A detailed, conductance-based computer model of intrinsic sensory neurons of the gastrointestinal tract

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Chambers JD, Bornstein JC, Gwynne RM, Koussoulas K, Thomas EA. A detailed, conductance-based computer model of intrinsic sensory neurons of the gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol 307: G517–G532, 2014. First published July 10, 2014; doi:10.1152/ajpgi.00228.2013.—Intrinsic sensory neurons (ISNs) of the enteric nervous system respond to stimuli such as muscle tension, muscle length, distortion of the mucosa, and the chemical content in the lumen. ISNs form recurrent networks that probably drive many intestinal motor patterns and reflexes. ISNs express a large number of voltage- and calcium-gated ion channels, some of which are modified by inflammation or repeated physiological stimuli, but how interactions between different ionic currents in ISNs produce both normal and pathological behaviors in the intestine remains unclear. We constructed a model of ISNs including voltage-gated sodium and potassium channels, N-type calcium channels, big conductance calcium-dependent potassium (BK) channels, calcium-dependent nonspecific cation channels (NSCa), intermediate conductance calcium-dependent potassium (IK) channels, hyperpolarization-activated cation (Ih) channels, and internal calcium dynamics. The model was based on data from the literature and our electrophysiological studies. The model reproduced responses to short or long depolarizing current pulses and responses to long hyperpolarizing current pulses. Sensitivity analysis showed that Ih, IK, NSCa, and BK have the largest influence on the number of action potentials observed during prolonged depolarizations. The model also predicts that changes to the voltage of activation for Ih have a large influence on excitability, but changes to the time constant of activation for Ih have a minor effect. Our model identifies how interactions between different ionic currents influence the excitability of ISNs and highlights an important role for Ih in enteric neuromicroscopy resulting from disease.

INTRINSIC SENSORY NEURONS (ISNs) of the enteric nervous system (ENS) respond to stimuli such as muscle contraction and stretch, distortion of the mucosa, and chemical changes in the lumen. Functionally, they are primary afferent neurons, morphologically they are Dogiel type II neurons, and electrophysiologically they are afterhyperpolarizing neurons (14). These neurons respond to the contents of the lumen and transmit this information to other neurons to produce the desired response, such as a particular motor pattern or reflex, secretion/absorption across the mucosa, or a change in blood flow (6, 14). Furthermore, the excitability of these neurons is markedly increased after prolonged electrical stimulation (39), during maintained stretch (18), and during or after a range of inflammatory insults (31, 32, 40–42). With such a crucial role in enteric function, it is not surprising that ISNs express a much larger range of ionic currents than other types of neurons in the enteric nervous system (12, 14). Ultimately, it is the interaction between these different ionic currents and their modulation that determines both the normal and the pathological behavior of the gut.

ISNs express a large number of both voltage- and calcium-gated ion channels. Sodium channels expressed in ISNs include Na1.3, Na1.7, and Na1.9 (46, 47). Na1.3 and Na1.7 conduct the tetrodotoxin-sensitive Na+ current of the action potential (AP), whereas Na1.9 conducts the TTX-insensitive Na+ current. The falling phase of the AP is carried by three outward currents: a nonactivating, Hodgkin-Huxley type delayed rectifier (Kdr); an inactivating A-type potassium (KA) current; and a large voltage- and calcium-dependent potassium (BK) current (15). During APs in ISNs there is also an inward calcium current (20, 39), which appears to be mediated by N-type channels (45). There may be a small contribution from R-type channels (45), but not from P/Q-type channels (45) or L-type channels (28). The calcium current can carry APs in its own right and contributes to the characteristic humps, or widening, of ISN APs.

There appear to be two calcium-dependent potassium conductances in the ISNs, which carry hyperpolarizing currents that produce an early afterhyperpolarization (AHP) and a slow AHP after each AP (14, 15). The early AHP lasts 50–100 ms and is continuous with the falling phase of the AP so it includes contributions from KA, Kdr, and BK currents. The current generating the slow AHP has a rapid onset, within a few milliseconds (52), and a duration of 2–30 s (20, 21). It is probably mediated by intermediate potassium (IK) channels since agents that block these channels suppress the late AHP (38).

There is a transient depolarization that occurs between the early and late AHPs. It is probably due to a calcium-activated, depolarizing, nonspecific cation (NSCa) current with a reversal potential of ~38 mV (11, 53). Furthermore, ~80% of ISNs have a hyperpolarizing-activated cation (Ih) current mediated by a hyperpolarization-activated, cyclic nucleotide-gated channel (16, 45).

These currents, and possibly others, govern the function of ISNs and determine their response to luminal stimuli. Furthermore, they can be modulated by neurotransmitters, hormones, and inflammatory mediators. Owing to the role of ISNs in coordinating intestinal behaviors, changes to ionic current have been implicated in a range of pathological states. For example, Ih has been shown to increase in inflammation (32). However, the complex nonlinear interactions between membrane conduc-
sodium current reversal potential. This analysis allowed us to predict which conductances, and which biophysical parameters of those conductances, have the largest influence on ISN function.

MATERIALS AND METHODS

A Detailed Model of the ISN

Using the NEURON simulation environment, we created a conductance-based model of ISNs in the gastrointestinal tract with different voltage- and Ca\(^{2+}\)-dependent currents. The model has a single cylindrical compartment to represent the soma with a diameter of 25 \(\mu\)m and a length of 49 \(\mu\)m (13). The membrane capacitance was 1 \(\mu\)F/cm and the leak current had a reversal potential of \(-62\) mV.

The general approach to model the properties of different ionic currents is based on a Hodgkin-Huxley-type formalism (22), where the voltage and time dependence of currents flowing through ion channels is governed by gating variables that determine the opening and closing of the channel pore. Each gating variable is governed by the differential equation

\[ \frac{dx}{dt} = \frac{x_s(V) - x}{\tau_x(V)} \]

where \(x_s\) denotes the steady-state gating equilibrium, \(\tau_x\) is the time constant of approach to the steady state, and \(x\) denotes the fraction of open gates. \(x_s\) and \(\tau_x\) are functions of voltage and in some cases internal calcium concentration.

Voltage-activated sodium currents. The predominant sodium channels expressed in sensory neurons are Na\(_{v}\).1.3, Na\(_{v}\).1.7, and Na\(_{v}\).1.9 (46, 47).

Na\(_{v}\).1.3 sodium current (\(I_{Na1.3}\)) was modeled by using the following kinetic scheme (37):

\[ I_{Na1.3} = gNa_{v}.1.3 \cdot m^3 \cdot h \cdot s \cdot (V - Ena) \]  
\[ m_a = \frac{0.4 \cdot (v + 31)}{1 - \exp\left(\frac{-(v + 31)}{4.5}\right)} \]  
\[ m_b = \frac{0.124 \cdot (-30 - v)}{1 - \exp\left(\frac{(v + 31)}{4.5}\right)} \]

where \(gNa_{v}.1.3\) is the maximum conductance, \(m\) is the activation gate, \(h\) is the fast-inactivation gate, \(s\) is the slow-inactivation gate, \(v\) is the membrane voltage, and \(Ena\) is the sodium current reversal potential.

Fast inactivation of this current is described mathematically as

\[ h_a = \frac{0.03 \cdot (-45 - v)}{1 - \exp\left(\frac{v + 45}{1.5}\right)} \]
\[ h_b = \frac{0.01 \cdot (-45 - v)}{1 - \exp\left(\frac{v + 45}{1.5}\right)} \]

\[ h_s = \frac{q_{t_{nav1.3}}}{h_a + h_b} \]
\[ h_m = \frac{1}{1 + \exp\left(\frac{v + 50}{4}\right)} \]

Slow inactivation is described by the following equations:

\[ s_a = \exp\left(\frac{1156.8 \cdot (v + 60)}{8.315 \cdot (273.16 + \text{celcius})}\right) \]
\[ s_b = \exp\left(\frac{1156.8 \cdot 0.2 \cdot (v + 60)}{8.315 \cdot (273.16 + \text{celcius})}\right) \]
\[ s_s = \frac{s_b}{3e - 4 \cdot (1 + s_a)} \]

Note that \(s_s\) has a minimum value of 10.

\[ s_y = \frac{1}{1 + \exp\left(\frac{v + 58}{2}\right)} \]
\[ s_x = s_y + ipc \cdot (1 - s_y) \]

where \(ipc\) is a constant that determines the maximum percentage of channel that can enter the slow inactivated state.

Na\(_{v}\).1.7 sodium current (\(I_{Na1.7}\)) was modeled as follows (49):

\[ I_{Na1.7} = gNa_{v},1.7 \cdot m^3 \cdot h \cdot s \cdot (v - Ena) \]

where \(gNa_{v},1.7\) is the maximum conductance, \(m\) is the activation variable, \(h\) is the fast-inactivation variable, and \(s\) is the slow-inactivation variable. These variables can be expressed as ordinary differential equations in the alpha and beta representation as

\[ m_a = \frac{15.5 \cdot \exp\left(\frac{v - 5}{-12.08}\right)}{1 + \exp\left(\frac{v - 5}{-12.08}\right)} \]
\[ m_b = \frac{35.2 \cdot \exp\left(\frac{v + 72.7}{16.7}\right)}{1 + \exp\left(\frac{v + 72.7}{16.7}\right)} \]

where \(q_{t_{nav1.3}}\) is the temperature sensitivity for \(I_{Na1.3}\) and is defined as

\[ q_{t_{nav1.3}} = 2^{\frac{celsius - 24}{10}} \]
\[ m_s = \frac{1}{m_a + m_b} \]  
\[ m_e = \frac{m_a}{m_a + m_b} \]  
Fast inactivation is described by
\[ h_a = \frac{0.38685}{1 + \exp\left(\frac{v + 122.35}{15.29}\right)} \]  
\[ h_b = \frac{2.00283}{1 + \exp\left(\frac{v + 5.5266}{-12.70195}\right)} - 0.00283 \]  
\[ h_i = \frac{1}{h_a + h_b} \]  
\[ h_e = \frac{h_a}{h_a + h_b} \]  
Slow inactivation is described by
\[ s_a = 0.00003 + \frac{0.00092}{1 + \exp\left(\frac{v + 93.9}{16.6}\right)} \]  
\[ s_b = 132.05 - \frac{0.00092}{1 + \exp\left(\frac{v + 93.9}{16.6}\right)} \]  
\[ s_i = \frac{1}{s_a + s_b} \]  
\[ s_e = \frac{s_a}{s_a + s_b} \]  
The \( N_a.19 \) channels produce a persistent sodium current, so we modeled this persistent current as follows (25, 51):
\[ I_{Na.19} = gNa.19 \cdot m \cdot (v - ena) \]  
where \( gNa.19 \) is the current conductance, \( m \) is the activation gate, \( v \) is the membrane voltage, and \( ena \) is the sodium ion reversal potential.
\[ m_a = \frac{1}{1 + \exp\left(-\frac{v - 30}{8}\right)} \]  
\[ m_e = \frac{1}{1 + \exp\left(-\frac{v - 32}{4.15}\right)} \]  
\[ m_r = 0.02 + 0.5 \cdot \exp\left(-\frac{v - 32}{4.15}\right) \]  
when \( v < -40 \) mV, otherwise
\[ m_t = 0.02 + 0.5 \cdot \exp\left(-\frac{v - 32}{4.15}\right) \]  
Voltage-gated potassium conductances. Our model has two voltage-gated potassium conductances, \( k_a \) and \( k_d \). The A-type potassium conductance was modeled as follows (17):
\[ I_{K_a} = gK_a \cdot m^3 \cdot h \cdot (v - ek) \]  
where \( gK_a \) is the maximum conductance, \( m \) is the activation variable, \( h \) is the inactivation variable, \( v \) is the membrane voltage, and \( ek \) is the potassium ion reversal potential.
\[ h_a = \frac{1}{1 + \exp\left(\frac{v + 80}{6}\right)} \]  
For the A-type potassium conductance, both \( m_a \) and \( h_e \) are constants. The values used were \( m_a = 0.5 \) and \( h_e = 15 \).
The delayed rectifier was modeled as follows (17):
\[ I_{K_d} = gK_d \cdot m^2 \cdot (v - ek) \]  
where \( gK_d \) is the maximum conductance, \( m \) is the activation variable, \( v \) is the membrane voltage, and \( ek \) is the potassium ion reversal potential.

Calcium conductance and internal calcium dynamics. During an AP there is inward calcium current (20, 39). It appears to be mediated by N-type channels (45) and there may be a small contribution from R-type channels (45), but not from P/Q-type (45) or L-type channels (28). Since N-type and R-type channels have similar kinetics (4), we have only modeled N-type calcium channels in this work.
The model for the N-type calcium conductance is as follows (2):
\[ I_{Ca_n} = gCa_n \cdot m^2 \cdot h \cdot (v - eca) \]  
where \( gCa_n \) is the maximum conductance, \( m \) is the activation variable, \( h \) is the inactivation variable, \( v \) is the membrane voltage, and \( eca \) is the calcium ion reversal potential. The calcium ion reversal potential is calculated at each time step by NEURON using the Nernst equation with the assumption the external concentration is constant at 2 mM and the internal calcium concentration varies as described below. Activation of the N-type calcium conductance is described by these rate functions:
\[ m_a = \frac{0.1 \cdot (v + 35)}{1 + \exp\left(-\frac{v - 35}{5}\right)} \]  
\[ m_b = 0.5 \cdot \exp\left(-\frac{v + 25}{2.5}\right) \]  
\[ m_e = \frac{m_a}{m_a + m_b} \]  
\[ m_r = \frac{1}{m_a + m_b} \]  
Calcium channel inactivation is described by the following rate functions:
\[ h_a = 0.01 \cdot \exp\left(-\frac{v + 50}{15}\right) \]  
\[ h_b = \frac{0.2}{1 + \exp\left(-\frac{v + 40}{17}\right)} \]  

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where $g_{BK}$ is the maximum conductance, $m$ is the activation gate for the voltage component, $v$ is the membrane voltage, and $e_k$ is the potassium current reversal potential.

$$m_n = \frac{0.28 \cdot [Ca^{2+}]}{[Ca^{2+}] + 0.48e^{-3} \cdot \exp(tadj + v)}$$

$$m_{\beta} = \frac{0.48}{1 + \exp(tadj + v) - 1 \cdot [Ca^{2+}]},$$

where $[Ca^{2+}]$ is the intracellular concentration of calcium and $tadj$ is the temperature sensitivity for the voltage-dependent and calcium-dependent components of the $BK$ channels, which is defined as

$$\text{tadj} = \frac{-0.96 \cdot F}{8.31/(273.15 + \text{celcius})}$$

where $F$ is Faraday’s constant.

IK channels are thought to mediate the slow AHP (38). The current underlying the slow AHP activates within a few milliseconds (52) and lasts 2–30 s (20, 21). We took a model of calcium-dependent potassium conductance from the central nervous system (43) that had been modified to allow variable time step (19) and adapted it to match the time course and shape for the AHP in ISNs:

$$I_{IK} = q_{kcas} \cdot g_{IK} \cdot n \cdot (v - e_k)$$

where $q_{kcas}$ is the temperature sensitivity for $IK$ rates, $g_{IK}$ is the current conductance, $n$ is the action gate, $v$ is the membrane voltage, and $e_k$ is the potassium ion reversal potential.

$$q_{kcas} = 2.3^{\text{electrons}/23}$$

$$n_{\alpha} = \frac{k_{\text{casA}}}{1 + \exp(1.5 \cdot e^{-4} - \frac{[Ca^{2+}]}{2e^{-5}})}$$

$$n_{\beta} = \text{kcasB}$$

where $k_{\text{casA}}$ and $k_{\text{casB}}$ are constants.

$$n_t = \frac{1}{n_{\alpha} + n_{\beta}}$$

$$n_n = \frac{n_{\alpha}}{n_{\alpha} + n_{\beta}}$$

**Calcium-dependent nonspecific cation conductance.** There is a transient depolarization that occurs between the early and late AHPs caused by a calcium-activated cation conductance (54). There is evidence suggesting that this current could be mediated via chloride ions (11), but this would have the same reversal potential and, therefore, make no difference in this conductance-based model. So this current was modeled in the same way similar currents have been modeled in the cortex (10), with an adjustment to the internal calcium sensitivity to produce the appropriate time course for our intracellular dynamics:

$$I_{NSCa} = g_{NSCa} \cdot m^2 \cdot (v - e_{rev})$$
\begin{equation}
  m_a = \frac{6e^{-2}}{1 + \exp\left(\frac{4e^{-4} - [\text{Ca}^{2+}]}{3e^{-5}}\right)} \quad (10.2)
\end{equation}

\begin{equation}
  m_b = 7.5e^{-4} \quad (10.3)
\end{equation}

\begin{equation}
  m_\gamma = \frac{1}{g_{Ican} m_a m_b} \quad (10.4)
\end{equation}

If \( m_\gamma \) is calculated as less than 0.1, it is set to 0.1. \( g_{Ican} \) is an adjustment for the temperature and is defined as:

\begin{equation}
  g_{Ican} = 3.0(e^{(celsius-22)/10}) \quad (10.5)
\end{equation}

\begin{equation}
  m_a = \frac{m_a}{m_a + m_b} \quad (10.6)
\end{equation}

\( I_b \) current. Approximately 80% of sensory neurons have an \( I_b \) current (14). We used a model based on \( I_b \) in ISNs of the guinea pig small intestine (45), which is modeled as follows:

\begin{equation}
  I_b = g_b m^n (v - e_{rev}) \quad (11.1)
\end{equation}

where \( g_b \) is the maximum conductance, \( m \) is the activation variable, \( v \) is the membrane voltage, and \( e_{rev} \) is the reversal potential for the combination of ions.

\begin{equation}
  m_t = 537 + \frac{qtI_b m^n 56}{\exp\left(\frac{v + 72}{11.9}\right) + \exp\left(-\frac{v + 72}{11.9}\right)} \quad (11.2)
\end{equation}

where \( qtI_b \) is the temperature dependence for \( I_b \), Rugiero and others did not perform experiments at different temperatures to calculate Q10 (the temperature coefficient), so we used the Q10 value from \( I_b \) studies in the central nervous system (33):

\begin{equation}
  qtI_b = 4.5(e^{(celsius-34)/10}) \quad (11.3)
\end{equation}

\begin{equation}
  m_e = \frac{1}{1 + \exp\left(\frac{v + iwhalf}{ihvslope}\right)} \quad (11.4)
\end{equation}

where \( iwhalf \) and \( ihvslope \) are constants.

Electrophysiological Recordings

Guinea pigs (180–380 g) of either sex were killed by stunning and having their carotid arteries and spinal cords severed. This procedure was approved by the University of Melbourne Animal Experimentation Ethics Committee. The abdominal cavity was opened, and segments of jejunum or ileum (5–10 cm in length) were removed, flushed clean, and placed in oxygenated (95% O2-5% CO2) physiological saline (composition in mM: NaCl 118, KCl 4.6, CaCl2 2.5, MgSO4 1, NaH2PO4 25, NaHCO3 1, 1 D-glucose) containing nicardipine (1.25 μM) and hyoscine (1 μM) to minimize contractions of any remaining circular muscle during intracellular recordings. The segment was opened along the mesenteric border and pinned flat in a dissecting dish lined with a silicone elastomer (Sylgard 184; Dow Corning, North Ryde, NSW, Australia). The mucosa, submucosa, and circular muscle were removed to reveal the myenteric plexus circumferentially adjacent to intact mucosa (27). The preparation was pinned into a recording bath (volume 1–2 ml), which was superfused with warmed physiological saline (35°C) at 5 ml/min and left to equilibrate for 1 h. Myenteric neurons were impaled with conventional intracellular recording techniques (5). The responses to depolarizing current pulses were recorded at resting membrane potential.

RESULTS

We constructed a detailed model of ISNs that included both voltage- and calcium-gated channels. We identified a physiologically relevant space for parameters within the model by making sure that the model reproduced published data and data we recorded in this study. Emphasis was placed on matching the AP firing patterns but also on ensuring that the other associated potentials had realistic amplitudes and durations. Once we constrained the model to this parameter space, we performed a sensitivity analysis on the total conductance for each channel to understand the influence each channel had on the excitability of ISNs. We also investigated the effect of varying the voltage of activation and time course of activation for \( I_b \) on the excitability of ISNs.

Comparing the ISN Model to Important Physiological Observations

Typical AP and after potentials in response to a brief stimulus. ISNs can be characterized by their unique electrophysiological properties such as a hump on the falling phase of the AP and a prominent AHP (14). To test whether the model could produce a realistic AP with associated after potentials we evoked an AP in the model by simulating a 2-msec current injection of 500 pA (Fig. 1). The upstroke of the AP is predominantly the result of a large inward current carried by Na\textsubscript{v1.3} (Fig. 1B). Na\textsubscript{v1.7} and Na\textsubscript{v1.9} provide only a small contribution to the upstroke (Fig. 1B). These contributions from the different sodium channels to the upstroke of the AP were constrained by the requirement that the model reproduce the AP amplitude, half-width for both single and multiple AP events.

Halfway through the upstroke of the AP, N-type calcium channels activate, which produces an inward current that raises the membrane potential above the reversal potential for sodium (Fig. 1C).

The downstroke of the AP results from a large outward current carried by the delayed rectifier (Fig. 1D). The A-type potassium channel also provides a small outward current. The calcium-activated BK channels provide a large outward current that is delayed because of its dependence on intracellular calcium concentration (Fig. 1D). The delayed rectifier and BK channels produce the early AHP (Fig. 1A), although the A-type potassium channel also contributes.

The inward calcium current increases the intracellular concentration of calcium (Fig. 1F). The calcium transient has three components: 1) an upstroke due to calcium entry, 2) a fast decay as calcium binds to a buffer, and 3) a slower decay involving saturation of the buffer, calcium-dependent calcium release, and calcium removal. The first two components are a consequence of the dynamics of the voltage-gated calcium channels and the buffering system. The internal calcium concentrations during the first two components were chosen to fit an average theoretical neuron (3). Calcium imaging studies of ISNs have reported a delay of ~300 ms before average maximum fluorescence across the whole cell is reached (18). These changes in fluorescence are unlikely to represent the changes in internal calcium due to direct entry through calcium channels located in the cell membrane but are more likely to be reporting calcium release from internal stores. The third component of the calcium transient in this model produces decays...
similar to these changes in fluorescence studies, and the calcium concentrations are within the physiological ranges reported (18).

The increase in intracellular calcium concentration then activates the $\text{NSCa}^2+$ and IK channels (Fig. 1G). $\text{NSCa}^2+$ activates quickly with the rise in intracellular calcium, so that initially it produces a small outward current while the membrane potential is less than the reversal potential of $-38 \text{ mV}$. However, during the downstroke of the AP, the $\text{NSCa}^2+$ channel produces a large inward current that decays in $\sim 500 \text{ ms}$. During its initial $50 \text{ ms}$, the $\text{NSCa}^2+$ current is larger than the IK current, thereby producing the afterdepolarizing potential (ADP) observed in ISNs (11, 54). After the first $50 \text{ ms}$, the outward IK current becomes larger than the $\text{NSCa}^2+$ current, leading to the late AHP observed in ISNs (Fig. 1E). This IK current is large and lasts seconds as it follows the decay of the intracellular calcium concentration.

$I_h$ channels are active at resting membrane potential producing a small outward current (Fig. 1H). During the upstroke of the AP, the membrane potential moves more positive than $-40$
mV [the reversal potential for \(I_h\) (45)] and the \(I_h\) channels produce an inward current (Fig. 1H). With the downstroke of the AP, this becomes an outward current once again during the early AHP. During the ADP, the \(I_h\) current drops again, but during the late ADP the \(I_h\) produces a large inward current.

Response to current pulses. Parameters of the AHP reported in the literature vary considerably and this appears to be the result of different stimulation protocols. For example, the amplitude of the AHP evoked by a single pulse is \(\sim 6\) mV (7, 20), whereas the amplitude evoked by three depolarizing pulses (at 50 Hz) is \(\sim 12\) mV (54). Therefore, to get an accurate description of the AHP parameters and how these parameters sum with the number of APs, we performed standard intracellular recordings while systematically varying the number of depolarizing pulses used to evoke the AHP (Table 1).

Our model was able to reproduce (to within one standard error of the means) the time to rise, peak amplitude, and half duration for the AHP evoked by 1, 3, and 5 depolarizing currents (Table 1). It was also noted that during the three- and five-pulse stimulus protocols that the neurons did not fire an AP on every depolarizing current pulse. On average, 2.1 APs were observed on every second depolarizing current pulse and our model replicated this (Fig. 2). Sometimes the APs were observed on every second depolarizing current pulse, and our model replicated this (Fig. 2). Sometimes the APs were observed on every second depolarizing current pulse and our model replicated this (Fig. 2). Sometimes the APs were observed on every second depolarizing current pulse and our model replicated this (Fig. 2). Sometimes the APs were observed on every second depolarizing current pulse and our model replicated this (Fig. 2). Sometimes the APs were observed on every second depolarizing current pulse and our model replicated this (Fig. 2).

However, the model was robust in being able to reproduce both the physiological example in terms of the number APs fired, the frequency of APs, and the fall in membrane potential during the later stages of the depolarizing current pulse (due to the late AHP). Our model did show a decrease in the amplitude of the APs, which is not observed in intracellular recordings. The decrease in the amplitude of the APs in our model was due to the calcium-dependent component of the BK current. This indicates that the calcium-dependent component of the BK conductance needs to be improved, which would require a detailed analysis of this current in ISNs, which is currently not available.

To reproduce number of APs, frequency of APs, and the fall in membrane potential during the later stages of the depolarizing current pulse, the model required a different parameter set from the one used to replicate responses to multiple short current pulses. Specifically, the prolonged depolarizing current pulse response required the BK conductance to be larger than the \(I_h\) and NSCa (for example, \(g_{IK} = 5e^{-4}\), \(g_h = 0.5e^{-4}\), \(g_{NSCa} = 1e^{-4}\)), the model fired more APs for a longer period of time. When the BK conductance was smaller relative to the \(I_h\) and NSCa (for example, \(g_{IK} = 4e^{-4}\), \(g_h = 0.5e^{-4}\), \(g_{NSCa} = 3e^{-4}\) or \(g_{IK} = 5e^{-5}\), \(g_h = 0.1e^{-4}\), \(g_{NSCa} = 1e^{-5}\)), the response to the multiple short depolarizing current pulses did not fire an AP on the fourth or fifth pulse and the amplitude of the late AHP was a lot larger than the physiological response. There was a parameter set that could reproduce the responses to both multiple short depolarizing current pulses and a prolonged depolarizing current pulse, but it required an unphysiologically large conductance for \(I_h\), which meant the response to a prolonged hyperpolarizing current pulse (see below) was unrealistic.

Response to prolonged hyperpolarizing current pulse. We investigated the response of the model to a series of 500 ms hyperpolarizing currents ranging from 20 to 100 pA (Fig. 4). The model was able to reproduce the characteristic sag in membrane potential during the hyperpolarizing current injection and the anode break APs for the larger current pulses. These properties and the amplitude of the hyperpolarization for a given current were largely determined by the parameters of the

Table 1. Comparing electrophysiological recordings to model output for the parameters of the afterhyperpolarization in intrinsic sensory neurons

<table>
<thead>
<tr>
<th>Number of Depolarizing Current Pulses</th>
<th>Parameter</th>
<th>Electrophysiological Data (n = 8)</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time to rise (ms)</td>
<td>0.82 ± 0.43</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Amplitude (mV)</td>
<td>7.4 ± 3.8</td>
<td>7.6</td>
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<td></td>
<td>Half-amplitude duration (s)</td>
<td>3.8 ± 1.7</td>
<td>4.4</td>
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<td>3</td>
<td>Time to rise (ms)</td>
<td>0.85 ± 0.73</td>
<td>0.77</td>
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<tr>
<td></td>
<td>Amplitude (mV)</td>
<td>11.5 ± 4.8</td>
<td>11.0</td>
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<tr>
<td></td>
<td>Half-amplitude duration (s)</td>
<td>5.2 ± 2.2</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>Time to rise (ms)</td>
<td>0.75 ± 0.45</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Amplitude (mV)</td>
<td>13.8 ± 4.1</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>Half-amplitude duration (s)</td>
<td>6.5 ± 2.2</td>
<td>6.2</td>
</tr>
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</table>
The only occasions when other channels affected these properties was when they had a large conductance active at resting membrane potential and basal internal calcium concentrations.

**Responses to voltage ramps.** Currents underlying the AHP can be analyzed by performing two sequential voltage ramps (starting at -120 mV and increasing to 0 mV) with a 2-s gap between them (45). The first voltage ramp evokes the late AHP and the second voltage ramp occurs during the AHP and, hence, the difference in current recording between the first and second voltage ramp indicates the current underlying the late AHP. We used the same protocol to test whether our model

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**Fig. 2.** Response to 1, 3, and 5 depolarizing current pulses. Depolarizing current pulses (250 pA at 50 Hz) were applied to an impaled intrinsic sensory neuron (ISN) (left, 1) or the computer model ISN (right, 2). The computer model was able to reproduce a similar AP firing pattern to the electrophysiological recordings. A: the response to a single pulse. B: the response to 3 pulses. C: the response to 5 pulses. D: the response to 5 pulses. D1 was the same neuron impaled for C1 running the same protocol again, indicating that sometimes the same cell would fire on different current pulses. This could be replicated in the model by increasing the total conductance of $I_h$ from $4e^{-4}$ (as in C2) to $5e^{-4}$ (D2).
produces realistic currents during the late AHP (Fig. 5). Parameter sets used to reproduce the physiological response to depolarizing currents (both prolonged and brief) and hyperpolarizing currents reproduced amplitude and the qualitative shape of changes in the current evoked by the voltage ramps during the first 3–4 s. However, toward the end of the voltage ramps the values in the model did not match the published data. This was caused by the IK conductance, which depends on the internal calcium concentration. To reproduce the last second of the voltage ramps, there has to be an additive effect (where the total current increases due to the second ramp activating the currents while some currents are still active from the first voltage ramp) with the IK conductance and internal calcium concentration during the last second of the second voltage ramp. This model could produce such an additive effect with the internal calcium concentration, but only when the intracellular calcium concentration was very small [on the order of 5–10 nM compared with the estimated 800 nM spike in internal calcium concentration (3)]. However, when the model was able to produce an additive effect between two voltage ramps seconds apart, it was not able to reproduce physiological responses to depolarizing currents because there were additive effects for BK and NSCa currents. Furthermore, the small additive effect with summation for the IK current in response to depolarizing current pulses could also not be reproduced.

Sensitivity Analysis of Total Conductance on Excitability of ISNs

Expression of ion channels is highly regulated in normal physiology and perturbed in disease states. To understand which conductances are most likely to be involved in pathological behavior we performed a sensitivity analysis on total conductance values using the prolonged current pulse stimulus. We used this stimulus because it is widely reported in the literature, especially in studies relating to inflammation.

Fig. 3. Response to a prolonged depolarizing current pulse. A 500-ms depolarizing current pulse was applied to an impaled ISN (left, 1) or the computer model ISN (right, 2). The computer model as able to reproduce the number of APs fired different current pulses. A: 150 pA depolarizing current. B: 250 pA depolarizing current. C: 350 pA depolarizing current.
performed a similar analysis on the activation parameters and time constant for $I_h$, because this current has been reported to be modified after gastrointestinal inflammation (23, 32).

Voltage-gated sodium and potassium channels. Changing the total conductance of the three sodium channels had only a small effect on AP firing and rate. The sodium current was largely carried by Nav1.3, but increasing $g_{\text{Nav1.3}}$ (Eq. 1.1) did not affect the number of APs nor the frequency of APs, but did increase the amplitude of the AP (Fig. 6A). Decreasing $g_{\text{Nav1.3}}$ did not alter the number of APs, but there was a reduction in the amplitude of the AP, which allowed the subsequent AP to occur after a shorter period of time. Whereas Nav1.9 had only a small contribution to the total sodium conductance, a large increase (1,000%) in $g_{\text{Nav1.9}}$ (Eq. 3.1) increased the number of APs from three to five with an increase in the duration of AP firing during the depolarizing current pulse (Fig. 6B). Decreasing $g_{\text{Nav1.9}}$ had no effect. Changes to Nav1.7 had very little effect on AP firing and frequency.

Fig. 4. Response to a prolonged hyperpolarizing current pulse. A series of hyperpolarizing current pulses (20–100 pA) was applied via patch-clamp recording from ISNs (A) or to the computer model (B). The computer model was able to reproduce a similar maximum hyperpolarization, sag during the hyperpolarizing current, and anode break APs. A is taken from Fig. 4A in Rugiero and coworkers, 2002 (45), by permission.

Fig. 5. Response to sequential voltage ramps. To quantify the current underlying the late AHP, the neuron was depolarized with 2 successive voltage ramps from −120 to 0 mV. Each voltage ramp was separated by 2 s. The first ramp (“1st”) induced calcium entry to activate the calcium late AHP. The second ramp (“2nd”) occurred while the late AHP was active, so the difference between the 2 current traces indicates the currents active during the late AHP. A: patch-clamp recordings from ISNs. A is taken from Fig. 9Aa in Rugiero and coworkers, 2002 (45), by permission. B: output from the computer model using the baseline parameters (see RESULTS and DISCUSSION). The computer model was able to reproduce similar currents during the initial stages of the voltage ramps, but not during the late stages for these parameters.
because Nav1.7 makes only a small contribution to the total sodium conductance. Nav1.7 had only a small contribution in this model, because it required a large total conductance for it to drive multiple APs by itself, which caused unrealistic increases in AP half-width and in resting membrane potential (due to an active conductance in the membrane potential ranges of -50 to -60 mV, the normal physiological range at rest).

Increasing the total conductance for Kdr (g_{Kdr} in Eq. 5.1) reduced the number of APs to 1 (Fig. 6C). This reduction in APs was due to the Kdr conductance preventing further depolarizations and also reduced the resting membrane potential before the depolarizing current pulse. Decreasing g_{Kdr} did not alter the number of APs observed but slightly decreased the frequency of the third AP.

Large changes to the total conductance of KA (g_{Ka} in Eq. 4.1) only produced minor changes in firing (Fig. 6D). An increase of 1,000% did not alter the number of APs but decreased the frequency of APs. This was a result of KA providing an outward current after the repolarization of the AP. A decrease to 10% of the control value resulted in a minor increase in the frequency of APs, but no change to the number of APs.

N-type calcium channels and calcium-gated channels. Small changes to the total conductance of N-type calcium channels (g_{CNa} in Eq. 6.1) resulted in large changes in the firing properties of ISNs (Fig. 7A). Decreasing g_{CNa} to 50% of control resulted in an increase in firing frequency and an increase in the number of APs from three to six but had little effect on the duration of AP firing (firing accommodated over a similar period). This was because the decreased N-type calcium conductance resulted in lower intracellular calcium concentration (28% reduction in peak concentration during the first AP), which in turn caused lower activation of BK and IK conductances. Increasing g_{CNa} to 200% of control value decreased the number of APs to 1. This was due to higher intracellular calcium concentration (83% increase in peak concentration during the first AP) and a large activation of BK conductance but also an interaction between the NSCa and IK conductances.

Increasing BK conductance resulted in a larger outward current after the AP, which reduced the number of APs from 3 to 2 (Fig. 7B). Decreasing the BK conductance to 10% of control did not alter the number of APs observed. The frequency of AP firing showed a decrease between first and second APs and an increase between the second and third APs. This complicated result is due to the BK conductance having a voltage-sensitive component and a calcium-sensitive component. Reducing the total conductance reduces the voltage-sensitive component during the falling phase of the AP, which increases the half-width of the AP and increases the intracellular calcium concentration. The increase in intracellular calcium concentration causes a larger BK conductance after the AP due to the calcium-sensitive component.

Changes in the total conductance of the calcium-dependent nonspecific cation conductance (NSCa) caused changes in the number of APs and frequency of APs (Fig. 7C). Decreasing g_{NSCa} (in Eq. 9.1) to 10% of control did not alter the number of APs and had only a very minor effect on the frequency of APs. Increasing g_{NSCa} to 200% of control did not alter the number of APs but increased the frequency of APs (data not...
Large increases in $g_{\text{NSCa}}$ reduced the number of APs to 1. This is because the reversal potential of $g_{\text{NSCa}}$ is $-38$ mV, so when it is strong enough ($\approx 600\%$ of control) it prevents further depolarization.

Changes to IK not only changed the number of APs but also dramatically changed the duration of AP firing during the depolarizing current pulse (Fig. 7D). These changes occurred because increasing the total conductance of IK decreased the time to rise, increased the amplitude, and increased the duration of the late AHP. Decreasing the total conductance of IK had the opposite effects on the late AHP. Changing the total conductance of IK changed the resting membrane potential by less than 2 mV, well within the normal physiological range (12, 14, 15). During the depolarizing current pulse, decreasing the total conductance of IK to 30% of control quadrupled the number of APs and increased the duration of AP firing from $\sim 70$ to 250 ms. Further decreasing the total conductance of IK to 10% of control prevented the accommodation of AP firing (Fig. 7D). Increasing the total conductance of IK to 100% of control reduced the number of APs to two. Increasing $g_{\text{IK}}$ to 1,000% of control increased the number of APs to four but had very little effect on the duration, or accommodation, of AP firing. If $g_{\text{IK}}$ was increased enough ($\approx 5,000\%$ of control), the model no longer showed accommodation of AP firing during the depolarizing current pulse.

Shifting the voltage of activation curve for $I_h$ (in half in Eq. 11.4) had a similar effect to changing the total conductance for $I_h$ (Fig. 8B). Shifting the voltage of activation by 10 mV in the positive direction increased the number of APs to 4 with an increase in the frequency. Shifting the voltage of activation in the positive direction causes $I_h$ to activate at more positive membrane potentials (for example, $-67$ mV instead of $-72$ mV), which means this current is more likely to depolarize the membrane potential toward the AP threshold. Shifting the voltage of activation by 10 mV in the negative direction did not alter the number of APs but decreased the frequency of APs. Shifting the voltage of activation had small effects (less than 5 mV) on resting membrane potential. Interestingly, these effects on resting membrane potential were smaller than those observed when the total conductance of $I_h$ was altered. For example, to increase the number of APs observed, the voltage of activation needed to shift by $+10$ mV, which produces an
changes in the total conductance of potential resulting from alterations in the voltage of activation membrane potential. Thus differences in resting membrane potential, which produces an increase in AP number \( g_{I_h} \) needed to increase 1,000%, which produces an \( \sim 10 \) mV increase in resting membrane potential. Thus differences in resting membrane potential resulting from alterations in the voltage of activation of \( I_h \) would be harder to observe than those resulting from changes in the total conductance of \( I_h \).

Changing the slope of the curve of activation \( \Delta h \) produced minor changes compared with shifting the voltage of activation (data not shown). Changing the time constant for \( I_h \) activation \( m_r \) in Eq. 11.2 did not have an effect on firing properties (Fig. 8C).

\(~5 \) mV increase in resting membrane potential, whereas to produce the same increase in AP number \( g_{I_h} \) needed to increase 1,000%, which produces an \( \sim 10 \) mV increase in resting membrane potential. Thus differences in resting membrane potential resulting from alterations in the voltage of activation of \( I_h \) would be harder to observe than those resulting from changes in the total conductance of \( I_h \).

Changing the slope of the curve of activation \( \Delta h \) in Eq. 11.4 produced minor changes compared with shifting the voltage of activation (data not shown). Changing the time constant for \( I_h \) activation \( m_r \) in Eq. 11.2 did not have an effect on firing properties (Fig. 8C).

**DISCUSSION**

We created a detailed, conductance-based model of enteric ISNs. The model includes voltage-gated sodium and potassium channels, N-type Ca\(^{2+}\) channels, BK channels, NSCa channels, IK channels, \( I_h \) channels, and internal calcium dynamics. The model reproduced experimental responses to depolarizing current pulses, prolonged depolarizing and hyperpolarizing current pulses, and voltage ramps. A sensitivity analysis for each conductance showed that \( I_h \) and IK had the largest influence on the number of APs observed during long depolarizations. The model also predicts that changes to the voltage of activation for \( I_h \) have a large influence on the number of APs but that altering the rate of activation for \( I_h \) has a minor effect. Our model identifies how interactions between different ionic currents influence the excitability of ISNs and indicates an important role for \( I_h \) in disease states.

**Reproducing Physiological Responses**

Our conductance-based model of enteric ISNs reproduced the characteristic AP with a hump on the falling phase and the associated early AHP, ADP, and late AHP. Quantitative properties of the late AHP in response to one, three, or five depolarizing current pulses could also be reproduced in the model. Our model indicated that NSCa and IK determine the time to rise of the late AHP; the amplitude of the late AHP was determined by interactions between NSCa, IK, and \( I_h \); and summation of the amplitude with multiple APs was largely determined by the properties of IK and the internal calcium dynamics. The duration of the late AHP was also largely determined by the properties of IK and internal calcium dynamics, but \( I_h \) could also modulate the duration. In response to five depolarizing current pulse stimuli, it was observed that AP usually fired on every second depolarizing current pulse, but sometimes the same cell would fire on different pulses, such as pulse 1, 2, and 4. Our model was able to reproduce both firing patterns with small changes in the magnitude of the total conductance for \( I_h \), BK, NSCa, \( K_A \), and/or IK. This suggests the natural variation observed within one neuron is determined by the availability of channels rather than the properties of the channels themselves. The availability of channels could be influenced by many factors, for example after prolonged electrical stimulation (39), during maintained stretch (18), or the presence/absence of neuromodulators.

In addition to reproducing physiological responses to short depolarizing current pulses, our model could also reproduce the physiological to a single prolonged depolarizing current pulse and a prolonged hyperpolarizing current pulse. Interestingly, the model could not produce all of these physiological responses with one parameter set. The differences between parameter sets were centered on the maximum conductances for IK, \( I_h \), and NSCa. The magnitude of the differences in the maximum conductance were similar to the changes required to alter the AP firing on different depolarizing current pulses, suggesting that these changes could be within the natural variation observed in these neurons. Although it is possible the different parameter sets indicate there is something missing within the model (such as an unidentified conductance or some other mechanism influencing channel availability), it appears that the most important features are present since the model can reproduce these physiological
observations. Indeed, it has been suggested that one model may not account for many observations because of the natural diversity in these sorts of biological systems, so that real neurons may not be capable of displaying all observations simultaneously or in one state under the same conditions (35). Instead, a range of models should be produced to give a better description of the natural diversity in the system (35).

To reproduce the published voltage-clamp analysis of currents active during the late AHP, our model required different intracellular calcium concentrations. This large difference indicates a limitation of the calcium dynamics in the model. We tried previously published models of calcium dynamics such as a simple calcium drive force to return calcium level to rest (51); a two-calcium pool system (44); and complex dynamics with buffering, diffusion, and internal accumulation (1). Using a simple calcium drive alone could not account for the summation of the AHP in ISNs. The two-calcium pool model reproduced summation of AHPs but failed to reproduce the time course of NSCa, because the first calcium pool was too rapid and the second calcium pool too slow. A search of the relevant parameter space failed to find parameters of this model that reproduced both datasets. The complex model of calcium dynamics worked well for short durations but produced unphysiologically high concentrations of internal calcium on longer time scales. The inability of previously published models of calcium dynamics to replicate the properties of ISNs highlights that ISNs are unique in this regard and in the complex afterpotentials associated with calcium entry. We believe our internal calcium dynamics (with a calcium drive to return internal calcium concentration to rest, a simple calcium-dependent calcium release, and a simple calcium buffering) to be a good combination of these published dynamics. However, our model does not, nor did any of the other published models we tried, include spatial localization of calcium ions within the soma. This may be crucial to accurately describe the internal calcium dynamics and subsequent activation of calcium-dependent currents because of the multiple locations of release and removal/reuptake/binding of internal calcium. Although our model does not account for the large differences between intracellular recordings and patch clamp recording of the AHP, this is probably due to differences in these methods. In the mouse myenteric plexus, intracellular recording and patch-clamp recordings produce different properties for the AHP in ISNs (34). When a neuron is impaled with an intracellular electrode there is a large spike in intracellular calcium (26), probably because the sharp electrode allows calcium entry into the cell where the electrode pierces the membrane. This large spike in intracellular calcium may underlie the different properties of the AHP for the different recording techniques. A large spike in intracellular calcium would reduce the effectiveness of the calcium buffering system because at least some of that calcium would be bound to the buffer. Interestingly, our model required very low levels of intracellular calcium to reproduce the patch-clamp recordings, which increases the effectiveness of the calcium buffering in the model. If changes to the internal calcium underlie the different results produced by intracellular and patch-clamp recordings, then no single model will be able to account for both sets of results without altering the initial and/or stable conditions of the model, hereby mimicking the physical changes resulting from the experimental technique.

Sensitivity Analysis for the Total Conductances of Each Channel

Diseases typically change a number of physiological variables. The value of models such as these is that they allow us to determine which of those changes cause symptoms and help identify “pressure points” in the system that could be therapeutically targeted. Of the sodium channel subunits, we found that changes in Na\textsubscript{v}1.3 conductance had the biggest influence on firing properties. Since Na\textsubscript{v}1.3 was largely carrying the upstroke of the AP, decreasing Na\textsubscript{v}1.3 could easily prevent the upstroke of the AP from occurring, and increasing the Na\textsubscript{v}1.3 total conductance would allow an AP to occur even in response to a very small stimulus. Increasing the total conductance of Na\textsubscript{v}1.9 increased the number and frequency of APs during a prolonged depolarizing current pulse. Na\textsubscript{v}1.9 current inactivates very slowly and so increases in this current could increase the excitability of ISNs by increasing AP number and frequency. Na\textsubscript{v}1.9 is upregulated by activation of neurokinin 3 receptors (9), which are involved with slow excitatory post-synaptic (EPSP) responses in ISNs (24). To investigate the role of this up regulation of the Na\textsubscript{v}1.9, we would need to include a slow EPSP in this model of ISNs.

The voltage-gated potassium currents had little effect on the excitability of our model, indicating that their main role is just to repolarize the membrane rather than control the number of APs. Although large changes in K\textsubscript{A} only influence AP frequency without modifying AP number, large increases in K\textsubscript{dr} reduced the number of APs, so this current could be a candidate for controlling the number of APs in other types of neurons. Compared with the voltage-gated sodium and potassium conductances, the N-type calcium conductance and calcium-gated currents had a larger influence on the excitability of model ISNs. Since ISNs are the only enteric neurons to express a widened AP (characteristic of calcium influx) and the late AHP, it makes sense that the calcium-dependent conductances largely determine their behavior. The only parameter to produce an increase in the duration of AP firing during a prolonged depolarizing current pulse was the total conductance of IK. Such changes in both firing and the AHP that depends on IK are seen in preparations that are under tonic circumferential stretch and these appear independent of alterations in the calcium component of the AP, although this has not been definitively tested (29). Similarly, prolonged (several minutes) low-frequency stimulation of synaptic inputs to ISNs produces long-lasting depolarizations accompanied reduced IK and increased durations of firing during prolonged depolarizing current pulses (8) without apparently changing the calcium component of the AP. In each case, there is an excellent match between the predictions of the model and the consequences of prolonged physiological input, via either sensory processes or synaptic activation, on the behavior of the ISNs. It has been reported that inflammation can increase the duration of AP firing during prolonged depolarizations (32). Although the authors reported a change in the characteristics of the late AHP, they concluded there was no change in the calcium-dependent potassium conductance (IK in our model) because there was no change in the input resistance or the
resting membrane potential, both of which are influenced by this current (45). They ascribed the altered firing that they saw to an alternation in \( I_h \). However, \( I_h \) has also been shown to influence resting membrane potential (45) and in our model changes to \( I_K \) or \( I_h \) altered resting membrane potential, but the changes observed were less than 5 mV, which is less than the experimentally observed variation in ISNs (15). Our model predicts that a change in duration of accommodation during a depolarizing current pulse will be associated with a reduced IK conductance and cannot be reproduced by changes to \( I_h \) alone.

We looked at all parameters of the \( I_h \) current because it has been implicated in the increased excitability of ISNs in inflammation of the colon (23, 32). We found that both an increase in total conductance and a shift in the steady-state voltage of activation increase the excitability of ISNs by increasing the number and frequency of APs observed in response to a depolarizing current injection. Changing the slope of voltage of activation and changing the time course of the current could only produce minor changes in the response to depolarizing current injections. Thus our model predicts that for \( I_h \) to increase the excitability of ISNs there must be a change in the maximum conductance or a shift in the voltage of activation. In our model, these increases in \( I_h \) suppress the amplitude and duration of the late AHP, which has been reported in inflammation of the colon (32), but do not increase the duration of AP firing (see above). Since there is nonlinear summation of the late AHP, if the \( I_h \) is strong enough to overcome the late AHP and allow AP firing after ~5 APs, then our model predicts that there will be no adaptation and the ISNs will continue to fire APs throughout the depolarizing current. Although the \( I_h \) cannot directly interfere with the late AHP to cause an increase in the duration of adaptation, it is possible there are more complex mechanisms to bring about this change. For example, if the \( I_h \) increases the excitability of ISNs, then more slow EPSP transmission between ISNs would be expected (30). These slow EPSPs can inhibit the IK current, meaning more APs may be required to produce a late AHP to cause adaption of firing. A model of a network of ISNs with slow EPSP transmission and an interaction between slow EPSPs and IK would be required to test such a mechanism.

In addition to the \( I_h \) and IK having a large effect on the excitability of ISN, the model has shown that the NSCa current can also affect the firing frequency and number of APs observed in response to the depolarizing current pulse. The ADP, which is caused by the NSCa current, observed in ISNs is significantly larger in developing myenteric neurons in mice (11). Although in developing mice there is evidence to suggest that the ADP is due to a chloride conductance (11), in adult guinea pigs it appears to be due to a nonspecific cation conductance (53). Since both the chloride conductance and the nonspecific cation conductance have similar reversal potentials, it makes no difference which ion carries the current in our conductance-based model. The ADP has a reversal potential of ~38 mV, which is below AP threshold, so it could increase or decrease the excitability of ISNs. Our model indicates that small increases in NSCa are likely to only increase the frequency of APs. Thus, given its time course, NSCa could be important for setting the firing properties of ISNs during the initial phases of slow EPSPs in a network of these neurons. However, our model indicates that large increases in NSCa probably decrease the excitability of ISNs by preventing multiple APs, which could be an important function of the large ADP observed in developing myenteric neurons in mice (11).

In conclusion, we have constructed a highly detailed model of ISNs incorporating most if not all the conductances that influence their behavior. This model provides insights into how the interactions between different ionic currents can influence behavior of ISNs and highlights an important role for \( I_h \), IK, NSCa, and BK in determining the excitability of ISNs. Our model has confirmed the suggestion that \( I_h \) can increase the excitability of ISNs in inflammation but also indicates that changes to IK must be involved too. Thus the model can provide key insights into the mechanisms underlying enteric neuroplasticity produced by a variety of physiological and pathophysiological stimuli.

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

AUTHOR CONTRIBUTIONS
J.D.C., J.C.B., and E.A.T. conception and design of research; J.D.C., R.M.G., and K.K. performed experiments; J.D.C., R.M.G., and K.K. analyzed data; J.D.C., J.C.B., and E.A.T. interpreted results of experiments; J.D.C. prepared figures; J.D.C. drafted manuscript; J.D.C., J.C.B., and E.A.T. revised manuscript; J.D.C., J.C.B., R.M.G., K.K., and E.A.T. approved final version of manuscript.

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