A Modeling Study Suggests Complementary Roles for GABA_A and NMDA Receptors and the SK Channel in Regulating the Firing Pattern in Midbrain Dopamine Neurons

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Komendantov, Alexander O., Olena G. Komendantova, Steven W. Johnson, and Carmen C. Canavier. A modeling study suggests complementary roles for GABA_A and NMDA receptors and the SK channel in regulating the firing pattern in midbrain dopamine neurons. J Neurophysiol 91: 346–357, 2004. First published September 17, 2003; 10.1152/jn.00062.2003. Midbrain dopaminergic (DA) neurons in vivo exhibit two major firing patterns: single-spike firing and burst firing. The firing pattern expressed is dependent on both the intrinsic properties of the neurons and their excitatory and inhibitory synaptic inputs. Experimental data suggest that the activation of N-methyl-D-aspartate (NMDA) and GABA_A receptors is a crucial contributor to the initiation and suppression of burst firing, respectively, and that blocking Ca^{2+}-activated potassium SK channels can facilitate burst firing. A multi-compartmental model of a DA neuron with a branching structure was developed and calibrated based on in vitro experimental data to explore the effects of different levels of activation of NMDA and GABA_A receptors as well as the modulation of the SK current on the firing activity. The simulated tonic activation of GABA_A receptors was calibrated by taking into account the difference in the electrototoxic properties in vivo versus in vitro. Although NMDA-evoked currents are required for burst generation in the model, currents evoked by GABA_A-receptor activation can also regulate the firing pattern. For example, the model predicts that increasing the level of NMDA receptor activation can produce excessive depolarization that prevents burst firing, but a concurrent increase in the activation of GABA_A receptors can restore burst firing. Another prediction of the model is that blocking the SK channel current in vivo will facilitate bursting, but not as robustly as blocking the GABA_A receptors.

INTRODUCTION

Midbrain dopaminergic (DA) neurons play an important role in voluntary movement, reward mediated learning, working memory, cognition, and motivation (Kitai et al. 1999; Schultz 1998; Spanagel and Weiss 1999). They are also implicated in neuropsychiatric disorders including schizophrenia (Weinberger 1987), drug abuse (Koob et al. 1987), and Parkinson’s disease (Obeso et al. 2000). This relatively homogeneous population of neurons (but see Liss et al. 2001; Neuhoff et al. 2002) is located in the ventral tegmental area (VTA or A10), the substantia nigra pars compacta (SNC or A9), and the retrorubral area (A8).

Midbrain DA neurons in vivo exhibit at least two major firing patterns: single-spike firing, which is often irregular, and burst firing (Freeman et al. 1985; Grace and Bunney 1984a,b; Tepper et al. 1995; Wilson et al. 1977). Activation of N-methyl-D-aspartate (NMDA) receptors has been implicated in burst firing in vivo (Chergui et al. 1993). The mechanisms by which the firing pattern is regulated are of interest because the firing pattern is likely to encode information that is transmitted to target neurons. Transitions between burst firing and single-spike firing have been observed in single-neuron recordings from behaving rats (Freeman et al. 1985). The transition to bursting appeared to be correlated with an orienting response to a sensory stimulus (Freeman et al. 1985). When spikes are clustered into bursts, the increases in levels of extracellular dopamine in the projection area are much larger per spike than those observed for regularly spaced trains of action potentials at the same average frequency due to nonlinear summation as release outpaces uptake (Wightman and Zimmerman 1990). Furthermore, a bursting pattern of stimulation of the medial forebrain bundle increased the expression of one immediate early gene (cfos) (Chergui et al. 1996) and increased the mRNA expression of several immediate early genes (Chergui et al. 1997) in the dopamine-innervated brain areas of the rat, whereas a regular single-spike stimulation pattern did not.

In contrast to the activity exhibited in vivo, dopamine neurons in vitro generally fire in a regular single-spike-firing pattern. Bath application of NMDA can induce burst firing in vitro under some circumstances (Johnson and North 1992), and increasing the activation of GABA_A receptors in vitro by applying the appropriate agonist can convert NMDA-induced burst firing to single spike firing (Johnson and Canavier 1998; Paladini et al. 1999b). Blocking the calcium-activated small conductance (SK) potassium channel facilitates bursting in vitro (Seutin et al. 1993) and can putatively induce burst firing in the absence of NMDA (Nedergaard et al. 1993; Ping and Shepard 1996) as well. Under other conditions, an SK channel blocker such as apamin can induce irregular single-spike firing (Ping and Shepard 1996).

Spontaneous synaptic potentials from excitatory amino acid (EAA) and GABAergic inputs are not usually observed in the slice preparation, although spontaneous events that appear to be GABA_A-mediated postsynaptic potentials (PSPs) can be observed if extracellular potassium concentration is increased (Johnson and North 1992). Thus the major difference between dopamine neurons in vivo versus in vitro is that the synaptic afferents are intact in vivo, leading to a much lower input...
resistance as measured with sharp electrodes [192 ± 12 (SE) MΩ (Johnson and North 1992), 183 ± 5 (SE) MΩ (Ping and Shepard 1996), 152 ± 14 (SE) MΩ (Sergoussis et al. 2001), 297 ± 11 (SE) MΩ (Seutin et al. 1997), and 117.5 ± 4.8 (SD) MΩ (Yung et al. 1991) versus 31.2 ± 7.4 (SD) MΩ (Grace and Bunney 1983)], and a shorter time constant [33 ± 2 (SE) ms (Johnson and North 1992) and 37.5 ± 1.9 (SD) ms (Yung et al. 1991) versus 12.1 ± 3.2 (SD) ms (Grace and Bunney 1983)]. We have assumed that the difference in input resistance is primarily due to the tonic activation of the GABA_A receptors in vivo because 70–90% of all the inputs to the DA neurons are GABAergic (Bolam and Smith 1990; Smith and Bolam 1990). The main sources of GABAergic inputs to DA neurons originate from the substantia nigra pars reticulata, striatum, and pallidum, which all exert their effects via GABA_A receptors (Celada et al. 1999).

The application of the GABA_A receptor antagonists such as picROTOXIN and bicculline strongly promotes burst firing in vivo (Paladini and Tepper 1999). However, bicculline also blocks the apamin-sensitive SK channel (Johnson and Seutin 1997), and blockade of this channel has also been recently shown to promote burst firing in vivo (Seutin et al. 2002). We used a multi-compartmental model of a DA neuron with a realistic morphological structure and electrophysiological properties to explore the effects of varying the levels of activation of NMDA and GABA_A receptors as well as the level of modulation of the SK current on the firing pattern because experimental data suggest that all three play a major role. Burst firing mechanisms (and oscillatory mechanisms in general) require a fast positive feedback process and a slow negative feedback process (Rinzel and Ermentrout 1998). As in previous models (Canavier 1999; Komendantov and Canavier 2002; Li et al. 1996), we assumed that the fast positive feedback process is the voltage-dependent opening and closing of NMDA receptor channels in the presence of tonic activation of those receptors, whereas the slow negative feedback is provided by the sodium activation of the sodium pump resulting from changes in sodium concentration in the dendrites due to sodium entry via NMDA receptors and sodium removal by the pump itself (Johnson and North 1992). This mechanism is controversial, but we believe it is the best available explanation of NMDA-induced burst firing in vitro and have incorporated it into our in vivo model as well, although we acknowledge that it may not be the sole bursting mechanism exhibited by these neurons (Amini et al. 1999). A preliminary report of our findings has been published in abstract form (Komendantov and Canavier 2001).

**Methods**

**Experimental procedures**

**Tissue preparation.** Male Sprague-Dawley rats (150–300 g; Bantin and Kingman, Seattle, WA) were anesthetized with halothane and killed by severing major thoracic vessels. The whole brain was quickly removed and submerged in ice-cold artificial cerebral fluid (ACSF). A vibrating microtome (Lancer) was used to cut slices of midbrain (300 μm) in the horizontal plane. A slice was then placed on a nylon mesh in a recording chamber (volume: 0.5 ml) and secured by two titanium electron microscopy grids that were held in place by small pieces of platinum wire (0.5 mm diam). The slice was submerged in continuously flowing (2 ml/min) ACSF of the following composition (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 2.4 CaCl₂, 10 glucose, and 18 NaHCO₃; gassed with 95% O₂-5% CO₂, pH 7.4, at 36°C.

**Intracellular recordings.** Microelectrodes were made from borosilicate capillary tubing (1.0 mm OD, 0.5 mm ID; Dagan) using a P-97 Flaming-Brown micropipette puller (Sutter Instrument, Novato, CA). Microelectrodes were filled with either 2 M potassium chloride or potassium acetate with resistances of 45–150 MΩ. Membrane voltage was amplified with an Axoclamp-2B amplifier (Axon Instruments, Foster, CA) and recorded on an IBM-compatible personal computer running Axotape (Axon Instruments) software. To ensure accurate measurement of voltage, recordings were made with an active bridge circuit that was frequently checked for proper balance by passing small (50 pA) current steps while voltage output was monitored on an oscilloscope. Membrane input resistance was calculated by measuring the change in membrane potential in response to small (20–100 pA) hyperpolarizing current pulses.

**Identification of dopamine neurons.** Using a dissection microscope, the VTA was identified as the region lateral to the fasiculus retroflexus and medial to the medial terminal nucleus of the accessory optic tract, whereas the SNC was located immediately rostral and caudal to the medial terminal nucleus of the accessory optic tract (Paxinos and Watson 1986). Neurons were identified as dopaminergic using well-established electrophysiological and pharmacological criteria (Grace and Onn 1989; Lacey et al. 1989; Yung et al. 1991). Briefly, dopamine-containing neurons were identified as such by their broad action potentials (>2 ms), spontaneous pacemaker-like firing pattern (1–5 Hz), by the “sag” in membrane potentials recorded during hyperpolarizing current pulses (signifying H current), and by 5- to 15-mV hyperpolarization evoked by superfusing the slice with dopamine (30 μM). Because we found no differences in results from neurons recorded in the SNC and VTA, data from these regions were pooled.

**Pharmacological materials.** All drugs were added to the superfusing ACSF. Drug solutions were prepared just before use and were subsequently diluted 1:1,000 in ACSF prior to use. We have previously shown that this concentration of dimethyl sulfoxide has no effect on burst firing (Johnson and Seutin 1997). Stock solutions of all other drugs were made in water and diluted 1:1,000 in ACSF prior to use. Bicuculline methiodide (BMI), BFB, NMDA, and isoguvacine were obtained from Research Biochemicals International (Natick, MA), whereas apamin, dopamine HCl, and tetrodotoxin (TTX) were obtained from Sigma Chemical (St. Louis, MO).

**Burst firing.** Dopamine neurons were induced to fire in bursts by perfusing the slice with NMDA (20 μM) and apamin (100 nM) as described previously (Johnson et al. 1992). Burst firing was defined as a repeating pattern in which each “burst” of action potentials, consisting of at least three spikes firing at a frequency of ≥10 Hz, is separated by an interburst hyperpolarization of ≥5 mV (Seutin et al. 1993). A constant hyperpolarizing current (50–250 pA) was passed though the electrode as needed to prevent excessive NMDA-induced depolarization.

**Modeling procedures**

**Model development.** We used a modified version of compartmental model of DA neuron as described by Komendantov and Canavier (2002) but with a more realistic morphological structure, which consists conceptually of a soma and four identical branched dendrites with a single proximal and two distal branches (Fig. 1) and allowed us to use realistic values for the passive properties in intensive units, while still matching the macroscopic properties such as the input resistance and the time constant. The distal portions of the dendrites can sustain an oscillation in current even when the soma is voltage clamped (Johnson and North 1992), so the need for the distal
Calcium dynamics were added to the soma, which is described by a calcium balance equation:

\[ \frac{d[Ca^{2+}]}{dt} = 2 \times f_{ca}(I_{Ca,i} + I_{Ca,k} + I_{Ca,n} + I_{Ca,p})d_{F} \]

where \( f_{ca} \) is the constant fraction of free calcium, and \( F \) is Faraday’s constant. The calcium dynamics include a low-threshold calcium current \( I_{Ca,L} = \frac{g_{Ca,L}}{2} \frac{dF}{dt}(V_{i} - E_{Ca}) \), an N-type calcium current \( I_{Ca,N} = \frac{g_{Ca,N}}{2} \frac{dF}{dt}(V_{i} - E_{Ca}) \), a calcium pump \( I_{Ca,P} = \left[ \frac{I_{Ca}[Ca^{2+}]}{[Ca^{2+}]_{o}} \right] + K_{M,Ca,P} \), a calcium leakage current \( I_{Ca,L} = \frac{g_{Ca,L}}{2} (V_{i} - E_{Ca}) \), and an amphoteric-sensitive current \( I_{Ca,SK} = \frac{g_{Ca,SK}}{2} \left[ I + (K_{M,SK} / [Ca^{2+}])^{2} \right] (V_{i} - E_{Ca}) \). For all gating variables \( X \) was described by \( \frac{dx}{dt} = (X_{m}(V_{i}) - x)/\tau_{x} \) where \( \tau_{x} \) is the time constant. The steady-state voltage dependence was determined using \( X_{m}(V_{i}) = 1.0/[1.0 + \exp(- (V_{i} - V_{half,i}]/\theta_{i})] \), where \( V_{half,i} \) is the half activation voltage for the gating variable \( X_{i} \), \( \theta_{i} \) is the slope factor for the variable. The time constants were described by \( \tau_{x} = \tau_{m} \exp(- (V_{i} - V_{half,i}]/\theta_{i})] + B_{i} \). For \( Z = I_{Ca,L} + I_{Ca,N} \) the steady-state calcium dependent inactivation function was modeled as \( Z_{i}([Ca^{2+}]) = K_{M,Z} / (K_{M,Z} + [Ca^{2+}]^{n}) \). Although voltage-activated calcium channels are present on the dendrites in these neurons (Wilson and Callaway 2000), we omitted calcium dynamics in the dendrites as the simplest way of implementing the segregation of calcium entry through NMDA receptors from SK channels that is observed experimentally (Canavio and Johnson 1999; Morikawa et al. 2003). The descriptions of the calcium currents were taken from Amini et al. (1999) with a few modifications as noted in the following text. Several currents included in Amini et al. (1999) were omitted for simplicity: a high-voltage threshold calcium current, the hyperpolarization-activated cation current, and the sodium-calcium exchanger. In addition, the slow component of \( I_{Ca,L} \) described by Amini et al. (1999) was ignored, the form of the calcium-dependency of \( I_{Ca,L} \) was changed to accommodate the data presented in this paper, and some parameters of \( I_{K,DR} \) and \( I_{Ca,L} \) were adjusted. The NMDA-induced current (\( I_{NMDA} \)) was simulated using a Goldman-Hodgkin-Katz equation (Canavio and Johnson 1999; Canavio and Canavier 2002) and an instantaneous magnesium block (\( \rho_{m} \)) described by \( \rho_{m} = 0.0225 + 0.9775 / \left( 1 + (\rho_{M} / (K_{m,Mg})) \right) \). Currents evoked by GABA\(_{A}\) receptors activation were modeled as a linear leak \( I_{GABA_A,s} = g_{GABA_A,s} \left( V_{i} - E_{Cl} \right) \). The simulated distribution of the GABA\(_{A}\) receptors in different compartments (soma, proximal dendrites, and distal dendrites) was based on the assumption that synapses on cell body are mainly inhibitory (Kandel and Siegelbaum 2000) and evidence of a preferential effect of GABA on the somata of dopamine neurons compared with the dendrites (Kalivas 1993). We used the following ratio between the conductances for GABA\(_{A}\)-induced currents in all simulations: \( g_{GABA_A,s} / g_{GABA_A,p} / g_{GABA_A,d} = 1.0 / 0.1 / 0.1 \). The leakage current \( I_{L} \) composed of calcium \( I_{L,Ca} \) in soma only, sodium \( I_{L,Na} \), and potassium \( I_{L,K} \) was adjusted along with other passive parameters to obtain better correspondence to the input resistance (215 M\( \Omega \)) (Johnson and North 1992; Ping and Shepard 1996), and time constant (\( \tau = 31 \) ms) (Johnson and North 1992) experimentally observable in vivo. These values were measured by applying 94 pA of hyperpolarizing current via a simulated electrode in the soma to hold the membrane potential at -66 mV to suppress the spontaneous SOP, then applying an additional 71 pA of...
TABLE 1. Model parameters

<table>
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<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
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<td>$\tau_{N}$</td>
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Conductance of this current (900 $\mu$S/cm$^2$) was set to produce agreement with experimental values. A simulated voltage-clamp experiment that corresponds to experimental data (Canavier and Johnson 1999) with a 350-ms voltage step to $-30$ mV from a holding potential $-60$ mV is shown in Fig. 2, A1. Simulated blockage of the SK channels eliminates the peak current of $\approx 1200$ pA with a latency of $\approx 200$ ms, and the peak tail current of 450 pA.

The parameters of model NMDA-induced current were fit to the experimental data of Wu and Johnson (1996) using a simulated voltage clamp in the soma. The conductances for the L-type calcium current ($g_{Ca,L}$) and the fast sodium current ($g_{Na,i}$) were set to zero to simulate the application of nifedipine and tetrodotoxin, respectively. The holding potential was $-60$ mV, the pulse duration was 800 ms, and the incremental voltage step was 10 mV. The control current was recorded at the end of the test potential. The series of experiments in the presence of 10 $\mu$M NMDA was reproduced with $P_{NMDA} = 0.4 \cdot 10^{-6}$ cm/s, and the recorded current was subtracted from the control current to obtain the difference current. The ratio of $P_{NMDA}$: $P_{NMDA} = 1:1$ on the distal and proximal dendrites produced the best fit to the experimental data. Figure 2A shows that the simulated NMDA difference currents (---) closely resemble those (- - -) obtained by Wu and Johnson (1996) in 10 $\mu$M NMDA. The region of negative slope in the current-voltage curve from $-90$ to $-50$ mV causes positive feedback, which is essential to the generation of the oscillation underlying burst firing (Mereu et al. 1991; Overton and Clark 1997; Wu and Johnson 1996).

The model produces spontaneous spiking in a simulation of an in vitro preparation in normal Ringer solution ($P_{NMDA} = 0$; $g_{GABA_A,i} = 0$; Fig. 2B1). The simulated application of apamin by setting $g_{K,Ca,SK} = 0$ (Fig. 2B2) decreased the afterhyperpolarization (AHP) after the action potential by 15 mV and increased spike frequency, which is consistent with experimental data (Johnson and Seutin 1997; Ping and Shepard 1996; Shepard and Bunney 1991). If application of TTX is simulated instead by setting $g_{K,i} = 0$, slow oscillations in membrane potential with an amplitude of 10 mV (Fig. 2B3) result from the interaction of Ca$^{2+}$ and Ca$^{2+}$-dependent conductances (Kang and Kitai 1993).

MODEL IMPLEMENTATION. As in previous research (Canavier 1999; Komendantov and Canavier 2002), the simulations and programs for analysis were coded in the C programming language. Numerical integrations of simulations were performed using the Fortran implementation of an implicit Runge-Kutta method of order five with variable step size (Harrer and Wanner 1996).

ANALYSIS OF MODEL ACTIVITY. The analysis of model responses used the time courses of $V$ and interspike intervals (ISIs); the first 30 s of a simulation was considered as a transient period and excluded. The next 20 s of simulations were recorded and analyzed. The control

![Figure 2](http://jn.physiology.org/Downloaded.from.http://jn.physiology.org/content/jn/91/1/349/F2)

FIG. 2. Model calibration: A: simulated voltage-clamp data. $A1$: simulated current recorded before, during, and after a simulated 350-ms step to $-30$ mV from holding potential of $-60$ mV in the absence (---) and simulated blockage of $g_{K,Ca,SK} = 0$ (- - -). $A2$: comparison of simulated N-methyl-D-aspartate (NMDA) difference currents recorded at the end of a simulated 800-ms step to the test potential from a holding potential of $-60$ mV. -- -- --, experimental data (Wu and Johnson 1996); ---, simulated data. All model conductances were equilibrated at $-60$ mV before each step. $B$: simulation of pacemaker activity in the absence of NMDA and GABA$A$ receptor activation ($P_{NMDA} = 0$; $g_{GABA_A,i} = 0$). -- -- --, experimental data (Wu and Johnson 1996); ---, simulated data. All model conductances were equilibrated at $-60$ mV before each step. $B1$: spiking activity in control. $B2$: spiking activity under simulated SK channel block ($g_{K,Ca,SK} = 0$). $B3$: slow oscillations of membrane potential under simulated TTX block of spike generation ($g_{K,i} = 0$).
parameters were the permeability for the NMDA-induced current in the dendrites ($P_{\text{NMDA}}$), corresponding to the level of excitation, and the conductance for the $\alpha$-GABA receptor current in soma ($g_{\text{GABA}_{A,\alpha}}$) corresponding to the level of inhibition. The automated determination of the mode of activity (regular and irregular spiking, regular and irregular bursting) was made according to algorithms used for the previous model (Komendantov and Canavier 2002) with minor changes to the evaluation criteria for burst determination. Bursts were defined as groups of at least two spikes terminated by an interburst interval of ≥160 ms (Grace and Bunney 1984b) with hyperpolarization of ≥5 mV (Johnson and Seutin 1997). The selection criteria for detection of irregularity in the model activity were determined empirically and confirmed by visual observation of the membrane potential waveform in many instances.

The dependence of input resistance on $g_{\text{GABA}_{A}}$ was calculated with the NMDA-induced permeability ($P_{\text{NMDA}}$) set to zero using the methods described in the preceding text. The values of the conductance for the $\alpha$-GABA receptor currents were varied from 0 to 2,750 $\mu$S/cm$^2$ for the soma and from 0 to 275 $\mu$S/cm$^2$ for the dendritic compartments. We selected the range of $g_{\text{GABA}_{A}}$ that produced a physiologically acceptable range of input resistance in vivo.

RESULTS

Effect of a GABA$\alpha$ agonist on the firing pattern in in vitro experiments

We used a concentration of 100 $\mu$M isoguvacine because preliminary studies showed that this concentration of this GABA$\alpha$ receptor agonist is near the EC$_{50}$ value for increasing conductance in DA neurons. In all dopamine neurons tested ($n = 3$), isoguvacine markedly inhibited NMDA-dependent burst firing and membrane oscillations, as seen in Fig. 3A.

When recording with electrodes filled with potassium acetate, isoguvacine converted burst firing to a single-spike pattern ($n = 3$) with little or no change in average membrane potential (see Fig. 3A). In contrast, this GABA$\alpha$ receptor agonist caused membrane depolarization (10–20 mV) and blocked all action potentials when recordings were made with microelectrodes filled with potassium chloride ($n = 4$). In the absence of NMDA and apamin, isoguvacine reduced the cellular input resistance by 58 ± 6% ($n = 3$). Isoguvacine acted within 3 min of starting superfusion. Recovery was observed 10–15 min after washout of isoguvacine.

Simulation of effects of GABA$\alpha$ receptor stimulation on bursting in vitro

Figure 3B shows that the simulated addition of isoguvacine in the bath in the simulated presence of both NMDA and apamin converts burst firing to single-spike firing as in the in vitro experiments (Fig. 3A, 1 and 2). The application of isoguvacine (100 $\mu$M) was simulated by adding an ohmic GABA$\alpha$ receptor current that reversed at −70 mV with $g_{\text{GABA}_{A,\alpha}} = 350$ $\mu$S/cm$^2$, $g_{\text{GABA}_{A,B}} = 35$ $\mu$S/cm$^2$, $g_{\text{GABA}_{A,D}} = 35$ $\mu$S/cm$^2$. The value of the reversal potential for this current was chosen based on its correspondence to the chloride reversal potential determined experimentally in DA neuron using micropipettes filled with potassium acetate (Johnson and North 1992). GABA$\alpha$ receptor-mediated depolarizations in dopamine neurons have been shown to be due to the shift in the Nernst potential for chloride, which is −72 ± 4 mV with potassium acetate-filled microelectrodes but shifts to −36 ± 6 mV when potassium chloride-filled microelectrodes raise the intracellular concentration of chloride (Johnson and North 1992).

The simulated application of isoguvacine in the absence of NMDA and apamin decreases the input resistance by 63% (from its control value of 215 to 80 M$\Omega$), which is within the range of the decrease in input resistance (58 ± 6%) that we observed experimentally. In both experiment and simulation, a constant hyperpolarizing current of 180 pA was applied, and the average membrane potential did not change significantly.

Joint modulation of the firing pattern by the activity of NMDA and GABA$\alpha$ receptors

The firing pattern of DA neurons depends on the activation of both excitatory inputs and inhibitory inputs. Komendantov and Canavier (2002) investigated the dependence of the firing pattern of an in vitro model of DA neuron on the simulated level of activation of the excitatory NMDA inputs. As $P_{\text{NMDA}}$ was increased, the firing pattern changed from low-frequency single spiking to bursting and then to high-frequency spiking. We observed similar transitions here. An increase in $P_{\text{NMDA}}$ leads to an increase in the firing frequency as well as transitions from low-frequency spiking through regular and irregular bursting to high-frequency spiking. These sequences correspond to the observed ones in vitro experiments as the concent-

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regulation of NMDA in the bath is increased (Johnson et al. 1992; Paladini et al. 1999b; Wang et al. 1994; Wu and Johnson 2001) except that those studies show depolarization block rather than high-frequency spiking.

The conductance for \( I_{\text{GABA}_A} \) was also varied at a fixed value of \( P_{\text{NMDA}} = 2.3 \cdot 10^{-6} \text{cm/s} \), which results in burst firing in the absence of \( \text{GABA}_A \) receptor activation. At this fixed value of \( P_{\text{NMDA}} \), the different regimes may be distinguished as \( g_{\text{GABA}_A} \). The following modes were observed: silence (hyperpolarization during any interburst interval), represents the deepest hyperpolarization during any burst and the lower branch represents the shallowest hyperpolarization. On the diagram, the minima corresponding to the AHPs of the slow wave decreased as \( g_{\text{GABA}_A} \) was increased: regular bursting (Fig. 4A, 1 and 3), irregular bursting (Fig. 4A2), single spiking (Fig. 4A, 4 and 5) and a silent regime \( g_{\text{GABA}_A} > 2.500 \mu \text{S/cm}^2 \). The amplitude of the slow wave decreased as \( g_{\text{GABA}_A} \) was increased (Fig. 4B). On the diagram, the minima corresponding to the AHPs after action potentials were extracted using the first and second time derivatives of the membrane potentials \( (dV/dt = 0, d^2V/dt^2 > 0) \). Of these minima, only the most and least hyperpolarized minima at each value of \( g_{\text{GABA}_A} \) were plotted on Fig. 4B, thus the upper branch represents the shallowest hyperpolarization during any burst and the lower branch represents the deepest hyperpolarization during any interburst interval.

We investigated numerically the dependence of the firing pattern on the activation levels of NMDA and \( \text{GABA}_A \) receptors simultaneously using a two-parameter bifurcation diagram (Fig. 5A). Each square on the diagram represents the type of activity that can be obtained at given values of \( g_{\text{GABA}_A} \) and \( P_{\text{NMDA}} \). The following modes were observed: silence (hyperpolarized, nonfiring), regular spiking (RS), irregular spiking (IS), regular bursting (RB), and irregular bursting (IB). Similar modes are also observed experimentally in vivo (Dai and Tepper 1998; Grace and Bunney 1984a,b).

Along the \( y \) axis where \( g_{\text{GABA}_A} \) is equal to zero, the firing pattern corresponds to the in vitro preparation in the absence of any tonic activation of \( \text{GABA}_A \) receptors.

**Calibration of the GABAergic inputs**

Grace and Bunney (1983) showed that nigral dopaminergic neurons have an input resistance in vivo in the range of 18–45 MΩ. Figure 5B shows the dependence of input resistance of model DA neuron on the value of conductance of the linear \( \text{GABA}_A \) receptor current, \( \tilde{g}_{\text{GABA}_A} \). The range of \( \tilde{g}_{\text{GABA}_A} \) from 850 to 2,750 \( \mu \text{S/cm}^2 \) (green bar) corresponds to the range of input resistance experimentally observable in vivo (blue bar). Thus we can use Fig. 5A to explain the effects of the application of \( \text{GABA}_A \) antagonists in vivo (Paladini and Tepper 1999; Paladini et al. 1999a). Every single instance of single-spike firing in the in vivo range (to the right of the dashed line) can be converted to burst firing when \( \tilde{g}_{\text{GABA}_A} \) is decreased by 41–65% of its original in vivo value, simulating the in vivo application of a \( \text{GABA}_A \) channel blocker such as picrotoxin. This is consistent with the results of Paladini and Tepper (1999) that revealed that application of \( \text{GABA}_A \) antagonists in vivo (picrotoxin, 200–1,000 \( \mu \text{M} \), and bicuculline methiodide, 200–400 \( \mu \text{M} \)) “caused a robust change to a bursty pattern regardless of the baseline firing pattern,” although the exact fraction of \( \text{GABA}_A \) receptors blocked and the change in input resistance after the application of picrotoxin in the in vivo experiments is not known precisely.

**Quantitative analysis of model activity**

We computed the average (Fig. 5C) spike frequency for each pair of values of the control parameters, \( g_{\text{GABA}_A} \) and \( P_{\text{NMDA}} \) for which the model demonstrated spiking or bursting spontaneous activity. Also, we counted the number spikes per burst (Fig. 5D) for each pair of the control parameters when the activity was determined as bursting. In the simulated physiological range of \( g_{\text{GABA}_A} \) from 850 to 2,750 \( \mu \text{S/cm}^2 \), average spiking frequencies are always less 6.5 Hz for low-frequency single-spike firing, whereas they range from 5.0 to 16 Hz for burst firing. Thus there is some correlation between firing pattern and average frequency (Fig. 5, A and C). On each diagram \( C \) and \( D \), the vertical dashed line bounds the area which corresponds to the putative in vivo of \( g_{\text{GABA}_A} \). In in vivo experiments, the average frequency has been reported as 0–7.1 Hz (Richards et al. 1997), 0 to 7.0–7.2 Hz (Grace and Bunney 1984a,b), and <10 Hz (Dai and Tepper 1998); the number spikes per burst lies in the range from 2 to 10, and the maximal number spikes per burst is 23 (Grace and Bunney 1984).

Figures 6, A1 and A2, shows two possible effects of blocking the \( I_{K,Ca,SK} \) current on the in vivo activity of DA neuron: a
transition from single spiking (A1) to bursting (A2) and a transition from short bursts that contain few spikes to longer bursts containing more spikes (A, 3 and 4).

Figure 6B1 reproduces Fig. 5A but only for $g_{\text{GABA}_{A}}$ from 850 to 2,750 $\mu$S/cm$^2$ and $P_{\text{NMDA}}$ from $1.0 \cdot 10^{-6}$ to $3.5 \cdot 10^{-6}$ cm/s. Figure 6B2 displays the transformation of this phase diagram when $g_{\text{K,Ca,SK}}$ is set to zero. This simulated application of an SK channel blocker converts 59 of the 270 instances of single-spike firing shown in B1, or ~22%, to burst firing and prolongs burst duration of the instances of burst firing. The number of instances of irregular spiking in the in vivo range of parameters increased by 60%, from 45 to 72.

**DISCUSSION**

**Relevance to behavior**

Dopamine neurotransmission probably operates on at least three time scales (Schultz 1998): reward responses on the order of tens and hundreds of milliseconds; the processing of a wide array of rewards, feeding, drinking, punishments, stress, and social behavior of a time scale of seconds to minutes; and an enabling function of a variety of motor, cognitive, and motivational processes on a slow, and possibly tonic, time scale.

Phasic firing in DA neurons increases within 100 ms after the onset of a salient sensory event (Horvitz 2002). Our results...
suggest that, due to the relatively slow rate of change of sodium concentration, a DA neuron must already be biased near the bursting range in order for a transient input to cause bursting in such a short interval. The changes in firing pattern we present here are representative of persistent activity after all transients dissipate, under conditions of inputs that remain nearly constant for seconds to minutes.

Role of different receptors in the activation of glutamatergic inputs

The main sources of EAA inputs (for a review, see Kitai et al. 1999; Overton and Clark 1997) to the midbrain DA neurons are the prefrontal cortex (PFC) (Sesack and Pickel 1992), the subthalamic nucleus (STN) (Kita and Kitai 1987), and the pedunculopontine nucleus (PPN) (Tokuno et al. 1988). Minor EAA projections include the amygdala (McDonald 1996; Phillipson 1979), the laterodorsal tegmental nucleus (Clements and Grant 1990; Gould et al. 1989), and the habenula nucleus (Matsuda and Fujiyama 1992). Recently a novel EAA projection from the bed nucleus of stria terminalis has been identified (Georges and Aston-Jones 2002). Although glutamate also activates non-NMDA ionotropic receptors (AMPA and kainate) and metabotropic receptors, we have chosen to focus initially on the NMDA receptor, based on the following evidence. The glutamate-induced excitation of DA neurons is more sensitive to the selective blockade of NMDA versus non-NMDA receptors because iontophoresis of glutamate and the selective NMDA receptor antagonist (H11006)-3-(-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) produced an 80% reduction of the glutamate-induced increase in firing (Christoffersen and Meltzer 1995), whereas glutamate and the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) produced only a 26% inhibition of the firing rate.
glutamate-induced excitation. Similarly, Chergui et al. (1993) showed that bursting was blocked by the local application (iontophoresis and pressure ejection) of the NMDA receptor antagonist 2-amino-5-phosphononovaleric acid (AP-5) but not by the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX) (see also Odorico and Clark 1997). The duration of the excitatory postsynaptic current (EPSP) evoked by AMPA is ~20 ms (Bonci and Malenka 1999), whereas that evoked by NMDA is much longer lasting (Mercuri et al. 1996; Odorico and Clark 1997), on the order of 600 ms (Destexhe et al. 1998). Glutamate affinity for AMPA receptors is lower (EC50 = 19 µM) than for NMDA receptors (EC50 = 2.3 µM) (Patneau and Mayer 1990), thus lower concentrations of glutamate in the cleft are required to activate NMDA receptors, which accounts at least in part for the longer duration of the activation of these receptors. Recent simulations (Canavier and Landry 2002) suggest that only EAA afferent firing frequencies at which NMDA but not AMPA inputs summate temporally are effective in evoking burst firing. Thus modeling the activation of NMDA receptors only is a reasonable first approximation of the effects of glutamatergic inputs.

In the model, we observed high-frequency bursts with a large number of spikes per burst at high values of PNMDA that have not been observed experimentally in vivo. We hypothesize that in vivo the presence of AMPA receptors prevents this pattern of activity. Similar activity with a large number of spikes per burst was obtained in vitro using bath application of NMDA (Wu and Johnson 2001). The inclusion of AMPA receptor activation in the model as a sum of linear leaks for Na+ and K+ (see Canavier 1999) converts this high-frequency spiking into depolarization block (for example, for PNMDA = 3.4 · 10−4 cm/s, gGABA,A = 0 µS/cm2, gAMPA,K = 30 µS/cm2, gAMPA,Na = 60 µS/cm2). We conclude that AMPA receptors play a more significant role at high levels of glutamate due to the lower affinity of AMPA receptors (Patneau and Mayer 1990) and suggest that AMPA receptors can cause depolarization block at high levels of glutamate rather than the very high-frequency spiking or bursting (~28 Hz) observed in the model.

The model predicts some correlation (Fig. 5, A and C) between firing pattern and firing frequency; however, the presence of such a correlation is controversial (see Freeman et al. 1985; Grace and Bunney 1984b; Paladini and Tepper 1999). Paladini and Tepper (1999) did not observe any correlation in vivo when the application of bicuculline or picrotoxin converted single-spike firing to burst firing. Our model, with its assumption of constant parameters that correspond to activation of NMDA and GABA_A receptors by the average concentration of transmitter in the cleft, cannot account for this lack of correlation. Therefore additional mechanisms must be postulated to account for it, and we hypothesize that the transient nature of the synaptic dynamics observed in vivo may provide these additional mechanisms.

Possible in vivo analogues of blockade of ampin-sensitive current

Although no endogenous neuromodulator is known to act directly on the receptor on the SK channel that binds apamin, the modulation of this current is nonetheless relevant to the modulation of the firing pattern of dopamine neurons by synaptic afferents. It is not necessary for a neuromodulator to act directly on this current to block it because reducing its access to calcium activation will effectively attenuate it. For example, muscarine (50 µM) reduces the amplitude of the slow AHP that follow action potentials in most substantia nigra DA neurons (Scruggs et al. 2001). The SK current is responsible for the AHP (Shepard and Bunney 1991), and muscarine is believed to exert its effects by reducing calcium entry. Another possible modulatory pathway involves inhibitory PSPs evoked by activation of metabotropic glutamate receptors (mGluR). This result in production of inositol triphosphate, IP3, which causes release of calcium from intracellular stores and, in turn, the activation of the SK channels (Fiorillo and Williams 1998). There is evidence that activation of a1 adrenergic receptors or M1 muscarinic receptors interferes with the release of calcium from intracellular stores (Fiorillo and Williams 2000; Paladini et al. 2001), decreasing the amount of SK current evoked by mGluR activation. Thus both the noradrenergic afferents from the locus ceruleus and the cholinergic afferents from the PPN may attenuate the apamin-sensitive SK current, and there have been suggestions (Brodie et al. 1999) that serotonin, possibly acting via IP3-coupled 5HT2 receptors, may attenuate the SK current as well, which would tend to promote burst firing. Also, dopamine, which is released somatodendritically by DA neurons, has some affinity for the a1 receptor. Thus the results of our simulations showing that blockage of Ca2+-sensitive potassium current may promote or facilitate bursting in DA neuron in vivo (Fig. 6) are probably physiologically relevant.

Recently, Wolfart and Roeppe (2002) showed that in response to 20-30 ns hybrid-clamp depolarizations that evoked action potentials, only calcium entering via the T-type calcium channel activates the apamin-sensitive current. Thus neuromodulators that reduce the T-type calcium current would also indirectly reduce the SK current under similar circumstances. It is possible that the experimental conditions did not mimic the activation pattern of calcium channels exhibited during spontaneous, pacemaker-like firing, which has a contribution from additional calcium currents, such as the L-type calcium current (Kang and Kitai 1993; Mercuri et al. 1994; Nederkaard et al. 1993; Takada et al. 2001). On the other hand, Wolfart and Roeppe have suggested that SK channels, T-type calcium channels, and intracellular calcium stores might be selectively colocalized, forming a specialized calcium-signaling complex in which T-type calcium channel influx triggers secondary calcium release that specifically activates SK channels, whereas other types of calcium influx would not. To address these issues, more detailed models that incorporate compartmentalization of calcium are required. Adding voltage-gated calcium channels and calcium dynamics to the dendritic compartments may enhance the ability of the SK channel current to suppress burst firing, as well as the ability of the model to exhibit irregular firing (Wilson and Callaway 2000) due to the desynchronization of calcium oscillations in the various compartments.

How the various manipulations induce or suppress burst firing

This is the first model in which we have combined sodium and calcium dynamics and can simulate regular, calcium-dependent spike firing as well as NMDA-induced burst firing and, as such, is our most complete model of the regulation of the firing pattern in dopamine neurons to date. This model is more focused on the interaction of the various synaptic and electrical properties of the cell, with less emphasis on the separation of these properties and further analysis of their individual effects. The model we describe has several important limitations. The exact role of each of these mechanisms is still unknown, and the model is not able to predict the effects of these manipulations on the in vivo responses. The model also does not incorporate the effects of postsynaptic dendritic spines or the effects of the dendritic spines' calcium dynamics. Finally, the model does not incorporate the effects of calcium oscillations in the various compartments.
intrinsic currents in producing single-spike firing versus bursting than other models of dopamine neurons (Kotter and Feizelmeier 1998; Li et al. 1996; Penney and Britton 2002; Wilson and Callaway 2000). In previous papers (Canavier 1999; Komendantov and Canavier 2002), we used nullcline analyses to gain insight into the mechanisms underlying NMDA-induced burst firing. Although it is not practical to use nullcline analysis on the complex model in this paper, many of the principles from our earlier work still apply. A regenerative, nonlinear current such as the NMDA-induced current is required for a bursting oscillation, whereas linear GABA\textsubscript{A} receptor currents oppose any oscillation. Therefore the many of the modeling results presented herein are likely to be robust and not highly dependent on the precise mechanism of burst repolarization.

It has been suggested (Paladini et al. 1999b) that GABA\textsubscript{A} receptor activation prevents the observation of bursting at the soma by a shunt mechanism. If the GABA\textsubscript{A} receptor current was merely acting as a shunt, that would imply that the distal dendrites continue to oscillate as they did before the GABA\textsubscript{A} agonist was applied but that the current leaks out of the GABA\textsubscript{A} channels and so does not reach the soma. In the model, the application of the excess linear current actually suppresses the oscillation in the dendrites, so there is no burst to spread passively (or actively) to the soma. By analogy to classical mechanics, we call this alternative mechanism linear damping. There are other considerations, such as bias. A higher level of NMDA receptor activation does not always lead to more bursting. Excessive depolarization causes the neuron to leave the voltage range in which the nonlinearity is active due to positive feedback is active because all available NMDA channels are already open. Sometimes hyperpolarization that is not excessive can facilitate bursting by bringing the neuron back into the range in which the positive feedback promotes an oscillation. Finally, the spikes themselves may promote or abolish burst firing via complex nonlinear mechanisms. Therefore increases in B\textsubscript{GABA\textsubscript{A}} evoke complex nonlinear activity (Fig. 4) that eventually leads to the suppression of bursting. Whereas in general increasing glutamatergic input may promote burst firing and in general increasing GABAergic input reduces burst firing, Fig. 5 shows that the ability of a change in one input to effect a change in pattern often depends on the value of the other input. An interesting possibility is that somatic hyperpolarization promotes or actually enables burst firing in the presence of dendritic glutamatergic input by countering excessive depolarization that prevents the dendrites from bursting.

The SK channel current is known to contribute to the regularity of single-spike firing in dopamine neurons because it mediates the repolarization of the slow oscillatory potential that underlies pacemaker-like firing, thus blocking this current disrupts regular pacemaker-like firing. The nonlinearity associated with the apamin-sensitive current is not regenerative and therefore works to suppress any burst generating nonlinearity (Amini et al. 1999).

The blockade by picrotoxin of GABA\textsubscript{A} receptors is maximally effective in inducing bursting. Bicuculline blocks not only GABA\textsubscript{A} receptors, but also the SK channels, the blockade of which also promotes burst firing both in vitro (Seutin et al. 1993) and in vivo (Seutin et al. 2002), so it is not surprising that bicuculline is also maximally effective in inducing burst firing in vivo (Paladini and Tepper 1999). A salient prediction of the model is that blocking the SK channel current in vivo will facilitate bursting but not as robustly as blocking GABA\textsubscript{A} receptors. If the model is shown to underestimate this effect, the contribution of the SK channel on the dendrites must be reconsidered.

DISCLOSURES

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