Multiple Firing Patterns in Deep Dorsal Horn Neurons of the Spinal Cord:
Computational Analysis of Mechanisms and Functional Implications

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Le Franc Y, Le Masson G. Multiple firing patterns in deep dorsal horn neurons of the spinal cord: computational analysis of mechanisms and functional implications. J Neurophysiol 104: 1978–1996, 2010. First published July 28, 2010; doi:10.1152/jn.00919.2009. Deep dorsal horn relay neurons (dDHNs) of the spinal cord are known to exhibit multiple firing patterns under the control of local metabotropic neuromodulation: tonic firing, plateau potential, and spontaneous oscillations. This work investigates the role of interactions between voltage-gated channels and the occurrence of different firing patterns and then correlates these two phenomena with their functional role in sensory information processing. We designed a conductance-based model using the NEURON software package, which successfully reproduced the classical features of plateau in dDHNs, including a wind-up of the neuronal response after repetitive stimulation. This modeling approach allowed us to systematically test the impact of conductance interactions on the firing patterns. We found that the expression of multiple firing patterns can be reproduced by changes in the balance between two currents (L-type calcium and potassium inward rectifier conductances). By investigating a possible generalization of the firing state switch, we found that the switch can also occur by varying the balance of any hyperpolarizing and depolarizing conductances. This result extends the control of the firing switch to neuromodulators or to network effects such as synaptic inhibition. We observed that the switch between different firing patterns occurs as a continuous function in the model, revealing a particular intermediate state called the accelerating mode. To characterize the functional effect of a firing switch on information transfer, we used correlation analysis between a model of peripheral nociceptive afference and the dDHN model. The simulation results indicate that the accelerating mode was the optimal firing state for information transfer.

INTRODUCTION

Spinal cord dorsal horn neurons relay peripheral sensory and nociceptive information. Afferent fibers from the periphery (skin, joints, or visceral receptors) project to different neuronal types that are segregated in different layers of the dorsal horn of the spinal cord (Rexed 1952) and form glutamatergic synapses (Besson and Chaouch 1987; Craig 2003; Millan 1999; Willis 1991). Different populations of dorsal horn spinal neurons project to supraspinal structures, including the thalamus and the brain stem (Craig 2003; Millan 1999; Willis 1991; Willis and Westlund 1997). One of the primary cellular types, the deep dorsal horn neurons (dDHNs), are primarily located in lamina V and are known to integrate innocuous and nociceptive signals before relaying the information to the somatosensory thalamus through the contralateral spinothalamic tract (Craig 2003; Millan 1999; Willis 1991).

Previous studies have demonstrated that dDHNs have an intrinsic property called plateau potential, wherein neurons produce nonlinear responses to short depolarizing pulses (Morisset and Nagy 1998; Russo and Hounsgaard 1996a,b, 1999). Plateau potentials are typically composed of a slow depolarization during the stimulus and then a progressive acceleration of the discharge, which is followed at the end of the pulse by a long-lasting firing called afterdischarge (Morisset and Nagy 1998). L-type, voltage-gated calcium channels control the tetrodotoxin (TTX)-resistant regenerative membrane property of dDHNs, as evidenced by its dihydropyridine sensitivity (Morisset and Nagy 1996, 1999; Russo and Hounsgaard 1996a). The following afterdischarge depends on intracellular calcium dynamics and the activation of a calcium-dependent mechanism that involves calcium-dependent cationic nonspecific conductance (CAN) (Morisset and Nagy 1999, 2000) and calcium-dependent potassium channels (SK) (see Russo and Hounsgaard 1999). A recent study has shown that a G-coupled potassium inward rectifier, the Kir 3.1 channel (Kir), is expressed in dDHNs and participates in the regulation of their excitability (Derjean et al. 2003).

Plateau firing is thought to participate in central integration processes in invertebrates as well as in mammals (Getting 1988, 1989; Llinás 1988; McCormick and Bal 1994) and may be involved in the amplification of afferent sensory and nociceptive signals (Derjean et al. 2003; Morisset and Nagy 1998; Russo and Hounsgaard 1996a). In addition, dDHNs are known to participate in central sensitization (Woolf 1983) by increasing their receptive field and becoming hyperexcitable (Cook et al. 1987; Laird and Bennett 1993; Millan 1999; Nagy et al. 1994; Woolf and Salter 2000). Although the enlargement of the receptive fields and the increase in excitability can be related to synaptic changes, the expression of plateau potential can also participate in this phenomenon. In support of this hypothesis, the expression of plateau properties in dDHNs has been shown to be a key component of wind-up (Morisset and Nagy 2000; Russo and Hounsgaard 1994), which is a local short-term form of sensitization that has been described both in vitro and in vivo (Chung et al. 1979; Dong et al. 1978; Mendell 1966). Wind-up consists of a progressive buildup of the dDHN response and excitability (Mendell 1966) during repetitive low-frequency C-fiber stimulation (Chung et al. 1979; Nagy et al. 1994; Thompson et al. 1990; Woolf et al. 1988; for a review, see Baranauskas and Nistri 1998; Herrero et al. 2000) and can be reproduced in vitro with a low-frequency stimulation of the...
dorsal roots and a current pulse injection ranging from 0.3 to 1 Hz (Morisset and Nagy 2000; Russo and Hounsgaard 1994). This phenomenon is generally used as a model of local sensitization, even if the mechanisms are thought to differ from central sensitization (Woolf 1983, 1996).

In vitro studies in rats and turtles have shown that the expression of plateau potential is controlled by both glutamatergic and GABAergic metabotropic modulation in deep dDHNs (Morisset and Nagy 1998; Russo et al. 1997, 1998). Derjean and colleagues (2003) have also shown that changes in modulatory control allow the dDHNs to switch between three main firing modes: tonic, plateau, and spontaneous bursting. This transition ultimately depends on the dynamic regulation of one particular potassium channel (Kir). In addition, dynamic clamp experiments have revealed that each firing pattern corresponds to one mode of information transfer (Derjean et al. 2003). Due to experimental limitations, it is not possible to systematically explore the effect of different conductance interactions on the firing switch and to quantify the different possible firing patterns of the dDHNs as well as their impact on sensory information processing.

To explore the role of different channels in the switch and their functional impact, we used a modeling approach that allows us to access different conductances and quantify their impact on the firing mode of the dDHNs and the resulting consequences with regard to the information transfer of peripheral inputs. Thus we constructed a two-compartment dDHN model that includes most of the conductances that are known to be involved in the plateau property. We tuned the model to reproduce the primary features of plateau firing. Interestingly, the model appeared to be capable of expressing the wind-up of its response to low frequency current injection, based on the model intrinsic properties. We used this model to explore some mechanisms that are involved in the genesis of plateau firing and to examine the impact of conductance regulation on firing behavior. We found that modifying the balance between the Kir and the L-type calcium conductance was sufficient to account for the experimentally described firing switch and that the model presented a continuum of firing. This exploration allowed us to characterize a new type of firing, referred to as the accelerating mode, which is at the edge between the tonic firing mode and the plateau mode. This particular type of firing corresponds to a controlled hyperexcitable state. We also correlated these firing changes to their functional roles in peripheral information transfer by connecting an Adelta (Aδ) fiber model to the dDHN model via an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) synapse. Correlation analysis revealed that the three known firing patterns correspond to the three information transfer states that have been experimentally described. Our analysis extends this result and demonstrates that the firing continuum corresponds to an information transfer state continuum. The primary finding therein is that the newly characterized accelerating firing mode corresponds to the optimal information transfer mode. Finally, we investigated a possible generalization of the switch by covarying many conductances. We found that changing the global balance between hyperpolarizing and depolarizing conductances led to a similar firing switch. The previous result extends the original finding of the firing switch to other modulators that have different target channels. Therein, the possible role of the network and, precisely, the effect of local inhibition on the regulation of the dDHN firing and sensory information processing can be elucidated.

**METHODS**

To study the role of ionic conductances on the switch between the different firing modes and on spike transfer processes, we designed conductance-based models that reproduced the known firing patterns for the two cellular types: the dDHN and the Aδ fiber. For this purpose, Hodgkin–Huxley formalism was used (Hodgkin and Huxley 1952) and models were developed with version 5.4 of the NEURON simulation package (Carnevale and Hines 2006; Hines and Carnevale 1997, 2001). For each model, the membrane potential trajectory is given by the following general equation

\[ C_m\frac{dV}{dt} = -\sum_i \cdot \cdot \cdot \text{(1)} \]

where \( C_m \) is the membrane capacitance (in \( \mu \)F), \( V \) is the membrane potential (in mV), and \( i \) represents the currents that influence the membrane potential (in nA). The Hodgkin–Huxley current description is as follows

\[ \cdot \cdot \cdot \text{(2)} \]

where \( g_{ion} \) represents the maximal ionic conductance and \( m(V, \cdot \cdot \cdot) \) and \( h(V, \cdot \cdot \cdot) \) are the activation and inactivation gating variables, respectively. \( E_{ion} \) is the ionic reversal potential.

We applied this simple description to most of our channels; however, it is well known that the activation of calcium channels leads to large changes in intracellular calcium concentration that, in turn, non-linearly modify the reversal potential of calcium (Hille 2001). To take these changes into account, we used the Goldman–Hodgkin–Katz formalism (GHK; Hodgkin 1949) given by the following equation

\[ \cdot \cdot \cdot \text{(3)} \]

where \( \cdot \cdot \cdot \) represents the maximal membrane permeability to calcium ions (in cm/s); \( m(V, \cdot \cdot \cdot) \) and \( h(V, \cdot \cdot \cdot) \) are the activation and inactivation gating variables, respectively; \([Ca^{2+}]_{\text{in}}\) and \([Ca^{2+}]_{\text{out}}\) are the inside and the outside calcium concentrations, respectively (in mM); \( Z \) is the valence of the ion; \( F \) is Faraday’s constant (in J · V · mol⁻¹); \( R \) is the perfect gas constant (in J · K⁻¹ · mol⁻¹); \( T \) is the temperature in Kelvin; \( V \) is the voltage in mV; and \( A = (ZFV)/(RT) \).

The dDHN model

Previous investigators have demonstrated both experimentally (Carlin et al. 2000; Svirskis et al. 2001) and theoretically (Booth et al. 1997; Elbasiouny et al. 2005; Svirskis et al. 2001) that dendrites play an important role in bistable behaviors, such as plateaus. Thus we constructed a two-compartment model, which is described in Fig. 1A. The model included a 56.25-μm wide spherical soma and an 800-μm long and 1-μm wide compact dendritic tree, for a total surface area of 12,454 μm² and an associated capacitance of 95 pF, as observed in physiological experiments (D. Derjean, unpublished data).

Soma description

We added the classical Na⁺/K⁺ channels that are responsible for action potentials (\( i_{Na} \) and \( i_{K_{trans}} \)) to the soma as well as a L-type calcium channel with an intermediate voltage threshold (\( i_{calc} \)). This calcium channel exhibits fast activation and slow inactivation kinetics (Voisin and Nagy 2001) and is known to be responsible for the fine-tuning of the action potential shape and the afterpotential (for a review, see Russo and Hounsgaard 1999). We inserted a calcium-dependent potassium conductance \( K \) (\( i_{k_{dep}} \)), which is known to be sensitive to apamin and to participate in the control of regenerative behavior in both motorneurons and dDHNs (Hounsgaard and Kiehn...
we inserted a G-protein-activated Kir 3.1 conductance (1989; Hounsgaard et al. 1988; Russo and Hounsgaard 1996a). Finally, the different conductances in dDHNs, we used and modified models from the model database ModelDB (http://senselab.med.yale.edu/ModelDB/default.asp; Davison et al. 2004; Hines et al. 2004; Migliore et al. 2003).

Based on this description, the somatic membrane potential is given by the following equation

\[ V = \mu F + \frac{1}{C_m} \int (i_{Na} - i_{KCa} - i_{Kt} - i_{L} + i_{m}) \, dt \]

where \( C_m \) is the membrane capacitance in \( \mu F \), \( V_{soma} \) is the somatic membrane potential in mV, and \( i_{m} \) is the leak current in nA/cm².

Due to a lack of quantitative experimental data regarding the gating of the different conductances in dDHNs, we used and modified models from the model database ModelDB (http://senselab.med.yale.edu/ModelDB/default.asp; Davison et al. 2004; Hines et al. 2004; Migliore et al. 2003).

The model of Na⁺/K⁺ currents that are responsible for the spike generation is based on a previous model, which was originally developed for NEURON by Destexhe et al. (1994) and has been used in several studies (Bazhenov et al. 1998; Destexhe et al. 1994, 1996a,b, 1998; Lytton et al. 1997; Wang et al. 2002).

We developed a calcium current model that is based on available data (Voisin and Nagy 2001). This calcium current activates at around −40 mV and exhibits fast activation and slow inactivation kinetics. The activation curve was extrapolated from the available data and inserted into a general model for calcium current (using the GHK formalism, Eq. 3) that was contained in the NEURON software package (Carnevale and Hines 2006) and has been used in other models (Poirazi et al. 2003a,b; Polsky et al. 2004; Rusakov et al. 1996, 1997). The extraction and fitting procedures are described in the APPENDIX and the resulting curves are shown in Supplemental Fig. S1, A–D.¹

The fitted activation function is given by the following equation

\[ m_a(V) = -0.0012 + (1 + e^{(-V + 14.3907)/3.1029})^{0.5289} \]  

where \( V \) is the membrane potential in mV.

It has been experimentally shown in dDHNs (Voisin and Nagy 2001) and in other in vitro preparations (Lipscombe 2002; Xu and Lipscombe 2001; for a review, see Lipscombe et al. 2004) that such currents are relatively fast. We then scaled the activation time constant to the range of 1 ms. The voltage dependence of the activation time constant is given by the following equation

\[ \tau_a = \tau_{a0} \cdot e^{-V/K_d} \]  

where the \( \tau_a \) is the scaling factor in ms and \( V \) is the voltage in mV.

Finally, because little is known regarding the voltage dependence of inactivation, we described this phenomenon as a sigmoidal function, as follows

\[ y = \frac{1}{1 + e^{(V - V_{half})/slope}} \]  

where \( V_{half} \) is the voltage for half-inactivation and slope is the slope of the curve.

To represent inactivation, we set \( V_{half} \) to 14 mV and slope to 4.03 mV.

The inactivation time constant was considered not to be voltage dependent, as was observed in a prior experiment (Voisin and Nagy 2001), and was therefore set to 1,500 ms to match the experimental values.

The model of the calcium-dependent potassium conductance, or SK conductance, was constructed on the basis of the experimentally defined calcium dependence (Sah 1996; Sah and Davies 2000; Sah and Faber 2002; Vergara et al. 1998). The SK current can be described with the general Hodgkin–Huxley current equation (Eq. 2) and the activation is given by the following equation

\[ m_a(C_a) = \frac{[C_a]}{[C_a] + K_d} \]  

where \( m(C_a) \) is the calcium-dependent activation variable, \( C_a \) is the intracellular calcium concentration in mM, and \( K_d \) is the half-activation calcium concentration in mM. In our model, \( K_d \) was tuned to 0.5 mM and the activation time constant was set at 10 ms, as previously described (Sah 1996; Sah and Davies 2000; Sah and Faber 2002).

Kir 3.1 current is known to be voltage independent and activated by metabotropic receptors (for a review, see Hibiino et al. 2010). Experimental results involving dDHNs indicate that metabotropic activation is directly linked to changes in the chord conductance of the current (Destjean et al. 2003). In addition, it has been proposed that the gating and current of Kir 3.1 channels are similar to those of Kir 2.1 channels (Hibiino et al. 2010). Therefore we decided to use Hodgkin–Huxley formalism to represent the Kir 3.1 current in a similar way to what has already been done for Kir 2.1 in previous models (Gruber et al. 2003; Mermelstein et al. 1998; Wessel et al. 1999; Wolf et al. 2005). The

¹ The online version of this article contains supplemental data.
activation gating is represented by a sigmoidal model (see Eq. 7),
in which the half-activation voltage is \(-65\) mV and the slope is 10 mV. The activation curve and the quantified current–voltage relation in the model are shown in Supplemental Fig. S1, E and F. It has been shown that inward rectifier channels exhibit fast activation (Constanti and Galvan 1983; Kiehn et al. 2000; Nichols and Lopatin 1997; Yamoah et al. 1998); thus we set the activation time constant in our model to 1 ms.

Finally, the leak current model is based on the general model that is provided in the NEURON environment (Carnevale and Hines 2006), with a reversal potential of \(-60\) mV.

The maximal conductance values of the dDHN model are shown in Table 1.

Dendrite description

The model of an active dendrite includes the following conductances: 1) a calcium L-type conductance (\(i_{CaL}\)) that exhibits a lower voltage activation (around \(-60\) mV) as well slower activation and inactivation compared with the soma, 2) a calcium-dependent cationic nonspecific conductance or CAN conductance (\(i_{CAN}\)), 3) an SK conductance that is similar to the somatic conductance, and 4) a leak conductance.

Because the spatial distribution of the CAN conductance is not clearly known in dDHNs, we elected to include this conductance in the dendrite only to reduce the model parameter space.

The global description of the dendritic membrane potential is then given by

\[
C_m(dV_{dendrite}/dt) = -i_{CaL} - i_{SK} - i_{CAN} - i_{leak}
\]

where \(C_m\) is the membrane capacitance in \(\mu F\), \(V_{dendrite}\) is the dendritic membrane potential in mV, and \(i_{leak}\) is the leak current in nA/cm².

The dendritic calcium current model is based on the method that is typically used for the somatic current. The activation curve was extracted from experimental data, as described in the APPENDIX, and is shown in Table 1.

Intracellular calcium dynamics

To link the calcium inflow through the L-type calcium channels to various calcium-gated conductances, we inserted a model of intracellular calcium concentration into the soma and the dendrite of the dDHN model as well as the afferent model (Koch 1998). This model represents intracellular calcium concentration variation by considering a shell beneath the cell membrane and is described by the following equation

\[
d[Ca^2+]_{i}/dt = -i_{Ca} \cdot k/2Fd - ([Ca^2+]/[Ca^2+]_{i})/\tau
\]

where \(i_{Ca}\) represents the calcium current in mA/cm², \(k\) is a unit conversion factor \((k = 10^{-7} \text{ nm} \cdot \text{cm}^{-1})\), \(F\) is Faraday’s constant in \(\text{coulomb} \cdot \text{mole}^{-1}\), \(d\) is the depth of the shell beneath the membrane in mm, \([Ca^2+]_{i}\) is the initial intracellular calcium concentration in mM, and \(\tau\) is the buffering time constant in ms. For all models, we considered a depth of 1 mm, a buffering time constant of 10 ms, and an initial concentration of \(5 \times 10^{-5} \text{ mM}\).

Affereence model

To produce a more physiologically relevant analysis for the functional role of various firing states, we chose to reproduce afferent spiking patterns with known temporal characteristics from primary nociceptive Aδ fibers. We used an experimental characterization from monkey nociceptive fibers (Slagg et al. 2000), which show an adaptation of the firing frequency of different afferences. We developed a single-compartment model (length = 100 \(\mu m\); diameter = 1 \(\mu m\)) to reproduce the aforementioned patterns by using the interaction between inward calcium currents and calcium-dependent potassium channels. Thus we added the Na⁺/K⁺ channels that are responsible for the generation of action potentials to the compartment, in addition to a leak current, an L-type calcium channel, and a calcium-dependent potassium conductance that is linked to the calcium inflow via a previously described model of intracellular calcium dynamics. The Na⁺/K⁺ conductance, leak current, and calcium channel models are similar to those that have been used in the dDHN model. Because the firing profile of the Aδ fibers exhibited an adaptation that included a double exponential, we chose to add two calcium-dependent potassium channels with two different activation time constants. This model is derived from Moczydlowski and Latorre (1983) and is provided in the NEURON software package. We modified the activation time constant of the two conductances to fit the instantaneous frequency profile (initial time constants were slowed by factors of 50 and 500). The maximal conductance parameters of the afferent model are given in Table 2.

It is well known that nociceptive fibers directly project to dDHNs through glutamatergic synapses that contain AMPA and N-methyl-d-aspartate (NMDA) components (Millan 1999; Willis 1991). To reduce the complexity of the model and the analysis, we chose to consider the AMPA component and used a model of AMPA synapses from Destexhe et al. (1994). The maximal synaptic conductance was set to 15 pA/cm² for the simulations, except when otherwise specified.

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**TABLE 1. DHN model parameters: control condition**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soma</th>
<th>Dendrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>gBar, mS/cm²</td>
<td>30.00</td>
<td>—</td>
</tr>
<tr>
<td>NaF</td>
<td>4.00</td>
<td>—</td>
</tr>
<tr>
<td>Kdr</td>
<td>0.03268</td>
<td>0.03</td>
</tr>
<tr>
<td>Leak</td>
<td>0.03</td>
<td>0.0185</td>
</tr>
<tr>
<td>K(Ca)</td>
<td>—</td>
<td>0.1275</td>
</tr>
<tr>
<td>CAN</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Kn</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Phar, (\mu m/s)</td>
<td>1.50</td>
<td>—</td>
</tr>
<tr>
<td>tCaL</td>
<td>—</td>
<td>0.20</td>
</tr>
<tr>
<td>tCa</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

| Reversal, mV| 50       | -90      | +140     | -60      |

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firing frequency ranging from 40 to 60 Hz; 2) persistent spiking activity after the end of the stimulation, which is called afterdischarge, with a variable duration; and 3) a nonlinear gain curve. The parameter set, given in Table 1, produced a smooth acceleration that resulted in a maximal frequency of 40 Hz in response to a somatic current pulse of 25 pA for 3 s (Fig. 1B). The stimulation induced an initial smooth depolarization of both the soma and the dendrite, leading to a sustained depolarization of the dendrite (from \(-70\) to \(-20\) mV) that directed an increase in somatic firing. At the end of the current pulse, the model continued to discharge with a lower frequency for approximately 10 s and then ceased to spontaneously fire.

We then visualized the gain curve of the model, by measuring the mean model response frequency to increasing current pulses that ranged from 0 to 30 pA (Fig. 1C). We observed a nonlinear relationship between the model response and the current intensity, demonstrating an apparent threshold for plateau generation. Magnification of the transition revealed a smoother transition with intermediate firing, which has not been experimentally described (see Fig. 1, A, inset and C). Indeed, for weak stimuli, the model demonstrated low-frequency responses with afterdepolarization potentials (ADPs), which do not lead to an afterdischarge (Fig. 1, B, arrow and C, inset). As the intensity was increased, the mean frequency reached high-frequency values that corresponded to the expression of the full plateau at a current threshold value of \(g_{Na}^{\text{threshold}} = 17\) pA. This transition was sharp and was observed as a small increase in the current intensity induced the expression of the plateau, even after the end of the stimulation, revealing the all-or-nothing nature of the plateau (see Fig. 3A, 17 pA, control condition). The gain then continued to linearly increase as both the acceleration maximal frequency and the afterdischarge duration increased (see Fig. 1C, inset 2, \(I_{\text{inj}} = 25\) pA). The next step was to validate our model by comparing its behavior to the well-known pharmacology of DHN.

Validation

VIRTUAL PHARMACOLOGY. To validate the model, we conducted “virtual” pharmacology experiments in our model by manually modifying the value of ionic conductances, known to be the target of the different channel blockers. Next, the DHN model voltage responses were compared with in vitro experimental results, wherein the DHNs were exposed to various channel blockers (Morisset and Nagy 1998, 1999; Russo and Hounsgaard 1996a, 1999).

First, we replicated the impact of sodium channel blockade by mimicking the application of TTX. For this purpose, we set the fast sodium maximal conductance (\(g_{Na}^{\text{fast}}\)) to zero, assuming a complete blockade, and we then stimulated our model with the same current pulse that was originally used to characterize the model (control; see Fig. 2A). The simulated application of TTX revealed a calcium plateau (Fig. 2B) that exhibited a longer afterdepolarization after the end of the stimulation. This increase in afterdischarge duration could be explained by the reduction of voltage-dependent potassium conductances (KDR and Kir 3.1), which are mostly activated during spike repolarization (not shown at this scale).

Second, we replicated the calcium dependence of the plateau potential by using a virtual application of a dihydropyridine, such as nifedipine, which is known to specifically block L-type

<p>| TABLE 2. Aδ fiber model parameters |</p>
<table>
<thead>
<tr>
<th>gBar, mS/cm²</th>
<th>Pbar, μm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF</td>
<td>56.50</td>
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<tr>
<td>Kdr</td>
<td>5.00</td>
</tr>
<tr>
<td>Leak</td>
<td>0.52</td>
</tr>
<tr>
<td>mK(Ca)</td>
<td>0.13</td>
</tr>
<tr>
<td>sK(Ca)</td>
<td>0.12</td>
</tr>
<tr>
<td>Na⁺</td>
<td>-90</td>
</tr>
<tr>
<td>K⁺</td>
<td>140</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>-70</td>
</tr>
<tr>
<td>Reversal, mV</td>
<td>50</td>
</tr>
</tbody>
</table>

The synapse was connected to the soma of the dDHN model to reflect the known projection of Aδ fibers in the layer IV–V of the spinal cord, where the dDHNs somata are located (Millan 1999; Willis 1991).

**Spike transfer quantification**

Spike train transfer was quantified using cross-correlation analysis (Derjean et al. 2003; Le Masson et al. 2002; Levine 1998). This method allowed us to measure the relation between two spike trains, \(g\) and \(h\), in a defined sliding time window \(\tau\) according to the following equation

\[
C(g, h) = \int_{-\infty}^{\infty} g(t + \tau)h(t)\,dt
\]

where \(g\) and \(h\) are sums of Dirac delta functions.

This analysis was performed based on the timings of the afferent and dDHN spikes. This time was measured as the time at which the voltage crosses the 0 mV threshold. Because the noise amplitude of the correlation depends on the firing rate, to properly compare the correlation between different combinations of conductance, we normalized the correlation distribution by the average correlation. This operation does not affect the shape of the correlation and enhances peak detection. The correlation distribution was normalized by the number of input spikes (afferent spike) to get the cross-correlation distribution and normalized by the number of output spikes (dDHN spikes) to get the contribution distribution. The cross-correlation represents the probability that an input spike triggers an output spike, whereas the contribution represents the probability that an output spike is due to an input spike.

**General simulation information**

Simulations were run with a fixed time step of 25 μs. To prevent any bias due to initial conditions, the simulations were run for 5,000 ms prior to any stimulation. This procedure allowed the model to reach a pseudosteady state and the corresponding voltage traces were removed from the graphs. The resting potential of the dDHN model was held at \(-59\) mV by using a constant current injection of 23.85 pA/cm² for all of the simulations.

The model and the code that were used to generate the figures will be made available in ModelDB.

**RESULTS**

**Model response to current injection**

The model parameters were adjusted to reproduce three fundamental plateau characteristics that have been observed in the experimental recordings of dDHNs (Morisset and Nagy 1996, 1998; Russo and Hounsgaard 1994, 1996a,b; for review, see Russo and Hounsgaard 1999): 1) a progressive acceleration of firing during the stimulation with a maximal instantaneous
channel with flufenamic acid (FFA) eliminates the afterdischarge without any notable modification of the firing acceleration during the stimulation. To confirm this role of the CAN channel in our model, the maximal conductance ($g_{\text{CAN}}$) was set to zero. The blockade of this conductance abolished the long afterdischarge without affecting the acceleration phase of the plateau (Fig. 2D).

Finally, we examined the effect of tetraethylammonium (TEA), which is a specific KDR blocker, on the membrane trajectory (Fig. 2E). To visualize the calcium plateau, the model was placed under TTX conditions ($g_{\text{NaF}} = 0$) and the KDR maximal conductance was then set to zero. Model stimulation revealed a slow increase in the potential until the activation of the plateau. Because the membrane potential cannot be repolarized, it then stabilizes in a sustained depolarized state. These results reveal the critical role of hyperpolarizing currents in the control of the plateau expression.

VOLTAGE DEPENDENCE OF THE PLATEAU PROPERTY. The expression of plateau potential is controlled by the membrane potential. This property depends on the voltage dependence of the ionic conductance that is involved in plateaugenesis, especially from the L-type calcium channel, and indirectly depends on the complex interactions between intracellular calcium dynamics and calcium-sensitive conductances. This voltage dependence affects different characteristics of the plateau, such as firing onset, acceleration, and afterdischarge duration. This property can be revealed by systematically varying the two primary stimulation parameters: the current intensity and the holding potential (Morisset and Nagy 1998).

To further validate our model, we simulated an increase of current pulse intensity (Fig. 3A) and a decrease of the holding potential (Fig. 3B). In the first simulation, a series of 3-s current pulses with intensities that ranged from 16.8 pA (see Fig. 3, 16.8 pA, control, as in Fig. 1C, inset 1B) to 25 pA (Fig. 3, 25 pA, control) was applied to the soma. During low-intensity current injections (Fig. 3A, $I_{\text{inj}} = 16.8$ pA), the model exhibited a long firing onset, followed by a short firing duration, which was terminated by an ADP (Fig. 3A, $I_{\text{inj}} = 16.8$ pA, arrow). Increasing the stimulation intensity dramatically reduced the firing onset and gave rise to a complete plateau with an accelerating phase and an afterdischarge (Fig. 3B, $I_{\text{inj}} = 25$ pA). For intermediate intensity injections (Fig. 3A, $I_{\text{inj}} = 17$ pA), the acceleration came after a longer onset compared with what was observed for the high-intensity injections. The acceleration in firing appeared to be independent of the stimulation and acceleration continued after the end of the current pulse, followed by an afterdischarge. This result demonstrates the previously described all-or-nothing nature of the calcium regenerative plateau process. This behavior was maintained under simulated TTX conditions (Fig. 3A, TTX, $g_{\text{NaF}} = 0$).

Next, we explored the effect of the holding potential by varying the model resting potential with a constant current pulse, which was released during the plateau triggering stimulation (Fig. 3B, 3-s duration and 25 pA intensity). The simulations exhibited a progressive reduction in the afterdischarge as the holding potential became more hyperpolarized (Fig. 3B, control). These results were not sensitive to the virtual application of TTX (Fig. 3B, TTX, $g_{\text{NaF}} = 0$).

FREQUENCY DEPENDENCE, WIND-UP OF THE RESPONSE. dDHNs have been shown to be involved in nociceptive sensitization by...
express a cumulative depolarization during the pulse train (data not shown); however, because plateau expression is voltage dependent, a pulse train with a reduced current amplitude ($I_{\text{nj}} = 15$ pA) was repeated when the model was depolarized to a resting potential of $-55$ mV. As can be observed in Fig. 4A, the model responded to the low-frequency stimulation, with a gradual increase in its firing frequency, leading to a plateau-like firing and followed by a long afterdischarge. The evolution of this process is quantified for each stimulation in Fig. 4D (black dots) and demonstrates a progressive increase in the action potential number, as well as a high frequency of firing and followed by a slow decrease of the firing, which corresponds to the repolarization phase of the plateau. This intrinsic wind-up was abolished when all of the calcium conductances were set to zero, which demonstrates the experimentally observed calcium dependence of the wind-up (Fig. 4, B and D, white dots) (Morisset and Nagy 1996, 2000).

Wind-up is often associated with a cumulative depolarization during stimulation (for a review, see Herrero et al. 2000). A relatively smaller cumulative depolarization was found in our model, as shown in Fig. 4C, and is quantified in Fig. 4E (black dots) and abolished when the calcium conductance was set to zero to mimic the effect of bath-applied nifedipine (Fig. 4E, white dots). This small cumulative depolarization was similar to that observed in experimental measurements (Morisset and Nagy 1999) and it is smaller than the depolarization that is observed for C-fiber-induced wind-up. Indeed, it is well known that NMDA activation and synaptic plasticity mechanisms participate in this phenomenon (for a review, see Herrero et al. 2000). The addition of this synaptic component will certainly lead to larger cumulative depolarizations, as has been observed in vivo.

**Analysis of dynamic conductance interactions that underlie plateau potential**

To assess the channel interactions that underlie plateau firing, Fig. 5 plots each conductance with the voltage response and intracellular calcium dynamics during current injection. At the beginning of the stimulation, the calcium conductance in the dendrite became activated as the membrane potential became depolarized with respect to the resting potential (Fig. 5, black arrow). On the other hand, the somatic conductance was maintained at resting values until the dendritic depolarization reached the somatic calcium activation threshold, leading to firing.

The acceleration of firing was controlled by the concomitant fast activation of dendritic SK, which clamped calcium activation by delaying dendritic depolarization. This effect was then overcome by two main mechanisms: 1) an increase in the intracellular calcium concentration induced the slow activation of inward CAN conductance (Fig. 5, star), which increased the depolarization of the dendrite and allowed the calcium channel to reach its activation threshold; and 2) SK conductance was saturated (Fig. 5, white arrow), which limited its hyperpolarizing effect on dendritic depolarization. These mechanisms allowed the nonlinear activation of the L-type channel, which supported high-frequency firing during the stimulation.

After injection offset, the calcium conductance decreased due to its slow inactivation dynamic. This reduction in calcium drive was compensated for by the CAN conductance, which sustained a slower firing frequency. An in-
creased inactivation of calcium channels led to a slow decrease in CAN activation.

Compared with this calcium-based mechanism, Kir conductance appeared to play a minor role during plateau expression because its activation was observed to rapidly decrease with membrane depolarization. The primary role of this conductance occurred during the repolarizing phase of each spike, wherein Kir helped to control the acceleration of firing frequency. In addition

**FIG. 4.** Model validation: frequency dependence. A: model response to somatic injection of 9 current pulses of 500-ms duration \( (I_{inj} = 15 \text{ pA}) \) with a frequency of 0.8 Hz. The holding potential was adjusted to \(-55 \text{ mV}\) by constant injection of 40 \text{ pA}. B: simulated pharmacological blockade of L-type calcium channel abolishes the wind-up of the response to the same frequency with stronger current pulses \( (I_{inj} = 50 \text{ pA}) \). C: visualization of the cumulative depolarization during the onset phase of the wind-up (5 first current injections in A). The voltage traces of the model from the end of the current injection (black arrow) to the beginning of the following current injection were taken and overlaid. D: spike count of the model response for each stimulus of the wind-up protocol, in control (black dots) and under simulated nifedipine condition (white dots). E: quantification of the cumulative depolarization: the voltage value \( (y\)-axis) at the middle of the interval between 2 current pulses was taken and plotted against the stimulus number \( (x\)-axis).

**FIG. 5.** From conductances to plateau property: description of the conductance activation dynamic in the different model compartments. Left: soma, Right: dendrite. Top traces: voltage traces; bottom traces: injected current \( (I_{inj} = 25 \text{ pA}) \). Dashed line represent the duration of the current pulse. The black arrow shows the fast activation of the dendritic calcium conductance. The white arrow shows the saturation of the SK conductance in the dendrite, which leads to the nonlinear activation of the calcium current in the dendrite. The black star represents the activation of the CAN conductance, sustaining the depolarization while the calcium conductance starts to inactivate.
to its role during spiking, the Kir conductance controlled the amount of depolarization that was needed to reach both the spiking and plateau thresholds. In summary, the observed plateau firing resulted from the nonlinear activation of L-type channels and a calcium autoregulated mechanism that involves calcium-dependent conductances. The plateau expression can be regulated by subthreshold conductances, such as Kir, which mask the plateau expression for low-intensity level current.

To further investigate the nonlinear effect of the calcium channel on plateau firing, we tested the impact of different nifedipine concentrations on the membrane potential trajectory under TTX conditions ($g_{NaF} = 0$, Fig. 6A). For this purpose, the initial calcium maximal conductances were systematically decreased to mimic an increase in nifedipine concentration (which is reported as a percentage of blockade). A decrease in the blockade revealed a slow plateau recovery (Fig. 6A, star) with an apparent threshold. A dose–response curve was established by measuring the percentage of increase in the area under the curve compared with that observed for a complete blockade situation (100%). The results are reported in Fig. 6C (continuous black line), wherein the squares correspond to the different conditions that are depicted in Fig. 6A. The curve (Fig. 6C, black curve) exhibits a nonlinear relation between the membrane trajectory and the reduction of the calcium conductance (nifedipine blockade). For a complete or a high percentage blockade, the relationship followed a quasi-linear increase, which corresponded to a slow increase in the active zone during the stimulation (Fig. 6A, star). Next, a break in this relationship, which appeared to relate to the threshold value of calcium conductance ($-75\%$ of the maximal conductances in our model) in connection to the active depolarizing phase of the plateau during stimulation and the development of an afterdischarge, was observed. After this nonlinearity, the relationship continued to follow a linear increase as a function of increasing afterdischarge duration. These results indicate that plateau expression depends nonlinearly on the calcium channel density. In addition, this exploration indicates that despite parameter changes, such as a $20\%$ decrease of calcium conductance, plateau firing can still occur in our robust model.

To quantify the impact of CAN conductance on the plateau, we proceeded to determine the effect of FFA concentration on the membrane trajectory (Fig. 6B) under TTX conditions ($g_{NaF} = 0$, Fig. 6B) via a dose–response curve (Fig. 6C, white dots). Our results indicate that the initial part of the plateau was not influenced by the reduction of $i_{CAN}$ (simulated FFA application, Fig. 6B, arrow), whereas the CAN conductance influenced the afterdischarge in a quasi-linear manner (see Fig. 6C).

**Role of the balance of calcium/Kir on firing behavior**

Plateau expression in dDHNs is controlled by both glutamatergic and GABAergic modulation in turtles (Russo et al. 1997, 1998) and rats (Morisset and Nagy 1996, 1998; Voisin and Nagy 2001). The balance between glutamatergic and GABAergic metabotropic activities has also been implicated in the firing switch in rat slices (Derjean et al. 2003). One of the local neuromodulation targets has been identified as a G-activated inward rectifier potassium Kir 3.1 channel (Kir). The application of the group I mGluR receptor agonist dihydroxyphenylglycine (DHPG) was observed to decrease the isolated Kir current, whereas the application of baclofen, which is a specific γ-amino butyric acid type B (GABA$_B$) receptor agonist, was observed to increase the current. In addition, the results of Voisin and Nagy (2001) demonstrate that the L-type calcium channel is a target of GABAergic modulation. The effect of mGluR1 on calcium current is controversial in some studies, wherein it shows no effect (Voisin and Nagy 2001), whereas others demonstrate that mGluR1 activation induces an up-regulation of L-type calcium channels in the spinal cord (Heinke and Sandkuhler 2005; for a review, see Perrier et al. 2002). In the following simulations, we assumed that the maximal conductance of the L-type calcium and Kir channels can be up- or down-regulated by either glutamate or GABA activity.

Is the variation of both Kir and L-type conductance sufficient to account for the firing switch between the tonic, plateau, and oscillatory modes? To answer this question, both calcium and Kir conductances in our original parameter set were set to 100% (see Table 1). Next, the conductance was systematically varied from 0 to 200% for Kir conductance and from 0 to 250% for L-type conductance (somatic and dendritic). The firing profile of the model (tonic, plateau, and bursting) in response to constant 3-s current pulses with 50-pA amplitudes is reported on the map depicted in Fig. 7. Therein, three distinct zones can be observed, which correspond to the three firing states: the tonic mode corresponds to a band from 0% calcium to 75% calcium and expands along the entire 200% range of
Kir variation; the plateau area begins at around 75% calcium and a low percentage of Kir and then slightly shifts toward 87.5% calcium to 100% calcium for high Kir values. The plateau mode spans the largest area in the map, except for when spontaneous oscillation occurred at around 100% calcium and 0% Kir, followed by a simultaneous increase in calcium and Kir conductance. Above 130% Kir conductance, the model exhibited only plateaus, even if the calcium conductance was 2.5-fold higher than that of the control condition. This screening indicates that each firing mode can be reproduced with a large range of conductance values.

A complete exploration of these qualitative stable zones revealed that the firing states can be further subdivided. For instance, in the tonic zone, two primary response profiles appeared, showing both a linear frequency profile during the stimulation and different firing frequencies (compare Fig. 7, top and middle left; white diamond = 50% calcium and 150% Kir; black diamond = 50% calcium and 50% Kir). Likewise, the plateau generated by 150% calcium and 150% Kir (Fig. 7, top right, black star) exhibited a faster acceleration and a longer afterdischarge compared with the control condition (Fig. 7, middle right, 100% calcium and 100% Kir, white star). The oscillatory state was characterized by a long period of firing (~15 s) and a slow bursting frequency (see Fig. 7, bottom right, 150% calcium and 50% Kir, white triangle).

A further investigation of the firing revealed the existence of a particular firing state between the tonic firing area and the plateau firing area. At the edge of the plateau parameters, we found a set of parameters wherein the instantaneous frequency profile increased and exhibited an ADP at the end of the stimulation (Fig. 7, bottom left, white circle, 75% calcium, 120% Kir, arrow). We elected to call this phenomenon an “accelerating” firing state. The key element that defined this particular state is the presence of an ADP and the acceleration of the firing frequency during stimulation. These two properties can vary in amplitude for different parameter values but are always present.

These results demonstrate that the dynamics of the neuronal intrinsic properties are embedded in a firing continuum, which, on one hand, makes the firing categorization task harder, whereas on the other, gives some insight into the experimental diversity of dDHN firing. Our data reveal that calcium conductance variation is primarily responsible for the existence of the different firing states and that Kir conductance modifies the state transition by increasing the amount of current that is needed to trigger regenerative firing, acting as a high-pass filter. The global effect of the balance of both glutamatergic and GABAergic modulation is best represented in this parameter space as the diagonal from low calcium and high Kir to high calcium and low Kir (that is, from the top left to the bottom right quadrant of Fig. 7).

Because the accelerating mode corresponds to the dynamic at the edge of the plateau expression, wherein the conductance parameters are below the plateau threshold, we investigated the impact of the stimulation parameters on plateau expression. We would expect that the model would express a full plateau if the stimulus intensity or duration were increased. To test this hypothesis and to demonstrate that this particular mode is not restricted to a particular stimulation parameter set, we investigated the impact of the stimulus parameters (intensity and duration) on the firing pattern. To restrict the parameter space to be explored, we investigated the firing pattern for the diagonal of the firing map depicted Fig. 7, which we consider to be equivalent to physiological conditions. We have reported the firing for each of the Kir/calcium pairings as a function of the intensity of the stimulus and the results are summarized in the map depicted in Fig. 7.
Fig. 8 for three different durations: 500, 1,500, and 2,500 ms. These results clearly show that the accelerating mode is reliably expressed, although the range of expression is a function of both parameters. The acceleration appears for lower values of calcium conductance as the stimulus intensity and/or duration is increased. Based on these results, we define an area of parameters where the accelerating mode can be expressed, depending on the stimulus intensity (Fig. 8, dashed box).

Generalization of the equilibrium

The previous simulations were based on the assumption that only the Kir and L-type conductances are involved in the firing switch; however, it is well known that many different modulatory pathways (5-HT, substance P, etc.) converge onto dDHN neurons, which involve many different targets (for a review, see Millan 2002).

To generalize the effect of the balance between the different conductances, the parameter space was then screened by taking into account the global balance of excitatory conductance versus hyperpolarizing conductance. We investigated whether the variation of this equilibrium would be able to trigger similar firing switches. For this purpose, we screened the parameters in the same way that was done for Fig. 7, except that we grouped conductance into depolarizing (L-type calcium conductance and CAN conductance) and hyperpolarizing conductances (Kir and SK conductances). It is important to note that the soma:dendrite ratios for the conductances were kept constant and that sodium conductance was kept fixed to get spikes from the model. We then varied the values of each group from 0 to 200% and reported the firing response of the model to the same current pulse onto a map (depolarizing percentage: x-axis; hyperpolarizing percentage: y-axis, Fig. 9).

The variations in the balance between the two primary drives were able to trigger a firing switch between the three modes, which revealed a very similar firing continuum, as previously reported. The tonic mode area appears to be reduced for low values of hyperpolarizing conductance compared with that observed for the case of Kir conductance alone and it expands in the territory of the plateau. This exploration indicates that it is possible to switch between the three primary firing modes, even without changing the calcium conductance (see Fig. 9 at 100% calcium: tonic mode from 200 to 170% of hyperpolarizing conductances; accelerating mode from 170 to 120% of hyperpolarizing conductance; plateau mode from 120 to 40% of hyperpolarizing conductances; oscillatory mode from 40 to 0% of hyperpolarizing conductances). This effect can be mediated either by the SK alone, revealing a calcium-dependent mechanism, or the collaboration of both the Kir and the SK.

For the same values presented in Fig. 7, we observed different tonic firings (Fig. 9, top left, white diamond, 50% depolarizing and 150% hyperpolarizing and black diamond, 50% depolarizing and 50% hyperpolarizing), with differences in the firing frequency of the model. We observed the same accelerating mode that was previously described by Fig. 7 (Fig. 9, bottom).
The existence of this firing state continuum raises the following question: does such a continuum exist for peripheral transmission states in our model of the Aβ fiber, which we stimulated with an increasing current pulse to quantify the gain curve (Fig. 10B). The model responses to four different current intensities are visualized in Fig. 10A. First, we compared the instantaneous firing profile of the model with experimental data (Fig. 10C). The instantaneous frequency profile (Fig. 10C, dotted line) fit well with the experimentally derived frequency values (Fig. 10C, white dots). The theoretical gain curve (Fig. 10B, dotted line) reproduced the experimental data (Fig. 10B, white dots), except for low mean frequency values where our model seemed to be slightly more excitable.

As previously described in vitro (Derjean et al. 2003), the three originally identified firing modes of dDHNs correspond to three spike transfer modes. The tonic mode exhibits a lower (low correlation) and more reliable (high contribution) spike transfer compared with the plateau property. Spontaneous bursting appeared to be the worst integration mode (low correlation and low contribution). To quantify the impact of conductance variation on the peripheral information processing in our model, we connected the Aδ afference model to the soma of the dDHN model via an AMPA synapse (Destexhe et al. 1994). For all the conductance pairs (%gKir/%gCaL), the input signal was triggered by a 20.75 pA current pulse with a 3 s duration, giving rise to a 35 Hz spike train. The AMPA maximal conductance was set to trigger the dDHN response to a 100% firing probability when the balance was set for 0% of calcium conductances and 200% of Kir conductances (g_{AMPA} = 15 pA/cm²). We measured both the peak cross-correlation and the contribution values for all of the Kir/calcium pairs of values depicted in Fig. 7. We reported the correlation index (CC) and...
The contribution index (CI) onto a map (Fig. 11, A and B; x-axis: \%gCaL, y-axis: \%gKir).

The results (Fig. 11, A and B) indicate that the areas corresponding either to the plateau or to the oscillatory modes (compare with Fig. 7) present a high CC with little variability together with a low CI (with values that are \(H_11021\) for extreme cases of oscillations; see Fig. 11 B, bottom right). Interestingly, the area corresponding to the tonic mode (see Fig. 7) presents two different regions: 1) low correlation and low contribution (top left quadrant: high Kir and low calcium conductances); and 2) high correlation and high contribution (bottom left quadrant: low Kir and calcium conductances). This second region can be explained by the relative higher excitability of the tonic mode for this set of parameters (Fig. 7, 50% Kir and 50% calcium, black diamond). Given that the tonic mode has been described with a slow adaptation (Derjean et al. 2003), this particular area of parameters is unlikely to represent the physiological reality. For this reason, we consider relevant for our analysis only the top left region corresponding to the case with higher Kir conductance and showing a slight adaptation.

In depth analysis of the model response for the different firing modes (data not shown; parameters from Fig. 7 white symbols) revealed that the variation of correlation is due to changes in

FIG. 10. The A6 fiber model. A: A6 model response to different current intensity stimulations (19.7, 19.8, 20, 20.75 pA) with a duration of 3 s. B: gain curve of the model (white dots), in response to increasing current amplitude ranging from 19.6 to 21 pA, compared with the experimentally described gain curve (dotted black line; Slugg et al. 2000). C: instantaneous frequency profile of the model (white dots) in response to a 3-s current pulse of 20 pA, compared with the experimentally described profile (dotted black line; Slugg et al. 2000).

FIG. 11. Exploration of the transfer. For each value shown in Fig. 7, the DHN model was stimulated by the same afferent input spike train (35 Hz, \(g_{AMPA} = 15 \) pA/cm\(^2\)). We calculated the cross-correlation and the contribution coefficients between the DHN and the afferent spike trains. We reported in a colored map the maximal value for each of the coefficients. A: cross-correlation. B: contribution, for each couple of values (%gKir/%gCa2). C: considering the left diagonal as the expected variation in physiological conditions, we reported both the cross-correlation and the contribution for pairs of values on the diagonal. Arrow indicate the value for plateau expression.
the spiking pattern generated by our model. The decrease of the correlation for the tonic mode is due to a decrease of the number of spikes (adaptation) as well as a reduced spike timing reliability (broadening of the correlation peak). The increase of correlation for both the plateau and the oscillatory mode corresponds to the emission of spike doublets or triplets, causing the strong decrease in the contribution. These results confirm the experimental data and constitute a strong validation of our model.

Further analysis of the correlation and contribution maps (Fig. 11, A and B) revealed the presence of an area with high correlation and contribution (relative to the tonic mode) at the limit between the tonic mode and the plateau mode. This thin band of values corresponds to the intermediate firing mode (accelerating; compare with Fig. 7), which was first described in our modeling study. We found that this particular mode could be a fundamental feature of dHFNs. Indeed, this firing mode appears to represent the optimal state for information transfer because both the correlation and the contribution coefficients were equal and high in value (CC = CI = 0.65).

Finally, we examined the variation of the integration properties along the diagonal of the map (from top left to bottom right), which was proposed to represent physiological conditions. In Fig. 11C, we visualized the variation of both the cross-correlation and the contribution for a different couple value in this diagonal. Apparently, both correlation and contribution decreased in the tonic area. As they approach the limit of the plateau expressing value (arrow), a sharp and strong jump in transmission occurs, which indicates a highly reliable transmission mode (both quantitatively and qualitatively). When the couple of conductance values reached the plateau and oscillatory values, the correlation became strong because many spikes were generated; however, the contribution decreased to low values, demonstrating an increase in transmission and a decrease of the meaning of the signal (Fig. 11C).

Next, we evaluated the effects of physiological synaptic parameters on the transmission of the afferent fiber model. We measured the correlations and contributions of the Kir/calcium values of the diagonal. Figure 12 depicts the different results for two different synaptic maximal conductances (\( g_{\text{syn}} = 12.5 \) and 17.5 pA/cm\(^2\)) and two afferent firing frequencies (15 and 40 Hz). For lower synaptic strengths (\( g_{\text{syn}} = 12 \) pA/cm\(^2\); Fig. 12, top), increasing the calcium to Kir ratio increased the postsynaptic neuron excitability, globally increased the correlation (more spikes are transmitted), and lowered the contribution (triggers intrinsic spikes that are not correlated to any inputs). What distinguishes this scenario from others is the ability of a higher afferent frequency to recruit the accelerating mode (the gray zone in Fig. 12), such that the afferent signal is optimally transmitted (high correlation with high contribution). With a high synaptic conductance (\( g_{\text{syn}} = 17.5 \) pA/cm\(^2\); Fig. 12, bottom panels), both frequencies were able to recruit this mode, demonstrating optimal transmission in both cases. Note how contribution dramatically decreased in all of the graphs when the calcium to Kir ratio set the neuron beyond the accelerating state to either the plateau or the oscillating mode. In these states, the intrinsically generated spikes reduce the information that postsynaptic spike train conveys about the presynaptic spike train. The average maximal conductance for the AMPA synapse in our model was set to 15 pA/cm\(^2\). Although the variation shown here was small, we did explore a much larger range of synaptic parameters (from \( g_{\text{syn}} = 0 \) to 20 pA/cm\(^2\)). Our circuit model appeared to be extremely sensitive to this parameter. A synaptic conductance that was <10 pA/cm\(^2\) was not strong enough to elicit any postsynaptic response. When the synaptic conductance was >20 pA/cm\(^2\), the response was saturated and a plateau was triggered for

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**FIG. 12.** Exploration of the interaction between the afference parameters and the information transfer. For each value of the pair \( \%g_{\text{Kir}}/\%g_{\text{Ca}^2+} \) found on the diagonal of Fig. 7, we calculated correlation and contribution coefficients for 2 different afferent frequencies (15 and 40 Hz) and synaptic maximal conductances (12.5 and 17.5 pA/cm\(^2\)). We reported for each pair of values (x-axis) the maximal peak of correlation (left) and contribution (right). The gray area corresponds to range of conductance values \( \%g_{\text{Kir}}/\%g_{\text{Ca}^2+} \) where the accelerating mode was found for different current injection parameters (intensity and duration; see Fig. 8, dotted rectangle). This area is correlated with an optimal information transfer.
lower values of calcium. This narrow band of parameters probably occurred because we used a single afferent fiber rather than multiple convergent fibers. More complex circuitry would be a natural continuation of this model.

The overall result of this study strengthens the finding that the subthreshold expression of regenerative properties (plateau firing state) is the optimal state for accurate peripheral nociceptive transmission.

**DISCUSSION**

In the present work, we investigated the ionic mechanisms involved in the firing switch that has been experimentally described in dDHNs (Derjean et al. 2003). Importantly, we explored the consequences of different firing modes on the transmission of afferent signals through the spinal cord. To address these two questions, we used a modeling approach and proposed a canonical monosynaptic model of the dorsal horn circuitry of the spinal cord. After an extensive validation, the exploration of this model revealed a general mechanism for the firing switch and the existence of a new firing mode that corresponds to an optimal information transfer state. These results shed new light on the functional aspects of sensory and nociceptive processing in the spinal cord.

*How realistic is our model?*

The dDHN model presented herein captures most of the experimental features that have been described in rat and turtle dDHNs (pharmacological and electrophysiological) and, furthermore, parameter variation reveals the robustness of the model’s response to small changes. In addition, this model was the first to produce a wind-up of its response to low-frequency current injection, based on the model intrinsic properties. Despite these strengths, the model is a simplified version of reality and further work is required to establish a better description of the ionic channels that are expressed in these neurons as well as the intracellular calcium dynamics that are involved in plateau expression. Finally, we must also take into account the morphology of these neurons.

In terms of conductance, we omitted other subthreshold conductances that are expressed by this particular type of neuron, such as H current as well as A- or D-type potassium currents that have been identified both in motoneurons and dDHNs (Bayliss et al. 1994; Schwindt 1984; Takahashi 1990a,b; for a review, see Russo and Hounsgaard 1999). We expect these different conductances to have an impact on the subthreshold behavior and to participate in the control of the calcium-dependent plateau firing. For example, the H current is known to be involved in rebound excitation as well as in the regulation of excitability during spike repolarization and bursting behavior. The lack of this conductance could explain the slight discrepancy between the oscillatory behavior of our model and experimental observation. Therefore, these conductances should be included in future models.

*Firing switch and generalization*

An investigation of the model reveals a possible generalization of the firing switch mechanisms as a balance between depolarizing (or inward) and hyperpolarizing (or outward) currents. This mechanism mimics an experimentally described case (Derjean et al. 2003) of Kir/calcium balance. An analysis of conductance interactions during the plateau reveals two different control mechanisms: a voltage-dependent mechanism and a calcium-dependent mechanism. The voltage-dependent mechanism has been linked to the Kir that acts as a high-pass filter, which masks plateau firing due to weak input. This control appeared in our exploration of the Kir/calcium balance (Figs. 7 and 8), wherein variations of the Kir conductance did not modify the transition but rather increased the amount of current needed to reveal the regenerative properties. This type of voltage-dependent plateau masking has already been described in subthalamic nuclei neurons (Beurrier et al. 1999, 2000) and provides a fast and reversible way to modify the excitability of neurons.

The second mechanism centers on calcium dynamics and has already been described in previous modeling work (Booth et al. 1997). This calcium-dependent mechanism is based on the interactions between the L-type calcium channels and the Kir channels, wherein SK conductance masks the plateau expression, resulting in a slow hyperpolarizing feedback to control the activation of calcium channels. Our analysis also revealed the impact of slow CAN conductance on plateau unmasking, which antagonized SK activation. These results explain the generalization of the switch and further reduce the firing switch to the calcium dynamic of the model. This phenomenon was confirmed by the fact that the three firing switches can be expressed with the same amount of depolarizing calcium conductance, emphasizing the strong impact of SK conductance.

Importantly, the generalization of the firing switch to a simple balance between inward and outward currents allowed us to control the firing with many possible mechanisms, such as intrinsic plasticity, neuromodulation, and network activity. This generalization establishes an important link between cellular mechanisms and network synaptic structure in the control of information processing. This link has been confirmed experimentally in vivo (Fossat et al. 2007). This study demonstrates the importance of the interplay between NMDA and inhibitory conductance (glycinergic and GABAergic) on the masking and unmasking of the wind-up properties of dDHNs and their dependence on intrinsic calcium dynamics.

*Working on the edge: the optimal solution?*

Further investigation of the firing dynamic of dDHNs allowed us to characterize a particular firing mode, the accelerating mode, which is at the edge of the transition between the tonic and the plateau modes. This accelerating mode corresponds to a subplateau threshold behavior and was characterized by an acceleration of the firing frequency during current injection and the presence of an ADP at the end of the stimulation. Throughout the screening, we found that these two characteristics were always present but with varying degrees of magnitude.

Importantly, this new firing mode corresponded to an optimal mode for peripheral information processing, showing high correlation and contribution coefficients. If this property—as well as the appearance of this particular mode—is related to stimulation parameters (intensity and duration for the current pulse, as well as input frequency and maximal synaptic conductance for the peripheral input), it always appears within a
specific balance between the intrinsic hyperpolarizing and depolarizing conductances. Therefore the accelerating mode appears to be a direct consequence of the general equilibrium of the previously discussed calcium dynamics (see firing switch and generalization) and corresponds to a critical point of this equilibrium. This critical point of the equilibrium serves a major functional role as an optimal information processing state.

An in-depth analysis of the model response to different peripheral inputs reveals that this information processing property is linked to its ability to generate spikes with better temporal resolutions compared with that of other modes. Indeed, if the equilibrium was shifted toward the hyperpolarizing contribution, then the spiking was influenced by a slow adaptation due to the activation of SK channels. Conversely, the shift toward a depolarizing conductance resulted in the occurrence of a strong accelerating mechanism, which added more spikes but in a less reliable matter. This functional property appears to be supported by an unstable equilibrium between slow calcium dynamic components and remains valid for a small range of parameters, which might not have been fully captured by our coarse parameter screening. However, the generalization of the firing switch and the existence of the accelerating mode indicate that the circuit can maintain this state using many different mechanisms (neuromodulation, intrinsic plasticity, or network synaptic properties) and suggests that the accelerating mode could be the physiological working mode for the optimal information processing of peripheral signals.

In the context of acute nociception, this equilibrium would be shifted toward the depolarized state by a strong barrage of afferent activity, triggering the expression of the plateau and unmasking its associated central sensitization state. This transition could be reversed by an increase in the inhibitory drive of the network, a decrease of the excitatory drive, a modification of the modulatory inputs (either local or descending), or any mixture of these mechanisms.

In a pathological case, irreversible modifications to the network properties and/or intrinsic plasticity mechanisms could lead to an unbalanced state that leans toward depolarization. Different experimental evidence supports this hypothesis. Indeed, it has been shown that in certain types of neuropathy, a large apoptosis occurs in the spinal cord, provoking a reduction of the inhibitory interneuron population in the spinal cord superficial layer (Moore et al. 2002; Scholz et al. 2005).

At the level of intrinsic plasticity, it has been shown that the expression profile of two particular types of L-type calcium channels changes after nerve injury (Dobremez et al. 2005). It has been shown that CaV1.2 becomes up-regulated and CaV1.3 down-regulated. Additionally, a more recent study in a rat model of neuropathy (spinal nerve ligation) has shown that neuropathy-associated mechanical hypersensitivity, hyperexcitability, and an increased responsiveness of dorsal horn neurons can be reversed by an antisense strategy that targets CaV1.2 channels (Fossat et al. 2010).

Finally, the existence of the accelerating mode appears to be a particular feature of a small subset of calcium conductances and calcium-dependent conductances that are known to be expressed in a large number of different neurons that are known to express plateau properties (subthalamic nuclei: Beurririer et al. 1999; entorhinal cortex: Fransen et al. 2006; Zhang et al. 2010; cingular cortex: Zhang and Séguela 2010; subiculum: Larimer and Strowbridge 2010; and cerebellum: Fernandez et al. 2007). This study could ultimately provide a new framework to study information processing in other nervous structures.

Conclusions

This model presented here is one of the few attempts to use modeling to describe neurons that are involved in nociception integration in the spinal cord (Aguirau et al. 2010; Melnick et al. 2004; Prescott and De Koninck 2002; Prescott et al. 2008). This model allows us to link variations in conductance and changes in the information processing of sensory and nociceptive information by spinothalamic relay neurons. Additionally, this model is the first to present a wind-up of the response that is exclusively based on intrinsic properties that have been experimentally uncovered (Morisset and Nagy 1999) and could be used as a basis to further explore central sensitization mechanisms, thereby extending previous theoretical studies (Aguirau et al. 2010; Farajidavar et al. 2006, 2008).

Importantly, our exploration of the developed model reveals the existence of a general mechanism based on hyperpolarization (inward) versus depolarization (outward) current ratio control, which facilitates a switching between different firing modes. This mechanism appears to be linked to the interaction between different calcium-dependent conductances.

As a result of this equilibrium, we have described a new firing state—the accelerating mode—which corresponds to an optimal information processing state. The accelerating mode appears to be a highly functionally relevant point in this particular equilibrium and could be maintained by any combination of regulatory mechanisms (intrinsic plasticity, changes in network activity, modification of local or descending neuromodulation). Such findings suggest that the accelerating mode could be the optimal working mode for sensory and nociceptive information processing in the spinal cord.

These particular features provide new insights for the investigation of normal and pathological nociceptive information processing by the spinal cord. Importantly, they provide a new framework for the investigation of pharmacological screening that aims to reduce the hyperexcitability state of neurons in neuropathic pain using virtual pharmacology (Aradi and Erdi 2006; Ferrante et al. 2008; Kiss and Erdi 2006).

Appendix

The L-type calcium conductance model: design from experimental data

Three different types of calcium dihydropyridine-sensitive channels have been characterized in dissociated cultures of dorsal horn relay neurons (Voisin and Nagy 2001). We chose to develop two models of calcium channels to introduce such diversity based on the data provided by D. Voisin. By using current–voltage (I–V) curves from voltage-clamp characterization of the calcium channels, we extrapolated the activation curve to reproduce the general behavior of the calcium channel. For this purpose, we calculated the conductance–voltage curve by dividing each value of the I–V curve by the difference between the voltage and the reversal potential. Because the experimental measurements were determined with EGTA (ethylene glycol tetraacetic acid) in a patch-pipette solution to prevent any intracellular calcium interference, it was difficult to estimate the...
experimental reversal potential. Therefore we decided to establish the reversal potential to the default calcium reversal in NEURON: +140 mV; however, because calcium reversal is not constant and is given by the field equation (Eq. 3 in METHODS), we should calculate all of the calcium values for each voltage step to achieve the best estimate of the current. We considered the maximal measured current to be equivalent to the maximal activation of the conductance; thus any decrease in current after the peak would mostly be due to a decrease in the difference between the voltage and calcium reversals. In this case, normalizing with a constant would change only the scale of the curve without changing the shape. In addition, we were mostly interested in fitting the particular voltage dependence of these channels for a more hyperpolarized voltage. Indeed, one of the L-type channels exhibits an activation curve that starts around −40 mV, whereas the second L-type channel exhibits a starting activation at −60 mV, which corresponds to the resting potential of the neurons.

Based on these assumptions, we thus calculated the conductance–voltage curve and normalized the curve with the maximal activation to generate a steady-state activation curve (Supplemental Fig. S1, A and B, black dots). Once these curves were calculated, we fitted them with a nonlinear regression function, a Boltzmann function, using SigmaPlot (version 8.0, SPSS). The best fit was obtained with a five parameter equation, as follows: y = y0 + a/[1 + e^−(V−V_{half}/b)]$, where V is the membrane potential in mV; $V_{half}$ is the half-activation in mV; b is the slope in mV$^{-1}$ and $y_0$, a, and c are scaling factors (Supplemental Fig. S1, A and C, red curve). The different values include the following: 1) $ICaL_0 = -0.0012$, $V_{half} = -14.3907$ mV, $a = 3.1029$ mV$^{-1}$, $c = 0.10022$ and $c = 0.5289$ ($R = 0.99$, SD = 0.0012) and 2) sCaL, $y_0 = -0.0048$, $V_{half} = -20.4565$ mV, $a = 4$ mV$^{-1}$, $c = 1.0257$, and $c = 0.4731$ ($R = 0.99$, SD = 0.0029). We then compared the experimental I−V to a simulated I−V curve (Supplemental Fig. S1, D and D), showing that our model fit the first part of the curve and showed a slope change for the more depolarized current. This discrepancy resulted from our primary assumption, but it should not affect our model because this voltage range will not be used for long periods of time during the simulation.

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References

Aguir PC, Sousa MM, Lima D. NMDA channels together with L-type calcium currents and calcium activated nonspecific cationic currents are sufficient to generate windup in WDR neurons. J Neurophysiol (June 16, 2010). doi:10.1152/jn.00834.2009.


