Modeling activity-dependent changes of axonal spike conduction in primary afferent C-nociceptors

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Activity-dependent changes in neuronal excitability are of key interest for a variety of diseases such as epilepsy or pain. While the plastic nature of signal processing at central synapses has been studied extensively, excitability changes in peripheral axons have received less attention. Pronounced activity-dependent slowing (ADS) of conduction velocity has been found to characterize axons of primary afferent C-nociceptors compared with non-nociceptors (Thalhammer et al. 1994), and it has been hypothesized that axonal characteristics would reflect the functional requirements of the particular primary sensory afferent (Thalhammer et al. 1994). Recently, a direct correlation between ADS of conduction velocity and neuronal excitability was also shown (De Col et al. 2012). In pain research, there is a current controversy whether spontaneous pain (Bennett 2012; Mogil 2012) and suprathreshold encoding behavior of nociceptors would have higher predictive value over studies of evoked pain read-outs, which mainly would detect changed activation thresholds from resting state. In this respect, activity-dependent modulation of conduction in C-fibers is of major importance. Most interestingly, reduced ADS in peripheral C-nociceptors, leading to reduced conduction failures of the axons at higher discharge frequencies, has been described in painful diabetic neuropathy in the rat (Sun et al. 2012). In addition, activation thresholds of the dorsal root ganglia (DRGs) were reduced and expression of NaV1.7 and NaV1.8 were increased in the neuropathic animals (Sun et al. 2012). A similar reduction of ADS in nociceptors accompanied by reduced conduction failure could also be induced experimentally by intradermal injections of nerve growth factor (NGF) in the pig (Obreja et al. 2011b). In humans, NGF injections led to increased pain ratings upon electrical skin stimulation (Obreja et al. 2011a; Rukwied et al. 2010) and reduced ADS of mechano-insensitive nociceptors (Namer B and Obreja O, unpublished data). Finally, in patients with neuropathic pain, reduced ADS of conduction has been assessed using micro-neurography (Schmidt et al. 2012; Serra et al. 2011), and recently a correlation between reduced ADS and spontaneous activity of nociceptors was described in pain patients (Kleggetveit et al. 2012). Therefore, understanding the physiological process of axonal action potential propagation is of considerable importance, particularly in relation to clinical pain states (Ørstavik et al. 2003; Serra et al. 2011) but also for neurodegenerative disorders (Kanai et al. 2006). Some of these changes can be determined experimentally in humans, offering a possibility to investigate diseases at the level of single human axons.

Unfortunately, intracellular recordings to determine the axonal membrane potential are difficult, since unmyelinated fibers are thin (<0.5 μm) and hard to locate in the tissue they innervate. Therefore, we set out to identify the process(es) underlying activity-dependent changes in excitability in single unmyelinated nerve fibers using computational modeling. The
Fig. 1. Model overview: geometry and temperature. A: model consisting of a terminal branch axon and a parent axon, connected via a cone. The branch and parent axons differ in length, diameter, and temperature. B: axon cross-section. The model consists of an intra-axonal space, a periaxonal space, and extracellular fluid (where concentrations are assumed to be unaffected by activity), modeled according to Scriven (1981). D, diameter of axon; θ, width of periaxonal space.

aim was to determine the likely contribution of each of an array of candidate molecular targets (ion channel subtypes, ion pumps) to changes in axonal membrane dynamics. Several nonspatial (single compartment) C-fiber mathematical models representing DRG somata have been used to study excitability in somatosensory neurons (Choi and Waxman 2011; Herzog et al. 2001; Kouranova et al. 2008; Maingret et al. 2008; Scriven 1981; Sheets et al. 2007). The current model presents a more realistic framework representing a segment (12.5 cm long) of a C-fiber including the peripheral terminal branches. The model is thereby to our knowledge the first to address axonal propagation of action potentials in cutaneous C-fibers.

Several features of activity-dependent changes in the conduction velocity of unmyelinated axons are used in the classification of C-fiber types (Campero et al. 2004; Weidner et al. 2000). In this work, we have focused on ADS of axonal conduction velocity (Obreja et al. 2010) and recovery cycles (RC) (Bostock et al. 2003; Weidner et al. 2000, 2002). For both ADS and RC, the underlying mechanisms have not been fully resolved (Blair and Bean 2003; Bostock et al. 2003; De Col et al. 2008; George et al. 2007; Serra et al. 2011, 2012; Weidner et al. 2000, 2002). However, the findings presented in this article point to the importance of the intra-axonal sodium concentration in modulating axonal excitability and conduction velocity during activity.

MATERIALS AND METHODS

To study activity-dependent changes of excitability in peripheral C-fibers, we constructed a biophysical model based on our own experimental data as well as previously published data. By virtue of their function, it is likely that the terminal region of C-fibers differs in some respects from the parent axon, which connects to the spinal cord. The main goal of the present study was to replicate and understand experimental data as well as previously published data. By virtue of the potential action, it is likely that the terminal region of C-fibers differs in some respects from the parent axon, which connects to the spinal cord.

The peripheral portion of a C-fiber was represented by a cylindrical membrane with length, diameter, and temperature as indicated in Fig. 1. Passive parameters of the membrane and the initial ion concentrations are listed in Table 1. We implemented dynamics for changes in sodium and potassium concentrations according to Scriven (1981), as illustrated in Fig. 1B (see Scriven 1981 for equations). To reduce periaxonal potassium accumulation, we doubled the periaxonal space to θ = 29 nm. Changes of ion concentration following an action potential can be seen in Fig. 2.

Ion Channel and Pump Models

Models for Hodgkin-Huxley-type ion channel currents were taken from the literature and are detailed below. Channel densities are presented in Table 2. Equations for ion channels and the pump are found in the Appendix. For the rate constants, Q10 was set at 2.5 for the voltage-dependent sodium (Na+) channels (Jonas 1989; Lux et al. 1990; Russ and Siemen 1996), 3.3 for the potassium channels (Russ and Siemen 1996), and 3 for the hyperpolarization-activated cyclic nucleotide-gated cation (HCN or h) channels (Pena et al. 2006).

$Na_{1.7}$ channel. Values for the $Na_{1.7}$ current were taken from Sheets et al. (2007).

$Na_{1.8}$ channel. Values for the $Na_{1.8}$ current were taken from Sheets et al. (2007) for activation ($m^+$) and fast inactivation ($h$), whereas equations and values for slow ($s$) and ultraslow ($u$) inactivation were taken from Maingret et al. (2008), according to $g = g_{max} m^+ h s u$, where $g_{max}$ is maximal channel conductance. For the simulations presented in Fig. 5F, an additional transition, from the

Table 1. Values of basic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_i$</td>
<td>35.4 $\Omega$ cm</td>
<td>Hodgkin and Huxley 1952</td>
</tr>
<tr>
<td>$C_m$</td>
<td>1 $\mu$F cm$^{-2}$</td>
<td>Hodgkin and Huxley 1952</td>
</tr>
<tr>
<td>$N_{asp}$ initial</td>
<td>11.4 mM</td>
<td>Ballanyi et al. 1983</td>
</tr>
<tr>
<td>$Na_{asp}$ initial</td>
<td>154 mM</td>
<td>Keynes and Ritchie 1965</td>
</tr>
<tr>
<td>$Na_{out}$ initial</td>
<td>154 mM</td>
<td>Keynes and Ritchie 1965</td>
</tr>
<tr>
<td>$K_{out}$ initial</td>
<td>121.7 mM</td>
<td>Ballanyi et al. 1983</td>
</tr>
<tr>
<td>$K_{asp}$ initial</td>
<td>5.6 mM</td>
<td>Keynes and Ritchie 1965</td>
</tr>
<tr>
<td>$K_{out}$ initial</td>
<td>5.6 mM</td>
<td>Keynes and Ritchie 1965</td>
</tr>
</tbody>
</table>

$R_i$, intracellular resistance; $C_m$, membrane capacitance; $N_{asp}$, intracellular (intra-axonal) sodium and potassium concentrations; $Na_{asp}$ and $K_{asp}$, periaxonal space concentrations; $Na_{out}$ and $K_{out}$, extracellular concentrations.
Table 2. Conductance and resting current densities (parent axon)

<table>
<thead>
<tr>
<th>Channels</th>
<th>Conductance Densities, mS/cm²</th>
<th>Resting Current Densities Before 1st AP, pA/cm²</th>
<th>Resting Current Densities Before 360th AP, pA/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump</td>
<td>0.0048</td>
<td>2,722.0</td>
<td>3,129.0</td>
</tr>
<tr>
<td>Na⁺,1.7</td>
<td>106.6439</td>
<td>−2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Na⁺,1.8</td>
<td>242.7124</td>
<td>−19.0</td>
<td>−5.0</td>
</tr>
<tr>
<td>Na⁺,1.9</td>
<td>0.0948</td>
<td>−63.0</td>
<td>−92.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>18.0017</td>
<td>116.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Kₐ</td>
<td>12.7555</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>K₉</td>
<td>6.9753</td>
<td>2,833.0</td>
<td>858.0</td>
</tr>
<tr>
<td>h (HCN)</td>
<td>2.5377</td>
<td>−4,313.0</td>
<td>−4,845.0</td>
</tr>
<tr>
<td>KNa,5</td>
<td>0.0012</td>
<td>57.0</td>
<td>1,958.0</td>
</tr>
</tbody>
</table>

AP, action potential. See text for descriptions of channel currents.

Open state of the fast activation gate to an inactivated state, was added. Rate constants for this transition were $a = 0.0043$ and $b = 0.00024$.

$Na_{v,1.9}$ channel. Values for the $Na_{v,1.9}$ current were taken from Herzog et al. (2001) and include three gates (activation, fast inactivation, and slow inactivation).

$K_{d,1}$ channel. Values for the $K_{d,1}$ current were taken from Sheets et al. (2007), with steady-state voltage dependence shifted by $-10$ mV to better fit the experimental data.

$K_{m,1}$ channel. The voltage dependence of steady-state activation for the $K_{m,1}$ current is from Maingret et al. (2008). The $K_{m,1}$ channel activation has a fast and a slow time constant as described by Passmore et al. (2003). To account for the two time constants, we implemented one fast ($n_{f,1}$) and one slow ($n_{s,1}$) gate, combined as follows:

$$g = g_{\text{bar}} \left( \frac{1}{4} \cdot n_f + \frac{3}{4} \cdot n_s \right).$$

Time constants were as follows:

$$\tau_{\text{inf}} = 1/(a + b),$$

with $a = 0.00395 \exp(V_m + 30)/40$ and $b = 0.00395 \exp[-(V_m + 30)/20]$, where $V_m$ is membrane potential.

$h$ channel. Values for the $h$ current were taken from Kouranova et al. (2008). which includes one fast ($m_{f,h}$) and one slow ($m_{s,h}$) activation gate, added as follows:

$$i_h = g_{\text{bar}} \cdot (0.5 \cdot m_{f,h} + 0.5 m_{s,h}).$$

$K_{m,1}$ channel. We modeled the $K_{m,1}$ current according to data from Bischoff et al. (1998), assuming an instantaneous effect of intracellular sodium concentration ($Na_{i,n}$) on gate opening:

$$g_{\text{KNa},5} = g_{\text{bar}} \left[ 1 + (38.7/Na_{i,n})^{3.5} \right].$$

Leak currents. Sodium and potassium leak (balancing) currents are included in the model to assure that the sum of potassium and sodium currents are each zero at the resting membrane potential ($V_{rest}$).

$$i_{\text{leak}} = g_{\text{Kleak}} \cdot (V_m - E_K) + g_{\text{Naleak}} \cdot (V_m - E_Na)$$

$$g_{\text{Kleak}} = - (in_{Na_{Nav,1.7}} + in_{Na_{Nav,1.9}} + in_{iNa_{Nav,1.8}} + in_{Na_{Kpump}})/(V_{rest} - E_Na)$$

$$g_{\text{Naleak}} = - (i_{k_{KM}} + i_{k_{KA}} + i_{k_{Kdr}} + i_{Na_{Kpump}} + i_{K_{Na}})/(V_{rest} - E_K).$$

Where $i_{\text{leak}}$ is leak current, $g_{\text{Kleak}}$ and $g_{\text{Naleak}}$ are conductances of potassium and sodium leak currents, $i_{k_{KM}}$ and $i_{k_{KA}}$ are the current component attributable to sodium and potassium, and $E_K$ and $E_{Na}$ are reversal potentials for $K$ and $Na$.

Na-K-ATPase pump. Values for the Na-K-ATPase pump model were taken from Scriven (1981) with a modified sodium dependence to account for recent C-fiber data (Hamada et al. 2003):

$$I_{K_{pump}} = g_{\text{bar}} \left[ (1 + 1/K_{pump})^3 - 1.62(1 + 6.7/(Na_{i,n} + 8)^3) \right] + 1.0/(1 + 67.6/(Na_{i,n} + 8)^3)$$

$$I_{Na_{pump}} = - \frac{3}{2} I_{K_{pump}}.$$

$$I_{pump} = I_{K_{pump}} + I_{Na_{pump}}.$$

Stimulation Protocols

To study activity-dependent changes of action potential conduction, an action potential was initiated in the distal-most compartment of the branch axon using current injection (5 ms, 0.1 nA). As in studies by Weidner et al. (1999), Campero et al. (2004), and Obreja et al. (2010), we used two different protocols to assess the amount of ADS: a high-frequency protocol (360 pulses at 2 Hz, followed by 60 pulses at 0.25 Hz) and a low-frequency protocol (20 pulses at 0.125 Hz, 20 pulses at 0.25 Hz, 30 pulses at 0.5 Hz, and 20 pulses at 0.25 Hz). Using these paradigms, we measured activity-dependent changes of the propagation latency (see Fig. 3). To examine recovery cycles, axons were stimulated at constant frequency until the conduction latency had stabilized, at which point additional electrical stimuli were interposed at varying intersinus intervals (ISI), similar to Weidner et al. (2000) and Bostock et al. (2003).

Extracellular Single-Fiber Recordings in Anesthetized Pigs

Extracellular recordings from the saphenous nerve were performed according to the experimental protocol described previously (Obreja et al. 2010). Experimental procedures were approved by the Ministry Department Baden-Württemberg in Karlsruhe (Aktenzeichen 35-9185.81/I/G-145/08) and by the central animal research unit at the University of Heidelberg. Action potentials were evoked by constant-current electrical stimulation (20 mA, 0.5 ms; DS7A, Digitimer, Hertfordshire, UK) applied at 0.25 Hz via intradermal uninsulated microneurography electrodes to the skin (FHC, Bowdoin, ME). The distance between stimulation needles and the recording electrode was used to determine conduction velocity (CV). When determined immediately after a 2-min pause, CV values for all fibers in this study were ≤5 m/s. Extracellular signals were amplified (model 5113 low-noise voltage preamplifier; Ametek, Oak Ridge, TN), filtered (bandwidth 100–3,000 Hz; model 3364 benchtop filters; Krohn-Hite, Brockton, MA), audio monitored, and digitized at a sampling rate of 32 kHz using DAPSYS 7.0, a joint hardware and software system designed for real-time acquisition, window discrimination, and latency measurements of action potentials (Obreja et al. 2010).

During ongoing intradermal electrical stimulation (0.25 Hz), mechanical stimuli (150 mN; Semmes-Weinstein calibrated monofilaments) were applied to the skin to localize the peripheral receptive field of the unit under study. At mechano-sensitive spots, action potentials induced by mechanical stimulation led to “marking” of the electrically evoked action potential, i.e., an increase in latency of the electrically evoked action potential (Schmelz et al. 1995; Trebjeruk and Hallin 1974). Fibers displaying marking on stimulation with 150 mN but not activated by stimulation with a paintbrush were classified as mechano-sensitive nociceptors (CM; Obreja et al. 2010). Receptive fields of mechano-insensitive nociceptors (CMI) were determined using collision techniques (Meyer and Campbell 1988).

Estimates of action potential width. Extracellularly recorded action potentials in slowly conducting nerve fibers typically have a triphasic shape (2 positive peaks and 1 large negative peak). Time-locked latencies and spike shape were used to discriminate action potentials. To determine action potential width, at least 20 time-locked, overlapp-
ping traces were averaged. Action potential width was calculated as the time between the 1st and 3rd (i.e., positive) peak.

Criteria Used to Set Model Parameters

Activity-dependent changes of conduction velocity have been measured in human control subjects (Namer et al. 2009; Weidner et al. 2000) as well as in patients with neuropathic pain (Kanai et al. 2006; Kleggetveit et al. 2012; Orstavik et al. 2003; Schmidt et al. 2012; Serra et al. 2011) with similar results in pig (Obreja et al. 2010). With the use of the available experimental data, the model was adjusted to meet the following six criteria: 1) the model axon should show a similar magnitude of conduction velocity slowing as human C-fibers, for both the low-frequency protocol and the high-frequency protocol (see below); 2) the model axon should hyperpolarize with activity in the high-frequency protocol (Moalem-Taylor et al. 2007); 3) the unstimulated (control) conduction velocity should lie between 0.6 and 1.3 m/s (Obreja et al. 2010); 4) the supernormal phase (SNP) magnitude should increase with increasing ADS (Bostock et al. 2003; Weidner et al. 2002); 5) the fiber should show an SNP when slowing has been induced (Weidner et al. 2002); and 6) preconditioning pulses should not further increase SNP (Bostock et al. 2003).

Several parameters used in the model, notably ion channel densities, have not currently been determined. For other parameters (e.g., ion channel half-activation voltage, \( V_{1/2} \)), values observed experimentally need to be adjusted to account for differences in ion concentration, temperature, etc. In the process of constructing a model that fulfills the functional criteria, replicating, for example, experimental characteristics of ADS and recovery cycles, a large number of simulations were performed. After each simulation, changes were made to parameter values based on several aspects of model output (output on which performance according to functional criteria can be assessed) or by the use of an optimizer algorithm (Tigerholm and Fransén 2011). In the development of the model, we initially set the ion channel conductances according to experimental data from DRG somata (Blair and Bean 2002, 2003; Gold et al. 1996; Hamada et al. 2003; Kouranova et al. 2008; Passmore et al. 2003; Winkelman et al. 2005). These and a set of other ion channel parameters were subsequently changed in order for the model to adequately replicate ADS and recovery cycles. We constructed the model in the following steps: basic propagation of action potentials, basic ADS pattern, basic RC pattern, improved action potential propagation and wave form, improved ADS pattern, improved RC pattern, improved action potential propagation. Criteria regarding basic electrophysiological properties are described below.

Basic Electrophysiological Properties

Resting membrane potential. The membrane potential (\( V_m \)) of human C-fibers in situ is not known and might vary among various fibers. We used \( V_m = -55 \) mV in this study, a value consistent with experimental data from rat DRG somata (−63 to −41 mV; Fang et al. 2005) and that has been recently confirmed by recordings from isolated neurites from rat DRG neurons suggesting a membrane potential of −60.2 mV (Vasylyev and Waxman 2012).

Passive membrane properties. The combination of passive membrane properties of ion channels and pumps described above results in a branch membrane time constant of \( \sim 1.5 \) ms and a length constant of \( \sim 3 \) mm at the resting membrane potential.

Action potential waveform. Details regarding the shape of the axonal action potential and the relative influence of the underlying currents in C-fiber axons are not known. However, the major sodium current during an action potential in DRG somata is TTX resistant (Blair and Bean 2002), most probably driven by the activation of Na\(_A\)/1.8 channels (Akopian et al. 1996; Renganathan et al. 2001). This observation was used as a criterion when the model was being tuned. Note that the TTX-sensitive Na\(_A\)/1.7 current also contributes to action potential generation and conduction velocity. A further criterion on the short time scale was that the action potential width should be less than 5 ms.

Results from our experimental investigations from pig nociceptive C-fibers showed that the extracellularly recorded action potential in C-type nociceptors was \( \sim 3 \) ms wide: CM, \( 3.0 \pm 0.22 \) (\( n = 26 \)); CM, \( 2.94 \pm 0.2 \) (\( n = 17 \)). The intracellular AP width in the model was \( \sim 3.5 \) ms measured at \(-40 \) mV; see also Figs. 2 and 4.

Refractory period. Preliminary experimental data from \( n = 4 \) pig polymodal nociceptors show that for \( 3 \) of \( 4 \) nociceptors, twin pulses applied with suprathreshold current pulses can generate two propagating action potentials at an ISI of \( 5 \) ms (at \( \sim 2 \) times current threshold) and \( 4 \) ms (at \( 15 \) times threshold), respectively. In the model, twin action potentials could be generated with ISI = \( 5 \) ms for suprathreshold (>1.4 times threshold) pulses and with ISI = \( 4 \) ms for very suprathreshold (>14 times threshold) pulses, which further supports the validity of our model.

RESULTS

In this work, we have generated a mathematical model of a peripheral C-fiber axon (Fig. 1) to investigate the mechanisms involved in activity-dependent changes of action potential propagation. The rationale for a computational approach was difficulties associated with experimental intra-axonal recordings that have thus far precluded direct determination of the intracellular membrane potential. The model replicates basic properties of the action potential (Fig. 2; see also MATERIALS AND METHODS, Basic Electrophysiological Properties, as well as the Appendix (see Fig. A1, top and middle) for expanded plots of the action potential). In particular, \( N_{A,1.8} \) and \( K_p \) are the dominating currents of the action potential (Fig. 2A). These currents differentially affect intracellular sodium and periaxonal potassium concentrations during the action potential in the branch vs. the parent axon (Fig. 2B).

Activity-Dependent Slowing

Simulations of latency changes during action potential propagation along the simulated C-fiber are shown in Fig. 3. The intracellular action potential amplitude decreases 10–15% (Fig. 3A); it becomes 10–15% wider and develops a 2- to 5-mV late depolarizing afterpotential during its propagation along the axon. For additional information about the action potential shape and its afterpotential, see also Figs. 2A, 6A, and the Appendix (Figs. A1, top and middle, and A3, top and middle). During repetitive stimulation the modeled axon conducts subsequent action potentials progressively slower, with the magnitude of the slowing of conduction latency being more pronounced at higher rates of stimulation (Fig. 3, B–D). During repetitive stimulation at 2 Hz for 180 s, the propagation latency increased from 212 ms (corresponding to a CV in the parent axon of 0.69 m/s) to 289 ms, corresponding to a 36% increase in propagation latency (Fig. 3, C and D, top). This is largely commensurate with experimentally obtained values for ADS in human and porcine C-fibers (i.e., 36.7 ± 2.2 and 30.1 ± 1.6%, respectively; Obreja et al. 2010). We also conducted a simulation with the low-frequency protocol (Fig. 3, C and D, bottom). The resulting ADS magnitude after a stimulation at 0.125 Hz (20 pulses), followed by 0.25 Hz (20 pulses) and 0.5 Hz (30 pulses), was \( \sim 8\% \), which also corresponds well to the experimental results (Obreja et al. 2010). The slowing of axonal conduction speed is also associated with changes in
action potential shape with the development of a prominent depolarizing afterpotential at the end of a 180-s period of 2 Hz stimulation (Figs. 3B and 4 and Appendix, Fig. A1, top and middle). The afterpotential is produced as an interplay between currents with slower kinetics (e.g., KM and h), currents that change when the sodium concentration changes (Na-K-ATPase, KNa), and currents that change over successive pulses due to accumulated inactivation or concentration change (see Appendix and Fig. A3, top, for further discussion).

In Fig. 4, changes in ionic currents and membrane potential during ADS are depicted. For information about the net membrane current during an action potential, see Appendix and Fig. A2. Compared with the first pulse (Fig. 4A), the membrane potential of the 360th pulse (Fig. 4C) is hyperpolarized and the overshoot of the action potential decreases. The ionic basis for these changes is shown in Fig. 4, C and D, with peak NaV1.8 current being much reduced. The magnitude of the NaV1.7 current also decreases, albeit to a lesser extent than that of the NaV1.8 current. Changes in currents before initiation of the AP are listed in Table 2. As shown in Table 2, the main difference is the increase in KNa current as a major factor contributing to membrane hyperpolarization. See also the Appendix and Fig. A1, top and middle, for additional information about the hyperpolarization.

**Mechanism of ADS: Accumulation of Intracellular Sodium**

The simulations indicate that the concentration of intracellular sodium may play a central role in ADS and thereby that changes in sodium reversal potential may be involved in the observed slowing. As shown in Fig. 5A, clamping the reversal potential for sodium (E_{Na}) to its initial value abrogates ADS and the response latency stays constant during 2-Hz stimulation. Note that in the model we are able to dissociate the reversal potential, which here was held fixed, from ion flux, which was unaltered and could affect other concentration-dependent processes such as KNa and the pump. Demonstrable changes in the concentration of intra-axonal sodium were observed during the ADS protocol (Fig. 5B, green line), presumably because the diameter (and thus volume) of the axon is small, and this results in a substantial reduction in E_{Na} (Fig. 5C). In contrast, periaxonal potassium remained relatively unaffected (Fig. 5B, black line). To examine the contribution of individual sodium conductances to the increase in intra-axonal sodium, the E_{Na} was clamped separately for the individual Na subtypes 1.7 and 1.8. The results are illustrated in Fig. 5D and suggest that the increase in intra-axonal sodium is largely attributable to the NaV1.8 current. With the reversal potential for NaV1.8 held constant, the amount of slowing is reduced by
80%, whereas clamping $E_{Na}$ for NaV1.7 results in an ~40% reduction in ADS.

Previous work has suggested that the entry of NaV channels into slow inactivated states can account for many of the features of ADS (Blair and Bean 2003; De Col et al. 2008; Snape et al. 2010). We examined this by adding a slow inactivation state to the NaV1.8 channel while clamping sodium and potassium concentrations (Fig. 5E, red and green traces). Importantly, activity slows axonal conduction under these conditions (Fig. 5E); however, slow inactivation alone...
can only generate about 25% of the slowing attributable to intra-axonal sodium accumulation (compare with blue traces in Fig. 5E). Note that the ADS amplitude attributable to Nav entry into slow inactivated states was more prominent at the cooler temperatures used experimentally in vitro. The rapid rise and early plateau of the slowing suggests that entry into slow inactivated states occurs rapidly in this model (Fig. 5E), in particular at body temperatures.

Direct intracellular recordings from DRG somata have shown that the minimum current amplitude needed to trigger an action potential increases during repetitive activation at 2 Hz (Snape et al. 2010). In the simulated C-fiber axon presented here, the minimum current required to trigger an action potential was found to increase linearly with the number of preceding action potentials (Fig. 5F, bottom). Moreover, we studied the relationship between slowing and the action potential threshold (Fig. 5F, top). The findings suggest that ADS is indicative of changed axonal excitability, with a reduction in axonal conduction velocity paralleling an increase in activation threshold (Fig. 5F, top).

Recovery Cycles

The recovery cycle refers to changes in either latency or threshold seen in the period up to about 300 ms following a single action potential in a peripheral axon. Simulations were performed using stimulus pulses at variable ISI values and at different base frequencies. Changes in membrane potential at the stimulation site (top) and at the end of the parent axon (bottom) are shown in Fig. 6A.

In Fig. 6B, characteristic changes in action potential conduction latency are shown for the model axon over the 250-ms period immediately following the passage of the first action potential. The basic form of the recovery cycle for the model axon is similar to that observed in recordings from peripheral unmyelinated axons in humans (Bostock et al. 2003; Weidner et al. 2002). An initial phase of reduced conduction velocity (i.e., subnormality) is followed by a period of supernormality (at ISI between 30 and 100 ms, consistent with the experimental range of 20–250 ms), and this subsequently reverses to a second late period of subnormality (Fig. 6B). The timing of these three phases varies according to axonal subtype, and for unmyelinated axons the extent and even the incidence of the supernormal phase is dependent on the base rate of stimulation (Bostock et al. 2003). For human C-fiber axons in situ, the magnitude of supernormality typically increases in proportion to the magnitude of ADS (Bostock et al. 2003, see their Fig. 6). Similarly, for the simulated axon an increase in the magnitude of the supernormal phase was observed with increasing repetition frequency (Fig. 6B).

Fig. 5. ADS is induced by accumulation of intracellular sodium. A: relative latency during the high-frequency protocol (2 Hz), control (blue) and with clamped reversal potentials (black). B: intracellular (intra-axonal) sodium concentration (green) and periaxonal potassium concentration (black). C: reversal potential of sodium. D: latency relative to the initial latency when the reversal potential was held constant, shown separately for NaV1.7 (green) and NaV1.8 (red) vs. control (blue). E: ADS resulting from NaV1.8 slow inactivation. Control condition (when the ADS developed from changes in Na concentration; blue) vs. ADS when sodium and potassium concentrations were held fixed and a slow inactivation transition was added to NaV1.8 (20°C, green; 37°C, red). F: relationship between minimum current injection and degree of latency change (top) and pulse number (bottom). The minimum current injection needed to trigger an action potential was measured over repetitive stimulations. Increased minimum current indicates decreased fiber excitability. Current injection was positioned on the midpoint of the branch segment.
Control Experiments

To validate the model, the behavior of the model was examined during a range of manipulations designed to replicate in vitro C-fiber experiments by using pharmacological agents and changes in ion concentrations and temperature. The results are shown in Fig. 7. We also performed a parameter sensitivity analysis, described in the Appendix (Fig. A3). Since most phases of the spike and post-spike periods show contributions from several currents, the model does not show indications of sensitivity. One exception is during the phase of the action potential where NaV1.8 and Kdr together amount to almost all variation, but this is really what is to be expected.

h-channel block. Since repetitive spiking-activity hyperpolarized the membrane potential, we assumed that hyperpolarization-activated channels (Ih), known to modulate the membrane potential, might change the amount or shape of ADS. We found that h-channel block resulted in an increased ADS (Fig. 7A), consistent with experimental data (Takigawa et al. 1998). In addition, full block of h-current decreased the initial latency and resulted in a steep rise in slowing for the first ~50 pulses, followed by a much slower, essentially linear increase in latency (see Fig. 7A, top left, magenta curve).

NaV1.7 and NaV1.8 channel block. Figure 7A also depicts ADS changes following blockade of the TTX-sensitive NaV1.7 and the TTX-resistant NaV1.8 channel. Blockade of NaV1.7 channels only slightly increased absolute latency and ADS, which is consistent with the results of De Col et al. (2008). NaV1.8 block, for which there is currently no experimental data for comparison, also increased latency, but it decreased ADS.

Changing extracellular ion concentrations. Reduction of the periaxonal (i.e., extracellular) sodium concentration (Fig. 7B) increased absolute latencies and increased ADS, consistent with De Col et al. (2008).

Changing temperature. A temperature reduction of 10°C, from 37 to 27°C, increased both the absolute conduction latency and the magnitude of ADS (Fig. 7C), in good agreement with De Col et al. (2008).

DISCUSSION

The model presented in this article offers a framework to examine activity-dependent changes in axonal excitability and conduction velocity in C-fibers. The model allows various contributing influences, such as membrane potential and ion concentrations, to be specifically and selectively dissociated from one another, a feature that is experimentally not possible. Moreover, the model provides a means of performing in silico pharmacology, which further allows for approaches that cannot be performed in real experiments such as changing reversal potentials for only a subset of ion channels. Arguments for the validity of the model include
the replication of a variety of independent experimental findings from single fiber recordings in human, rat, and pig. The key result is the identification of a specific contribution of intra-axonal sodium accumulation to activity-dependent changes in the conduction velocity of C-fibers. In addition, the model provides insight into the relationship between axonal excitability and ADS, with the degree of slowing being indicative of magnitude of stimulation current needed to evoke an action potential.

**Accumulation of Intracellular Sodium Can Contribute to ADS**

There have been several hypotheses regarding the mechanistic basis of ADS. For some time, axonal hyperpolarization driven by increased Na-K-ATPase activity accompanying action potential activity in unmyelinated axons (Rang and Ritchie 1960) was thought to result in the slowing of conduction velocity. Although changes in membrane potential most certainly contribute to activity-dependent changes in axonal conduction velocity, pharmacological blockade of the Na-K-ATPase increased the magnitude of ADS (De Col et al. 2008), casting doubt on a causal link between pump activity and ADS. A contribution of sodium channel entry into slow inactivated states was proposed to contribute to ADS (Blair and Bean 2003; De Col et al. 2008) and was confirmed using current threshold measurements to examine excitability in DRG somata (Snape et al. 2010). Our simulations support the contribution of NaV channel inactivation to ADS (Fig. 5E) but indicate that additional mechanisms are needed to fully explain the quantitative nature of ADS.

The results of the presently simulated C-fiber axon contend that an accumulation of intracellular sodium, which would reduce the current passing through sodium channels, prominently affects axonal conduction velocity. Recordings from DRG somata are less affected by action potential-mediated changes in intracellular ion concentrations, and experimental approaches to examine sodium concentration in axons have not yet been realized but have been suggested by Endres et al. (1986). Decreased extracellular sodium reduces the sodium driving force and results in increased latency (Donnelly et al. 1998). Experimentally, indirect evidence for accumulation of intracellular sodium as a mechanism for increased ADS is provided by increased slowing following block of the Na-K-ATPase activity by low extracellular potassium or by replacing extracellular sodium with lithium, which is not extruded by the Na-K-ATPase (De Col et al. 2008). Indeed, reducing extracellular sodium resulted in an increase in ADS in single afferent axons in the rat (De Col et al. 2008), consistent with
the observed increase in ADS accompanying lowered extracellular sodium in our model.

The physiological role of ADS remains speculative. Raymond (1979) showed that the electrical activation threshold of myelinated frog axons increases in proportion to ADS, results recently confirmed in porcine cutaneous C-fibers, where a strong correlation between activity-induced increased electrical thresholds, propagation failure, and amount of ADS was documented (Obreja et al. 2011b). This has recently been corroborated with the use of dynamic mechanical stimuli in single afferent axons in the rat cranial dura, which showed that mechanical activation threshold increased monotonically with axonal conduction latency (De Col et al. 2012). An increase in intracellular sodium concentration leading to a measurable slowing of axonal conduction might be regarded as a form of accommodation, i.e., a negative feedback mechanism that prevents excessive discharge, in particular for small C-fiber neurons and their terminals exposed to restricted metabolic conditions.

**Implications of Conduction Velocity, Recovery Cycles, and ADS for Pain**

Standard experimental assessment of neuronal hyperexcitability mainly relies on excitation thresholds rather than on suprathreshold discharge. However, under clinical conditions, spontaneous activity and discharge frequencies upon suprathreshold activation are crucial. In this respect, reduced ADS in nociceptors in human pain patients (Ørstavik 2003, 2006; Serra et al. 2012), and in particular the correlation between reduced ADS and spontaneous activity (Kleggetveit et al. 2012), suggests that reduced ADS can contribute to chronic pain states. Because mechanistic studies in humans are limited, important contributions have been provided by animal models of neuropathic pain. Reduced ADS and lower conduction failure in the nociceptive primary afferents nociceptors, in combination with increased expression of Na\textsubscript{v}1.7 and Na\textsubscript{v}1.8 in the corresponding DRG, were found in a rodent model of diabetic neuropathy (Sun et al. 2012). Our model suggests that ADS is mainly determined by sodium influx via Na\textsubscript{v}1.8. The model also shows that Na\textsubscript{v} 1.7 in fact opposes ADS, and thus that decreased ADS would be associated with relatively larger increases in Na\textsubscript{v}1.7 compared with Na\textsubscript{v}1.8. This is consistent with the increased protein levels found in the DRG (Sun et al. 2012). Furthermore, the model also predicts that additional changes are required to explain the decreased ADS in the hyperalgesic animals, such as increased sodium potassium pump activity or depolarization of the peripheral axons. Thus specific contributions of channel subtypes in combination with membrane potential and pump activity to neuronal hyperexcitability can be identified. This is of particular value to predict the effects of specific blockers and thus increase the probability for advances in drug development from a better understanding of mechanism of action. Future studies will link the model more closely to experimental data from pathophysiological states such as NGF-induced reduction of ADS (Obreja et al. 2011b). Ultimately, spontaneous activity (Kleggetveit 2012) and increased discharge frequencies of nociceptors (Schmidt 2012; Serra 2012) as the key source of clinical pain in the peripheral nervous system have to be modeled.

**APPENDIX A**

**Equations for Ionic Currents**

**Na\textsubscript{v}1.7 current.**

\[ I_{\text{Nav}1.7} = g_{\text{Nav}1.7} \cdot m^3 h s u \cdot (V_m - E_{\text{Na}}) \]

\[ m = m + \left[ 1 - \exp\left( - \frac{dt}{\tau_m} \right) \right] \left( m_{\text{inf}} - m \right) \]

\[ \alpha_m = 15.5 / \{1 + \exp[(V_m - 5) / (-12.08)]\} \]

\[ \beta_m = 35.2 / \{1 + \exp[(V_m + 72.7) / 16.7]\} \]

\[ \tau_m = 1 / (\alpha_m + \beta_m) \cdot Q_{10_{\text{Na}}} \]

\[ m_{\text{inf}} = \alpha_m / (\alpha_m + \beta_m) \]

\[ h = h + \left[ 1 - \exp\left( - \frac{dt}{\tau_h} \right) \right] \cdot (h_{\text{inf}} - h) \]

\[ \alpha_h = 0.38685 / \{1 + \exp[(V_m + 122.35) / 15.29]\} \]

\[ \beta_h = -0.00283 + 2.00283 / \{1 + \exp[(V_m + 5.5266) / (-12.70195)]\} \]

\[ \tau_h = 1 / (\alpha_h + \beta_h) \cdot Q_{10_{\text{Na}}} \]

\[ h_{\text{inf}} = \alpha_h / (\alpha_h + \beta_h) \]

\[ s = s + \left[ 1 - \exp\left( - \frac{dt}{\tau_s} \right) \right] \cdot (s_{\text{inf}} - s) \]

\[ \alpha_s = 0.00003 + 0.00092 / \{1 + \exp[(V_m + 93.9) / 16.6]\} \]

\[ \beta_s = 132.05 - 132.05 / \{1 + \exp[(V_m + 384.9) / 28.5]\} \]

\[ \tau_s = 1 / (\alpha_s + \beta_s) \cdot Q_{10_{\text{Na}}} \]

\[ s_{\text{inf}} = \alpha_s / (\alpha_s + \beta_s) \]

**Na\textsubscript{v}1.8 current.**

\[ I_{\text{Nav}1.8} = g_{\text{Nav}1.8} \cdot m^3 h s u \cdot (V_m - E_{\text{Na}}) \]

\[ m = m + \left[ 1 - \exp\left( - \frac{dt}{\tau_m} \right) \right] \left( m_{\text{inf}} - m \right) \]

\[ \alpha_m = 2.85 - 2.839 / \{1 + \exp[(V_m - 1.159) / 13.95]\} \]

\[ \beta_m = 7.6205 / \{1 + \exp[(V_m + 46.463) / 8.8289]\} \]

\[ \tau_m = 1 / (\alpha_m + \beta_m) \cdot Q_{10_{\text{Na}}} \]

\[ m_{\text{inf}} = \alpha_m / (\alpha_m + \beta_m) \]

\[ h = h + \left[ 1 - \exp\left( - \frac{dt}{\tau_h} \right) \right] \cdot (h_{\text{inf}} - h) \]

\[ \tau_h = 1.218 + 42.043 \cdot \exp\left[ - \left[ \frac{(V_m + 38.1)^2}{2 \cdot 15.19^2} \right] \right] \cdot Q_{10_{\text{Na}}} \]

\[ h_{\text{inf}} = 1 / \{1 + \exp[(V_m + 32.2) / 4]\} \]

\[ ds / dt = (s_{\text{inf}} - s) / \tau_s \]

\[ s_{\text{inf}} = 1 / \{1 + \exp[(V_m + 45) / 8]\} \]

\[ \tau_s = 1 / (\alpha_s + \beta_s) \cdot Q_{10_{\text{Na}}} \]

\[ \alpha_s = 0.001 \cdot 5.4203 / \{1 + \exp[(V_m + 79.816) / 16.269]\} \]

\[ \beta_s = 0.001 \cdot 5.0757 / \{1 + \exp[-(V_m + 15.968) / 11.542]\} \]

\[ du / dt = (u_{\text{inf}} - u) / \tau_u \]

\[ u_{\text{inf}} = 1 / \{1 + \exp[(V_m + 51) / 8]\} \]

\[ \tau_u = 1 / (\alpha_u + \beta_u) \cdot Q_{10_{\text{Na}}} \]

\[ \alpha_u = 0.0002 \cdot 2.0434 / \{1 + \exp[(V_m + 67.499) / 19.51]\} \]

\[ \beta_u = 0.0002 \cdot 1.9952 / \{1 + \exp[-(V_m + 30.963) / 14.792]\} \]
**NaV1.9 current.**

\[
I_{\text{Nav}1.9} = g_{\text{Nav}1.9} \cdot m_h s \cdot (V_m - E_{Na})
\]

\[
\alpha_m = \frac{1.032}{1 - \exp\left(\frac{V_m + 6.99}{14.87115}\right)}
\]

\[
\beta_m = \frac{5.79}{1 + \exp\left(\frac{V_m + 130.4}{22.9}\right)}
\]

\[
\alpha_h = \frac{0.06435}{1 + \exp\left((V_m + 73.26415)/3.71928\right)}
\]

\[
\beta_h = \frac{0.13496}{1 + \exp\left((V_m + 10.27853)/(29.09334)\right)}
\]

\[
\alpha_s = 0.0000016 \cdot \exp\left(-\frac{V_m}{12}\right)
\]

\[
\beta_s = 0.0005/\left(1 + \exp\left[-\frac{(V_m + 32)}{23}\right]\right)
\]

\[
m_{\text{inf}} = \frac{\alpha_m}{\alpha_m + \beta_m}
\]

\[
\tau_m = \frac{1}{(\alpha_m + \beta_m) \cdot \text{Q10}_\text{Na}}
\]

\[
h_{\text{inf}} = \frac{\alpha_h}{\alpha_h + \beta_h}
\]

\[
\tau_h = \frac{1}{(\alpha_h + \beta_h) \cdot \text{Q10}_\text{Na}}
\]

\[
s_{\text{inf}} = \frac{\alpha_s}{\alpha_s + \beta_s}
\]

\[
\tau_s = \frac{1}{(\alpha_s + \beta_s) \cdot \text{Q10}_\text{Na}}
\]

**K_{dr} current.**

\[
I_{K_{dr}} = g_{K_{dr}} \cdot n^4 \cdot (V_m - E_{K})
\]

\[
dn/dt = (n_{\text{inf}} - n)/\tau_n
\]

\[
n_{\text{inf}} = 1/\left(1 + \exp\left[-\frac{(V_m + 45)}{15.4}\right]\right)
\]

\[
\tau_n = 1.000 \cdot (0.000688 + 1/\left(\exp\left((V_m + 75.2)/6.5\right) + \exp\left((V_m - 131.5)/-34.8\right)\right)) \cdot \text{Q10}_\text{K}, \text{ if } V_m \leq 31 \text{ mV}
\]

\[
\tau_n = 0.16 + 0.8 \cdot \exp\left[-0.0267 \cdot (V_m + 11)\right] \cdot \text{Q10}_\text{K}, \text{ if } V_m > -31 \text{ mV}
\]

**K_M current.**

\[
I_{K_M} = g_{K_M} \cdot (n/4 + 3n/4) \cdot (V_m - E_{K})
\]

\[
dn_s/dt = (n_{\text{inf}} - n)/\tau_{ns}
\]

\[
dn_i/dt = (n_{\text{inf}} - n)/\tau_{nf}
\]

\[
n_{\text{inf}} = 1/\left(1 + \exp\left[-\frac{(V_m + 30)}{6}\right]\right)
\]

if \(V_m < -60\) \(\{\tau_{ns} = 219 \cdot \text{Q10}_K\}, \text{ else}\)

\[
\{\tau_{ns} = 13 \cdot V_m + 1,000 \cdot \text{Q10}_K\}
\]

**Fig. A1.** Action potential for 1st and 360th pulse. Action potential shapes are shown for the 1st (left) and 360th pulse (right) in the parent axon. Intracellular \(V_m\) is plotted on a long (400 ms; top), medium (10 ms; middle), and short time scale (0.6 ms; bottom).
$\tau_{\text{inf}} = 1/(\alpha + \beta)$, with $\alpha = 0.00395 \cdot \exp\left(\left(V_m + 30\right)/40\right)$
and $\beta = 0.00395 \cdot \exp\left(-\left(V_m + 30\right)/20\right)$, Q10K

**$K_A$ current.**

$I_{K_A} = g_{K_A} \cdot n_h \cdot (V_m - E_K)$

$dn/dt = (n_{inf} - n)/\tau_n$

$dh/dt = (h_{inf} - h)/\tau_h$

$n_{inf} = (1/[1 + \exp(-(V_m + 5.4 + 15)/16.4)])^4$

$\tau_n = 0.25 + 10.04 \cdot \exp\left(-\left(V_m + 24.67\right)^2\right)/(2 \cdot 34.8^2)$, Q10K

$h_{inf} = 1/[1 + \exp((V_m + 49.9 + 15)/4.6)]$

$\tau_h = 20 + 50 \cdot \exp\left(-\left(V_m + 40\right)^2\right)/(2 \cdot 40^2)$, Q10K

if $\tau_h < 5$ then $\tau_h = 5$

**$h$ current.**

$I_{h,Na} = 0.5 \cdot g_h \cdot (0.5n_s + 0.5n_f) \cdot (V_m + E_{Na})$

$I_{h,K} = 0.5 \cdot g_h \cdot (0.5n_s + 0.5n_f) \cdot (V_m + E_K)$

$n_s = 1/[1 + \exp((V_m + 87.2)/9.7)]$

$n_f = 1/[1 + \exp((V_m + 87.2)/9.7)]$

$dn_s/dt = (n_{inf,s} - n_s)/\tau_{n,s}$

$dn_f/dt = (n_{inf,f} - n_f)/\tau_{n,f}$

$\tau_{n,s} = 300 + 542 \cdot \exp((V_m + 25)/20)$, Q10H if $V_m > -70$

$\tau_{n,f} = 2,500 + 100 \cdot \exp((V_m + 240)/50)$, Q10H

if $V_m < -70$

$\tau_{n,f} = 140 + 50 \cdot \exp((V_m + 25)/-20)$, Q10H if $V_m > -70$

$\tau_{n,f} = 250 + 12 \cdot \exp((V_m + 240)/50)$, Q10H if $V_m < -70$

**Sensitivity Analysis**

This model replicates basic properties of the action potential. For expanded plots of the action potential, see Fig. A1, *top* and *middle*. For information about the net membrane current (sum of all transmembrane currents) during an action potential, see Fig. A2.

The relative influences of a range of currents during action potential phases are shown in Fig. A3 as first-order sensitivity indexes (Homma and Saltelli 1996; Sobol 1990), the contribution to the variation from a current. See Petersson 2012 for an in-depth discussion. As shown in Fig. A3, different currents contribute differently to the action potential (short time scale), afterpotential (intermediate time scale), and ADS (long time scale) phenomena. Since most phases show contributions from several currents, the model does not show indications of sensitivity. One exception is during a phase of the action potential where $Na_{Nav1.8}$ and $K_{dr}$ together account for almost all variation, but this is really what is to be expected.

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![Fig. A2](http://jn.physiology.org/)

**Fig. A2.** Net membrane current ($I_{m}$; sum of all transmembrane currents) for 1st and 360th pulse. Net action potential current is shown for the 1st (left) and 360th pulse (right) in the parent axon. Transmembrane current is plotted on a long (400 ms; top) and medium time scale (10 ms; bottom).
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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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ACTIVITY-DEPENDENT CONDUCTION IN C-NOCICEPTORS


