Simulation in Sensory Neurons Reveals a Key Role for Delayed Na\(^+\) Current in Subthreshold Oscillations and Ectopic Discharge: Implications for Neuropathic Pain

Yifat Kovalsky, Ron Amir, and Marshall Devor

Department of Cell and Developmental Biology, Institute of Life Sciences and Center for Research on Pain, The Hebrew University of Jerusalem, Jerusalem, Israel

Submitted 5 January 2009; accepted in final form 25 June 2009

Kovalsky Y, Amir R, Devor M. Simulation in sensory neurons reveals a key role for delayed Na\(^+\) current in subthreshold oscillations and ectopic discharge: implications for neuropathic pain. *J Neurophysiol* 102: 1430–1442, 2009. First published July 1, 2009; doi:10.1152/jn.00005.2009. Somata of primary sensory neurons are thought to contribute to the ectopic neural discharge that is implicated as a cause of some forms of neuropathic pain. Spiking is triggered by subthreshold membrane potential oscillations that reach threshold. Oscillations, in turn, appear to result from reciprocation of a fast active tetrodotoxin-sensitive Na\(^+\) current (\(I_{Na}\)) and a passive outward I\(_K\) current. We previously simulated oscillatory behavior using a transient Hodgkin–Huxley-type voltage-dependent \(I_{Na}\) and ohmic leak. This model, however, diverged from oscillatory parameters seen in live cells and failed to produce characteristic ectopic discharge patterns. Here we show that use of a more complete set of Na conductances—which includes several delayed components—enables simulation of the entire repertoire of oscillation-triggered electrogenic phenomena seen in live dorsal root ganglion (DRG) neurons. This includes a physiological window of induction and natural patterns of spike discharge. An \(I_{Na}\) component at 2–20 ms was particularly important, even though it represented only a small fraction of overall \(I_{Na}\) amplitude. With the addition of a delayed rectifier I\(_K\) the singlet firing seen in some DRG neurons can also be simulated. The model reveals the key conductances that underlie afferent ectopia, conductances that are potentially attractive targets in the search for more effective treatments of neuropathic pain.

**INTRODUCTION**

Repetitive discharge that develops ectopically in injured sensory neurons after nerve trauma is thought to contribute significantly to chronic neuropathic dysesthesias and pain (Devor 2006). The repetitive discharge, in turn, appears to be triggered by the depolarizing limb of intrinsic subthreshold membrane potential oscillations (Amir et al. 1999; Kovalsky et al. 2008; Yang et al. 2009). Here we explore the conductances that sustain the oscillatory behavior and resulting spike discharge. A simulation approach was taken in the absence of adequate pharmacological tools for controlling the relevant conductances in live cells.

Voltage-dependent Na\(^+\) conductance (\(g_{Na}\)) contributes to the depolarizing phase of the oscillations in live cells (Amir et al. 1999; Xing et al. 2001). Blocking voltage-sensitive K\(^+\) channels does not eliminate oscillations or spiking, although shifting the K\(^+\) reversal potential to neutralize all K\(^+\) channels does. This indicates a role for a passive \(g_{K}\) in the repolarizing phase of oscillations. However, in contrast to live cells, in numerical simulations that included only a classical Hodgkin–Huxley-type voltage-dependent \(g_{Na}\) and leak \(g_{K}\), the oscillations formed never triggered spikes and the range of membrane potentials within which oscillation could be induced was very narrow (Amir et al. 2002a). This suggests that additional \(g_{Na}\) components may be required. Indeed, dorsal root ganglion (DRG) cells are known to express many types of Na\(^+\) channels. These can be subdivided by their tetrodotoxin (TTX) sensitivity and their relative contribution to transient and more sustained Na\(^+\) currents (Amir et al. 2006). Our goal here was to find a set of Na\(^+\) conductances that, combined with a physiologically realistic leak current, is capable of inducing oscillations and triggering spikes within a “window” of membrane potentials compatible with live DRG neurons.

Our work was based largely on that of Baker and Bostock (Baker 2000; Baker and Bostock 1997, 1998) who presented evidence that in addition to the known fast inactivating transient \(g_{Na}\), large-diameter DRG neurons in adult rats also generate a delayed current, including “late” and “persistent” components. The delayed current is TTX sensitive (TTX-S), it activates rapidly, but inactivates slowly. Baker and Bostock described the late current using the sum of two exponentials as it decays according to intermediate and slow time constants (\(\tau\)). The component that inactivates at the intermediate rate was termed “late1” and the component that undergoes slow inactivation, “late2.” They also recorded a component that never fully decays to zero, termed the “persistent” current (\(I_{Na}^{P}\)). The decay rates \(\tau\) of late1 and late2 currents are in the range of tens and hundreds of milliseconds, respectively. Delayed and, particularly, persistent Na\(^+\) currents are known to mediate subthreshold oscillations and to participate in burst generation in many brain cell types (e.g., Agrawal et al. 2001; Wu et al. 2005). The aim of the present study was to find whether the addition of late and persistent Na\(^+\) currents to the fast transient \(g_{Na}\) used in the earlier DRG simulation would significantly enhance oscillatory behavior and ectopic electrogenesis.

**METHODS**

We constructed a mathematical model of an isolated large-diameter DRG sensory cell soma based on quantitative voltage-clamp data recorded from dissociated neurons from the rat. The simulation used the NEURON programming environment (version 5.8; Hines and Carnevale 1997), running under Windows XP. We modeled a sensory cell soma that was designed to be simple, with as few unconstrained...
variables as possible. We used the Crank–Nicholson second-order accuracy method for integration (dr = 0.01 ms) with one computational compartment. Simulated impulses were monitored in the middle of the compartment. Prolonged step depolarizing stimuli (3–5 s) were applied to a single compartment. Simulated impulses were monitored in the middle of the stimulation step. Spike frequency was computed as the inverse of the stimulation step. Spike frequency was computed as the inverse of the stimulation step. Spike frequency was computed as the inverse of the stimulation step.

We focused on somata of medium- to large-diameter neurons with myelinated Aδ afferent axons because these are the main contributors to ongoing spike discharge originating in DRG neurons in a variety of animal models of neuropathic pain, especially around the time of onset of mechanical hypersensitivity (Amir et al. 1999; Boucher and McMahon 2001; Devor 2006; Liu C-N et al. 2000a; Liu X et al. 2000b; Tal et al. 2006). Although these neurons normally signal touch and vibration sense, in the event of neuropathy they are widely believed to contribute to pain (tactile alldynia; Campbell et al. 1988; Devor 2009a; Kolzenburg et al. 1994). This modularity change is thought to reflect response to: 1) the presence of central sensitization and/or 2) a phenotypic switch that causes the neurons to begin expressing and releasing neurotransmitters that are normally exclusive to nociceptors and that are capable of driving postsynaptic pain signaling pathways and inducing spinal central sensitization (Devor 2006, 2009a; Malcangio et al. 2000; Noguchi et al. 1995; Weissner et al. 2006; Woolf and Salter 2000).

The parameters of interest were oscillatory behavior and the generation of spikes, spike bursts, and repetitive (sustained) firing. The oscillation amplitude was measured peak to peak. “Repetitive firing” was defined as firing that, once initiated, persisted at least until the end of the stimulation step. Spike frequency was computed as the inverse of the last interspike interval (ISI). Where possible oscillation frequency was computed by averaging over the entire trace. When firing interrupted oscillations, the average oscillation frequency was computed over the longest run of oscillations available between spike bursts. The presence of oscillations and spiking complicated the evaluation of membrane potential (\(V_m\)) during stimulation traces. When oscillations were present \(V_m\) was computed as the average of the peak and trough of the oscillations. \(V_m\) was not defined during periods of spiking.

As in live DRG neurons, sustained oscillations (i.e., oscillations that persisted throughout the prolonged stimulus pulse) occurred only within a defined “window” of membrane potentials. At potentials more hyperpolarized or more depolarized than this window, oscillations were either not present or they damped out. The boundaries of this window depended on parameters of the model cell. Under some conditions oscillations led to spiking within the range of membrane potentials that supported oscillations. The “threshold” for single spike generation was defined as the potential at which \(I_{\text{Na}^+} = I_K + I_{\text{leak}}\) within a sharp voltage peak, where \(I_{\text{leak}}\) refers to the sum of all of the individual \(\text{Na}^+\) currents present.

In live DRG neurons, sustained oscillations (i.e., oscillations that persisted throughout the prolonged stimulus pulse) occurred only within a defined “window” of membrane potentials. At potentials more hyperpolarized or more depolarized than this window, oscillations were either not present or they damped out. The boundaries of this window depended on parameters of the model cell. Under some conditions oscillations led to spiking within the range of membrane potentials that supported oscillations. The “threshold” for single spike generation was defined as the potential at which \(I_{\text{Na}^+} = I_K + I_{\text{leak}}\) within a sharp voltage peak, where \(I_{\text{leak}}\) refers to the sum of all of the individual \(\text{Na}^+\) currents present.

The intermediate and the late currents both derive from the two delayed conductances, late1 and late2. Following Baker and Bostock (1998) we term the “persistent” (steady-state) current during the period 2–200 ms the “intermediate” \(I_{\text{leak}}\) and the declining current from 20 to 200 ms the “late” \(I_{\text{leak}}\). The intermediate and the late currents both derive from the two delayed conductances, late1 and late2. Following Baker and Bostock (1998) we term the “persistent” (steady-state) current remaining after 200 ms \(I_{\text{leak}}\). It derives from all three conductances (fast, late1, and late2). In the model, the maximal value of \(I_{\text{leak}}\) is controlled exclusively by the maximal value of the three declining currents. It could not be adjusted independently (Fig. 1).

The modeling process began by systematically varying parameters \(\left(I_{\text{Na}^+}^\text{max}, g_{\text{Na}^+}\right)\) and the values of infinity activation \(m_{\text{a}}\) and inactivation \(h_{\text{a}}\) of the three \(\text{Na}^+\) currents within a range that produced an integrated peak \(I_{\text{Na}^+}\) and current–voltage dependence, consistent with measurements made in live DRG cells (peak \(I_{\text{Na}}^\text{v} = -126 nA;\) see RESULTS). The objective was to find a combination of values that yielded oscillations and oscillation-evoked spike discharges resembling those of live cells as closely as possible. The values identified in this way were used for the “baseline model.” The effects on the baseline model of perturbations from these values are shown in RESULTS. For convenience, we call \(I_{\text{leak}}\) the declining current during the period 2–200 ms the “intermediate” \(I_{\text{leak}}\) and the declining current from 20 to 200 ms the “late” \(I_{\text{leak}}\).

Membrane properties of the model neuron

An isopotential cylinder with 50-μm diameter and length was used to model a DRG neuron. Specific membrane capacitance was 1 μF/cm² and temperature was 20°C. The model was fast, intermediate, and slow inactivating \(g_{\text{Na}^+}\), each with transient and persistent components. These were meant to emulate experimentally recorded TTX-S \(\text{Na}^+\) currents in the cells of interest. We did not aim to model specific \(\text{Na}^+\) channel types. In particular, there was no Na1.9-like current that yields \(I_{\text{Na}^+}\) without a fast \(I_{\text{leak}}\) component. Na1.9 is selective to small-diameter DRG neurons and is not present in the cells modeled (Waxman et al. 1999).

There was also a voltage-insensitive linear leakage current, defined as \(I_{\text{leak}} = g_{\text{leak}}(V_m - E_{\text{leak}})\), where \(g_{\text{leak}}\) is the leakage conductance and \(E_{\text{leak}}\) is the reversal potential of the leak current. \(E_{\text{leak}}\) was set at \(-65.5 mV\), yielding \(V_m = -57.4 mV\). This approximates \(V_m\) of live DRG neurons treated with blockers of voltage-sensitive \(K^+\) channels (Amir et al. 2002a). The leak conductance was set at 1.42 mS/cm² (calculated from Scroggs et al. 1994). To keep the model simple we generally excluded voltage-sensitive \(K^+\) conductances (\(g_{K^+}\)). We have previously shown in excised rat DRGs that \(K^+\) channel blockers do not prevent oscillatory behavior or spiking. In fact, they are facilitatory in the sense of allowing oscillations at membrane potentials closer to \(V_m\) and at lower frequencies (Amir et al. 2002a).

Voltage-dependent \(\text{Na}^+\) currents

Our objective was to modify the model used by Amir et al. (2002a) in a manner that would generate oscillatory behavior over a broader, more physiological range of membrane potentials and that would also yield oscillation-triggered spikes and spike bursts. Amir et al. (2002a) used a single transient TTX-S-type voltage-sensitive \(\text{Na}^+\) conductance with kinetics of the squid giant axon \(\text{Na}^+\) channel (Hodgkin and Huxley 1952; hereafter H-H), but with maximal \(\text{Na}^+\) conductance \((g_{\text{Na}^+}^\text{max})\) increased to more closely resemble that of mammalian DRG neurons. Here we replaced this with fast activating and fast inactivating \(\text{Na}^+\) currents based on DRG neurons (\(I_{\text{Na}^+}\); fast; see following text). In addition, we included two types of fast activating and slow inactivating “delayed” \(\text{Na}^+\) conductances, known to play a role in large- to medium-diameter DRG neurons. Emulating live DRG neurons, both of these conductances (late1 and late2) were described by an H-H-type equation that generated a fast transient current as well as delayed and persistent currents.

The current that occupies the first 2 ms from the beginning of the stimulus pulse, referred to here as the “fast transient” \(I_{\text{Na}^+}\), derives almost exclusively from the fast and late1 conductances. From 2 to nearly 200 ms the current declines to a steady-state “persistent” value.

The membrane potential \(V_m\) was set at -65.5 mV (Baker and Bostock 1998). The three \(\text{Na}^+\) currents were as follows (summarized in Table 1).

1. Fast inactivating \(\text{Na}^+\) current \((I_{\text{Na}^+}\text{fast})\).

- \(I_{\text{Na}^+}\text{fast}\) was fast based on the conventional H-H model (with modified values): \(I_{\text{Na}^+}\text{fast} = g_{\text{Na}^+}\text{fast}^\text{max} \times \text{mmh}(V_m - E_{\text{Na}})\), where \(g_{\text{Na}^+}\text{fast}^\text{max}\) is the maximal fast conductance, which was set at 25 mS/cm² in the baseline model. The values of \(m_k\) and \(h_k\) were modified to fall within the range...
present in large-diameter DRG neurons in rats. For $m_{\alpha}$ we set $V_{1/2}$ to $-34.12$ mV and the slope factor to 9.14. In live cells $V_{1/2}$ of $m_{\alpha}$ lies between $-35$ and $-25$ mV and the slope factor between 3.1 and 12 (Cummins et al. 1999; Hong and Wiley 2006; Yoshimura et al. 1996; Yu et al. 2005). The activation time constant ($\tau_{m}$), chosen to simulate fast activation, was voltage sensitive, but always remained $\leq 0.11$ ms (Kostyuk et al. 1981; Nowycky 1992). $\tau_{m} = 0.1092 \exp[-0.5(V_{m} + 28.71)/25.5]^{1.8}$. For $h_{\alpha}$ we set $V_{1/2}$ to $-56.39$ mV and the slope factor to 7.22. In live cells of small and large diameter $V_{1/2}$ of $h_{\alpha}$ has been reported to lie between $-69$ and $-56$ mV and the slope factor between 7.3 and 16.5 (Baker and Bostock 1997, 1998; Cummins et al. 1999; Hong and Wiley 2006; Yoshimura et al. 1996). We acknowledge that the values chosen are low in the physiological range. The inactivation time constant ($\tau_{h}$) was voltage sensitive and always

### TABLE 1. Parameters of the conductances used for the baseline model

<table>
<thead>
<tr>
<th>Conductance</th>
<th>Midpoint Potential of $m_{\alpha}$, mV</th>
<th>Activation Slope Factor</th>
<th>Midpoint Potential of $h_{\alpha}$, mV</th>
<th>Activation Slope Factor</th>
<th>$\tau_{m}$ ms</th>
<th>$g_{\text{max}}$, mS/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{\text{Na}^+}^\text{fast}$</td>
<td>-34.12</td>
<td>9.146</td>
<td>-56.39</td>
<td>7.22</td>
<td>$\leq 0.11$</td>
<td>25.0</td>
</tr>
<tr>
<td>$g_{\text{Na}^+}^\text{late1}$</td>
<td>-25.29</td>
<td>9.052</td>
<td>-72.50</td>
<td>8.0</td>
<td>0.0</td>
<td>27.0</td>
</tr>
<tr>
<td>$g_{\text{Na}^+}^\text{late2}$</td>
<td>-51.80</td>
<td>4.6</td>
<td>-55.67</td>
<td>6.552</td>
<td>0.0</td>
<td>0.128</td>
</tr>
<tr>
<td>$g_{\text{Leak}}$</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1.42</td>
</tr>
</tbody>
</table>

**FIG. 1.** Characteristics of the simulated sodium currents. A: total current, summing all of the $I_{\text{Na}^+}$ components, evoked by 200-ms voltage-clamp steps ($-80$ to $10$ mV in $10$-mV increments) from a prepulse potential of $-110$ mV. Inset: enlargement of the first $120$ ms better shows the delayed currents. B–D: current-voltage (I–V) relation for the total $I_{\text{Na}^+}$ shown in A. Curves plot the fast transient current peak (B), the current present $30$ ms after the step depolarization (C), and the current present $200$ ms after the step depolarization (D). The stimulation protocol for D differed from that of B and C, as indicated in the text. E–G: the individual components of the total $I_{\text{Na}^+}$: $I_{\text{Na}^+}^\text{fast}$ (E), $I_{\text{Na}^+}^\text{late1}$ (F), and $I_{\text{Na}^+}^\text{late2}$ (G). Calibration in F is as in E. Note the different scale in G.
remained ≈2 ms. $\tau_n = 0.246 + 1.63 \exp[-0.5(V_m + 61.87)/15.25^2]$). Using these parameters $I_{Na}$ fast decayed to a small residual persistent current as in live cells (Chen et al. 2000); it did not fall to absolute zero (Fig. 1E).

2. Intermediate-inactivating $\text{Na}^+$ current ($I_{Na\text{-}late}$). $I_{Na\text{-}late}$ was calculated as: $I_{Na\text{-}late} = g_{Na\text{-}late}I_{Na\text{-}late,max} \times m(V_m - E_{Na})$, where $g_{Na\text{-}late}I_{Na\text{-}late,max}$ is the maximal late current conductance. The variable $m$ was used to the first rather than the third power because this gave a better fit to the biological data. $I_{Na\text{-}late}$ is known to be fast activating (Baker and Bostock 1998). However, since no direct measurements of its activation characteristics are available we set $\tau_m = 0$ in the baseline model and explored the impact of increasing this parameter by simulation. For $m$, we set $V_{Na} \leq -72.5$ mV and the slope factor to 8.0, slightly modified from Baker et al. (1992). Using these parameters $I_{Na\text{-}late}$ decayed more slowly than $I_{Na\text{-}fast}$, and, like $I_{Na\text{-}fast}$, it did not reach zero at steady state, but rather ended in a small residual persistent current (Fig. 1F).

3. Slow-inactivating $\text{Na}^+$ current ($I_{Na\text{-}late2}$). $I_{Na\text{-}late2} = g_{Na\text{-}late2}I_{Na\text{-}late2,max} \times m(V_m - E_{Na})$. The physiological value of $g_{Na\text{-}late2}I_{Na\text{-}late2,max}$ has not been measured directly. We set $g_{Na\text{-}late2}I_{Na\text{-}late2,max}$ in the baseline model to 0.128 mS/cm$^2$, which resulted in a maximal total persistent current of $\approx 1.68$ nA when all three contributions were summed (at 200 ms; Fig. 1D). This is near the upper end of the range ($0.4 \text{ to } 27$ mS/cm$^2$) reported by Baker and Bostock (1997). The values of $m_a$ and $h_a$ are taken from Baker (2000): $m_a = 1/(1 + \exp[(V_m + 51.8 - V_{Na})/4.61])$, $h_a = 0.9827/(1 + \exp[-(V_m + 55.67)/-6.552])$. Baker and Bostock (1997) had difficulty quantifying $m_\tau$, although their data show that the current activates very quickly. Thus as for $I_{Na\text{-}late1}$, we set $\tau_m$ as instantaneous in the baseline model and explored this parameter space by simulation. The variable $m_a$ derived from Baker and Bostock (1998), was set to $\tau_m = 1/(0.04 \exp(V_m/25.5))$ + 63.2 (yielding 100–200 ms depending on $V_m$). Using these parameters $I_{Na\text{-}late2}$ decayed to a small residual persistent current; it did not fall to absolute zero (Fig. 1G).

4. Persistent $\text{Na}^+$ current ($I_{Na\text{-}p}$). As noted, there was no independent $I_{Na\text{-}p}$. The persistent current constituted the persistent residues of the fast and two late $\text{Na}^+$ currents. The time point of transition between the late (but still decaying) currents $I_{Na\text{-}late1}$ and $I_{Na\text{-}late2}$, and true $I_{Na\text{-}p}$, late-derived persistent current is arbitrary. As noted, the boundary was set at 200 ms (Fig. 1D). $I_{Na\text{-}p}$ declined by only an additional 50% when tracked out to 5 s.

Delayed rectifier potassium current ($I_{K\text{-}R}$)

The potassium K-current was modeled as $I_{K\text{-}R} = g_{K\text{-}max} \times m(V_m - E_K)$, where $n$ is the activation variable. The first rather than the fourth power of $n$ was used to improve the fit to the biological data. $g_{K\text{-}max}$ used was 1.5 mS/cm$^2$, which is within the physiological range (0.7–2.6 mS/cm$^2$), calculated from Everill et al. (1998) and Rolà et al. (2003). $V_{K\text{-}R}$ of activation and the slope factor were taken from small- and medium-sized DRG neurons (Rolà et al. 2003) in the absence of data from large DRG neurons. We used these values to set $n_a = 1/(1 + \exp[(V_m + 9.2)/-16])$, $\tau_n = -23 + 69.46 \exp(-0.0142V_m)$ following Fedulova et al. (1998). $E_K$ was set at $-94$ mV (Scroggs et al. 1994). Although derived from various sources, the voltage-clamp plot using these values fits nicely to that of large DRG cells (Everill et al. 1998).

RESULTS

Subthreshold oscillations and ectopic discharge in the baseline model

In current clamp we injected the cell with a series of 3-s depolarizing steps of gradually increasing amplitude. This evoked a characteristic range of oscillatory and spiking behavior quite similar to that observed in live DRG cells (see the following text). The model cell did not oscillate at rest ($V_m = -57.4$, Fig. 2A). However, a small depolarization evoked damped oscillations (Fig. 2B) and a slightly increased current evoked sustained oscillations (stimulus = $0.012$ nA; $V_m = -57.2$ mV; oscillation frequency = 44.7 Hz; amplitude = $2.3$ mV, Fig. 2C). Another increment in stimulus amplitude induced oscillations that triggered repetitive spike bursts (Fig. 2D) and a further increment yielded a spike burst at stimulus onset, without prior oscillations, that was followed by repetitive bursting (Fig. 2E). Subthreshold oscillations were present between the bursts. During the course of burst spike amplitude declined slightly as did within-burst frequency. The duration of bursts and the interval between bursts were voltage sensitive; increasing stimulus current increased burst duration, whereas the interval between bursts decreased (Fig. 2, D and E). Finally, with a stimulus of $0.063$ nA, the cell fired in a sustained manner for the duration of the 3-s pulse (at 36 Hz, Fig. 2F).

Firing frequency proceeded to increase with further depolarization, reaching a maximum of 99.3 Hz with stimuli of $1.615$ nA. With still greater depolarization, spikes (including spike bursts) ceased. In their place sustained oscillations emerged at about the frequency of the spikes. Further depolarization caused these oscillations to gradually decrease in amplitude and increase in frequency up to a maximum of $110.5$ Hz at $V_m = -36.67$ mV (Fig. 2G). Oscillations ceased when the cell was still further depolarized.

Overall, oscillatory behavior began at $V_m = -57.21$ mV and ended at $-36.67$ mV, with a range of about 20 mV within which oscillations were generated. This span of potentials is the “window” for generating subthreshold oscillations. Spikes were generated within a part of this window. The breadth of the window proved to be dependent on parameters of the model cell as described in the following text. The patterns of spiking behavior and the breadth of the oscillatory window were similar to those previously described in live DRG neurons, including in neurons in which voltage-activated $K^+$ channels had been blocked pharmacologically (Amir et al. 2002a). That is, oscillations transitioned into oscillation-triggered bursting and tonic firing and then back to oscillations of gradually declining amplitude, much as in live cells (Fig. 3). In these respects the baseline model was much more realistic than the H–H–based model reported by Amir et al. (2002a).

Beyond general patterns of activity, many of the specific parameters of oscillatory and spiking behavior in the baseline model were lifelike (Fig. 3). This includes the general range of oscillation frequencies (45–110 Hz in the simulation and 32–62 Hz in live cells in the presence of $g_{K\text{-}R}$-block), the membrane potentials at which oscillations began and ceased ($-57$ to $-37$ mV in the simulation; $-55$ to $-22$ mV in live cells in the presence of $g_{K\text{-}R}$-block), the range of frequencies of spike discharge during bursts and tonic firing.
output of the baseline model with physiological values, particularly checking parameters of the total $I_{Na^-}$. We applied to the model the same stimulation protocol that Baker and Bostock (1998) used to define the three $I_{Na^-}$ components in live cells. Specifically, the model was held at a prepulse of $-110 \text{ mV}$ and stepped for $200 \text{ ms}$ to potentials ranging from $-80$ to $10 \text{ mV}$ in $5$-mV increments. Overall, total $I_{Na^-}$ behaved very much like that in live cells (Fig. 1, A and E–G). The only prominent difference was that $I_{Na^-}$ late1 inactivated in about $20 \text{ ms}$, more rapidly than observed by Baker and Bostock (1998). With respect to the integrated fast transient Na$^+$ current, $I_{Na^-}$ fast contributed about $25.5\%$ of the current peak ($V_m = -10 \text{ mV}$), $I_{Na^-}$ late1 about $74\%$, and $I_{Na^-}$ late2 only about $0.5\%$. With respect to the integrated $I_{Na^-}$, the decaying component of $I_{Na^-}$ (2–200 ms, excluding $I_{Na^-}$) was contributed entirely by $I_{Na^-}$ late1 and $I_{Na^-}$ late2, with relative proportions varying with time.

Current–voltage (I–V) curves. Also consistent with experimental data the integrated peak $I_{Na^-}$ in the simulation started to activate at around $-60 \text{ mV}$ and reached a max-

Validation of current parameters

Total Na$^+$ current. Since some of the kinetic parameters used were not based on explicit data, we compared the
mum at \(-10\) mV (Fig. 1B). This is within the expected voltage range for large DRG cells (Abdulla and Smith 2002; Baker and Bostock 1998; Hong and Wiley 2006; Peng et al. 2002; Shah et al. 2004). The peak transient inward current in the model was \(126\) nA. Although high, this value is not inconsistent with physiological values for large DRG neurons, especially after axotomy. Baker and Bostock (1997, 1998) did not provide a direct measure of their peak \(I_{\text{Na}}\), although they stated that the current amplitude after 200 ms, as much as \(-1.7\) nA, was about 1.1% of the transient peak. Extrapolating, the peak transient current was as much as \(-155\) nA. Current measurements from excised membrane patches were consistent with this value (\(-80\) to \(-120\) nA, derived from Baker and Bostock 1998; Caffrey et al. 1992). Direct measurements from large-diameter DRG neurons in nerve-injured rats showed peak \(I_{\text{Na}}\) of up to about \(-100\) nA, reflecting a 1.6-fold increase following axotomy (Abdulla and Smith 2002; also see Rizzo et al. 1995).

Baker and Bostock (1997) adopted an alternative voltage-clamp protocol for isolating \(I_{\text{Na}}\). The membrane was held at a prepulse potential of \(-20\) mV for 200 ms and then stepped to a series of values between \(-80\) and 10 mV, in increments of 5 mV, for 100 ms. Applying this protocol to our baseline model yielded an \(-V\) plot, 200 ms after the beginning of the pulse, that represents the integrated \(I_{\text{Na}}\) (Fig. 1D). The peak inward current was \(-1.68\) nA at \(-30\) mV, a value similar to that recorded by Baker and Bostock (1997; \(-1.7\) nA, their Fig. 1A).

Comparison to Amir et al. (2002a). Plotting voltage-clamp records from the model cell of Amir et al. (2002a) shows that the total \(I_{\text{Na}}\) also begins to activate at \(V_m = -60\) mV. However, the peak inward current (\(-63\) nA) was obtained at

![Output of the baseline model (B1–B4) resembles that of live dorsal root ganglion (DRG) neurons (A1–A4). Tracings of the live cell come from the study of Amir et al. (2002a). A1 and B1: \(V_r = -61\) mV for A1, \(-57.4\) mV for B1. A2 and B2: sustained subthreshold oscillations; \(V_m = -58\) mV for A2, \(-57.21\) mV for B2. Fourier analysis of the traces A2 (live) and B2 (model) are shown in the inset. A3 and B3: oscillation-triggered burst discharge; \(V_m = -57.0\) mV for A3, \(-57.14\) mV for B3. The onset of one burst is shown at higher gain below (spikes are truncated). A4 and B4: repetitive firing (spikes are truncated). Scale bars: 4 mV/100 ms for A1 and A2 and B1 and B2; 16 mV/2 s for A3, top trace, 10 mV/100 ms for A3, bottom trace; 20 mV/500 ms for B3, top trace, 20 mV/100 ms for B3, bottom trace; 10 mV/50 ms for A4; 10 mV/200 ms for B4.](http://jn.physiology.org/102/9/1435/subfig.png)
$V_m = 0 \text{ mV}$, which is somewhat positive to the present value ($-10 \text{ mV}$) and to the potential range seen in live cells ($-30$ to $-10 \text{ mV}$; Abdulla and Smith 2002; Baker and Bostock 1998; Hong and Wiley 2006; Peng et al. 2002; Shah et al. 2004). Plotting the persistent current from the Amir et al. (2002a) model yields a peak of $-1.93 \text{ nA}$ at $V_m = -30 \text{ mV}$. This value is slightly higher than that obtained in the current baseline model ($-1.68 \text{ nA}$) and out of the range measured in live cells ($-0.4$ to $-1.7 \text{ nA}$; Baker 2000; Baker and Bostock 1997).

Overall, the fundamental difference between the present and the earlier model is the presence of the late currents. Each $\text{Na}^+$ conductance plays a distinctive role in generating ectopic activity.

We assessed the functional contribution of the fast and the two late $\text{Na}^+$ conductances individually by running simulations in which $g_{\text{Na}_\text{max}}$ of each was gradually reduced, whereas the others either maintained their original values or were augmented to compensate for the loss of specific $I_{\text{Na}^-}$ components. Focus was on the ability of the system to generate oscillations and spikes, the membrane potential “window” within which oscillations and spikes were present, and their frequency.

Changing $g_{\text{Na}_{\text{fast}}}$. Reducing $g_{\text{Na}_{\text{fast}}_{\text{max}}}$ from its initial value of $25 \text{ mS/cm}^2$ reduced the voltage window, within which sustained oscillations and spiking occurred, and the amplitude of both oscillation sinusoids and spikes at any given membrane potential. This is illustrated in the state diagram in Fig. 5A, which shows the pattern of activity observed over a range of membrane potentials as the value of $g_{\text{Na}_\text{fast}}_{\text{max}}$ was varied. In the diagrams in Fig. 5 the full repertoire of activity patterns noted earlier—oscillations, spike bursting, and sustained firing (Fig. 2)—is expressed as different shading fills. Observed by reading Fig. 5A horizontally for each value on the $y$-axis, all of the activity patterns were present until $g_{\text{Na}_\text{fast}}_{\text{max}}$ fell to $<18 \text{ mS/cm}^2$. For $g_{\text{Na}_{\text{fast}}_{\text{max}}} = 17 \text{ mS/cm}^2$ oscillations and oscillation-evoked bursts continued to be present, but tonic firing was no longer evoked. When $g_{\text{Na}_{\text{fast}}_{\text{max}}}$ was reduced still further, into the range of $13–16 \text{ mS/cm}^2$, repetitive bursting also ceased. Depolarizing steps now evoked a brief spike burst at the beginning of the pulse that then decayed into sustained subthreshold oscillations. After the initial spike burst no subsequent spikes were generated irrespective of the stimulation amplitude or duration.

Interestingly, the frequency of subthreshold oscillations, about $45 \text{ Hz}$ at liminal stimulus amplitude, and the threshold for evoking oscillations (about $-57 \text{ mV}$) remained virtually unchanged as $g_{\text{Na}_{\text{fast}}_{\text{max}}}$ was reduced from $25$ to $13 \text{ mS/cm}^2$ (Figs. 5A and 6). When $g_{\text{Na}_{\text{fast}}_{\text{max}}}$ was reduced to $<13 \text{ mS/cm}^2$ the initial spike burst gave way to subthreshold oscillations whose amplitude damped out to zero during the course of the stimulus pulse. Sustained oscillations no longer occurred. Thus the window within which oscillations could be evoked narrowed progressively through the range $25–13 \text{ mS/cm}^2$, with its center shifting progressively toward the threshold potential for evoking oscillations ($-57 \text{ mV}$). The use of weak stimuli or further reducing $g_{\text{Na}_{\text{fast}}_{\text{max}}}$ yielded a progressively shorter initial burst until spiking ceased altogether, yielding damped oscillations at the beginning of the step in the absence of spiking (as in Fig. 2B). We conclude that $g_{\text{Na}_{\text{fast}}}$ is
important for burst and sustained spike discharge because the most prominent effect of reducing it was to eliminate spiking. It is less critical for generating oscillations.

In light of the suppressive effect of reducing $g_{Na^{+}}^{fast_{max}}$ on spike bursting, we evaluated how this change affects the postspike DAP, an element thought to be critical for maintaining spike bursts (Amir et al. 2002). As anticipated, the amplitude of the DAP that followed single spikes evoked by 1-ms depolarizing steps was highly sensitive to reduction of $g_{Na^{+}}^{fast_{max}}$.

Changing $g_{Na^{+}}^{late_{1}}$. All of the patterns of activity illustrated in Fig. 2 continued to be present as $g_{Na^{+}}^{late_{1_{max}}}$ was reduced from its baseline value (27 mS/cm²) to 22 mS/cm², although the potential window in which oscillations and spiking occurred narrowed (Fig. 5B) and spike and oscillation amplitude decreased. Burst duration remained unchanged. Moving into the range 21–17 mS/cm² spiking ceased, but subthreshold oscillations persisted albeit within a window that gradually narrowed, with its center shifting toward less negative potentials (Fig. 5B). Throughout the range 17–27 mS/cm², lowering $g_{Na^{+}}^{late_{1_{max}}}$ also increased the oscillation frequency (Fig. 6, triangles). The loss of tonic and burst firing was accompanied by substantial reduction in the amplitude of the DAP that followed single evoked spikes. For $g_{Na^{+}}^{late_{1_{max}}}$ ≤ 16 mS/cm² strong stimuli induced an initial spike or spike burst followed by damped oscillations, whereas weaker stimuli induced damped oscillations.

$g_{Na^{+}}^{fast_{max}}$ to < 13 mS/cm² or $g_{Na^{+}}^{late_{1_{max}}}$ to < 17 mS/cm² eliminated spiking and oscillations. However, reducing $g_{Na^{+}}^{late_{2_{max}}}$ to zero still permitted burst firing and oscillations, albeit within a narrow range of membrane potentials.

**FIG. 6.** The frequency of subthreshold oscillations is affected by $g_{Na^{+}}^{max}$ . The minimum frequency at which oscillations can be generated increased as $g_{Na^{+}}^{late_{1_{max}}}$ or $g_{Na^{+}}^{late_{2_{max}}}$ was reduced. Reducing $g_{Na^{+}}^{fast_{max}}$, in contrast, had no effect on the minimal oscillation frequency. Note that in each case, including $g_{Na^{+}}^{fast_{max}}$, stimulus strength must be progressively increased in order to evoke oscillations.
osillations without an initial spike. With $g_{Na^-}^{late1}_{max} < 10$ mS/cm$^2$ damped oscillations were no longer induced even following pulses strong enough to evoke an initial spike.

A substantial proportion of the $I_{Na^-}$ at 2–20 ms, the time of the “intermediate” current, is carried by the $I_{Na^-}^{late1}$. Reducing $I_{Na^-}^{late1}_{max}$ by less than half was enough to dramatically reduce the ability of the system to generate both oscillations and spikes. To test whether this effect was due to the simultaneous reduction of the fast transient and persistent conductances of $I_{Na^-}$, we eliminated $g_{Na^-}^{late1}$ and restored the fast transient and persistent currents to baseline values by augmenting $g_{Na^-}^{fast}_{max}$. This did not restore spiking or oscillations. We conclude from these results that the “intermediate” current is essential for generating oscillations.

Changing $g_{Na^-}^{late2}$. Reducing $g_{Na^-}^{late2}_{max}$ from the baseline value of 0.128 mS/cm$^2$ reduced the already narrow window within which bursting occurred and increased burst duration (Fig. 5C). When $g_{Na^-}^{late2}_{max}$ reached 0.095 mS/cm$^2$ bursting ceased entirely. As the cell was further depolarized, oscillations transitioned directly into tonic firing and then back to the oscillatory pattern. With continued reduction in $g_{Na^-}^{late2}_{max}$ the window within which these patterns of activity occurred gradually narrowed with its center shifting toward less negative potentials. This behavior persisted until $g_{Na^-}^{late2}_{max}$ was reduced to about 0.01 mS/cm$^2$, at which point oscillations were no longer generated at potentials $<-42$ mV, although they continued to appear at potentials $>-40$ mV. Rather, suprathreshold stimuli directly evoked tonic spiking. This continued to be the case when $g_{Na^-}^{late2}_{max}$ was set to zero. Throughout, lowering $g_{Na^-}^{late2}_{max}$ increased the frequency of oscillations (Fig. 6, squares).

With $g_{Na^-}^{late2}_{max}$ set to zero, returning the persistent current to baseline by augmenting $g_{Na^-}^{fast}$ had little effect, presumably because this maneuver did not restore the intermediate current. In contrast, when the persistent current was returned to baseline by augmenting $g_{Na^-}^{late1}$, which does restore the intermediate current, oscillations could once again be generated by weak stimuli, although burst firing remained absent. We conclude that although the intermediate component of $g_{Na^-}^{late2}$ contributes to oscillatory behavior, the main contribution of this conductance is to interrupt tonic firing and enable bursting.

To gain insight into the mechanism whereby $g_{Na^-}^{late2}$ facilitates bursting, we plotted the status of the inactivation gates of all three conductances in the baseline model (Fig. 7, A and B) and in the absence of repetitive bursting (Fig. 7, C and D). This analysis revealed that, because of its prolonged $\tau_{inact}$ inactivation of the late and persistent components of $I_{Na^-}^{late2}$ summates during the course of a burst, closing slowly. This has the effect of damping the firing. During the subsequent interval inactivation of $g_{Na^-}^{late2}$ slowly fades, permitting oscillations to augment in amplitude and eventually to trigger a new spike burst (Fig. 7, A and B). The effect of the slow inactivation of

---

**FIG. 7.** The status of inactivation of $g_{Na^-}^{late2}$ controls the cycle of bursting. A: burst firing using parameters of the baseline model. B: the status of the inactivation (h) gates of the 3 Na$^+$ conductances during the course of the bursts shown in A. Due to their relatively small $\tau_{inact}$ of $g_{Na^-}^{fast}$ and $g_{Na^-}^{late1}$, the reciprocate between relatively low (closed) and relatively high (open) values with each spike and oscillation sinusoid. Zero inactivation reflects closed h-gates (the maximally open value is 1.0). Inactivation of $g_{Na^-}^{late2}$, on the other hand, is slow. For this reason its inactivation is integrated over time, rising and falling slowly. The rate of rise and fall determines the burst cycle. C and D: reducing $g_{Na^-}^{fast}_{max}$ to 16 mS/cm$^2$ eliminated repetitive bursting. The step depolarization shown in C evoked a single spike burst followed by sustained subthreshold oscillations. The corresponding plot of h-gate inactivation of the 3 Na$^+$ conductances (D) shows that, as in B, inactivation of $g_{Na^-}^{fast}$ and $g_{Na^-}^{late1}$ track each spike and oscillation sinusoid. Inactivation of $g_{Na^-}^{late2}$ falls slowly during the burst, terminating it, and then slowly stabilizes without triggering a second burst.
gNa-late2 is also evident in the presence of a single burst, accomplished by reducing gNa-fastmax (Fig. 7, C and D). Inactivation of gNa-late2 during the burst was more pronounced than that in the baseline model and its recovery was less complete. The reason is that stable oscillations appeared at less negative membrane potentials than those in the baseline model. As a result the membrane oscillates at relatively depolarized potentials, causing the inactivation (h-) gate of gNa-late2 to be closed and thus unable to initiate a subsequent burst. Studying the mesencephalic trigeminal nucleus (MesV), a brain stem DRG homolog, Enomoto et al. (2006) also showed that slowly inactivating components of INa+ contribute significantly to burst termination.

Role of the fast activating phase of INa-late1 and INa-late2

In our baseline model both gNa-late1 and gNa-late2 activated very rapidly, consistent with Baker and Bostock (1998). The fast activating component of these currents summed with the fast activating gNa-fast to generate the total fast transient INa+ peak. Since the specific parameters of activation of these conductances in vivo are unknown, we examined the effect of slowing activation rate on oscillations and spike electrogenesis. In the baseline model cell integrated INa+ activation was rapid (≤0.11 ms), in accord with measurements from live cells (Kostyuk et al. 1981; Nowycky 1992). Using stimuli of 5-s duration we gradually increased τm of gNa-late1 and gNa-late2 (from 0 in steps of 0.1 ms) while maintaining τm of gNa-fast unchanged. This narrowed the range of membrane potentials at which oscillations and tonic spiking were generated (Fig. 8). The window for burst firing expanded. At τm > 0.5 ms spiking, tonic and burst firing ceased altogether and, as τm approached 1.0 ms, oscillations were no longer generated. At membrane potentials that supported oscillations, the oscillation frequency fell as τm increased.

Increasing τm of gNa-late1 had the additional effect of reducing the peak of the fast transient INa+, by about 20% when τm was set to 0.5 ms and by about 25% when it was set to 1.0 ms. Restoring the peak to baseline by increasing gNa-fast restored the ability of the system to generate spikes and oscillations. We conclude that in addition to the other contributions of the lat Na+ currents, most notably the intermediate current at 2–20 ms, rapid activation of gNa-late1 is also important for oscillations and spike electrogenesis.

DISCUSSION

Nerve block using local anesthetics generally eliminates neuropathic pain caused by distal ectopic pacemaker sources such as neuromas, at least for the duration of the block. When the block is placed proximal to the DRG (foraminal or spinal block) the likelihood of pain relief is higher still. Ectopic discharge generated in the DRG appears to be triggered by subthreshold membrane potential oscillations generated in primary afferent neurons. Oscillations may also contribute to discharge generated in axons at the nerve injury site (Devor 2006; Kapoor et al. 1997). We investigated which characteristics of INa+ foster oscillations and spiking. In the absence of pharmacological tools capable of independently modulating the various kinetic components of INa+ we used a computational approach. In live DRG cells total INa+ is the integral of fast and delayed currents. Our model included a range of INa+ components: INa-fast, INa-late1, INa-late2, and INa-p, along with ohmic K+ leak. This system generated both oscillatory behavior and oscillation-driven spiking, which resembled recordings from live DRG neurons in experimental models of neuropathic pain.

We found that the fast, delayed, and persistent Na+ currents make different and distinctive contributions to repetitive firing capability. gNa-fast is important for burst and sustained spike discharge. gNa-late1 appears to be particularly important for the generation of subthreshold oscillations. Since sustained burst spiking requires oscillations, the intermediate component of gNa-late1 (2–20 ms) also seems to be the key for enabling spiking in the presence of gNa-fast. gNa-late2 also provides some current in the 2- to 20-ms range, thus facilitating oscillatory behavior, although its contribution here is much smaller than that of gNa-late1. The main contribution of gNa-late2 is its later component (20–200 ms), which is important for burst firing. Our ability to test the contribution of INa-p was limited because this current could not be manipulated independently of the other currents.

The parameter space we studied most closely was channel inactivation, but we also looked at channel activation. In our baseline model τm of activation for the fast current was about 0.1 ms in the voltage range typical of subthreshold oscillations. Physiological values for activation τm of the late currents are not known with much certainty, but for gNa-late1, at least, τm appears to be very fast (Baker and Bostock 1998). In our simulations, oscillations and spiking proved to be fairly sensitive to the onset kinetics of the late Na+ conductances, failing for τm > 1.0 ms. This observation implies that in vivo the late currents have a rapid onset (<0.5 ms). Thus if our model is correct they are unlikely to depend solely on Na+ channel types with slow onset kinetics (see following text).

Passive leak was the second type of conductance present. In contrast to the model of Amir et al. (2002a) the reversal potential of Ileak was not at the K+ reversal potential (EK+), but
more depolarized. This was because setting $E_{\text{leak}}$ at $E_{K_+}$ yielded an unrealistically hyperpolarized $V_r$. Voltage-sensitive $K^+$ channels were not included because they are not required for oscillations or oscillation-evoked spiking in DRG neurons, although they do affect oscillation and spike discharge frequency (Amir et al. 2002a). However, just to be sure, we incorporated them in a few simulations. The effect on oscillatory behavior was minimal, although they did enable single-spike firing.

The basic features of in vivo ectopic spike patterning were well captured by the current simulation (Fig. 3). However, not all features were present. For example, in many live cells $V_m$ shifts several millivolts in the hyperpolarizing direction during the course of a burst, contributing to burst termination. This behavior, at least partly attributable to Ca$^{2+}$ entry during spiking and activation of a Ca$^{2+}$-activated K$^+$ conductance (Amir and Devor 1997), was not observed because neither conductance was included in the baseline model. Bursting was nonetheless present, indicating that other mechanisms may also contribute to the arrest of tonic firing (Fig. 7). In vivo recordings from neopuna afferents sometimes show exotic firing patterns also not observed in our simulations. Examples are regularly cycling modulations in spike frequency or repeated cycling between staccato and prolonged bursts (see Fig. 3.2 in Devor 1989). These patterns no doubt also reflect additional, idiosyncratic conductances present in particular cell types.

**Role of delayed $I_{Na^+}$ in repetitive firing**

Delayed and persistent inward current carried by TTX-resistant (TTX-R) Na$^+$ channel $\alpha$-subunits is thought to contribute to hyperexcitability of afferent neurons, particularly in the event of tissue inflammation (Elliott 1997; Herzog et al. 2002; Roza et al. 2003; Waxman 2002). Delayed $I_{Na^+}$ has also been described at nodes of Ranvier of myelinated peripheral nerve fibers (Dubois and Bergman 1975) and subthreshold oscillations have been observed in intraxonal recordings from such fibers (Kapoor et al. 1997). Finally, large-diameter primary sensory neurons in MesV also show delayed TTX-S $I_{Na^+}$. In these cells there are two components, one with $\tau$ of several seconds and another that is truly persistent (noninactivating).

In vitro observations and simulations suggest that both contribute to cell resonance, subthreshold oscillations, and repetitive firing (Enomoto et al. 2006; Wu et al. 2001, 2005).

Delayed Na$^+$ currents are also known to contribute to repetitive firing in other neuronal types (Crill 1996). For example, Pennartz et al. (1997) described in suprachiasmatic nucleus neurons a rapidly activating, slowly inactivating TTX-S current resembling $g_{Na^+}\text{late1}$. This contributes significantly to the slope of the depolarizing ramp following spikes and thus to spontaneous firing rate. Delayed $I_{Na^+}$ may also facilitate electrogenesis in the CNS through other mechanisms, such as enhancement of oscillatory behavior (Schindler et al. 2006; Spampanato and Mody 2007). Using slow ramp depolarizations (100 mV/s) Kononenko et al. (2004a,b) revealed in suprachiasmatic neurons a TTX-S current with inactivation similar to that of $g_{Na^+}\text{late2}$ ($\tau \approx 50–250$ ms at its maximum). This current was a necessary supplement to the fast $I_{Na^+}$ in the generation of sustained firing. Likewise, a delayed TTX-S current similar to that of $g_{Na^+}\text{late2}$ contributes to repetitive firing in neurons of the entorhinal (Agrawal et al. 2001) and frontal agranular cortices (Urbani and Belluzzi 2000). Finally, in spinal motorneurons depolarized with slow ramps Kuo et al. (2006) found that the persistent component of the total $I_{Na^+}$ imparts an acceleration to the depolarization required to overcome membrane accommodation and to permit repetitive firing.

**Source of delayed $I_{Na^+}$**

The identity of the ion channel(s) underlying late and persistent Na$^+$ currents in large DRG neurons has not yet been determined. Indeed, these currents might well be generated by a repertoire of gating states of channels that also generate the fast transient $I_{Na^+}$ (Baker and Bostock 1998; Crill 1996). The delayed currents are almost certainly not generated by the Na$_{1.9}$ Na$^+$ channel because this channel is TTX-R, whereas the currents in question are TTX-S. In any event Na$_{1,9}$ is not expressed in large DRG neurons (Cummins et al. 1999). Likewise, the Na$_{1,8}$ channel generates delayed and persistent $I_{Na^+}$, but these too are TTX-R (Renganathan et al. 2000). Both TTX-R channels have slow onset kinetics incompatible with oscillations and spiking (Fig. 8) and both are down-regulated following axotomy. Na$_{1.5}$ generates a delayed $I_{Na^+}$ in cardiac myocytes (Noble and Noble 2006) and it has recently been identified in adult DRG neurons (Kerr et al. 2007). At both locations, however, Na$_{1.5}$ is TTX-R.

Na$_{1.1}$ and Na$_{1.3}$, both TTX-S Na$^+$ channels, generate a fast transient Na$^+$ current when coexpressed with accessory $\beta_1$ and $\beta_2$ subunits. In addition, however, they can generate delayed $I_{Na^+}$ for certain Na$_{1,1}$ alleles $\tau_{slow} = 5–10$ ms (Lossin et al. 2003; Montegazza et al. 2005); for human Na$_{1,3}$ expressed in HEK cells, $\tau_{slow} > 150$ ms (Chen et al. 2000)). Na$_{1.1}$ is expressed in normal adult DRG neurons, but Na$_{1,3}$ and Na$_{1,4}$ is not and thus cannot contribute to the oscillations that occur in (a small fraction of) intact DRG neurons. However, Na$_{1.3}$ is up-regulated in DRG neurons after axotomy (Waxman et al. 1994). It is also up-regulated in dorsal horn neurons after spinal cord injury and in the latter case up-regulation has been shown to contribute to both enhanced firing during ramp depolarization, hyperexcitability, and pain behavior (Hains et al. 2003; Lampert et al. 2006). Na$_{1,2}$ and Na$_{1,6}$ are also TTX-S, but when expressed in isolation they generate little if any current with intermediate kinetics (Shirahata et al. 2006; Weisner et al. 2006). Na$_{1,7}$ is TTX-S and generates fast transient and delayed $I_{Na^+}$, but essentially no $I_{Na^+}$, at least when expressed in isolation (Cummins et al. 2004; Herzog et al. 2003). Overall, these considerations point to Na$_{1,1}$, Na$_{1,3}$, and Na$_{1,7}$ as the most likely contributors of the key delayed TTX-S $I_{Na^+}$ component in large-diameter DRG neurons.

Mitigating against Na$_{1,1}$ and Na$_{1,7}$ as essential carriers of delayed $I_{Na^+}$ is the fact that these transcripts are down-regulated in DRG neurons following axotomy (Chung et al. 2003). Axotomy, however, is known to strongly enhance oscillatory behavior and spike electrogenesis in DRG neurons (Liu C-N et al. 2000a). This factor enhances the candidacy of Na$_{1,3}$, which is up-regulated following axotomy (Waxman et al. 1994). However, deletion of Na$_{1,3}$ does not apparently interfere with repetitive firing (Nassar et al. 2006) or oscillatory behavior in large-diameter DRG neurons (our unpublished...
observations). Thus, no single Na\textsuperscript{+} type appears to be the key to ectopic electrogenesis in these neurons.

Gene regulation notwithstanding, little is known about the effect of axotomy on actual delayed and persistent Na\textsuperscript{+} currents, although Abdulla and Smith (2002) reported an increase in $I_{\text{Na}^-}$ at intermediate latencies (10 ms) in large-diameter DRG neurons. Interestingly, this change occurred in animals that showed pain behavior (autotomy) after nerve injury, but not in nerve-injured animals without pain behavior. A variety of mediators present in injured nerves are able to alter whole cell $I_{\text{Na}^-}$ kinetics, but it is uncertain whether this reflects a shift in the relative populations of the specific contributing Na\textsuperscript{+} channel types present or a change in the kinetics of individual channels (Bevan and Storey 2002).

**Potential implications for pain and analgesic drugs**

Our main conclusion is that the “intermediate” component of $I_{\text{Na}^-}$ (2–20 ms) is a key contributor to sustained firing capability, despite the fact that the maximal amplitude of this current is only about 2% of the peak transient $I_{\text{Na}^-}$ that generates the spike itself. In principle it should be possible to develop agents that act on the delayed component of the integrated whole cell $I_{\text{Na}^-}$ by enhancing the inactivation kinetics of a variety of channel types rather than by blocking a particular channel. An example is riluzole (2-aminoo-6-trifluoromethoxy benzothiazole), a neuroprotective agent used for the treatment of amyotrophic lateral sclerosis. Riluzole blocks delayed $I_{\text{Na}^-}$ including $I_{\text{Na}^-}$p (Song et al. 1997; Urbani and Belluzzi 2000; Wu et al. 2005). As expected, it also suppresses subthreshold membrane potential oscillations and bursting in CNS neurons (Reboreda et al. 2003; Wu et al. 2005). Unfortunately, it is brain permeant and, at clinically tolerated doses, does not provide useful pain relief (Galer et al. 2000). Yang et al. (2009) reported recently that gabapentin suppresses subthreshold oscillations and repetitive firing in (live) medium to large DRG neurons by suppressing delayed Na\textsuperscript{+} current. Indeed, this may be the drug’s primary mode of action in the relief of neuropathic pain (Devor 2009b). The effect was attributed to suppression of persistent $I_{\text{Na}^-}$p. True $I_{\text{Na}^-}$p, however, was not adequately dissociated from other delayed Na\textsuperscript{+} currents. Suppression of an $I_{\text{Na}^-}$ with the intermediate kinetics of $I_{\text{Na}^-}$late1 might in fact be the effective target. An agent that selectively suppresses the intermediate current in afferent neurons, especially if it were excluded from the CNS, might have improved efficacy and specificity as a reliever of neuropathic pain.

**Acknowledgments**

We thank C.-N. Liu for permission to use the live cell data in Fig. 3.

**Grants**

This work was supported by the United States–Israel Binational Science Foundation, the National Institute for Psychobiology in Israel, and the Hebrew University Center for Research on Pain.

**References**


Herzog RI, Cummins TR, Ghassemi F, Dib-Hajj SD, Waxman SG. Distinct repressing and closed-state inactivation kinetics of Nav1.6 and Nav1.7


Waxman SG, Kocsis JD, Black JA. Type III sodium channel mRNA is expressed in embryonic but not in adult spinal sensory neurons, and is reexpressed following axotomy. J Neurophysiol 72: 466–470, 1994.


