Computational Model Predicts a Role for ERG Current in Repolarizing Plateau Potentials in Dopamine Neurons: Implications for Modulation of Neuronal Activity

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1Neuroscience Center and Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, Louisiana; 2Department of Physics and Astronomy, College of Charleston, Charleston, South Carolina; 3Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, Tennessee; and 4Maryland Psychiatric Research Center and the Department of Psychiatry, University of Maryland School of Medicine, Baltimore, Maryland

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Canavier CC, Oprisan SA, Callaway JC, Ji H, Shepard PD. Computational model predicts a role for ERG current in repolarizing plateau potentials in dopamine neurons: implications for modulation of neuronal activity. J Neurophysiol 98: 3006–3022, 2007. First published August 15, 2007; doi:10.1152/jn.00422.2007. Blocking the small-conductance (SK) calcium-activated potassium channel promotes burst firing in dopamine neurons both in vivo and in vitro. In vitro, the bursting is unusual in that spiking persists during the hyperpolarized trough and frequently terminates by depolarization block during the plateau. We focus on the underlying plateau potential oscillations generated in the presence of both apamin and TTX, so that action potentials are not considered. We find that although the plateau potentials are mediated by a voltage-gated Ca2+ current, they do not depend on the accumulation of cytosolic Ca2+, then use a computational model to test the hypothesis that the slowly voltage-activated ether-a-go-go-related gene (ERG) potassium current repolarizes the plateaus. The model, which includes a material balance on calcium, is able to reproduce the time course of both membrane potential and somatic calcium concentration, and can also mimic the induction of plateau potentials by the calcium chelator BAPTA. The principle of separation of timescales was used to gain insight into the mechanisms of oscillation and its modulation using nullclines in the phase space. The model predicts that the plateau will be elongated and ultimately result in a persistent depolarization as the ERG current is reduced. This study suggests that the ERG current may play a role in burst termination and the relief of depolarization block in vivo.

INTRODUCTION

Midbrain dopamine neurons, which are involved in motivation and the control of movement, have been implicated in various pathologies such as Parkinson’s disease (Bernheimer et al. 1973), schizophrenia (Weinberger et al. 1987), and drug abuse (Koob et al. 1987). As a result, considerable effort has been devoted to the study of dopamine signaling. The firing pattern in these neurons influences the extracellular concentration of dopamine in projection areas, and a burst firing pattern produces a greater transient increase in dopamine concentration than a tonic one (Chergui et al. 1996; Gonon 1988; Heien and Wightman 2006). Bursts in dopamine neurons are thought to convey signals pertaining to reward prediction and attribution of salience (Schultz 2006). An understanding of dopaminergic signaling must include an appreciation of how the firing pattern is regulated.

Dopamine (DA) neurons in the presence of their afferent inputs can exhibit one of several firing modes: silence, regular single-spike firing, irregular single-spike firing, and bursting (Grace and Bunney 1984a,b; Hyland et al. 2002). By contrast, dopamine neurons in brain slice preparations exhibit a homogeneous pacemaker-like firing pattern that appears to result from an intrinsic slow oscillatory potential (SOP) (Fujimura and Matsuda 1989; Harris et al. 1989; Kang and Kitai 1993a; Yung et al. 1991). Local application of the selective SK channel blocker apamin converts the SOP to an oscillatory plateau potential resembling a square wave. Apamin, applied in the absence of tetrodotoxin (TTX), induces bursting activity that is driven by these plateau oscillations (Ping and Shepard 1996). Johnson and Wu (2004) replicated these results and were also able to convert pacemaker firing to bursting by the application of Bay-K-8644 [3-pyridinecarboxylic acid (1,4-dihydro-2,6-dimethyl-5-nitro-4-(2-(trifluoromethyl)phenyl) methyl ester], which potentiates the opening of L-type Ca2+ channels (Nowycky et al. 1985). In some cases, application of apamin in the absence of TTX induced irregular firing instead of bursting (Ping and Shepard 1996), but if a small applied current was injected, bursting could be established (Johnson and Wu 2004). The bursting observed in the two studies was qualitatively similar, with slow spiking during the trough of the oscillation that accelerates and diminishes in amplitude during the upstroke of the plateau. Spiking often ceases during the plateau, presumably as a result of inactivation of fast Na+ channels. Plateau potentials similar to those observed in vitro may underlie burst firing in vivo as a result of endogenous neuromodulators acting to restrict access of the small-conductance (SK) channel to intracellular calcium (Brodie et al. 1999; Fiorillo and Williams 2000; Paladini et al. 2001) or by second-messenger cascades that alter the affinity of the channel for Ca2+ (Allen et al. 2007; Bildt et al. 2004).

Nifedipine blocks the plateau potential oscillations (Johnson and Wu 2004; Nedergaard et al. 1993; Shepard and Stumpf 1999). Thus it appears that the L-type calcium channel is responsible not only for the depolarizing phase of the SOP (Mercuri et al. 1994; Nedergaard et al. 1993), but also for the...
plateau potentials. Although the mechanism responsible for terminating the bursting plateau potentials observed in apamin has yet to be established, it could involve cytosolic Ca$^{2+}$-dependent or -independent mechanisms. Potential cytosolic Ca$^{2+}$-dependent candidates include the Ca$^{2+}$-dependent inactivation of a Ca$^{2+}$ current, an electrogenic Ca$^{2+}$/H$^{+}$ pump, apamin-insensitive Ca$^{2+}$-activated K channel, or Ca$^{2+}$-activated chloride channel. Alternatively, recent studies by Nedergaard (2004) suggest that a slow, cytosolic calcium-independent outward current resembling an ether-a-go-go-related gene (ERG) current might be involved in termination of plateau potentials. Additional evidence for the presence of this current is the clear ERG1 antibody labeling observed in the substantia nigra pars compacta (SNC; Papa et al. 2003). Notably, ERG currents in the heart and CNS are potently blocked by a wide variety of antipsychotic drugs including haloperidol (Kongsamut et al. 2002; Suessbrich et al. 1997). In the present study, an experimental approach was used to assess the contribution of cytosolic Ca$^{2+}$-dependent mechanisms to termination of plateau potential oscillations exhibited by DA neurons. In addition, we incorporated an ERG conductance into an existing computational model of oscillatory activity (Amini et al. 1999) to determine whether the kinetics of the conductance is consistent with its hypothesized role in terminating the plateau potentials. Furthermore, we examined both the sequential kinetic scheme postulated for the ERG current and an independent kinetic scheme with a similar steady-state open fraction to determine the unique contribution of the unusual sequential kinetic scheme.

METH O DS

Experimental procedures

Intracellular recording and Ca$^{2+}$ imaging techniques are described in detail elsewhere (Wilson and Callaway 2000). Briefly, coronal tissue slices (300 μm) were obtained from male Sprague–Dawley rats [postnatal day (PND) 13–21] and submerged in an artificial cerebrospinal fluid (aCSF) consisting of (in mM) 124 NaCl, 4.0 KCl, 1.25 NaH$_2$PO$_4$, 0.1–1.2 MgSO$_4$, 25.7 NaHCO$_3$, 2.00–2.45 CaCl$_2$, and 11 glucose (pH 7.35, 295–305 mOsm). Whole cell recordings were made from neurons in the SNC visualized (×40 water-immersion objective) by infrared differential interference contrast video microscopy using an Olympus fixed stage microscope equipped with a CCD camera. Patch pipettes were prepared from standard wall borosilicate glass tubing (1.5 mm OD) using a P-97 Flaming-Brown micropipette puller. Patch pipettes were prepared from standard wall borosilicate glass tubing (1.5 mm OD) using a P-97 Flaming-Brown micropipette puller and filled with a solution containing (in mM): 131 K-glucuronate, 9 KCl, 20 Hapes, 0.1 EGTA, 5 Mg-ATP, and 0.5 GTP Tris (pH 7.2; 280–290 mOsm). Ca$^{2+}$ imaging studies were conducted using a modified patch solution consisting of (in mM): 135 K-glucuronate, 5 KCl, 4 NaCl, 10 Hapes, 1 Mg-ATP, 1 Mg-ATP, 0.3 Na-GTP and 0.1 fura-2 (K salt) (pH 7.4). Current-clamp recordings were made using a bridge amplifier and digitized at 10 kHz. Optical measurements were made in frame transfer mode (20–50 Hz) using a cooled CCD camera (Photometrics EEV37) and were synchronized with electrical recordings. Baseline ratio metric measurements, made during application of a hyperpolarizing bias current that prevented oscillations in membrane potential, were converted to Ca$^{2+}$ concentration using the method described by Grynkiewicz et al. (1985). Autofluorescence correction was performed by subtracting a background value from a region adjacent to the area targeted for measurement.

Experiments comparing the effects of apamin and BAPTA [1,2-bis(o-aminophenox)ethane-N,N',N'',N'''-tetraacetic acid] on plateau potentials were conducted using a modified patch solution containing 91 mM of K-glucuronate and 10 mM BAPTA-K$_2$. The prototypical SK pore blocker, apamin (200–300 nM) or the novel SK channel negative modulator N-(1R)-1,2,3,4-tetrahydro-1-naphthalenyl)-1H-benimidazo[2-amine hydrochloride (NS8593, 3 μM; Strobaek et al. 2006) was applied directly to the aCSF. Some experiments were conducted in the presence of TTX (1–2 μM).

Computational and mathematical procedures

EQUIVALENT CIRCUIT. A minimal, single-compartment Hodgkin–Huxley (HH)-type parallel conductance membrane model was constructed to capture the essential mechanisms underlying the SOP and plateau potential oscillations. The model has six state variables, including membrane potential, free cytosolic calcium concentration, and four HH-type gating variables. The differential equation for membrane potential is

$$C_m (dV/dt) = I_{ion} - I_{Ca} - I_{Cl} - I_{SK} - I_{ERG} - I_{L} - I_{R} \quad (1)$$

where $C_m$ is the membrane capacitance (Amini et al. 1999; Kang and Kitai 1993b). $I_{ion}$ is the external bias current, $I_{Ca}$ is the L-type calcium current, $I_{Cl}$ is the background calcium leak current, $I_{SK}$ is the SK current, $I_{ERG}$ is the ether-a-go-go-related current, $I_{L}$ is the leakage current, and $I_{R}$ is the hyperpolarization-activated current. Numerical integration of the model equations was performed on an Apple G4 using an implicit fifth-order Runge–Kutta method with variable step size (Hairer and Wanner 1990). The model does not include the fast sodium current or the delayed rectifier because the subthreshold oscillations that are simulated herein were experimentally observed in the presence of TTX and often tetraethylammonium (TEA) as well.

The gating variables for $I_{Ca}$ and $I_{ERG}$ were mathematically described by the solutions of a first-order differential equation

$$dz/V(V)dt = [z_{ss}(V) - z(V,t)]/\tau(z)(V) \quad (2)$$

where $z_{ss}(V)$ is the steady-state value of the generic gating variable $z$ at membrane potential $V$. A similar scheme was used as one of the two kinetic schemes investigated for $I_{ERG}$. The steady-state gating variables $z_{ss}(V)$ are sigmoidal functions given by

$$z_{ss}(V) = 1/[1 + \exp(-(V - V_{zss})/\Delta V_{zss})] \quad (3)$$

where $V_{zss}$ is the half-activation potential and $\Delta V_{zss}$ is the corresponding voltage slope. Model parameters that are not given in the text are contained in Table 1.

The description for the nonspecific hyperpolarization-activated current $I_{R}$ was taken directly from Amini et al. (1999). The equation for $I_{R}$ is $I_{R} = g_{R}m_{R}(V - E_{R})$. To match the amplitudes of the currents evoked under voltage clamp as shown in Fig. 1C of Amini et al. (1999), a conductance of about 8 nS is required, and that value was used here. The equation for the time constant is $\tau_R(V) = 26.21 + 3,136.0/(1 + \exp(-(V - 22.686)/(29.597)))$.

The description of L-type current was the same as the description of the current referred to as N-type (see discussion for an explanation of the change in nomenclature) in Amini et al. (1999). The reversal potential for the calcium currents was constant at 50 mV (Amini et al. 1999; Kang and Kitai 1993b). To match the amplitudes of the currents evoked under voltage clamp as shown in Fig. 1E of Amini et al. (1999), a conductance of 3.0 nS is required, and a slightly different value of 2.3 nS was used in the simulations presented herein. The description of Amini et al. (1999) included calcium-mediated inactivation, although calcium-mediated inactivation was not included in our model, nor in the particular cell originally used for the fit to the voltage-clamp data. The specific equation for $I_{CaL}$ is $I_{CaL} = \gamma_{CaL}g_{CaL}(V - E_{Ca})$, where $\gamma_{CaL}$ is the time-dependent activation of L-type current with first-order kinetics with the parameters listed in Table 1. The time constant for

$^{\text{1}}$Expression for $\tau_R$ was incorrect in Amini et al. (1999).
TABLE 1. Control parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{half,ERG}}$</td>
<td>$-35.0$ mV</td>
</tr>
<tr>
<td>$I_{\text{ERG}}$</td>
<td>$15.0$ nS</td>
</tr>
<tr>
<td>$g_{\text{ERG}}$</td>
<td>$1.0$ nS</td>
</tr>
<tr>
<td>$I_{\text{P}}$</td>
<td>$8.0$ nS</td>
</tr>
<tr>
<td>$g_{\text{SK}}$</td>
<td>$2.3$ nS</td>
</tr>
<tr>
<td>$g_{\text{L}}$</td>
<td>$5.5$ nS</td>
</tr>
<tr>
<td>$g_{\text{CaB}}$</td>
<td>$0.2$ nS</td>
</tr>
<tr>
<td>$I_{\text{CaP,max}}$</td>
<td>$500$ pA</td>
</tr>
<tr>
<td>$I_{\text{ICaP}}$</td>
<td>$100$ pA</td>
</tr>
<tr>
<td>$V_{\text{half,h,ERG}}$</td>
<td>$-70.0$ mV</td>
</tr>
<tr>
<td>$V_{\text{half,CaL}}$</td>
<td>$-20.0$ mV</td>
</tr>
<tr>
<td>$K_{\text{CaP}}$</td>
<td>$19.0$ mV</td>
</tr>
<tr>
<td>$K_{\text{sk}}$</td>
<td>$190$ nM</td>
</tr>
<tr>
<td>$E_{\text{sk}}$</td>
<td>$-29.0$ mV</td>
</tr>
<tr>
<td>$E_{\text{CaL}}$</td>
<td>$50.0$ mV</td>
</tr>
<tr>
<td>$E_{\text{Ll}}$</td>
<td>$-65.5$ mV</td>
</tr>
<tr>
<td>$F$</td>
<td>$596.5$ mV</td>
</tr>
</tbody>
</table>

Top row applies to independent kinetic scheme only.

$I_{\text{CaP}}$ was described by a Gaussian relationship $\tau_{\text{CaP}}(V) = 0.30 + 18.0 \exp(-(V - 70.0)/25.0)^2$, again taken directly from Amin et al. (1999).

The description of the amino-sensitive current was taken from Komendarov et al. (2004). $I_{\text{SK}}$ in the model has a Michaelis–Menten dependence on intracellular free calcium with a half-activation at $K_{\text{SK}} = 190$ nM and a Hill coefficient of 4 (Kohler et al. 1996). The explicit equation for the SK current is

$$I_{\text{sk}} = g_{\text{sk}} p_{\text{sk}} (V - E_{\text{sk}}) = 1/[1 + (K_{\text{sk}}/([Ca^{2+}]^2))]$$

The model includes a nonspecific linear background current $I_{\text{L}} = g_{\text{L}} (V - E_{\text{L}})$. This current is the main component of the input resistance of the model as measured. The input resistance of the model, measured using 250-ms hyperpolarizing pulses with an amplitude of 10 pA at −60 mV, was 250 MΩ in the simulated presence of apamin ($g_{\text{sk}} = 0$) and with $g_{\text{sk}}$ set to its usual value of 1.0 nS. Experimentally, the input resistance of dopamine cells (n = 20) was measured in the presence of apamin and TTX from the voltage deflection produced by small-amplitude (0.01 nA) rectangular current pulses (250-ms duration). The experimentally measured input resistance was in the range 97.64 to 465.50 MΩ (mean: 233.60 MΩ; SD: 98.27 MΩ).

CALCIUM BALANCE. The differential equation for the rate of change of intracellular calcium is

$$\frac{d[Ca^{2+}]}{dt} = -f (I_{\text{CaL}} + I_{\text{CaP}} + I_{\text{ICaP}}) (2volf)$$

where $f$ is the ratio of free to total calcium, $vol$ is the intracellular volume of the spherical soma of radius $r$, and $F$ is Faraday’s constant (see Table 1). Calcium extrusion was modeled using a pump. For a nonelectrogenic pump, the term for calcium extrusion appears only in the calcium balance (Eq. 4) and not in the equation for membrane potential (Eq. 1). The pump is not included in Eq. 1 because it is assumed to be nonelectrogenic (see discussion). The calcium pump was described by the equation $I_{\text{CaP}} = I_{\text{CaP,max}} ([Ca^{2+}]^0/([Ca^{2+}] + K_{\text{CaP}})$. The calcium balance necessitated the introduction of a small background calcium leak, $I_{\text{CaL}} = g_{\text{CaL}} (V - E_{\text{CaL}})$. Buffering was modeled simply by assuming that only a fixed fraction of the total calcium remained free in the cytosol and that the addition of an exogenous buffer reduced this fraction.

Independent versus sequential kinetic schemes for ERG current. The inclusion of an ERG current in a model of a dopamine neuron is novel. The rationale for its inclusion was provided by observations of a slow, calcium-independent afterhyperpolarization (AHP) in these cells (Nedergaard 2004; Wolfart et al. 2001). This slow AHP is distinct from the medium AHP mediated by the SK channel (Shepard and Bunney 1991). The current underlying the slow AHP was tentatively identified as an ERG (ether-a-go-go–related gene) potassium channel (Nedergaard 2004). The ERG current is characterized by a slow, voltage-dependent activation and a fast, voltage-dependent inactivation (Lecchi et al. 2002; Wang et al. 1997).

The kinetics of the ERG channel are unusual in that activation and inactivation are not independent, but rather sequential (Wang et al. 1997) in that a closed channel must pass through the open state before it can inactivate and an inactivated channel must pass through the open state before it can close. To examine the impact of this unusual kinetic scheme, a head-to-head comparison was made with an independent kinetic scheme as follows. In this section and the next, the ERG subscript for the gating variables will be dropped when it is clear that we are referring only to the gating of the ERG current.

The independent kinetic system is presented visually in scheme A and the sequential system in scheme B. In a kinetic scheme in which the activation and inactivation gates in each channel are independent (Hodgkin and Huxley 1952), the probability of an open channel (o) is given by the product (m)h and the probability that it is not inactivated (h). On the other hand, in the sequential scheme, o is the open fraction that is both activated and deinactivated, is the fraction inactivated, and the fraction of channels in the closed, deinactivated state is given by 1 - o - i. (To avoid confusion with current, all currents in this study are indicated by a capital I.)

$$\begin{align*}
\text{Scheme A} & \\
1 - m & = m \\
1 - o - i & = o + i \\
\text{Scheme B} & \\
1 - h & = 1 - h \\
\end{align*}$$

whereas those for scheme B are

$$\begin{align*}
dm/dt & = a_o (1 - m) - \beta_m m \\
dh/dt & = \beta_i (1 - h) - a_h h \\
di/dt & = a_o, a_i, \beta_m, \beta_i, \alpha_o, \beta_o, \beta_i
\end{align*}$$

Note that the system of equations in Eq. 5 is exactly equivalent to the system given in Eqs. 2 and 3 that provided that $\tau_{\text{ERG}} = 1/(\alpha_o + \beta_o)$ and $\tau_{\text{ERG}} = 1/(\alpha_i + \beta_i)$. The expression for the current in scheme A is $I_{\text{ERG}} = g_{\text{ERG}} m_r h$ (Eq. V - E_{K}) and in scheme B it is $I_{\text{ERG}} = g_{\text{ERG}} o (V - E_{K})$.

Selection of parameters for the sequential kinetic scheme. The ERG current was calibrated according to the data on the slow AHP observed experimentally in these neurons, which requires >10 s to activate fully, has a half decay time of about 5 s and reversed near the Nernst potential for potassium (Nedergaard 2004). The AHP was activated at potentials as hyperpolarized as −55 mV and continued to activate at least until −40 mV (Nedergaard 2004), consistent with the published half-activation voltages (Saganih et al. 2001; Schwann et al. 1999). The characteristics of activation and inactivation were matched to Sacco et al. (2003), who observed a $V_{\text{half}}$ of about −50 mV for activation and −70 mV for inactivation, and a $V_{\text{slope}}$ of about 5 mV for activation and 24 mV for inactivation. A $V_{\text{half}}$ of −50 mV for activation produced a current that activated substantially below −55 mV; therefore $V_{\text{half}}$ was shifted to −35 mV for the independent kinetic scheme, which is quite reasonable because the presence of physiological levels of extracellular calcium shifts the voltage dependence of activation (but not inactivation) in a depolarizing direction (Johnson et al. 2001).

The head-to-head comparison was achieved by selecting the parameters for the channel kinetics to keep the steady-state fraction of open channels the same in each scheme. The resultant kinetic equa-
kinetics are much faster than the slow activation kinetics (Spector et al. 1997) at all potentials in the range of interest (Fig. 1, bottom traces), then returned to a potential of ~60 mV. The maximum activation at more depolarized potentials is masked by the rapid inactivation and is apparent only in the tail currents that were all measured at ~60 mV. The apparent activation and deactivation rates were slower in the sequential kinetic scheme. During activation, the closed pool was smaller due to sequestration of some channels in the inactivated state that were no longer available for the transition to the open state and, during deactivation, the pool of open channels was continually replenished by the inactivated pool. The relative slowing was more marked at larger time constants. Unless otherwise noted, the sequential kinetic scheme was used in all simulations.

PHASE SPACE ANALYSIS. The nullcline analyses are based on two assumptions: 1) that for each oscillation there are two relevant timescales, a fast one for voltage plus a second slow one; and 2) that the voltage nullcline has a region of positive feedback when all variables that are fast are set to their steady-state variables as a function of voltage and all slow variables slower than the slow variable are assumed to be essentially constant.

Calcium-voltage nullclines for the SOP. In this case, $V$ is the fast variable and $Ca^{2+}$ is the slow one. The following variables were set to their steady-state value as a function of membrane potential: $m_{Ca^0}$ and $m_{Ca^+}$. Because the ERG current varies slowly compared with $Ca^{2+}$, the fraction of open channels $o$ was held constant at the value at the fixed point (0.058136) using the sequential kinetic scheme. Then the nullclines were obtained by solving at each value of $V$ for the value of $Ca^{2+}$ at which Eq. 4 for $d[Ca^{2+}] / dt$

$$Ca^{2+} = -K_{Ca}(I_{Ca} + I_{Ca}^B)(I_{Ca} + I_{Ca}^B)$$

and Eq. 1 for $dV/dt$

$$Ca^{2+} = K_{Ca}(I_{Ca} - I_{Ca}^B)(I_{Ca} - I_{Ca}^B)$$

respectively equal zero.

Nullclines for the apamin-induced plateau potentials. In all cases, $V$ is the fast variable. Because the pool $o + i$ changes slowly, the nullcline analysis now requires that the slow variable be $o + i$, the sum of the channels that are not closed, but may be open or inactivated. To find the appropriate voltage nullcline, first we find the value of $o$ that is required to make $dV/dt = 0$ using Eq. 1 at each value of membrane potential and the expression for $I_{ERG}$ described earlier for the sequential kinetic scheme.

be slower than $m_{ERG}$, due to the large concentration of fast-acting buffer, and was held constant at its value at the fixed point (136.163 nM). The analysis given earlier for the apamin-induced plateau potentials was repeated with this change in calcium handling as well as a change in the value of $g_{SK}$ from 0 to 1 nS.

**Nullcline analysis for the independent kinetic scheme.** Membrane potential is again the fast variable, but the slow activation $m_{ERG}$ is the relevant slow variable. The following variables were set to their steady-state value as a function of membrane potential: $m_{CaL}$, $m_{h}$, and $h_{ERG}$. In addition, $Ca^{2+}$ was presumed to be faster than $m_{ERG}$ and set to its steady-state value as a function of membrane potential using Eq. 7. The voltage nullcline was obtained by solving Eq. 1 for the value of $m_{ERG}$ that results in $dV/dt = 0$ for each value of membrane potential

$$m_{ERG} = (I_{on} - I_{SE} - I_{CaL} - I_{h} - I_{f})$$

where $I_{on}$ is the fast variable and the slow pool ($o$) to compute the value of the slow pool ($o + i$). The nullcline for the slow pool at each value of membrane potential is given by the expression $o + i = (\alpha_o \beta_i + \alpha_i \alpha_o)/(\alpha_o \alpha_i + \alpha_i \beta_i + \beta_i \beta_o)$. If the slow pool is designated $p$, where $p = o + i$, the differential equation for the slow variable in the reduced system is

$$dp/dt = \alpha_p(1 - p) - \beta_p[p/\beta_i(\alpha_i + \beta_i)]$$

where $p = [\beta_i(\alpha_i + \beta_i)]$.

**Nullclines for BAPTA-induced plateau potentials.** In this case, $V$ is the fast variable and the slow pool ($o + i$) is again the slow one. The following variables were again set to their steady-state value as a function of membrane potential: $m_{CaL}$, and $m_{h}$. $Ca^{2+}$ was presumed to

**RESULTS**

**Simultaneous calcium imaging and electrophysiological recordings during plateau potentials**

Figure 3 gives two examples of simultaneous electrophysiological recordings of the membrane potential (black trace) and fluorescent imaging of changes in intracellular calcium concentration (gray trace) in a nigral dopamine neuron during oscillatory plateau potentials induced in the presence of apamin (200 nM). Although TTX was not applied in this case, the amplitude of the oscillation was insufficient to evoke spiking, and thus the underlying plateau potential oscillation can be studied in isolation. The vertical dashed line indicates the point on the voltage trace sometimes called a “knee,” at which plateau repolarization becomes rapid and presumably regenerative. The regenerative nature is hypothesized to be due to the positive feedback of L-type channel closing that results in hyperpolarization, which results in further channel closings. Location of the knee was determined by drawing a slanted dashed line that captures the slope during the fast repolarization and determining at what point in time the line is first aligned with the voltage waveform. The variation in calcium concentration does not appear to be driving the time course of membrane potential, but rather calcium seems to follow volt.

In an effort to determine whether $Ca^{2+}$-dependent mechanism(s) contributed to repolarization of plateau potentials in

**BAPTA mimics the induction of plateau potentials by apamin**
DA neurons, experiments were conducted to determine whether plateau oscillations similar to those observed in apamin could be elicited by chelation of intracellular Ca$^{2+}$. As illustrated in Fig. 4, addition of 10 mM BAPTA to the patch solution resulted in the emergence of plateau oscillations identical to those observed in response to application of the SK pore blocker apamin (300 nM). The duration of the plateaus recorded using BAPTA-filled pipettes did not differ significantly from those obtained in the presence of apamin (APA: 2.73 ± 0.2 s, n = 36; BAPTA: 3.03 ± 0.2 s, n = 44). We hypothesize that the large concentration of BAPTA (10 mM) essentially clamps the somatic calcium concentration at a fixed value, thus clamping the SK channel current at a fixed value, thereby preventing any depolarization-induced increases in the SK current. This eliminates the role of SK in pacemaking (Amini et al. 1999; Ping and Shepard 1996; Wilson and Callaway 2000) and allows the L-type calcium channel to produce a regenerative depolarization when these channels open because they are no longer opposed by the increase in SK channel current.

Elongation of the plateaus by haloperidol but not sulpiride

Results from Nedergaard (2004) suggesting that dopamine neurons express an ERG-like potassium conductance prompted us to determine whether this current contributes to repolarization of plateau oscillations exhibited by these neurons in brain slices. The plateau potentials, which often last for seconds and depolarize the neuron to about −40 mV, would be expected to activate ERG. In our initial series of experiments, we compared the effects of haloperidol (5 μM) and sulpiride (2 μM) on the duration of spontaneous plateau potentials recorded in the presence of TTX. At these concentrations, both drugs effectively antagonize D2 dopamine receptors on dopamine neurons. However, as a potent ERG channel blocker (IC$_{50}$ = 1 μM; Suessbrich et al. 1997), haloperidol also reduces ERG K$^+$ current activated by the oscillation. As illustrated in the example presented in Fig. 5, A and B, haloperidol, but not sulpiride, increased the duration of spontaneous plateau oscillations. The response of 10 dopamine neurons to bath application of haloperidol (5 μM) is illustrated in the bar graph in Fig. 5C. The average duration of plateau potentials recorded 30–60 min following the drug exceeded those recorded from the same group of neurons under control conditions [paired $t_{(9)}$ 3.34, $P < 0.01$]. Although some variability was observed in the response of individual cells to haloperidol, 8 of the 10 dopamine neurons tested showed an increase in plateau potential duration exceeding 50% of control values.
Simulated conversion of the SOP to plateau potential oscillations

The model simulated the production of SOP at 1.6 Hz (Fig. 6A) with model parameters set to their values in Table 1. This SOP is similar to that observed by Ping and Shepard (1996) in TTX and TEA. The effect of haloperidol on the ERG current was simulated by reducing \( g_{\text{ERG}} \) by 50% (Fig. 6B, solid curve). The slight increase in frequency to 1.8 Hz could be reversed (Fig. 6B, dotted curve) by the application of 4 pA of hyperpolarizing current. On the other hand, simulating block of \( I_H \) by setting \( g_H = 0 \) decreased the frequency to 1.1 Hz (Fig. 6C). The change in frequency could be offset by the injection of a 6-pA depolarizing current. Application of the SK channel blocker apamin was modeled by setting \( g_{SK} = 0 \) in Fig. 6D and resulted in plateau potential oscillations at 0.2 Hz, similar to those observed in Fig. 3 and by Ping and Shepard (1996) as a result of blocking the SK channel. Application of BAPTA (Fig. 6E) was simulated by reducing the fraction of calcium that remains free in the cytosol from 0.025 to 0.00025 in the presence of control levels of \( g_{SK} \) (1 nS). This manipulation also induced plateau potential oscillations similar to those observed in Fig. 4 and previously observed by Ping and Shepard (1997).

Proposed ionic basis for the plateau potential oscillations

The ionic basis for the plateau potential oscillation is shown in Fig. 7. Figure 7A contains an expanded version of the membrane potential (black curve) during a single cycle from Fig. 6D as well as the variation in free calcium concentration (gray curve), which lags the voltage waveform as in Fig. 3. Note that calcium reaches a peak long before the plateau ends and, in fact, declines toward the end of the plateau, consistent with Callaway et al. (2000). We hypothesize that calcium peaks as it reaches its steady-state value as a function of potential, then declines as potential continues to hyperpolarize, causing the steady-state value of calcium to decline. Figure 7B shows the time course of the L-type calcium current (dashed curve), which turns on rapidly and regeneratively to initiate a plateau and turns off rapidly and regeneratively to terminate the plateau, as well as the time course of the ERG current (solid curve). Variation in this current is so small as to be invisible on the same scale as the L-type calcium current (see expanded version in Fig. 7D). Figure 7C reveals the importance of this current by showing the time course of open (solid curve) and inactivated (dashed line) channel pools. The conductance associated with this current in Table 1 may seem large, but the current is never activated to >5% of its maximal value. During the hyperpolarized phase, \( I_{\text{ERG}} \) turns off gradually until sufficient depolarization occurs for \( I_{CaL} \) to turn on regeneratively.

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**FIG. 5.** Representative examples of the effects \((\text{black traces})\) of sulpiride \((2 \mu\text{m})\) \((A)\) and haloperidol \((5 \mu\text{m})\) \((B)\) on plateau oscillations \((\text{control, red traces})\). All recordings made in the presence of TTX \((1 \mu\text{M})\) and apamin \((300 \text{nM})\). C: summary data obtained from 10 dopamine neurons before \((\text{open bar})\) and after \((\text{shaded bar})\) both application of haloperidol.

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On the upstroke of the plateau there is a quick decrease in the number of open channels due to fast inactivation, followed by a slow increase that continues until it causes enough hyperpolarization for $I_{Ca,L}$ to turn off regeneratively. Then there is a rapid increase in $I_{ERG}$ after plateau termination due to fast deinactivation and the cycle repeats.

Variation in duty cycle and frequency as a function of applied current

The plateaus observed in the presence of BAPTA and SK blockers could not be measured in the same neuron due to experimental constraints, but instead plateaus obtained in different neurons were compared. The plateau potential oscillation observed experimentally resembles a relaxation oscillator (Perko 1991) and those in the model can certainly be characterized as such. In different neurons, different amounts of applied current may be necessary to observe the plateau potentials (Johnson and Wu 2004; Ping and Shepard 1996). The duty cycle (fraction of the cycle above a certain threshold, here set to $-45$ mV) and frequency of a relaxation oscillator can be highly dependent on the applied current, as shown in Fig. 8 for the simulated plateau potentials induced by setting $g_{SK}$ to zero. The duty cycle increases monotonically with increasing depolarization, whereas the frequency reaches a peak near a duty cycle of 0.5 (Fig. 8A2), where the plateau and trough are of approximately equal duration. Hyperpolarization from this point (Fig. 8A1) decreases the frequency by preferentially elongating the trough, whereas depolarization (Fig. 8, A3 and A4) decreases the frequency by preferentially elongating the plateau. Therefore the bias point of the model neuron determines the duty cycle and the observed plateau (and trough) durations, with the plateaus increasing with more depolarizing bias current as shown in Fig. 8B. This prediction was tested experimentally in the presence of the SK channel negative modulator NS8593 (3 μM) as shown in Fig. 8, C and D. In this experiment, TTX was not applied so a burst of spikes is visible during the depolarization preceding the plateau. As in the model neuron, plateau duration (Fig. 8D) and duty cycle increased with the injection of increasing amounts of depolarizing current, although the real neuron supported plateau potential oscillations over a much broader range of values of injected current. The comparison of plateau durations will be affected by the variability in the bias point of the neuron.

Comparison of the sequential and independent kinetic schemes

A head-to-head comparison of the two kinetic schemes described in METHODS was performed by comparing the simulation results at the parameter setting in Table 1 except that $g_{SK} = 0$. The sequential kinetic scheme (Fig. 9A, solid curve) has a slower time course than the independent scheme (dot-dashed line). This effect was consistent across the entire range of values of applied current that supported an oscillation; Fig. 9B plots the trough versus plateau duration for the sequential scheme (open circles) and the independent scheme (open triangles). These effects are highly dependent on the exact shape of the curve for the time constant of activation (Fig. 1B), more pronounced the slower the activation time constant, and can be limited to the plateau or the trough if the activation is slow only in the voltage range corresponding to that phase of the oscillation.

Comparison of simulated plateau durations evoked by SK block or BAPTA

Given that the plateau duration can vary greatly as a result of simply changing the applied current, the plateaus generated by simulated SK block or simulated BAPTA were compared across the range of applied currents that support plateau potential oscillations. Plateaus of similar duration for SK block (Fig. 10A, solid curve, 0 pA) and BAPTA (dot-dashed curve, 23 pA) could be observed at different duty cycles, although if everything else was held constant the period and plateau were shorter in BAPTA compared with during SK block. There was a significant region of overlap (between dotted lines) in the distribution of plateau durations for SK block (open circles) and BAPTA (plus signs), which encompasses the durations of the plateaus observed experimentally in Fig. 4 (2–4 s). This is a possible explanation for why the observed plateau durations compared across the two populations in Fig. 4 were not shorter for BAPTA than for SK block. Surprisingly, both the plateaus and the troughs in the presence of BAPTA (see Fig. 11A) tend to have a shorter duration in the model despite the complete
absence of a calcium-dependent dynamics in the model. The effect is due to the presence of the SK conductance in the BAPTA experiments and not those in which SK channels were blocked by apamin and to the conversion of the SK conductance to essentially a linear leak in the presence of nearly constant calcium concentration. This linear conductance reduces the contribution of the nonlinear conductance due to the L-type calcium channel, thus reducing the amplitude of the oscillations and increasing their frequency (see Nullcline analysis).

Although the value of $g_{SK}$ in the simulated presence of BAPTA is nearly constant at a given value of applied current, it varies substantially over the range of applied current. As depolarization increases, the steady-state value of calcium and thus SK conductance increases, requiring more depolarizing applied current to achieve the same fraction of slow pool channels on the voltage nullcline, thus extending the range of applied current values that will support the oscillation in a depolarizing direction (Fig. 11B).

Simulated effect of blocking the ERG current

In the model, complete block of the ERG current together with SK block results in a persistent depolarized plateau. A partial block can elongate the plateau or stop the oscillation completely such that a depolarized plateau again results. At a constant level of applied current (−7 pA), 50% block of the ERG conductance (Fig. 12A) results in a greatly elongated plateau (dot-dashed curve) compared with control (solid curve). When there was no applied current (0 pA), the control oscillation (Fig. 12B, solid curve) is converted to persistent depolarization block (dotted line) when the ERG conductance is reduced by half, but injection of −7 pA hyperpolarizing current restores the oscillation (dot-dashed line) albeit with a longer plateau duration. The plateaus and troughs are consistently shifted to larger values when the ERG conductance is partially blocked (Fig. 11B). The critical importance of this conductance in the oscillatory mechanism is indicated by a dramatic narrowing of the range of values of applied current that will support an oscillation when this current is partially blocked (Fig. 11B).

Simulated effect of blocking $I_H$

At hyperpolarized values of the applied current (−11 pA; see Fig. 10B), a mixed-mode oscillation (Diener 1984; Drover 2004) can be observed as the plateau potential oscillation emerges, with one or more small-amplitude oscillations alternating with a single large-amplitude one (see Fig. 13A, top). The small-amplitude oscillations are greatly magnified in the bottom trace of Fig. 13A. This type of oscillation can be observed when there are two slow vari-

FIG. 8. Effects of $I_{stim}$ on the oscillatory plateau potentials evoked by small-conductance (SK) block. A1–A4: variability of the oscillatory waveform with applied current. B: plateau durations increase as the magnitude of the injected depolarizing current increases. C1–C4: representative traces from an individual dopamine cell illustrating the effects of intracellular injection of increasing levels of positive bias current (0–0.8 nA) on the duration of plateau potentials elicited by bath application of NS8593 (3 μM). D: summary data from the cell illustrated in C1–C4. Each point represents the duration of an individual plateau recorded in one of 2 consecutive 40-s trials of continuous current injection. Note that in addition to increasing plateau duration, the number of plateau potentials increased as a function of applied current. Arrows in C3 denote the area used to measure plateau potential duration.
ables (in this case the activation of $I_{ERG}$ and of $I_H$), but the parameter region becomes vanishingly small in the case of only one slow variable. Accordingly, if the activation variable for $I_H$ is set to its steady-state variable, eliminating its time dependence, the mixed-mode solutions are replaced by a constant hyperpolarization (dotted line Fig. 13A, top). Small-amplitude oscillations in between plateaus resembling those seen in the model have been observed experimentally (see Fig. 5, red trace). This phenomenon allows lower frequencies than would be possible without the second slow variable, and these mixed-mode solutions are not amenable to the nullcline analyses subsequently given precisely because of the significant contributions of two slow variables.

With SK block and no applied current (0 pA), blocking $I_H$ decreases the frequency of the plateau potential oscillation (Fig. 13B). In one respect, $I_H$ is the opposite of $I_{CaL}$ because the former is an inward current that turns on with increasing hyperpolarization, whereas the latter is an inward current that turns on with increasing depolarization. Therefore $I_H$, like the SK current, has a tendency to dampen the region of positive slope in the potential nullcline (see following text) and thus the amplitude of the oscillation, which tends to decrease the frequency across the range of parameters that support oscillation (see Fig. 11, A and B). The range of values of applied current that support the oscillation (open circles in Fig. 11B) in the presence of $I_H$ are slightly shifted in a depolarized direction when $I_H$ is blocked (open triangles in Fig. 11B).

**Nullcline analysis**

The reduction of an oscillation to a two-dimensional (2D) space can provide great insight into the mechanisms and modulation of the oscillation. The limitations are that the analysis is approximate only when there are more than two variables and can fail if two of the slow variables are sufficiently slow with respect to the others. The full model has six dimensions and a different reduction to two dimensions is applied to the model under different circumstances. Nullcline analysis strictly applies to only a 2D system, and the trajectories for both the reduced system and the full model are both shown. The trajectory of the reduced system is constrained to travel along the potential nullcline and to switch quickly between branches when stability is lost at the knee. The trajectory of the full system is not so constrained, but the reduction helps to clarify the essential underlying mechanism of the oscillation.

**FIG. 10.** Comparison of BAPTA and SK-induced plateau potentials. A: similar plateau durations can be observed in the simulated SK block case (solid curve) at $I_{stim} = 0$ pA and the simulated BAPTA case (dot-dashed curve) at $I_{stim} = 23$ pA. B: plateau durations across the range of $I_{stim}$ that supports oscillations are given for the simulated SK block (open circles) and BAPTA (plus signs) schemes. Dotted lines bracket the region in which plateau durations overlap.
The first case considered (Fig. 14A) was the slow oscillatory potential (SOP) and corresponds to the oscillation in Fig. 6A. The analysis here is quite similar to that given in Amini et al. (1999). Free cytosolic calcium is the relevant slow variable. The calcium nullcline (blue curve) gives the steady-state calcium concentration for a given potential, where calcium influx balances efflux. Approximate agreement was obtained with the experimentally observed calcium nullcline in Fig. 9 of Wilson and Callaway (2000), although the maximum value in our nullcline (300 nM) is somewhat lower than the maximum value that they obtained. The potential nullcline (green curve) gives the value of calcium at each potential that renders the total inward current equal to the total outward current. The fixed point is indicated by the intersection of the calcium nullcline and the potential nullcline. At the fixed point, the rate of change of membrane potential and calcium is zero. However, the potential does not rest here because of the positive feedback provided by $I_{CaL}$. On or near the middle branch of the potential nullcline, which has a positive slope, the L-type calcium channels close or open regeneratively depending on the direction of the trajectory (black curve) in the space defined by voltage and calcium concentration. The trajectory circles the fixed point with the direction indicated by the arrow due to the slow negative feedback produced by the activation of the

![Graph of Effects on Plateau and Trough](image)

**Fig. 11.** Summary of the effects of various manipulations on frequency and plateau and trough duration. A: trough and plateau duration across the range of applied current values that support the oscillation with parameters as in Table 1 except that for BAPTA (plus signs) $I_{stim} = 0.00025$, for SK block (open circles) $g_{SK} = 0$, for SK and H block (open triangles) $g_{SK}$ and $g_{H} = 0$, and for SK and 50% ERG block (filled squares) $g_{SK} = 0$ and $g_{ERG} = 7.5 \text{nS}$. B: frequency as function of applied current under the same manipulations as in A.

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![Graph of Effects on Frequency as a Function of Current](image)

**Fig. 12.** Effects of partial ERG current block. A: at a constant level of $I_{stim}$ (−7 pA), 50% block of $I_{ERG}$ (dot-dashed curve) greatly elongates the plateau duration compared with the control (solid curve). Parameters as in Table 1 except $g_{SK} = 0$. B: at $I_{stim} = 0$ pA, the control oscillation (solid curve) disappears (dotted line) with 50% block of $I_{ERG}$, but can be restored (dot-dashed curve) with the application of −7 pA, albeit with a longer plateau duration.

![Graph of Effect of $I_H$](image)

**Fig. 13.** Effect of $I_H$. A: mixed mode oscillations at $I_{stim} = −11$ pA are enabled by the slow kinetics of $I_H$ interacting with those of $I_{ERG}$ (solid curve) and disappear when $I_H$ activation is made instantaneous (dotted line). Insert: magnification of the small amplitude portion of the oscillation. B: at a constant level of $I_{stim}$ (0 pA), blocking $I_H$ (dotted curve) decreases the frequency compared with control (solid curve). Parameters as in Table 1 unless otherwise noted.
SK channel. In Fig. 14A, the reduced system with the values given in Table 1 does not oscillate because the timescale for cytosolic Ca\(^{2+}\) is not sufficiently slow to destabilize the fixed point. The reduced system trajectory (yellow) is shown for \(f = 0.00025\) to illustrate the relaxation oscillation (Perko 1991) in the reduced system when the time course of cytosolic Ca\(^{2+}\) is sufficiently slow. In the trajectory for the full system (black), \(f = 0.025\) as in Table 1, but the influence of the other four dimensions destabilizes the fixed point to induce Ca\(^{2+}\)-driven oscillations.

The second case (Fig. 14B) corresponds to the plateau potentials induced by SK block (Fig. 6D). It is novel because the slow variable is not the activated fraction of the ERG current, as one might expect, but rather the slow pool, which consists of all the channels in either the open or the inactivated state. This is because the rate of inactivation is so fast compared with activation that the inactivated fraction remains at steady state as a function of the open fraction (see METHODS). The membrane potential nullcline is shown in green as before, but whereas in Fig. 14A calcium was treated as a slow variable, here it is fast relative to the ERG slow pool and is therefore set to its steady-state value as a function of voltage. The slow pool nullcline is given in red and the trajectory in black. The trajectory again circles around the fixed point, although, in this case the negative feedback is produced by the slow activation of the ERG potassium current. The trajectory follows the membrane potential nullcline far more closely than in Fig. 14A, and in fact tends to jump from branch to branch at the “knees” to avoid the unstable middle branch. This is a characteristic of a relaxation oscillator (Perko 1991). At the hyperpolarized end, there is some overshoot of the potential nullcline by the trajectory in the full model due to the slow contribution of \(I_{HF}\), but no overshoot in the reduced model trajectory (yellow).

Nullcline analysis predicts a plateau potential oscillation if the slow pool and potential nullclines intersect in the middle branch of the potential nullcline, a persistent hyperpolarization if they intersect in the left branch, and a persistent depolarization if they intersect in the right branch.

These predictions are in general accurate with one exception. At very hyperpolarized values of the applied current, a persistent hyperpolarization is predicted, but a mixed-mode solution as described earlier is observed due to the slow H current. When the time dependence of this current is removed, the predictions are correct.

The nullcline analysis explains the variation in frequency and duty cycle observed in Fig. 8: At a duty cycle of 0.5, the fixed point is not near the right or the left branch of the potential nullcline. The plateau tracks the right branch, whereas the trough tracks the left branch. The applied current moves the position of the fixed point, with a hyperpolarization moving it closer to the left branch and the trough, and a depolarization closer to the right branch and the plateau. Near the fixed point, the rate of change of all variables is near zero and thus the trajectory moves slowly. Thus a depolarization from the 0.5 duty cycle point elongates the plateau and a hyperpolarization elongates the trough.

The third case, included for completeness, corresponds to the plateau potentials induced by SK block represented by the
The ERG current activation. In Fig. 14, the dot-dashed curve in Fig. 9A and is analogous to the second case except that independent rather than sequential kinetics was considered. The fraction of activated channels is $d_{ERG}$ is the relevant slow variable in this case (see Fig. 14C). The expressions for the potential nullcline (green curve) given in these two cases [independent (Fig. 14C) and sequential (Fig. 14B)] are derived differently, but because $h_{er}/(\alpha_r + \beta_r)$ are exactly equivalent. On the other hand, although the expressions for the slow pool (Fig. 14B, red curve) and the $d_{ERG}$ nullclines (Fig. 14C, orange curve) are extremely similar because of the calibration illustrated in Fig. 1A, they are not identical. This small difference accounts for the slightly different range of values of applied current that will support oscillations in the two cases. The analysis produces very similar results as in the sequential case, but the full model trajectory (black curve in Fig. 14C) does not track the potential nullcline as closely, possibly because the effective kinetics is not as slow.

The fourth case examines the BAPTA-induced plateau potentials, with sequential kinetics as shown in Fig. 6E. The model provides an explanation for the BAPTA-induced plateau potentials. The mechanism is the same as that of the apamin-induced plateau potentials. The slow pool nullcline (red curve) is exactly the same as in Fig. 14B. The potential nullcline (green curve) in Fig. 14D was done in a similar fashion as in Fig. 14B, except that instead of assuming calcium relaxes rapidly to its value on the calcium nullcline, calcium now varies so slowly that it can be considered constant at its value at the fixed point (136 nM). In fact, the average value of calcium does vary by a few nanomoles during the plateau potential oscillations, thus explaining the deviation from the nullclines shown by the full model trajectory (black curve) in Fig. 14D, but not the reduced model trajectory (yellow). The reduction in amplitude of the positive slope middle branch region of the potential nullcline (green curve) is evident compared with Fig. 14B. As explained earlier, the calcium concentration is approximately clamped to a constant value, so this current functions as a linear leak, reducing the input resistance of the cell and reducing the amplitude and period of the plateau potential oscillation.

The nullcline portraits for the SOP in Fig. 14A and the BAPTA-induced oscillatory potentials in Fig. 14D produce the same equilibrium, or fixed point ([Ca]$^\infty = 136.163$ nM, $o = 0.014571$, $o + i = 0.58136$, $V = -48.227$ mV), but the dynamics are vastly different. Only a single parameter, the fraction of free calcium ($f$), has been changed, but the relevant timescales are completely different—thus the different 2D portraits that capture essentials of the dynamics. Decreasing $f$ reverses the relative timescales of calcium concentration and the ERG current activation. In Fig. 14A, calcium is slow compared with voltage, but the activation of the ERG current is much slower, although in Fig. 14D, the converse is true. The nullcline analyses improve the ability of the model to provide mechanisms that explain its predictions.

**Discussion**

**Calcium versus voltage dependence of the plateau repolarization**

An important conclusion of this paper is that the plateau potential oscillations, unlike the slow oscillatory potentials that drive pacemaking, are not driven by an oscillation in free cytosolic calcium concentration. The most salient prediction of mathematical models in which an oscillation in cytosolic calcium drives an oscillation in voltage is that calcium concentration rises slowly during the depolarized phase and declines slowly during the hyperpolarized phase (Amini et al. 1999; Chay and Keizer 1983). When it was discovered that cytosolic calcium did not consistently rise slowly during the bursting phase in pancreatic beta cells (Valdeomilnos et al. 1989), and in fact sometimes declined during the burst, the hypothesis that an oscillation in cytosolic calcium concentration drove the burst was discarded (Bertram and Sherman 2005).

Additional evidence against a calcium-dependent mechanism is provided by the finding that the application of BAPTA in the absence of SK blockers suffices to eliminate the SOP and produce oscillatory plateau potentials. The model provides a simple explanation of this phenomenon: in both cases, SK block and the application of BAPTA, the SK conductance is essentially set to a constant value, zero in the first case and nonzero in the second, such that the SK conductance can no longer pace the SOP. In both cases a slower oscillation results when the ERG current provides the repolarizing drive instead of the SK current.

**Role of $I_H$**

In the model, blocking $I_H$ decreased the frequency of the SOP by about 30% (Fig. 6C). This implies that the same manipulation should reduce the frequency of pacemaker-like firing, although the reduction may be smaller in the presence of the sodium current, which also contributes to the SOP (Ping and Shepard 1996). On the other hand, Mercuri et al. (1995) did not observe an effect of blocking $I_H$ with external cesium on the frequency of pacemaking. However, a more recent study (Seutin et al. 2001) using a more specific blocker [4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-methylamino)pyridinium chloride (ZD7288)] did report a decrease of ≤40% in the firing rate in some neurons, but yet another study (Neuhoff et al. 2002) reported that the effect of $I_H$ on pacemaking was limited to a subset of SNC neurons and not found in VTA neurons. An even more recent study found that not only did blocking $I_H$ reduce the pacemaker frequency in VTA neurons but also suggested that the excitatory effect of ethanol on VTA firing was mediated by an augmentation of $I_H$ (Okamoto et al. 2006). Thus it seems likely that $I_H$ has some stimulatory effect on pacemaking, but that the dopamine neuron population is heterogeneous with respect to the contribution of this current. The model also predicts that blocking $I_H$ reduces the frequency of the plateau potentials, although this effect may be much less pronounced in subpopulations in which the contribution of this current is not as robust.

**Role of the L-type and other calcium currents**

We included a single calcium current in our model. Durante et al. (2004) concluded that current conducted through these putative L-type class D channels makes up the bulk of the calcium current activated by small depolarizations such as those observed during the SOP and, in our estimation, the plateau potentials; thus this was the only calcium current included in the minimal model. The L-type calcium current has
two subtypes: the class C subtype that is sensitive to dihydropyridines and the class D subtype that is sensitive to both ω-conotoxin and dihydropyridines (Williams et al. 1992). The description used here for the L-type current was calibrated using data from Kang and Kitai (1993b) for the persistent low-voltage–activated (LVA) calcium current that was blocked by 1 μM ω-conotoxin, but not by 10 μM nifedipine. There has been some confusion about the identification of this current: a previous modeling study identified it as an N-type current (Amini et al. 1999) because it was blocked by 1 μM ω-conotoxin. However, a similar current was identified by Durante et al. (2004) that was blocked by 2 μM nimodipine, but was not blocked by relatively low levels (50 nM) of ω-conotoxin, consistent with their conclusion that the persistent LVA calcium current is not an N-type but rather a class D L-type current. It is not clear why nifedipine did not block the persistent LVA in the earlier experiment by Kang and Kitai (1993b), although it has been suggested that the block was masked by nonspecific effects of nifedipine on other currents (Takada et al. 2001).

The presumption that all current blocked by ω-conotoxin in dopamine neurons necessarily represents the class B subtype associated with the N-type calcium current is further weakened by the fact that no immunoreactivity against the class B α1 subunit was detected in the substantia nigra pars compacta (SNC) in adult rats, whereas intense immunoreactivity was detected against the class D subunit and a subpopulation of dopamine neurons displayed weak to moderate immunoreactivity against the class C subunit (Takada et al. 2001). The class D subtype of the L-type calcium current can have a lower threshold of activation than that of the class C subtype (Fisher and Bourque 1996), consistent with the relatively low voltage activation of the calcium current observed by Durante et al. (2004) and Kang and Kitai (1993b).

Despite the primary role postulated for the class D L-type current in the oscillations displayed by dopamine neurons, it is very likely that other calcium currents can also contribute. In addition to the persistent LVA current, dopamine neurons generate a transient LVA (Kang and Kitai 1993b) calcium current (presumably T-type) as well as several components of the HVA (high-voltage–activated) calcium current (Cardozo and Bean 1995; Durante et al. 2004).

Role of the calcium pump

Little is known about the mechanism of calcium removal in dopamine neurons, although here we presume that the calcium pump rather than an exchanger is primarily responsible (Waneverbecq et al. 2003). One piece of evidence argues against completely electrogenic calcium removal, in which the removal of calcium ion from the cell results in the net removal of two positive charges from the cell. For a voltage-clamp step of sufficient duration, the cytosolic Ca\(^{2+}\) buffers equilibrate and the Ca\(^{2+}\) concentration reaches a steady level (Wilson and Callaway 2000). At this point the rates of calcium influx and efflux are balanced and, if the calcium removal process were completely electrogenic, the rates of charge influx and efflux would also be balanced. An equal exchange of charge would contradict the observation that the cells have a persistent inward calcium current at depolarized voltages (Kang and Kitai 1993b). Some studies have shown that protons can enter the cell as calcium is pumped out, reducing the electrogenicity of the pump (Salvador et al. 1998). An early study using sharp electrodes (Ping and Shepard 1997) indicated that the plateaus evoked by intracellular chelation of calcium lengthened the plateaus compared with those observed in apamin, which might indicate that another calcium-mediated process also contributes to plateau repolarization, although more recent data with patch-clamp electrodes suggest that there is no significant difference (Fig. 4). This study predicts that blocking the calcium pump with vanadate would not affect the plateau potentials observed in the presence of SK blockers.

Role of the SK channel

In this study, block of the SK channel accentuates the contribution of \(I_{ERG}\). Its contribution may also be accentuated under certain conditions in vivo as a result of a low expression of the SK channel or indirect neuromodulation of this channel. The SK channel is expressed less strongly in the ventral tegmental area (VTA) than in the SNC and the precision of the firing is higher the more strongly the SK channel expressed (Wolfart et al. 2001). In addition to the activation of the SK channel by a rise in cytosolic calcium concentration (Wilson and Callaway 2000) that results from calcium influx by voltage-gated channels, the nigral SK channel is activated in response to calcium release from internal stores, for example as a result of metabotropic glutamate receptor (mGluR) activation (Fiorillo and Williams 1998) or as a spontaneous event in young rats (Seutin et al. 2000). It is not necessary for a neuromodulator to act directly on this current but only to reduce its access to calcium activation to effectively attenuate it. For example, muscarine (50 μM) reduces the amplitude of the medium SK-mediated AHP (Scruggs et al. 1997), presumably by reducing calcium entry. Alternatively, the activation of α1 adrenergic receptors or M1 muscarinic receptors can interfere with the release of calcium from intracellular stores (Fiorillo and Williams 2000; Paladini et al. 2001). Thus noradrenergic afferents from the locus ceruleus, the cholinergic afferents from the pedunculopontine nucleus, or even somatodendrically released dopamine, which has some affinity for the α1 receptor, or serotonin acting by IP3-coupled 5HT2 receptors (Brodie et al. 1999) could attenuate the SK current evoked by mGluR activation and promote plateau potentials in vivo. It is also conceivable that SK current is regulated by neuromodulators capable of altering the sensitivity of calmodulin for Ca\(^{2+}\) (Allen et al. 2007; Bildl et al. 2004).

Role of the ERG current and possible therapeutic implications

A 50% block of \(I_{ERG}\) in the model increased the frequency of the SOP by about 12% (Fig. 6B). Haloperidol, which partially blocks ERG channels (Suessbrich et al. 1996) and reduces by almost half the \(I_{ERG}\)-like, apamin-insensitive AHPs in these neurons, has been observed to increase the firing frequency of dopamine cells in vitro by 20% on average (Nedergaard 2004). However, as yet we cannot exclude the possibility that this effect was mediated by blockade of D2 receptors (Pucak and Grace 1996; Werkman et al. 2001).

The effect of ERG block on the plateau potentials in the model is much more prominent because the mechanism for the...
oscillation is completely dependent on this current. This implies that in the presence of spiking, where bursting is observed rather than just a plateau potential oscillation, the ERG current may play a central role in bursting. The bursts evoked by SK block in vitro are unusual because the spiking occurs during the hyperpolarized phase, then a high-frequency burst occurs on the upstroke of the plateau, and usually depolarization block occurs during the final portion of the plateau. The contribution of $I_{ERG}$ to burst termination does not require depolarization block, just a sustained depolarizing wave like the one observed to underlie burst firing in vivo (Grace and Bunney 1984b). This is a novel suggested role for $I_{ERG}$.

Figure 5 shows that haloperidol elongates the depolarized plateaus observed in the presence of apamin and TTX, and that this effect is not mediated by D2 receptors. Instead, we hypothesize that the ERG current helps to relieve depolarization block and may relieve it in vivo as well. Activation of the ERG current is too slow to contribute to spike frequency adaptation, which in dopamine neurons is most pronounced in the interval between the first spike and the second spike (Richards et al. 1997; Shepard and Bunney 1991). However, this current does contribute to the poststimulus inhibitory period (Nedergaard 2004) immediately after a prolonged depolarization, which supports the hypothesis that this current is activated by a long depolarization and therefore able to contribute to relief of depolarization block. Bursts in dopamine cells recorded intracellularly in vivo have been shown “to ride on a depolarizing wave, which often extended beyond the last spike in the burst” (Grace and Bunney 1994b) and could, at least in the case of rats treated chronically with haloperidol, “bring the membrane to the inactivation level” (Grace and Bunney 1986), that is, depolarization block. When dopamine neurons are depolarized excessively, such as during the plateaus of apamin-induced burst firing (Ping and Shepard 1996), inactivation of the fast sodium current cannot be removed between spikes and spiking ceases until the neuron is sufficiently hyperpolarized to relieve this inactivation. High concentrations of glutamate induce cessation of firing presumably due to depolarization block in vivo (Kiyatkin and Rebec 1998); L-glutamate has also been shown to be capable of inducing depolarization block in vitro (Wang and French 1993) and thus depolarization block may also occur in vivo under conditions of excessive glutamatergic stimulation.

Most antipsychotic drugs (Witchel et al. 2003) have the side effect of blocking ERG channels, including the cardiac isoform. Blockade of the cardiac ERG channel is responsible for some cardiac arrhythmias and may contribute to sudden death in individuals without sufficient repolarization reserve. In this study, we show that by repolarizing the plateau potentials, the ERG current likely contributes to relief from depolarization block in dopamine neurons under conditions of apamin-induced block of the SK channel in a slice preparation. It is also possible that the ERG acts to relieve depolarization block in vivo, under normal physiological conditions. The postulated role of depolarization block (Grace et al. 1997) in the action of antipsychotic drugs used to treat schizophrenia combined with our suggestion that the ERG channel may also relieve depolarization block under physiological conditions in vivo implies that antipsychotics may derive some of their therapeutic benefits from their effects on the neural ERG channel.

In summary, the experimental results presented here strongly support the contention that the plateau potential oscillations induced by SK block are not driven by an oscillation in somatic calcium because the calcium concentration appears to merely lag the membrane voltage and the oscillations can also be induced by BAPTA with no significant difference in the plateau duration. We have proposed a model in which the plateau termination mechanism is purely voltage dependent and slow, small-amplitude changes in the ERG current combine with the action of the L-type calcium current to produce the oscillation. The model predictions can be summarized as follows: 1) The sequential kinetic scheme is not essential to the oscillatory mechanism, but can preferentially slow activation and deactivation in the voltage range in which the time constant is the slowest. 2) The relaxation oscillator model predicts that the duty cycle will be strongly dependent on the applied current and will preferentially elongate the plateau at the depolarized end of the range and the trough at the hyperpolarized end. This prediction was then confirmed experimentally. 3) The application of BAPTA converts the SK conductance to an essentially linear leak conductance with a magnitude that depends on the level of applied current that determines the steady-state calcium concentration. If all other parameters are held constant, the frequency in the case of BAPTA application should be faster than that in the case of SK block. 4) The H current enables mixed-mode oscillations and $I_H$ block decreases the frequency of the oscillation. 5) Partial block of the ERG current elongates the plateaus. This prediction was also confirmed experimentally. The model further predicts that partial block greatly reduces the range of applied current that supports the plateau potential oscillations and that complete block results in a persistent depolarized plateau. Partial block can also result in a persistent depolarized plateau, but if the block is not sufficiently complete, the application of a hyperpolarizing current can restore the oscillation. The ERG current may be a key determinant of burst firing in vivo by virtue of its predicted contributions to burst termination and relief of depolarization block.

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REFERENCES


