) elicited depolarizations similar to that produced by dopamine. ***B***, Summary time course plot of the effect of pretreatment with the selective $D_1$-like receptor antagonist SCH23390 (10 $\mu$M) or the $D_2$-like receptor antagonist eticlopride (1 $\mu$M). Both antagonists blocked the dopamine-induced membrane depolarization. ***C***, Bar graph of averaged membrane potential changes induced by dopamine, SKF81297, quinpirole, dopamine in the presence of SCH23390 or eticlopride, and SKF81297 plus quinpirole. RMP, Resting membrane potential; SKF, SKF81297; Quin, quinpirole; SCH, SCH23390; ETI, eticlopride; DA, dopamine. $^{**}p < 0.01$.

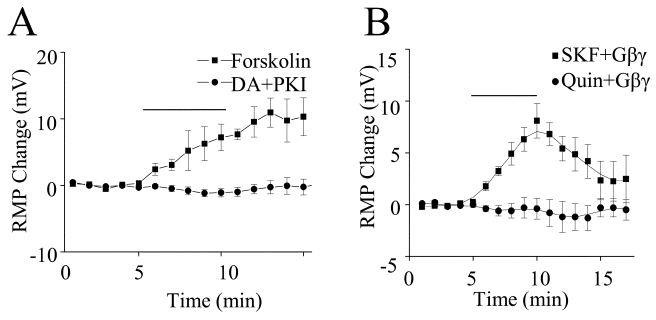


**Figure 4.** Requirement of cAMP-dependent PKA activation for dopamine-induced membrane depolarization. ***A***, Forskolin (10 $\mu$M), a potent nonspecific adenylyl cyclase activator, produced a significant membrane depolarization. The effect of forskolin was persistent and difficult to reverse after washing. The addition of 1 $\mu$M PKA inhibitory peptide (PKI) in the recording pipette blocked the dopamine (DA)-induced depolarization. In the PKI experiments, dopamine was applied at least 15 min after achieving the whole-cell configuration to allow diffusion of the peptide into the cell. The perfusion of PKI itself did not induce significant membrane potential changes. ***B***, Effect of including purified bovine brain G-protein $\beta\gamma$ (G$\beta\gamma$; 20 nM) in the recording pipette. The $D_1$-like receptor agonist SKF81297 (SKF; 1 $\mu$M) produced a significant depolarization when G$\beta\gamma$ was included in the recording pipette; the $D_2$-like antagonist quinpirole (Quin; 10 $\mu$M) did not. These results strongly suggest that synergistic activation of $D_1$ and $D_2$ receptors by dopamine produces a depolarization of layer I interneurons via a mechanism involving G$\beta\gamma$ and cAMP signaling. RMP, Resting membrane potential.

al., 2003). G$\beta\gamma$ subunits released from the $G_{i/o}$-linked $D_2$ receptor in combination with $G_{\alpha s}$ from $D_1$ receptors activate specific adenylyl cyclase subtypes. This kind of $G_{\alpha s}$–G$\beta\gamma$ interaction may allow $D_2$-linked $G_{i/o}$ receptors to enhance, rather than oppose, activation of the AC–PKA signaling pathway. If dopamine-induced depolarizations require cooperative interaction of both $D_1$ and $D_2$ receptors as described above, activation of $D_1$ receptors should produce a depolarization if exogenous G$\beta\gamma$ subunits are included in the patch pipette. As shown in Figure 4*B*, when purified bovine brain G$\beta\gamma$ was included in the recording pipette, the $D_1$-like receptor agonist SKF81297 (1 $\mu$M), but not the $D_2$-like agonist quinpirole (10 $\mu$M), produced a significant depolarization (SKF 81297: 8.1 ± 2 mV, $n = 7$, $p < 0.01$; 0.4 ± 1.0 mV, $n = 4$, $p > 0.05$ vs control).

### Role of hyperpolarization-activated currents in dopamine-induced depolarizations

Layer I interneurons display large sag responses during hyperpolarization, indicative of hyperpolarization-activated, nonspecific cation channel activity. $I_h$, the current underlying sag responses, is modulated by several neurotransmitters and has also been suggested to play a role in regulating the resting membrane potential in some cells (Robinson and Siegelbaum, 2003). We reasoned that if $I_h$ was responsible for the dopamine-induced depolarization, bath application of 4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride (ZD7288), a specific blocker of $I_h$ channels (Harris and Constanti, 1995), should occlude the action of dopamine. When ZD7288 was applied, a small but reproducible hyperpolarization (−6.5 ± 2 mV; $n = 4$) was observed. The voltage sag observed with hyperpolarizing current pulses was abolished. An example of the hyperpolarization produced by ZD7288 application is shown in Figure 5*A*. In the presence of ZD7288, dopamine did not depolarize the cell (Fig. 5*A*). The results from a group of cells are summarized in Figure 5*B*. These results strongly suggest that dopamine depolarizes layer I neurons via an action on $I_h$.

### Dopamine enhances $I_h$ in layer I interneurons

To test whether dopamine was affecting $I_h$, voltage-clamp studies of $I_h$ were conducted. Figure 6*A1* shows a typical recording of a hyperpolarization-activated inward current in a layer I interneuron. The neuron was voltage clamped at −50 mV, and a series of hyperpolarizing voltage commands from −110 to −50 in 10 mV steps were applied. A time- and voltage-dependent inward current was activated by steps more negative than −70 mV. In the presence of 30 $\mu$M dopamine (Fig. 6*A2*), $I_h$ amplitude was enhanced and there was a shift in the activation threshold (−70 mV). These changes were partially reversible after washing. Measurements of both the instantaneous (ins) and steady-state (ss) currents were made at the times indicated by the arrows in Figure 6*A1*. Current–voltage plots are shown in Figure 6*B1*, where it can be seen that the instantaneous current was linear and not affected by dopamine. The steady-state current showed marked inward rectification and was enhanced by dopamine. The instantaneous current was subtracted from the steady-state current and is plotted in Figure 6*B2*. A clear enhancement by dopamine is seen. Activation curves for $I_h$ are shown in Figure 6*C* for a population of cells. Dopamine shifted the curve to the right by changing the $V_{1/2}$ from −86.0 ± 0.6 mV to −78.9 ± 0.4 mV ($n = 19$; $p < 0.01$).

The inward relaxation attributable to $I_h$ was fitted to a single-exponential function. The activation time constant was voltage dependent. The voltage dependence of $I_h$ activation is shown in Figure 6*D*. In the presence of dopamine, $I_h$ activated more rapidly than control at membrane potentials more positive than −100 mV.

## Discussion

The present results demonstrate that dopamine depolarizes layer I interneurons by enhancing the hyperpolarization-activated, nonspecific cationic current $I_h$. This occurs as a result of a dopamine-induced depolarizing shift in the $I_h$ activation curve. This enhancing action of dopamine requires synergistic activation of $D_1$ and $D_2$ receptors and is blocked by either $D_1$ or $D_2$ receptor antagonists. Intracellularly, the action of dopamine involves G$\beta\gamma$ and cAMP-dependent processes. These results indi-

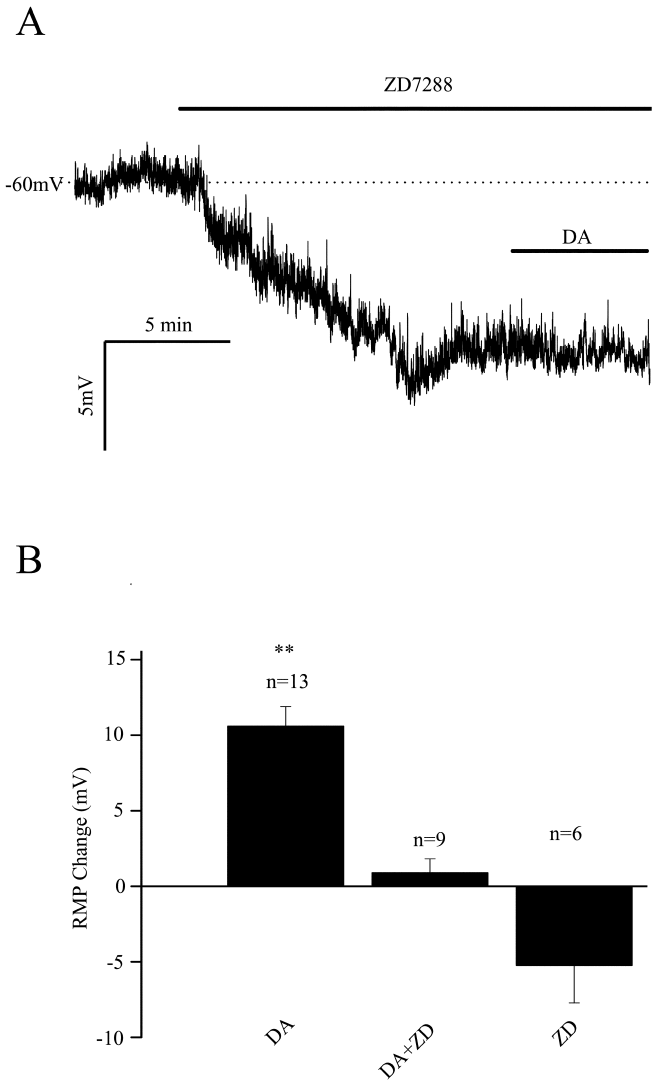


**Figure 5.** Effects of ZD7288, a specific $I_h$ blocker, on dopamine-induced membrane depolarizations. ***A***, A typical whole-cell current-clamp recording shows the effects of $I_h$ inhibition. When 30 $\mu$M ZD7288 was bath applied, a reproducible hyperpolarization was observed, indicating that $I_h$ contributes to the resting membrane potential of these cells. Application of dopamine in the presence of ZD7288 did not produce a depolarization. ***B***, Summary plot of ZD7288 effects. $I_h$ inhibition hyperpolarized interneurons and occluded the depolarizing effect of dopamine. DA, Dopamine; RMP, resting membrane potential; ZD, ZD7288. $p < 0.01$.

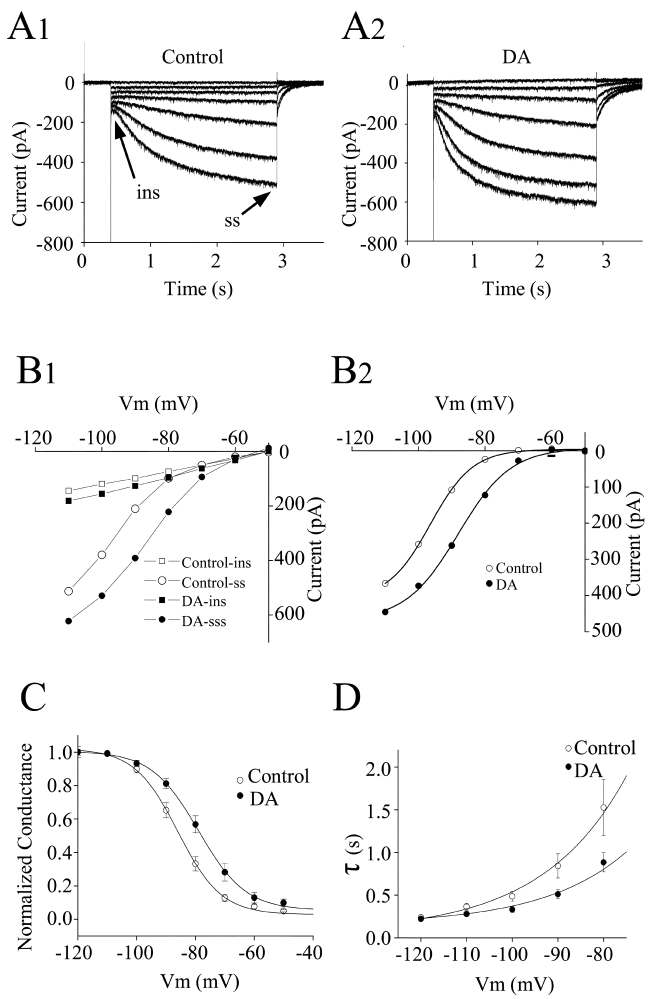


**Figure 6.** Voltage-clamp analysis of the effects of dopamine on $I_h$ activation. ***A1***, Voltage-clamp recordings of $I_h$. The neuron was voltage clamped at −50 mV, and a series of hyperpolarizing voltage commands from −110 to −50 in 10 mV steps were applied. A time- and voltage-dependent inward current was activated by steps more negative than −70 mV. The instantaneous current (arrow) was measured just after the decay of the capacitive transient, and the steady-state current (arrow) was measured near the end of each voltage commend. ***A2***, In the presence of 30 $\mu$M dopamine, $I_h$ amplitude was enhanced and there was a depolarizing shift in activation threshold (−60 mV). After dopamine application, the holding current increased ~65 pA corresponding to the depolarization seen in current-clamp recordings. The holding current change has been subtracted. ***B1***, Current–voltage curve showing the instantaneous (■ and □) and steady-state (○ and ●) currents as a function of the commend voltage in control and in the presence of 30 $\mu$M dopamine. Dopamine increased the steady-state current at all voltages negative to −60 mV and had little effect on the instantaneous current. ***B2***, Plot of the $I_h$ amplitude measured as the difference between steady-state and instantaneous current under control conditions (●) and in the presence of dopamine (○). ***C***, Dopamine caused a depolarizing shift in the voltage dependence of $I_h$ activation. $I_h$ amplitude from 19 interneurons was converted to conductance ( $g$ ) with the equation $g = I_h/(E - E_{rev})$, where $E$ is the command voltage and $E_{rev}$ is the reversal potential of $I_h$; the value of $E_{rev}$ is −27 mV by calculating the tail current. The conductance $g$ for each cell was normalized by $G_{max}$ ( $g/G_{max}$); $G$ was taken to be the value of $g$ at −110 mV, which was assumed to reach the maximum activation. The normalized conductance ( $g/G_{max} \pm$ SE) in control (■) and dopamine (●) were plotted as a function of voltage. The data were fitted with the Boltzman equation [$g/G = 1/(1 + e^{(v - v1/2)/K})$]; dopamine shifted the curve to the right by changing the $V_{1/2}$ from −86.0 ± 0.6 mV to −78.9 ± 0.4 mV ($n = 19$; $p < 0.01$). ***D***, Plot of the rate of $I_h$ activation as a function of voltage. ins, Instantaneous current; ss, steady-state current; DA, dopamine; $V_m$, voltage.

cate that $I_h$ contributes to the resting potential of layer I interneurons and that modulation of this current alters tonic inhibition in the neocortex.

### $I_h$ in layer I neurons

Hyperpolarization-activated, nonspecific cation currents, termed $I_h$, have been described in a variety of mammalian cell types (Pape, 1996). During early cortical development, neurons in layer I, including interneurons and Cajal-Retzius cells, express a prominent $I_h$ (Zhou and Hablitz, 1995; Kilb and Luhmann, 2000). Similar currents are observed in mature neurons, but with smaller amplitudes. In the present study, we have shown that $I_h$ is also present in P7–P11 layer I interneurons, where it contributes to the resting potential of these cells. In current-clamp recordings, a hyperpolarization-activated voltage sag was observed when the membrane potential reached a value near −70 mV. Stronger hyperpolarizations gave rise to more marked voltage sags and rebound excitation after pulse offset, presumably because of persistent $I_h$ activation. These sag responses and rebound excitations were reduced or blocked by the selective $I_h$ blocker ZD7288. This compound also routinely and reliably produced a

membrane hyperpolarization, indicating $I_h$ was active at rest. This is in contrast to Cajal-Retzius cells in layer I where $I_h$ does not contribute to the resting membrane potential (Kilb and Luhmann, 2000). This differential effect on resting potential may represent developmental changes in $I_h$ properties or intrinsic differences between Cajal-Retzius cells and other layer I interneurons.

The present results are based on somatic recordings from layer I interneurons. The dopamine-induced depolarization of these cells results in increased interneuron excitability and spontaneous firing, producing a robust enhancement of spontaneous IPSCs and a tonic inhibition of layer II/III pyramidal cells (Zhou and Hablitz, 1999). In the hippocampus, $I_h$ current density increases markedly from the soma to distal dendrites (Magee, 1998). $I_h$ in distal dendrites serves to dampen dendritic excitability and alters the pattern of local dendritic synaptic integration (Williams and Stuart, 2000a). The presence of $I_h$ currents in the dendrites of layer I interneurons and possible modulation by dopamine has not been examined. If present, dendritic $I_h$ currents would add another level of complexity to the control of interneuron excitability.

In the present study, $I_h$ activation was well described by a single exponential with a time constant of 700 ms at −90 mV. This value is in the range for $I_h$ in thalamic neurons (Santoro et al., 2000), hippocampal interneurons (Santoro et al., 2000), and neocortical pyramidal cells (Williams and Stuart, 2000b) and is consistent with mediation by HCN1–HCN2 subunits. Considerably faster time constants have been described in hippocampal CA1 pyramidal cells (Magee, 1998). HCN1–HCN4 subunits exhibit distinct patterns of activation and inactivation and varying sensitivities to cyclic nucleotides (Santoro et al., 2000; Wainger et al., 2001). HCN1–HCN2 are also highly expressed in layer I and sensitive to cAMP modulation, whereas HCN3–HCN4 are less prominent (Takuya, 2004). The $I_h$ activation time constants and forskolin results in layer I interneurons is consistent with mediation by channels containing HCN1–HCN2 subunits.

### Dopamine modulation of intrinsic excitability in the prefrontal cortex

Various effects of dopamine on the membrane properties of individual prefrontal cortex neurons have been reported. An early *in vivo* study showed that a membrane depolarization accompanied dopamine inhibition of cortical neurons (Bernardi et al., 1982). The number of spikes elicited by depolarizing current pulses in prefrontal cortex neurons *in vitro* has been reported to be decreased by low concentrations (0.1–10 $\mu$M) of dopamine (Geijo-Barrientos and Pastore, 1995). Higher dopamine concentrations (20–100 $\mu$M) produce an increase in excitability (Penit-Soria et al., 1987; Shi et al., 1997), perhaps via $D_1$ receptors (Yang and Seamans, 1996). Dopamine also decreases excitability of layer V prefrontal cortex neurons via $D_2$ receptor activation (Gulledge and Jaffe, 1998) but has variable effects on layer II/III prefrontal cortex pyramidal cells (Gonzalez-Islas and Hablitz, 2001).

In contrast to the varied effects on pyramidal cells, dopamine consistently increases the excitability GABAergic interneurons (Zhou and Hablitz, 1999; Gorelova et al., 2002). The present results clearly demonstrate the dopamine-induced increased excitability of layer I neurons is attributable to an enhancement of $I_h$, whereas a $Ba^{2+}$-sensitive inward rectifier current was not affected. Dopamine depolarizes and increases the excitability of fast-spiking interneurons in layers II–V via an effect on a $Cs^+$-sensitive inward rectifier $K^+$ current and a resting leak $K^+$ current; other classes of interneurons were not significantly affected (Gorelova et al., 2002). This indicates that dopamine has multiple effects on interneuron excitability that vary by cell type and ion channel. These differences presumably relate to the function of the individual interneuron subtypes in regulation of excitability in local neocortical circuits.

Dopamine-induced depolarization of layer I interneurons required synergistic activation of $D_1$ and $D_2$ receptors. In addition, the effects of dopamine were mimicked by forskolin and blocked by the PKA inhibitory peptide, indicating involvement of cAMP-dependent PKA activation. Because $D_1$ and $D_2$ receptors are conventionally thought to positively and negatively, respectively, couple to cAMP production (Missale et al., 1998), it has been difficult to understand how synergistic activation of both receptors could lead to increased cAMP levels via traditional $G_S$- and $G_{I/O}$-coupled mechanisms. An alternate signaling pathway involves AC stimulation by $G\beta\gamma$ subunits released from $G_{I/O}$ proteins (Taussig et al., 1993; Watts and Neve, 1997). This allows $G_{I/O}$-linked $D_2$ receptors to add to the $D_1$ activation of the PKA pathway. Cooperative activation of $D_1$ and $D_2$ receptors involving $G\beta\gamma$ subunits has also been described in nucleus accumbens neurons (Hopf et al., 2003) and may represent a widespread mechanism whereby dopamine modulates intrinsic excitability.

### Physiological significance

Information concerning the function and connectivity of layer I interneurons is expanding. Layer I neurons are connected via electrical and chemical synapses (Chu et al., 2003). In addition, layer I cells provide inhibitory inputs to pyramidal cells (Chu et al., 2003). *In vivo* recordings have shown that ascending sensory information is rapidly relayed to layer I neurons, arriving 5–7 ms after whisker stimulation (Zhu and Zhu, 2004). Excitatory inputs to the apical tufts of pyramidal cells reaching layer I can trigger dendritic action potentials that affect pyramidal cell activity (Schiller et al., 1997; Zhu, 2000). Distal dendritic zones have unique coincidence detection mechanisms that allow subthreshold EPSPs to trigger bursts of calcium-dependent action potentials when it coincides with a back-propagating action potential. This $Ca^{2+}$ spike firing could be abolished by appropriately timed IPSPs. Thus, the increase in IPSPs resulting from dopamine-induced depolarization could alter distal dendritic integration as well as enhance inhibition in other layers.

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