NMDA-induced burst firing in a model subthalamic nucleus neuron

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Kubota S, Rubin JE. NMDA-induced burst firing in a model subthalamic nucleus neuron. J Neurophysiol 106: 527–537, 2011. First published May 11, 2011; doi:10.1152/jn.01127.2010.—Experiments in rat brain slice show that hyperpolarized subthalamic nucleus (STN) neurons engage in slow, regular burst firing when treated with an N-methyl-D-aspartate (NMDA) bath. A depolarization-activated inward current (DIC) has been hypothesized to contribute to this bursting activity. To explore the mechanism for STN burst firing in this setting, we augmented a previously published conductance-based computational model for single rat STN neurons to include both DIC and NMDA currents, fit to data from published electrophysiological recordings. Simulations show that with these additions, the model engages in bursting activity at <1 Hz in response to hyperpolarizing current injection and that this bursting exhibits several features observed experimentally in STN. Furthermore, a reduced model is used to show that the combination of NMDA and DIC currents, but not either alone, suffices to generate oscillations under hyperpolarizing current injection. STN neurons show enhanced burstiness in Parkinson’s disease patients and experimental models of parkinsonism, and the burst mechanism studied presently could contribute to this effect.

IN THE SUBTHALAMIC NUCLEUS (STN), an increased incidence of oscillations and burst firing, as well as higher overall spike rates, are observed in experimental (Bergman et al. 1994; Mallet et al. 2008) and clinical parkinsonian states (Brown et al. 2001; Levy et al. 2001; Magnin et al. 2000), relative to normal conditions. These parkinsonian alterations in STN outputs may arise through reciprocal synaptic interactions of STN neurons with neurons in the external segment of the globus pallidus (Bevan et al. 2002a, 2002b; Plenz and Kitai 1999; Smith et al. 1998; Terman et al. 2002). STN neurons also receive glutamatergic inputs from other brain areas, however, including cortical inputs via the so-called hyperdirect pathway (Feger et al. 1997; Magill et al. 2001, 2004a; Mallet et al. 2008; Nambu et al. 1996, 2002), offering an alternative source for the modulation of STN activity. Individual STN neurons can also fire bursts in rat STN slice preparations, particularly under various manipulations such as injection of hyperpolarizing current or N-methyl-D-aspartate (NMDA) receptor stimulation (Beurrier et al. 1999; Loucif et al. 2008; Loucif et al. 2005; Overton and Greenfield 1995; Rouzaire-Dubois and Scarnati 1987; Zhu et al. 2004, 2005).

Zhu et al. (2004, 2005) characterized a depolarization-activated inward current (DIC) that contributes to slow (0.4 Hz mean frequency) burst firing in hyperpolarized STN neurons in a rat brain slice bathed with NMDA. They performed a variety of experiments to elucidate the features of the currents and bursting they observed. The DIC itself was activated by calcium (Ca²⁺) influx specifically through NMDA channels and inactivated by voltage. The presence of sodium (Na⁺) in the perfusate was required for DIC expression, suggesting that this is a Ca²⁺-activated nonselective cationic current. Bursting, or membrane potential oscillations, persisted under application of the Ca²⁺-activated potassium (K⁺) channel blocker apamin or the Na⁺ channel blocker tetrodotoxin (TTX), as well as in conditions of low magnesium (Mg²⁺), which releases NMDA channels from their voltage-dependent block, or low K⁺. Activity was lost, however, when Mg²⁺ levels were high or sodium levels were low. Together, these results suggest that hyperpolarized STN neurons can be induced to fire at high frequencies in an NMDA bath due to the combined actions of their NMDA and DIC currents, but they leave open the question of how high-frequency activity is terminated such that bursting oscillations result.

Computational analysis offers an opportunity to explore the mechanisms underlying bursting in individual STN neurons. In this article, we present the results of such a computational study, performed by augmenting an existing conductance-based, single-compartment computational model for an STN neuron (Terman et al. 2002) to include NMDA and DIC currents, fit to the data of Zhu et al. (2005). We characterize the activity of this model under different levels of current injection, with or without NMDA exposure, and show that the model responds to various manipulations in a way that is consistent with experimental observations (Zhu et al. 2005). Notably, we find that without any additional parameter tuning, the combined effects of hyperpolarizing current injection, the NMDA current, and the DIC current are sufficient to yield STN bursting that is consistent with the experimental results of Zhu et al. (2004), without invoking any additional currents or other features. We also present a reduced model that includes only these minimal components to illustrate the fundamental features of the underlying oscillation, and we discuss the possible relevance of these results to parkinsonian conditions.

METHODS

STN neuron model. We constructed a conductance-based STN neuron to analyze activity patterns resulting from NMDA application. We incorporated the active currents used in the STN neuron model of Terman et al. (2002), which exhibits firing properties characteristic of rat STN neurons observed in in vitro recordings (Beurrier et al. 2000; Bevan and Wilson 1999; Bevan et al. 2000): voltage-dependent Na⁺/K⁺ currents (I_Na and I_K), a low-threshold T-type Ca²⁺ current (I_P), a high-threshold Ca²⁺ current (I_{Ca}), and a Ca²⁺-activated K⁺ current (I_{AHP}) (Appendix). Furthermore, we developed a model of the NMDA receptor (NMDAR)-mediated currents (I_{NMDA}) and DIC (I_{DIC}) that were measured in whole cell patch recordings in rat brain.
slices (Zhu et al. 2004, 2005). The membrane potential (V) of our neuron model obeys

\[ C_m \frac{dV}{dt} = -I_{\text{leak}} - I_{\text{Na}} - I_K - I_T - I_{\text{Ca}} - I_{\text{AHP}} - I_{\text{NMDA}} - I_{\text{DIC}} + I_{\text{inj}}, \]

where \( C_m = 1 \ \mu F/cm^2 \) is the specific membrane capacitance; the leak current \( I_{\text{leak}} = g_l(V - V_i) \) with the leak conductance \( g_l = 2.25 \ mS/cm^2 \) and the leak reversal potential \( V_i = -60 \ mV \) (Terman et al. 2002); and \( I_{\text{inj}} \) is the injected current. Other currents have been characterized in STN neurons (Atherton et al. 2010; Barraza et al. 2009; Beurrier et al. 1999; Gillies and Willshaw 2006; Hahn and McIntyre 2010; Overton and Greenfield 1995; Nakashiba et al. 1987), but since they were found not to be necessary for the reproduction of the experimental results that we studied (Zhu et al. 2004, 2005), they were omitted from the model.

Functional NMDARs are heterotetramers consisting of obligatory NR1 subunits and modulatory NR2 (NR2A–NR2D) subunits. The NMDARs in STN neurons appear to mainly comprise NR2D-containing NMDARs, which are expressed in quite limited regions in the adult brain (Monyer et al. 1994; Standaert et al. 1994; Wenzel et al. 1996). NMDARs having NR2D subunits are characterized by a voltage dependence provided by Mg\(^{2+}\) block, which is weaker than that of cortical NMDARs containing NR2A and NR2B subunits, and also by strong Ca\(^{2+}\)-dependent desensitization (Krupp et al. 1996; Monyer et al. 1994). Therefore, we consider that the conductance of the NMDAR current depends on the membrane voltage and, additionally, that the kinetics of channel opening relies on the mechanism of NMDA-induced activation as well as Ca\(^{2+}\)-dependent inactivation. With this assumption, the NMDA current can be described as follows:

\[ I_{\text{NMDA}} = G_{\text{NMDA}}(V, m_N, h_N)(V - V_N), \]

\[ G_{\text{NMDA}}(V, m_N, h_N) = \frac{g_{\text{N}}m_Nh_N}{1 + \eta[Mg]\exp(-\gamma V)}, \]

\[ \frac{dh_N}{dt} = -h_N + h_N^\infty([Ca_N]), \]

where the peak conductance \( g_N = 20 \ mS/cm^2 \) unless otherwise stated, \( V_N = -20 \ mV \) is the reversal potential (Zhu et al. 2005), [Mg] = 1.3 mM is the Mg\(^{2+}\) ion concentration (Zhu et al. 2005), \( \eta = 0.33 \ mM^{-1}\), and \( \gamma = 0.05 \ mV^{-1} \) (Jahr and Stevens 1990). The voltage dependence in Eq. 3 is modified from Jahr and Stevens (1990) to reproduce the data of in vitro STN cells (Zhu et al. 2005; Fig. 1). The activation variable \( m_N \) is set to 1, unless otherwise stated, to reproduce the experimental conditions of Zhu et al. (2004, 2005) using bath application of NMDA with a constant concentration. The inactivation variable \( h_N \) evolves according to Eq. 4, where \( h_N^\infty([Ca_N]) = 1/[1 + \exp(([Ca_N] - \theta_N^0)/\theta_N^0)] \) is a decreasing function of the NMDAR-mediated Ca\(^{2+}\) concentration, \([Ca_N]\) (Fig. 1A), and the inactivation time constant \( \tau_N = 3.000 \ ms \) (Krupp et al. 1996). The Ca\(^{2+}\) dynamics related to NMDAR inactivation is described by the first-order kinetics:

\[ \frac{d[Ca_N]}{dt} = -[Ca_N] - I_{\text{NMDA}}, \]

where the Ca\(^{2+}\) influx through NMDARs is \( \xi_{\text{NMDA}} = k_{\text{NMDA}}G_{\text{NMDA}}(V, m_N, h_N)(V - V_{Ca}), \) the time constant \( \tau_{Ca,N} = 80 \ ms \), and the parameters \( k_1 = 0.005 \) and \( V_{Ca} = 120 \ mV \) (Terman et al. 2002). This model reflects experimental findings (Zhu et al. 2005) suggesting that the...
inactivation of the NMDA current depends on the increase in intracellular Ca2+ mediated specifically by NMDARs (but not T-, L-, or N-type voltage-gated Ca2+ channels).

The same set of experiments indicate that prolonged hyperpolarization (e.g., at V = −100 mV) strongly activates DIC in a calcium-dependent way, whereas the subsequent depolarization to a fixed holding voltage slowly inactivates it, with inactivation kinetics dependent on the holding voltage. To incorporate these experimental observations, we modeled DIC as follows:

\[ I_{DIC} = g_D m_D^p [(\text{Ca})_N] h_D (V - V_D). \]  

In Eq. 6, the peak conductance g_D is set to 20 mS/cm^2 unless otherwise stated, the activation variable depends on the NMDA-mediated Ca2+ concentration, m_D = ([(\text{Ca})_N]^{1/2}[[((\text{Ca})_N)^2] + k_D^2]) (Fig. 1A) with k_D = 1.15 μM, and the reversal potential V_D = −18 mV (Zhu et al. 2005). The inactivation variable h_D obeys the following equation:

\[ \frac{dh_D}{dr} = -h_D + h_D^p (V) \tau_D (V), \]  

where the steady-state value and the time constant of h_D are h_D^0 (V) = 1/[1 + exp(V - \theta_D)/\sigma_D] and \tau_D (V) = 1/[1 + exp(V - \theta_D)/\sigma_D], respectively (Fig. 1B). The parameter values for I_DIC and I_NMDA, that are not mentioned above are summarized as: \theta_D = 0.7 μM, \sigma_D = 0.05 μM, \sigma_D = 300 ms, \sigma_D = 350 ms, \theta_D = −95 mV, \sigma_D = 14 mV, \theta_D = −60 mV, and \sigma_D = 3 mV. These values were selected to reproduce the response of STN neurons and their membrane currents observed in the in vitro recordings (Zhu et al. 2004, 2005), i.e., the voltage and hyperpolarization dependence of I_DIC and I_NMDA (Fig. 1C–G) and the effects of specific ion channel blockers on the firing pattern (Figs. 2 and 3; see RESULTS). All other parameter values and functions included in the model were taken from Termman et al. (2002), with a small number of adjustments, and are described in the appendix.

The neuron model was coded either in C++ programming language or for compilation by XPPAUT software (Ermont 2002) and integrated using the fourth-order Runge-Kutta method with the standard time step size of 0.05 ms. XPPAUT was also used for bifurcation analysis (see Fig. 6).

Three-compartment model. Since NMDA channels are located at dendritic sites, to explore the interaction of dendritic oscillations at the soma, we also constructed a three-compartment STN neuron model consisting of two dendritic compartments coupled electrically (with coupling strength scaled appropriately by membrane area) to a common somatic compartment. We included NMDA currents and DIC in the somatic compartment but not the somatic one; for simplicity, all other currents were the same in all three compartments, with parameters as in the single-compartment model, and we neglected propagation delays. With this assumption, the membrane potentials V_s and V_d,i (i = 1, 2), of the somatic and two dendritic compartments, obey the following equations:

\[ \frac{dV_s}{dr} = -I_{\text{leak}} - I_{Na} - I_K - I_Ca - I_{\text{AHP}} + I_{\text{inj}} \]
\[ + \sum_{i=1}^{2} g_{d,i} (V_{\text{d,i}} - V_s), \]  

\[ \frac{dV_{\text{d,1}}}{dr} = -I_{\text{leak}} - I_{Na} - I_K - I_Ca - I_{\text{AHP}} - I_{\text{NMDA}} - I_{\text{DIC}} \]
\[ + I_{\text{inj}} + \frac{g_{d,1}}{P_{d,1}} (V_s - V_{\text{d,1}}), \]  

\[ \frac{dV_{\text{d,2}}}{dr} = -I_{\text{leak}} - I_{Na} - I_K - I_Ca - I_{\text{AHP}} + I_{\text{NMDA}} - I_{\text{DIC}} \]
\[ + I_{\text{inj}} + \frac{g_{d,2}}{P_{d,2}} (V_s - V_{\text{d,2}}), \]  

where g_{d,i} denotes the coupling conductance between the soma and the ith dendrite and is set to g_{d,1} = g_{d,2} = 1 mS/cm^2. p_s = 0.05 and p_{d,1} = p_{d,2} = 0.475 represent the membrane area of the soma and two dendrites, which are normalized by the total cell membrane area so that p_s + p_{d,1} + p_{d,2} = 1. By construction, the activity of each dendritic compartment in isolation matched that of the single-compartment model. When the three compartments were coupled, random initial conditions were used for all compartments.

Reduced model and its nullclines. To analyze slow membrane oscillations underlying burst activity emerging through coactivation of I_NMDA and I_DIC (Fig. 2), we constructed a reduced model STN neuron. Since the Ca2+ time constant τ_Ca2+ (80 ms) is relatively fast compared with the period of oscillation (~1.3 s) (Fig. 2H), we simply consider a case where [Ca]_N converges to its equilibrium value in Eq. 5: [Ca]_N = [Ca]_N(V, h_N)(1 - k_D^0 G_{NMDA}(V, m_N, h_N(V - V_Ca))). Using this simplification, we can obtain from Eq. 1, with the currents other than I_{leak}, I_NMDA, and I_DIC removed, and Eq. 4 the following expressions:

\[ C_m \frac{dV}{dr} = -g_L (V - V_l) - g_D m_D^p \{ [Ca]_d (V, h_N) \} h_D (V - V_D) \]
\[ - G_{NMDA}(V, m_N, h_N(V - V_N)) + I_{\text{inj}}, \]  

\[ \frac{dh_N}{dr} = \frac{-h_N + h_N^p \{ [Ca]_d (V, h_N) \}}{\tau_{h_N}}. \]  

These equations combined with Eq. 7 constitute the reduced model that we will use for the analysis of NMDA- and DIC-dependent membrane oscillations. Note that I_{leak} does not contribute an additional variable to the model but does affect its quantitative performance, so we include it in the reduced model. We plotted the nullclines of this model, which are the curves of values where the velocity of each state variable equals 0 (see Fig. 7B). In the three-dimensional space spanned by V, h_N, and h_D, it is informative to plot the curved surface obtained from solving dV/dr = 0 from Eq. 10 together with the curve obtained from simultaneously solving dh_D/dr = 0 and dh_N/dr = 0 from Eqs. 7 and 11:

\[ \frac{dV}{dr} = 0 \Leftrightarrow h_D = \frac{-g_L (V - V_l) - G_{NMDA}(V, m_N, h_N(V - V_N)) + I_{\text{inj}}}{g_D m_D^p \{ [Ca]_d (V, h_N) \}} \]
\[ \frac{dh_N}{dr} = \frac{dh_D}{dr} \Leftrightarrow h_N = h_N^p \{ [Ca]_d (V, h_N) \}, \]  

where h_D = h_N = h_D^0(V). We refer to the graphs of the solutions of Eqs. 12 and 13 as the V-nullsurface and the h-nullcline, respectively. The nullsurface and nullcline were plotted and analyzed using MATLAB software.

RESULTS

NMDA-induced burst firing in STN neurons. The activation of the NMDA current and the DIC has been suggested to underlie NMDA-induced bursting in STN neurons (Zhu et al. 2005). To investigate how the interaction of these currents could lead to bursting, we constructed models of the NMDA current and the DIC that reproduce the voltage-dependent kinetics of these currents in in vitro recordings (Zhu et al. 2004, 2005; see METHODS and Fig. 1). The DIC in our model is rapidly activated by the Ca2+ influx evoked by the voltage-dependent NMDAR conductance and is slowly deactivated through membrane hyperpolarization. Thus DIC is evoked most strongly when the membrane is depolarized to about −50 mV after being held at an initial hyperpolarizing potential (~100 mV; Fig. 1, C–E), as in Zhu et al. (2005), and this current exhibits its peak near the voltage at which the NMDA current takes its peak value (Fig. 1, F and G). The decay kinetics of DIC depends on the membrane potential, with longer durations...
occurring at moderate depolarization (about −60 mV; Fig. 1E; Zhu et al. 2005). However, we found that the details of its kinetics are not critical, since our key results (e.g., NMDA and hyperpolarization dependence of burstiness; see Figs. 2 and 3) do not change even when $g_N$ (Fig. 1B), which determines the inactivation time course of DIC (gray line) corresponding to burst firing. $E$: time courses of the inactivation variables for the NMDA current ($h_N$, solid line) and DIC ($h_D$, dashed line). $F$: firing profile of the model in the absence of AHP current ($I_{AHP}$; i.e., $g_{AHP} = 0$), with hyperpolarizing current of −80 μA/cm². $G$: burst duration becomes longer with the blockade of AHP (gray line) than in the presence of AHP (black line). $H$: slow membrane oscillation emerges under a blockade of Na⁺ current ($g_{Na} = 0$; solid line) or with selective activation of DIC and NMDA currents ($g_N = g_K = g_Ca = g_T = g_{AHP} = 0$; dashed line). $I$: blocking either DIC ($g_D = 0$; solid line) or NMDA current ($g_N = 0$; dashed line) eliminates the membrane oscillation and yields convergence to an equilibrium point. Parameter values are $g_N = 16.5$ mS/cm² and $g_D = 14$ mS/cm², except when NMDA or DIC is blocked, with hyperpolarizing current −80 μA/cm² for D–I.

Fig. 2. Firing properties of the subthalamic nucleus (STN) model neuron. $A$: firing response of the model in the absence (left) or presence (right) of NMDA application [i.e., $m_N = 0$ (left) or 1 (right)] without hyperpolarizing current ($I_{inj}$) injection. $B$: model firing response with hyperpolarizing current of −40 μA/cm². $C$: model firing response with hyperpolarizing current of −80 μA/cm². $D$: time course of the NMDA-mediated Ca²⁺ concentration ([Ca]N, black line) and that of the activation variable for DIC ($m_D$, gray line) corresponding to burst firing. $E$: time courses of the inactivation variables for the NMDA current ($h_N$, solid line) and DIC ($h_D$, dashed line). $F$: firing profile of the model in the absence of AHP current ($I_{AHP}$; i.e., $g_{AHP} = 0$), with hyperpolarizing current of −80 μA/cm². $G$: burst duration becomes longer with the blockade of AHP (gray line) than in the presence of AHP (black line). $H$: slow membrane oscillation emerges under a blockade of Na⁺ current ($g_{Na} = 0$; solid line) or with selective activation of DIC and NMDA currents ($g_N = g_K = g_Ca = g_T = g_{AHP} = 0$; dashed line). $I$: blocking either DIC ($g_D = 0$; solid line) or NMDA current ($g_N = 0$; dashed line) eliminates the membrane oscillation and yields convergence to an equilibrium point. Parameter values are $g_N = 16.5$ mS/cm² and $g_D = 14$ mS/cm², except when NMDA or DIC is blocked, with hyperpolarizing current −80 μA/cm² for D–I.

Incorporating the model NMDA current and DIC into a model STN neuron (Terman et al. 2002) yields NMDA-induced burst firing in the presence of hyperpolarizing inputs, which matches experimental observations (Fig. 2, A–E; Zhu et al. 2004). Without hyperpolarization, the neuron exhibits regular single-spike firing independent of whether NMDA is applied (Zhu et al. 2004), but the firing rate is much higher under NMDA application, which evokes depolarizing $I_{NMDA}$ and $I_{DIC}$ (Figs. 2A and 3A). With NMDA application, increased hyperpolarization decreases the firing rate and then increases it again for a wide range of $g_N$ (Fig. 3A). This increased firing
activity is accompanied by strong burstiness, featuring sequences of spikes on top of depolarizing plateaus alternating with periods of quiescence (Fig. 2C), which results in a higher coefficient of variation (CV) of interspike intervals (ISIs) (Fig. 3B). The dependence of NMDA-induced bursting on hyperpolarizing inputs is consistent with experimental results (Zhu et al. 2004) and reflects the voltage-dependent kinetics of DIC, which is deinactivated through hyperpolarization (Zhu et al. 2005; Fig. 1). Typical values of the interburst frequency (i.e., the frequency of occurrence of the active spiking phase) and intraburst frequency (i.e., the frequency of action potentials within each active phase), obtained at a high level of hyperpolarizing input ($I_{inj} = -80 \mu A/cm^2$; Fig. 2C, right), are 0.70 and 41 Hz, respectively. These particular frequencies exceed those reported by Zhu et al. (2004) (0.4 and 13.9 Hz, respectively), although the intraburst frequency is lower than that of some STN bursts arising in other conditions (e.g., Atherton et al. 2010, Bevan et al. 2002a), and the values observed can be significantly modified by the magnitude of the synaptic conductance and of the hyperpolarizing input (see below; Fig. 4).

The NMDA-induced bursting in the present model exhibits several characteristics that are observed in STN neurons (Zhu et al. 2004). Removal of $I_T$ from the model, by setting $g_T = 0$ mS/cm$^2$, has essentially no effect on firing rate or on CVs of ISIs (data not shown). This finding agrees with the fact that mibefradil, a specific T-type Ca$^{2+}$ channel blocker, does not significantly affect STN bursts (Zhu et al. 2004). Blocking $I_{AHP}$ increases the burst duration (i.e., duration of active spiking phase within each burst) from the control value of 219.6 ms to 329.5 ms (relative change of 50.0%) and slightly decreases the interburst frequency, at $I_{inj} = -80 \mu A/cm^2$, from 0.70 Hz to 0.67 Hz (relative change of 3.9%) (Fig. 2, F and G). This result is similar to the experimentally observed effect of apamin on STN neurons, which considerably increases the duration of each burst without significantly altering the interburst frequency (Zhu et al. 2004). The blockade of $I_{Na}$ in the presence of NMDA reveals a slow membrane oscillation (0.75 Hz) that underlies burst activity (Fig. 2H, solid line), as observed in STN neurons recorded in the presence of TTX (Zhu et al. 2004). A similar membrane oscillation occurs even when the active currents other than NMDA and DIC are removed (Fig. 2H, dashed line). However, blocking either of these two currents eliminates the slow oscillation (Fig. 2I).
increases in frequency, leaving duty cycle relatively unaffected. In contrast, bursts (see also Fig. 3 lead to prolonged burst durations and faster spiking within A–D), respectively. More hyperpolarizing current injections A–D burst frequency as a function of these two parameters (Fig. 4, duration to full burst period), intraburst spike frequency, and within each burst), duty cycle (i.e., ratio of active phase computed burst duration (i.e., duration of active spiking phase to a common somatic compartment. In isolation, each of the three compartments were identical to the single-compartment model, consisting of two dendritic compartments coupled electrically to a soma, is plotted for various values of NMDA conductances in the 2 dendrites (as shown in the x- and y-axes) in the presence of hyperpolarizing inputs of \(-80 \, \mu A/cm^2\). The peak conductance for DIC is fixed at 14 mS/cm\(^2\) for both of the dendrites.

suggesting the involvement of the coactivation of \(I_{NMDA}\) and \(I_{DIC}\) in the membrane oscillation. This finding is consistent with the experimental observation that the NMDA-induced oscillation occurring during TTX treatment can be abolished by the effect of flufenamic acid, which blocks DIC (Zhu et al. 2004, 2005).

**Dendritic interactions.** Since NMDA channels are located at dendritic sites, we also simulated a three-compartment model consisting of two dendritic compartments coupled electrically to a common somatic compartment. In isolation, each of the three compartments were identical to the single-compartment model except that the NMDA current and DIC were included in the dendritic compartments but not the somatic one (see METHODS), and hence only the dendritic compartments exhibited bursting (Figs. 2 and 3).

Quantitative features of model bursts depend on its NMDA conductance, which could differ across different dendrites, as well as the injected current level. In a single dendritic compartment (equivalent to the single-compartment model), we computed burst duration (i.e., duration of active spiking phase within each burst), duty cycle (i.e., ratio of active phase duration to full burst period), intraburst spike frequency, and burst frequency as a function of these two parameters (Fig. 4, A–D, respectively). More hyperpolarizing current injections lead to prolonged burst durations and faster spiking within bursts (see also Fig. 3A). They also lower the overall burst frequency, leaving duty cycle relatively unaffected. In contrast, increases in \(g_N\) cause small changes in burst duration and intraburst frequency, relative to those induced by increases in hyperpolarizing inputs, but significantly increase burst frequency and duty cycle. Thus changes in dendritic NMDA conductances could be used to regulate these aspects of STN bursting.

We used the three-compartment model to test the interaction of dendritic oscillations at a soma not capable of generating bursts intrinsically. Simulations using dendritic parameter values associated with bursting in the single-compartment model, including a wide range of \(g_N\) values, yielded periodic bursting in the soma of the three-compartment model (Fig. 5), suggesting that a lack of burst-generating currents at the soma would not prevent transmission of bursts originating at the dendrites. Consistent with past results on bursting model cells coupled by gap junctions (De Vries et al. 1998; Smolen et al. 1993), the somatic burst frequency always fell between the intrinsic burst frequencies of the two dendrites when these were unequal (Fig. 5).

Analyzing the slow oscillation of the membrane potential. To explore the mechanism of \(I_{NMDA}\) and \(I_{DIC}\)-dependent membrane oscillations underlying burst activity, we performed bifurcation analysis using a model that includes only the minimal set of currents required for the oscillatory behavior \([i.e., \, C_m(dV/dt) = -I_{leak} - I_{NMDA} - I_{DIC} + I_{inj}; \, \text{see Fig. 6}].\)

We found that under an increase in the magnitude of hyperpolarizing inputs, a limit cycle oscillation arises from an equilibrium point via a supercritical Hopf bifurcation (Fig. 6, B and C), as can be expected from the occurrence of bursts with

![Fig. 5. Bursting oscillations in 2 dendritic compartments interact to control somatic burst frequency. The interburst frequency obtained using a 3-compartment model, consisting of 2 dendrites interacting with a soma, is plotted for various values of NMDA conductances in the 2 dendrites (as shown in the x- and y-axes) in the presence of hyperpolarizing inputs of \(-80 \, \mu A/cm^2\). The peak conductance for DIC is fixed at 14 mS/cm\(^2\) for both of the dendrites.](http://jn.physiology.org/)

![Fig. 6. Bifurcation analysis of the membrane potential oscillation. A: examples of the membrane potential oscillation for 2 different values of NMDA conductance, \(g_N = 15\) (black line) or \(30\) mS/cm\(^2\) (red line), with injected current of \(-85 \, \mu A/cm^2\). Larger \(g_N\) augments the temporal average of the membrane potential (dashed lines; \(-74.0\) and \(-66.0\) mV for 15 and 30 mS/cm\(^2\) conductances, respectively). B: membrane oscillation arises from a supercritical Hopf bifurcation when the hyperpolarizing current injection is strengthened. Filled circles represent the maximum and minimum values of membrane potential during oscillation, the lines (solid and dashed) show the equilibria, and the label of s (u) stands for stable (unstable) dynamics (NMDA conductance is color coded as in A). C: frequency of oscillation varies with injected current, as shown for 4 different NMDA conductances, \(g_N = 15\) (black line), 20 (red line), 25 (blue line), or \(30\) mS/cm\(^2\) (green line). D: Hopf bifurcation curves in the \(g_N\) vs. \(I_{inj}\) plane for DIC conductances, \(g_D = 15\) (black line), 20 (red line), 25 (blue line), or \(30\) mS/cm\(^2\) (green line). Oscillations arise for parameter values above and to the left of the curves.](http://jn.physiology.org/)}
strong hyperpolarization (Fig. 2, A–C). Larger hyperpolarization produced an increase in the amplitude and a decrease in the frequency of the membrane oscillation (Fig. 6, B and C), similar to experimental observations (Louif et al. 2008). Although larger NMDAR conductances \( g_N \) decreased the amplitude of the oscillation, this change was accompanied by an elevation of the average membrane potential (Fig. 6, A and B), which would underlie the increased firing rate seen with larger \( g_N \) in Fig. 3A. Furthermore, the two-parameter bifurcation diagrams in Fig. 6, D and E, show that the occurrence of the limit cycle oscillation generally requires the combination of a sufficiently strong hyperpolarization, a sufficiently large \( g_N \), and a sufficiently large \( g_D \). This finding is consistent with the conditions under which burst firing occurs, as shown in Fig. 2. Additional two-parameter bifurcation analysis (data not shown) demonstrates that once \( I_{\text{inj}} \) is below some level (roughly \(-23 \, \mu A/cm^2\)), oscillations will occur, but they require the leak conductance \( g_l \) to be in the right range, and this range depends on the value of \( I_{\text{inj}} \). More specifically, a transition from a stable rest state to stable oscillations happens as \( g_l \) is decreased, with the bifurcation occurring at a larger \( g_l \) as \( I_{\text{inj}} \) becomes more negative (e.g., the bifurcations in Fig. 6B occur with \( I_{\text{inj}} \approx -70 \, \mu A/cm^2 \) and \( g_l = 2.25 \, mS/cm^2 \)). If \( g_l \) becomes too small, however, then oscillations are lost and a stable hyperpolarized rest state emerges; that is, enough leak current is needed to help pull the membrane potential up toward the leak reversal potential in the presence of a sufficiently strong hyperpolarizing injected current.

To further understand the oscillatory dynamics, we constructed a reduced three-dimensional model STN neuron (Eqs. 7, 10, and 11; see METHODS), which includes the membrane potential and the inactivation variables of the NMDA current and the DIC (\( h_N \) and \( h_D \), respectively) as the state variables (Fig. 7A). We plotted the trajectory of the reduced model with its \( V \)-nullsurface and \( h \)-nullcline (Eqs. 12 and 13) in Fig. 7B, with parameters fixed at their values from the full model shown in Fig. 2C, right. The \( V \)-nullsurface has two fold curves (Fig. 7B, pink lines), at which the value of \( h_D \) takes a local maximum or minimum with respect to changes in \( V \). As shown in Fig. 7B, when the model generates oscillations, the corresponding trajectory of the system (Fig. 7B, red and blue lines), starting from point \( a \), slides up along the \( V \)-nullsurface until it reaches the fold curve at point \( b \). At this moment, the variable \( V \) rapidly jumps and the trajectory rapidly transitions to point \( c \), since the change in \( V \) is much faster than that of \( h_N \) and \( h_D \), which are associated with slower desensitization processes. After that, the system slides down the \( V \)-nullsurface again as \( I_{\text{DIC}} \) inactivates, and at point \( d \), it jumps to point \( a \), thereby completing one oscillation.

For the oscillation to arise via the Hopf bifurcation, the equilibrium point where the \( V \)-nullsurface and \( h \)-nullcline (defined as the curve where the time derivatives of both \( h_N \) and \( h_D \) are 0) intersect (Fig. 7B, red cross) should lie between the two fold curves (Fig. 7B, pink lines) in a position where it is unstable (Izhikevich 2007). With parameters tuned to this regime and the neuron starting in a sufficiently hyperpolarized state that \( I_{\text{DIC}} \) is deinactivated (Fig. 1), the application of NMDA activates \( I_{\text{NMDA}} \) and this effect in turn activates \( I_{\text{DIC}} \) through NMDAR-mediated Ca\(^{2+}\) influx. However, since \( I_{\text{NMDA}} \) is an inward current in this voltage range, it is not obvious why its activation alone is not sufficient to elicit membrane oscillations and, instead, the concurrent activation of the DIC is required in both the model (Fig. 6E) and the experiments (Zhu et al. 2004). To explain this observation, we plotted the changes in the amplitudes of \( I_{\text{NMDA}} \) and \( I_{\text{DIC}} \) and their sum at the equilibrium point of the reduced model as one of the parameters \( g_N \) or \( g_D \) was varied and the other was kept at a relatively small fixed value (Fig. 8, A and B). We found that increasing \( g_N \) produces saturation of \( I_{\text{NMDA}} \) through the Ca\(^{2+}\)-dependent desensitization (Eq. 4) and thereby causes the saturation of \( I_{\text{DIC}} \) (Fig. 8A). On the other hand, increasing \( g_D \) yields saturation of \( I_{\text{NMDA}} \) but not of \( I_{\text{DIC}} \) (Fig. 8B). This is because increases in \( g_D \) directly amplify \( I_{\text{DIC}} \), even though NMDA saturation disables further enhancement of NMDAR-
mediated Ca\(^{2+}\) influx, as can be expected from Eq. 6. Therefore, the depolarizing input provided by \(I_{\text{NMDA}}\) and \(I_{\text{DIC}}\) can be enhanced more, yielding a larger equilibrium value of membrane potential (\(V_{\text{eq}}\)), through increases in \(g_D\) than by increases in \(g_N\) (Fig. 8C). In particular, a sufficiently large \(g_D\) is required to push \(V_{\text{eq}}\) through the Hopf bifurcation (e.g., compare Figs. 7B and 8C) and achieve oscillations (assuming that \(g_I\) is in the appropriate range, as in the reduced model analyzed in Fig. 6).

In summary, to obtain oscillations, the NMDA current is required to activate the DIC, and the DIC must be sufficiently strong to destabilize the low-voltage rest state associated with the hyperpolarizing current injection.

**DISCUSSION**

STN neurons mostly exhibit irregular tonic spiking in normal conditions, whereas parkinsonism in patients and experimental models is characterized by an increased prevalence of oscillations and bursting and higher overall firing rates in STN (Bergman et al. 1994; Brown et al. 2001; Gatev et al. 2006; Levy et al. 2001; Magnin et al. 2000; Mallet et al. 2008). To explore one possible mechanism for bursting in STN neurons, we have extended a previously published conductance-based computational model for STN neuron activity (Terman et al. 2002), calibrated to rodent slice data, to include additional currents subsequently observed and characterized in rat brain slice (Zhu et al. 2005). When parameters associated with these currents are fit to experimental data, the resulting model generates robust bursting oscillations under a constant hyperpolarizing current injection and complete NMDA activation. Moreover, this bursting activity responds to manipulations representing the application of apamin or of TTX in a way that is consistent with experimental results (Zhu et al. 2004). Interestingly, the bursting oscillations observed in the model, given sustained NMDA current activation, arise predominantly from repeated cycles of activation of a nonspecific cationic current, \(I_{\text{DIC}}\), by Ca\(^{2+}\) influx through NMDA channels, followed by voltage-dependent inactivation of \(I_{\text{DIC}}\). Although a small amount of slow, Ca\(^{2+}\)-dependent NMDA desensitization (Krupp et al. 1996; Monyer et al. 1994) waxes and wanes during the oscillations, no additional elements are needed for this bursting activity to occur. This oscillation mechanism is similar to that arising in previous modeling of the interaction of NMDA current and an outward Na\(^{+}\)-pump current (Li et al. 1996), but it was not obvious that the specific combination of current magnitudes and kinetics identified experimentally in STN neurons would yield such oscillations, as our work shows.

Our model extends an earlier published model (Terman et al. 2002) to include the NMDA and DIC currents hypothesized by Zhu et al. (2004, 2005) to contribute to this form of STN bursting activity. Therefore, our model can still reproduce the characteristic firing properties of STN cells shown in the original model (Terman et al. 2002), such as the intrinsic spontaneous discharge and postinhibitory rebound bursting (Bevan and Wilson 1999; Bevan et al. 2000), which depends on T-type Ca\(^{2+}\) currents. Other currents identified in STN neurons (Atherton et al. 2010; Barraza et al. 2009; Beurrier et al. 1999; Gillies and Willshaw 2006; Hahn and McIntyre 2010; Overton et al. 1995; Nakanishi et al. 1987) were omitted to maintain a focus on a specific putative burst mechanism, and our results suggest that these additional currents are not necessary for the experimentally observed STN bursting to occur, although they may affect its properties. In particular, we omit HCN currents that have been identified in STN neurons (Atherton et al. 2010), since they are activated by synaptic inhibition and act by limiting the availability of low-threshold T-type Ca\(^{2+}\) current, neither of which is an important feature in our study. Zhu et al. (2004) provided evidence that a variety of voltage-dependent Ca\(^{2+}\) currents are not significantly involved in STN bursting, justifying their omission from our model for this particular study. Loucif et al. (2005) observed a significant increase in robustness of bursting when NMDA application was supplemented by apamin administration, a finding that we have not attempted to explain with our model, yet this result was obtained without strong membrane hyperpolarization, and thus its relation to our model is not clear. With membrane hyperpolarization, Zhu et al. (2004) showed that apamin application lengthened bursts and interburst intervals but did not qualitatively alter bursting, and our simulations agree with these results. Beyond apamin, additional \(K^+\) currents (Barraza et al. 2009) may contribute to burst characteristics, and the effects of these currents may help explain why the bursts we observed were briefer (0.22 vs. 1.8 s) and faster (0.7 vs. 0.4 Hz) than those reported by Zhu et al. (2004). Drastic reductions of bath \(K^+\) concentrations were found to increase burst durations and to enhance the amplitude of membrane potential hyperpolarizations between bursts, supporting the involvement of \(K^+\) currents in the observed bursting, although effects of \(K^+\)
reductions on interburst interval durations were not reported (Zhu et al. 2004).

The bursting that we have studied occurred experimentally under combined hyperpolarizing current injection and NMDA bath application. These are rather extreme conditions, and their relevance to parkinsonian states in vivo depends on whether or not STN cells are subjected to significant sources of hyperpolarization and NMDA inputs in that setting. In parkinsonian conditions, STN neurons do experience bouts of strong inhibitory inputs from GPi neurons (Baufreton et al. 2001, 2005; Bevan et al. 2002a, 2002b; Terman et al. 2002). In STN neurons that are voltage clamped at $-60$ mV, Baufreton et al. (2001) observed hyperpolarizing currents of $117 \pm 14 \, \mu A/cm^2$ induced by application of the GABA agonist muscimol at 10 \mu M (with half-maximal response at 5.2 \mu M), suggesting that the currents injected to induce the bursting that we have discussed are of physiologically relevant amplitudes (but see Loucif et al. 2005). Dopamine depletion, a key feature of parkinsonism, is also known to lead directly to hyperpolarized STN membrane potentials (Cragg et al. 2004, Loucif et al. 2008), and the absence of dopamine enhances GABA-mediated outward currents (Shen and Johnson 2005), suggesting that the loss of dopamine associated with parkinsonism may help promote the hyperpolarization that can serve as a backdrop for STN bursting. Consistent with this idea, Loucif et al. (2008) found that STN burst firing that is induced in slices by NMDA and apamin administration can be switched to tonic, regular firing by dopamine application. These forms of hyperpolarization would not match the intensity of sustained hyperpolarization achieved by experimental current injection, however, and simulation studies of how these effects can contribute to bursting are still needed.

Positing that membrane hyperpolarization is an important component of STN bursting leads to the prediction that counteracting STN hyperpolarization may be therapeutic for parkinsonism. This prediction should not be interpreted as a prescription for elimination of sources of inhibition to STN, however. Lesions in GPe neurons of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys have been found to worsen parkinsonian symptoms (Zhang et al. 2006). Complete elimination or block of GPe activity after the induction of a dopamine-depleted state could yield such effects by promoting at least somewhat excessive STN firing as well as reduced inhibition to GPI neurons (leading to enhanced GPI firing as also reported in Zhang et al. 2006), even if it did reduce STN bursting, effectively substituting one form of pathology for another. On the other hand, less extreme interventions that lower the levels of inhibition to STN more modestly would be expected to reduce bursting without such negative side effects.

As for the activation of STN NMDA receptors, although it was found that the ratio of AMPA current to NMDA current was significantly increased by 6-hydroxydopamine (6-OHDA) lesions in rats, NMDA currents remain strong after lesions, with the ratio near one (Shen and Johnson 2005). A natural source for NMDA activation is the delivery of cortical glutamatergic inputs to STN via the hyperdirect pathway, and cortical stimulation is indeed known to elicit STN responses (Magill et al. 2004a). Experiments show that slow cortical oscillations in anesthetized rats are associated with and temporally coupled with enhanced STN bursting and that peaks in STN bursting and cortical oscillations are nearly coincident in this setting (Magill et al. 2001, 2004b). Furthermore, dopamine depletion strengthened the cortical influence on STN activity, whereas STN bursting in these experiments was eliminated by cortical ablation (Magill et al. 2001). Of course, cortically induced NMDA activation would not match the sustained activation from experimental bath application of NMDA, and these more physiological mechanisms remain to be explored computationally. Interestingly, the administration of various NMDA antagonists, such as amantadine and memantine, yields improvements in motor signs in Parkinson’s disease patients (Blandini et al. 2001; Mettan et al. 1998; Varanese et al. 2010). Experiments have also been done to investigate how particular NMDA antagonists affect NMDA currents in parkinsonian conditions. In extracellular recordings in 6-OHDA rats, the NMDA antagonist MK801 did not significantly modify the incidence of 3- to 8-Hz oscillations but did decrease spectral power in this frequency range (Allers et al. 2005). Furthermore, coapplication of a dopamine D1 receptor agonist eliminated all bursting, consistent with the idea that a weak dopamine signal together with strong NMDA currents supports STN bursting. In contrast, amantadine significantly increased firing rates but had surprisingly little effect on bursting in lesioned animals, although it eliminated 3- to 8-Hz oscillations in intact animals (Allers et al. 2005). Clearly, additional experiments are needed to sort out the changes in NMDA currents that occur under parkinsonian conditions and how these changes impact STN activity patterns.

APPENDIX: DESCRIPTION OF ACTIVE CURRENTS OTHER THAN $I_{\text{NMDA}}$ AND $I_{\text{IC}}$

As mentioned in Methods, with the exception of $I_{\text{NMDA}}$ and $I_{\text{IC}}$, the currents in Eq. 1 have been taken from a previous model (Terman et al. 2002). These currents are described as $I_{\text{gNa}} = g_{\text{Na}}[\text{Na}]^V(I(V-V_{\text{Na}}))$, $I_{\text{gK}} = g_{\text{K}}[\text{K}]^V(I(V-V_{\text{K}}))$, $I_{\text{gL}} = g_{\text{L}}(V-V_{\text{L}})$, and $I_{\text{AHP}} = g_{\text{AHP}}(V-V_{\text{AHP}})$, where $V_{\text{Na}} = 55 \, \text{mV}$, $V_{\text{K}} = -80 \, \text{mV}$, $\theta_0 = 30 \, \text{mV}$, $\sigma_0 = -14.5 \, \text{mV}$ ($-15.0 \, \text{mV}$), $\sigma_b = -39 \, \text{mV}$, $\sigma_c = 3.1 \, \text{mV}$, $\sigma_s = 0.3 \, \text{mV}$, $\sigma_b = -8 \, \text{mV}$, $\theta = -67 \, \text{mV}$, $\sigma_f = 2 \, \text{mV}$, $\theta_0 = -63 \, \text{mV}$, $\sigma_f = -7.8 \, \text{mV}$, $\theta_b = 0.4 \, \text{mV}$, $\sigma_b = -0.1 \, \text{mV}$, $\theta = -39 \, \text{mV}$, $\sigma_f = -8 \, \text{mV}$, $\sigma_b = 1 \, \text{ms}$, $\tau_f = 500 \, \text{ms}$, $\theta_0 = -85 \, \text{mV}$ ($-57 \, \text{mV}$), $\sigma_f = 3 \, \text{mV}$, $\tau_f = 1 \, \text{ms}$, $\theta = 100 \, \text{ms}$, $\theta_0 = -80 \, \text{mV}$, $\sigma_f = 26 \, \text{mV}$, $\sigma_b = 40 \, \text{ms}$, $\tau_f = 17.5 \, \text{ms}$, $\theta_0 = 68 \, \text{mV}$, $\sigma_f = 2.2 \, \text{mV}$, $\theta_0 = 0.75 \, \theta_0 = 0.2, k_1 = 7.5 \, \mu \text{M}$ ($15 \, \mu \text{M}$), $g = 3.75 \times 10^{-5} \, \text{mM-s}^{-1}(\mu \text{A-cm}^{-2})^{-1}$, and $\gamma_{\text{Ga}} = 22.5 \, \mu \text{A-cm}^{-2} \text{um}^{-1}$.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


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