Dynamic excitation states and firing patterns are controlled by sodium channel kinetics in myenteric neurons: a simulation study

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Enteric neurons located in the gastro-intestinal tract are of particular importance to control digestive functions such as motility and secretion. In our recent publication, we showed that mouse myenteric neurons exhibit 2 types of tetrodotoxin-resistant Na\(^+\) currents: a fast inactivating Na\(^+\) current produced by Nav1.5 channels, present in nearly all myenteric neurons, and a persistent Na\(^+\) current attributed to Nav1.9 channels, restricted to the intrinsic primary afferent neurons (IPANs). By combination of experimental recording and computer simulation we found that Nav1.5 contributed to the upstroke velocity of action potentials (APs), whereas Nav1.9 opposed AP repolarization. Here, we detailed the Na\(^+\), Ca\(^{2+}\) and K\(^+\) currents used in our computational model of IPAN. We refined the prototype cell to reproduce the sustained firing pattern recorded in situ. As shown in experimental conditions we demonstrated that Nav1.9 channels critically determine the up-state life-time and thus, are essential to sustain tonic firing.

Introduction

The action potentials (APs) are elementary signals by which neurons of the enteric nervous system (ENS) communicate with each other and with the principal cells of the ENS-controlled gastro-intestinal tract.\(^{1}\) The generation of APs in enteric neurons, including sensory, interneurons, and motor neurons, involves plethora of ion channels,\(^{2}\) among which excitatory channels are of special interest as main producers of the membrane depolarization. Knowledge of normal and pathologically modified properties of these channels is required for a better understanding of the ENS and GI tract normal function in health and dysfunction in disease. To meet this request, in recent combined experimental and computer simulation studies\(^{3}\) we showed that tetrodotoxin-resistant sodium channels are key determinants of electrical responsiveness of mouse myenteric neurons. These channels conducted 2 types of Na\(^+\) currents, persistent (I\(_{\text{NaP}}\)) and early inactivating transient (I\(_{\text{NaT}}\)). The I\(_{\text{NaT}}\) flowing via the Scn5a-encoded “cardiac” Nav1.5 channels was encountered in all myenteric neurons, whereas the I\(_{\text{NaP}}\) attributed to the Scn11a-encoded Nav1.9 channels was preferentially found in Dogiel type II sensory neurons. Using current-clamp and dynamic AP voltage-clamp protocols we specified the role of Nav1.5 and Nav1.9 channels in electrophysiology of enteric neurons. The fast activating Nav1.5 was related to the upstroke velocity of AP, whereas the slowly inactivating Nav1.9 remained active during the falling phase of AP and thus opposed AP repolarization. In this “followed-up” work, performed on the earlier described biologically based model of the Dogiel type II myenteric neuron,\(^{3}\) we detail the biophysical properties of the channels conducting inward Na\(^+\) and Ca\(^{2+}\) currents in the prototype cells and relate them to the neuron’s electrophysiology. In particular, we, for the first time, show dynamic current-voltage relations (I-Vs) characteristic of voltage-gated ion channels conducting sodium currents of 3 types (fast inactivating tetrodotoxin-sensitive, NaTTX-S, and tetrodotoxin-resistant,
Nav1.5 and Nav1.9) and N-type calcium current. From these I-Vs we derive the dynamics of the depolarization up-state as a key factor determining the electrical signature of myenteric neurons, relate this dynamics to the presence and ratio of partial ion conductances, and demonstrate that Nav1.9 channels critically determine the up-state life-time and thus the overall pattern of AP firing. These findings disclose fine biophysical mechanisms of the enteric neuron excitability that are important for gaining insights into ENS functioning in health and disease.

Results

Inward currents rule dynamics of momentary I-Vs of the myenteric neuron membrane

In the in situ myenteric neurons, voltage-dependent sodium currents via Nav1.5 and Nav1.9 channels differently contributed to the waveform of single APs evoked by short stimulus as well as to multi-AP firing response to a longer depolarizing current step. The Nav1.5 current was confined to the AP upstroke with negligible effect on spike duration, whereas Nav1.9 supported the membrane depolarization during the AP down-stroke and was necessary for persistent firing during maintained stimulus. Here we detail biophysical mechanisms of such electrophysiological properties that involve the mentioned above and other inward currents present in myenteric neurons, in particular those conducted via tetrodotoxin-sensitive sodium channels (NaTTX-S) and N-type calcium channels (CaN). For that, we used our earlier described model of the Dogiel type II myenteric neuron except that we adjusted the maximal conductance of INaTTX-S to 20 mS/cm² to match the amplitude of the recorded current. Using step-wise voltage-clamp protocol we recorded current families generated at different voltages (Fig. 1A) and further derived momentary I-Vs of partial currents and total current at different time moments after the step onset (Fig. 1B). This allowed revealing dynamic properties of the channels and relating them to firing patterns observed in the in situ experiments. With post-step time, the peak activation of all partial inward currents first increased (till 0.7 ms for INav1.5, 0.8 ms for INaTTX-S, 2.2 ms for ICaN, and 25.9 ms for INav1.9) and then decreased with progressive shift to more negative voltages. The total peak current increased till 0.8 ms that is similar to parameters of INav1.5 and INaTTX-S showing their kinetic prevalence.

Such changes resulted in convergence, at different rates, of the momentary I-Vs to corresponding steady-state I-Vs (not illustrated). The momentary I-Vs became close to steady-state ones in about 2 to 4 ms for INaTTX-S and INav1.5, 50 ms for ICaN, and more than 300 ms for INav1.9. In these characteristic times, INav1.5 and INav1.9 acquired typical properties of “window” currents (although at very different rates, in about 9 and 240 ms, respectively (Fig. 1B, second and third rows)) defined by the overlap of the steady-state activation and inactivation variables.

Noteworthy, before converging to the positively sloped steady-state I-V with zero current at about rest potential, the momentary I-Vs of the total current were N-shaped and crossed the voltage axis initially at 3 points (until 3rd ms) and later at a single point (during next 2 ms) that approached to the rest potential (Fig. 1B, bottom). The N-shaped momentary I-V having an inward current-induced limb of negative slope and 3 zero-current points is well known to be indicative of the membrane property to generate a self-maintained (regenerative) depolarization that is necessary for the AP. Indeed, a regenerative AP recorded from simulated myenteric lasts approximately as long (about 3 ms) as the momentary I-V of the total current remains N-shaped with 3 zero current points (Fig. 1B, bottom).

INav1.9 determines AP firing response to prolonged depolarization

The simulated myenteric neuron with the original parameters of partial currents (see Methods) responded to a short suprathreshold depolarizing current by generation of a single AP that bore principal features of the response recorded from prototype related to contributions of Nav1.5 and Nav1.9 currents (Figs. 7A, 8, and 9 in3). However, changes in ratios but not kinetics of the partial ion conductances were required for enabling the model to generate a train of APs during application of prolonged depolarizing current similar to what was recorded in the prototype neuron and used here as a command voltage in the following dynamic-clamp simulations (Fig. 2A, see also Fig. 7C in3). A 10-fold decrease in the maximum conductivity of IKM current (to 3 mS/cm²) was sufficient for changing a single-spike to AP train response but not for reproducing the typical behavior of amplitudes of the consecutive spikes in the train (not illustrated). A target 8-AP sequence mimicking the prototype response to 500-ms 60-pA current step (extracted from a family of WT traces shown in Fig. 7C of in3) with spike amplitude reduced and adapted at a certain level (Fig. 2B) was obtained when maximum conductances of other component currents were also changed: from 20 to 47.740 mS/cm² for INaTTX-S, 4.7748 to 1.9 mS/cm² for ICaN, 1 to 0.8 mS/cm² for INav1.5, 0.47748 to 0.33424 mS/cm² for ICaN, and 250 to 25 mS/cm² for IKM. Noteworthy, whatever was the amplitude of depolarizing current step a single AP followed by a steady depolarization was the only possible type of response generated when Nav1.9 current was blocked totally (Fig. 2C) or partially (the maximum conductivity <0.2 mS/cm², not illustrated).

In both the dynamic voltage clamp and current clamp modes, the sequential spikes in the train (upper traces in Fig. 2A and B, respectively) were accompanied by changes in partial inward currents (ibid. lower traces). In the both cases, peak values of the currents decreased in magnitude to a certain, almost constant level; peak INav1.5 and INaTTX-S were associated with the upstroke while INav1.9 and ICaN climbed up during AP down-stroke of an individual AP. In the both cases, according to the rates of adaptation (inactivation), the currents were ordered in the following sequence INav1.9 > INav1.5 > ICaN > INaTTX-S from slow to fast. The peak partial currents demonstrated a characteristic spike-to-spike adaptation which reflects the entry into inactivation of the channels.
A noteworthy difference compared to the dynamic clamp case was observed in the behavior of INav1.9 current accompanying ramp depolarization of the membrane potential during inter-spike intervals. In the present case, a post-spike increase of this current was very slow during the first 30 ms, then accelerated and shortly before the next AP upstroke reached the level that was about 40% of the peak value observed during AP downstroke. This differed noticeably from what was observed in the dynamic clamp mode during approximately the same inter-spike intervals, when activation of this current started immediately after the preceding AP and, before the next upstroke, reached a much greater, 75%-level of the peak timed to the next down-stroke. This was essentially due to the different membrane potential reached during the fast afterhyperpolarization and following ramp depolarization.

INav1.9 rules life-time of the membrane depolarization up-state and thus the cell electroresponsiveness

Momentary I-Vs of the transmembrane current and their change with time on depolarization since long ago are proved to be informative descriptor of non-linear electrical properties of excitable membranes. Information of particular importance is carried by behavior of cross-points of these time-evolving I-Vs with the voltage axis as these points (of zero current) indicate the membrane characteristic electrical states and their stability (see e.g., Fig. 8.12 in6). Momentary I-Vs of the membrane capable of generating APs has 3 such points. The left-hand cross-point belonging to the I-Vs limb of positive slope reports about electrical state close to the rest potential (now often named as “down-state”) telling that it is stable, because deflection of the membrane potential in either direction from this point produces current returning the membrane potential to the original level. The middle point on the limb of negative slope reports about unstable depolarization state such that any deflection of the membrane potential produces currents increasing the deflection. This electrical instability enables the membrane to change its potential regeneratively. The right-hand cross-point also belongs to the limb of positive slope and reports about high-depolarization state (“up-state”), which, in principle, tends to be stabilized by currents eliminating the deflections. However, more or less long lasting high depolarization inactivates inward currents so that the cross-point shifts leftward and ultimately disappear. The time interval between occurrence (shortly after onset of applied depolarization voltage step) and disappearance of that cross-point can be considered as life-time of the membrane electrical upstate. Unlike truly stable down-state that can last infinitely long, the upstate has a finite life-time and, by analogy with other physical systems, can be qualified as “metastable.”

To retrieve such information we employed similar approach6 and performed a detailed quantitative analysis the momentary I-Vs of the total current in the conditions where Nav1.9 channels were either “on” or “off” (Fig. 2B and C).
From the momentary I-Vs (e.g., left plots in Fig. 3A and B, respectively), voltages corresponding to zero transmembrane current were retrieved and plotted versus time moments, when each momentary I-V was measured (right plots in Fig. 3A and B). On the latter plots, the lower and upper chains of closed circles (D and U) depict zero-current points of momentary I-Vs on their, respectively, left-hand and right-hand limbs of positive slope, that correspond to the stable downstate and metastable upstate of the membrane potential. The middle chain of open circles (M) depicts zero-current points of the same I-V but on the limb of negative slope that corresponds to unstable medium depolarization state. The latter is associated with the upstate (U) as they both occur and disappear simultaneously. Noteworthy is the significant, more than one order difference in the life-time of the high depolarization upstates in the cases of Nav1.9 channels “on” and “off” (horizontal extent of U-chains in Fig. 2A and B, right). As U-chains report, in the both cases, the dynamic upstate occurring shortly after the voltage step onset grows during 0.65 ms, then relaxes at similar rates till about 4th ms (first slowly and then faster). Thereafter the relaxation continues at noticeably different rates, slower in the “on” case and faster in the “off” case until the upstate disappears together with the associated depolarization midstate (M-chains of open circles). The lifetime so defined in the “on” case is 159 ms, and that in the “off” case is 8.65 ms. Comparing diagrams A and B highlights the biophysical mechanism determining the impact of Nav1.9 current on the observed electroresponsiveness of myenteric neurons. This current significantly prolongs the life time of the high depolarization upstate and thus promotes continuous discharge in response to applied excitatory current.

Discussion

This work is an addendum to our last article which demonstrated the presence of Nav1.5 and Nav1.9 sodium channels in myenteric neurons from intact mouse ganglia and related currents through these...
channels to the AP shape and firing modes generated in response to depolarizing stimuli. Here we focus on the provenance of the firing modes described in the Dogiel type II neurons, which were unique in expressing both Nav1.5 and Nav1.9 channels and normally generated a train of APs during application of depolarizing current, but responded to such stimulus by a single AP if Nav1.9 channels were knocked-out. Focusing on this aspect was motivated by the fact that latter neurons are likely intrinsic primary afferent neurons (IPANs), which address their firing to all other type myenteric neurons and thus influence the patterns of motility, secretion of fluid across the mucosal epithelium and local blood flow in the intestine. We particularly detail the biophysical properties of a set of channels found to conduct inward currents and specify their roles in electrical states of the neuronal membrane critical for switching between the firing modes. For that we use families of simulated momentary I-Vs of the total transmembrane current and its inward components that allow detecting types and life-times of the dynamical states, which determine firing signature of the neurons under study.

**Insight from momentary I-Vs**

Various firing patterns and their principal elements, APs, are distinguished by time course of shifts of the membrane potential from resting level to depolarization and back that are driven by respectively inward and outward currents. Insightful are rates (kinetics) and power (dynamics) of these drives. All partial conductivities (except the leakage $G_{\text{leak}}$) included in our virtual Dogiel type II myenteric neuron change in time and so does, the balance of inward and outward currents in the total current. In the context of this study, 3 issues are noteworthy. First is the temporal behavior of each zero-current point of the momentary I-V, i.e., a point at which inward and outward component currents are temporarily balanced. Second is the slope conductance in the vicinity of that zero-current point indicating whether the balance of currents at the given potential tends to be maintained (positive slope) or not (negative slope). Third is differential contribution of different partial currents of the same driving potential, they drive the membrane potential with the same power but at different rates. For instance, different types of sodium currents ($I_{\text{Na-TTX}}$, $I_{\text{Nav1.5}}$, and $I_{\text{Nav1.9}}$) have the same driving potential but drive the membrane potential at different rates that are reflected in different contributions of their momentary I-Vs to that of the total current.

**Contributions of inward components to kinetics of the total membrane current**

In the neuron under study, the 4 inward currents have different kinetics, which are reflected in their momentary I-Vs, particularly in the characteristic behavior of the peak currents. Such currents altogether, if big enough, endow the negative slope to the total current I-V making the latter N-shaped. It is the case in our simulated neuron: there is an initial

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**Figure 3.** Contribution of Nav1.9 channels critically determines life-time of dynamic depolarization up-states and related electroresponsiveness of modeled myenteric neuron. (A and B) Evolution of zero-current points of instantaneous I-Vs of the total transmembrane current representing dynamic electrical states of the cell membrane related to the responses shown in Figure 2 Band C, with Nav1.9 channels "on" and "off," respectively. For each case, the $I_{\text{tot}}$ traces were obtained and a family of 3,000 instantaneous I-Vs were measured every 0.1 ms during 300 ms voltage step as exemplified in Figure 1, bottom plots. For each k-th I-V at time instance $t_k$, the voltages corresponding to zero current $I_{\text{tot}}$ were determined on the limbs of positive and negative slope (left, closed and open circles, respectively) and plotted versus $t_k$ on the diagram (right) in semi-logarithmic scale. In the latter, the upper and lower chains of closed circles (U and D, respectively) represent dynamics of the electrical upstate and downstate, respectively, whereas the chain of open circles (M) represents that of the mid-state. The horizontal extent of U and M chains represent the finite life-time of the metastable upstate and related unstable mid-state. The D chain of infinitely long extent represents the stable down-state close to the rest potential.
interval of time, during which inward, depolarizing currents dominate over outward, hyperpolarizing ones in a certain voltage range; with time this range is gradually narrowing and vanishes. (Fig. 1B, bottom plot).

Such domination of activation kinetics of the fastest sodium current components having equal driving potentials points to the impact of their conductance ratio relative to slower components. Indeed, in the present case (Fig. 1), the maximum conductivities of Na\textsubscript{TTX-S} and Nav1.5 channels (20 and 4.8 mS/cm\textsuperscript{2}, respectively) exceed that of Nav1.9 channels (1.0 mS/cm\textsuperscript{2}) as well as that of the calcium N-type current (0.47 mS/cm\textsuperscript{2})\textsuperscript{3}. This is the likely reason why, with this ratio, the inward currents can provide the regenerative membrane depolarization shorter than needed for multiple AP firing (see below). Nevertheless, even with this ratio the partial currents clearly demonstrate kinetic relations between them and with changing membrane potential during generation of multiple APs in the dynamic clamp mode (Fig. 2A).

In response to all consecutive “command” APs pre-recorded during supra-threshold 500-ms depolarization step, the Nav1.5 and Na\textsubscript{TTX-S} currents with their fastest activation kinetics as well as the slower N-type calcium current are most pronounced during upstroke, whereas Nav1.9 is overwhelming during downstroke, like observed in response to a single AP evoked by a short stimulus (Fig. 9 in \textsuperscript{3}). Noteworthy are spike-to-spike changes in partial inward currents reporting about different kinetics of “availability” of channels. The AP commands revealed most rapid adaptation (from 55 to 70 ms) to a lower level in peaks of I\textsubscript{Na\textsubscript{TTX-S}}, I\textsubscript{CAN}, and I\textsubscript{Nav1.5}. Adaptation of I\textsubscript{Nav1.9} peak continued till the end of AP train being slower during downstroke compared to upstroke phases (Fig. 2A, lower plot). The kinetic properties of a given channels remain the same whatever is their conductivity. Therefore, the kinetic changes in the currents responses to a multi-AP command observed in the dynamic clamp mode can be (and actually was in this study) a target feature for adjusting ratios of currents some of which, particularly potassium ones, were very much variable in biological experiments.

Nav1.9 channels as a molecular switch between firing modes of myenteric neurons

Simulations in the current clamp mode highlighted the roles played in the firing patterns by partial currents among which the Nav1.9 current was “starring.” Switching Nav1.9 channels “on” or “off” enabled or disabled the multi-AP firing in response: 500-ms depolarizing stimulus (Fig. 2B and C). Closer approaching the pre-recorded multi-AP firing pattern in terms of number of spikes (firing frequency) and spike-to-spike changes of AP amplitude required adjusting ratios of simulated partial currents by changing maximum partial conductivities. These were associated with characteristic changes in the momentary I-Vs of the total membrane current, particularly in the post-onset time, during which inward currents dominated over outward ones (Fig. 3A).

Switching between firing modes can be achieved by changing contribution of different channel types. For example, inhibition of M-type potassium current switched the response to depolarizing step from single-AP to burst mode in DRG small sensory neurons\textsuperscript{11-13} and induced spontaneous ongoing firing in A-delta nociceptive afferents.\textsuperscript{14} In our model myenteric sensory neuron, we were able to get a similar effect. Decrease in the maximum conductivity of solely M-type potassium channels (an uncertain parameter in the recorded prototype cells), from 30 to 3 mS/cm\textsuperscript{2}, was sufficient for switching from single-AP to multi-AP firing mode. However, this did not allow reproducing other important features of the prototype response, such as shapes of APs and adaptation. The multi-AP response bearing main features of the prototype was obtained in other way: by substantial (10-fold) changes in the conductivity of I\textsubscript{Na\textsubscript{TTX-S}} (increase) and I\textsubscript{Kdr} (decrease) currents, accompanied with some decrease in other conductivities (I\textsubscript{CAN} and I\textsubscript{Nav1.5}; no change in others). Variation of maximum conductivity of Nav1.9 channels demonstrated their crucial role as a molecular switch between firing modes in myenteric sensory neurons. Possible effects of down-regulation, normal expression, and up-regulation of these channels can be predicted from the model responses in different ranges of Nav1.9 conductivity: from single-AP response followed by a steady depolarization at small values (<0.15 mS/cm\textsuperscript{2}, down to zero; Fig. 2C) or by sub-threshold low frequency oscillations at medium values (form about 0.15 to 0.48 mS/cm\textsuperscript{2}, not illustrated) to multi-AP firing at higher values (> 0.5 mS/cm\textsuperscript{2}; Fig. 2B).

Nav1.9 current controls firing pattern via life-time of the membrane depolarization upstate

The momentary I-Vs of the total membrane current were shown informative with regard to fine dynamic organization of electrical states of the membrane populated by ion channels of different types.\textsuperscript{15} Considering momentary I-Vs derived from current traces recorded in the step-wise voltage clamp mode allows observing the time evolution of the high depolarization state, the “upstate,” and representing this on the electrical state diagrams (Fig. 3). The diagrams plotted for the myenteric neuron channel population show that switching Nav1.9 channels “on” or “off” (Fig. 3A and B) leads to more than one order change in the upstate life-time. The long-living high-depolarization upstate (Fig. 3A, right) is a biophysical prerequisite for a long-lasting firing response that is observed in the “on” case (Fig. 2B). The upstate longevity is estimated by stability of the corresponding I-V zero-current point that means existence of intrinsic mechanisms tending to eliminate any deflection of the membrane potential from the level corresponding to this point and thus restore the disturbed balance (equality) of inward and outward currents. In the neuronal membrane such mechanism is provided by “compensating” currents which are depolarizing when the deflection is toward hyperpolarization and vice versa.\textsuperscript{6} Unlike the steady I-V with a truly stable upstate that can persist infinitely long, the I-V of the myenteric neuron in our cases is dynamic, non-steady; its upstate relaxes until vanishing, i.e., it lives a finite time and therefore can be qualified as “meta-stable.” Ultimately it “annihilates"
with living for the same time unstable zero-current point on the I-V limb of negative slope. The mechanism operates so that relaxing depolarization that is deviation toward hyperpolarization induces ‘compensatory’ depolarizing currents but they are smaller than needed to eliminate the relaxation because of inactivation of the involved channels. The relaxation kinetics is determined by the kinetic properties of the total current reflected in the momentary I-Vs, which in their turn are dictated by kinetic properties of dominating inward currents (see above). Here it is the current through Nav1.9 channels which becomes dominating and is more pronounced if Nav1.9 are more numerous (have greater maximum conductivity).

In conclusion, our computational model demonstrates that different ion channels determine the electrical states of IPANs, among which Nav1.9 acts as a molecular switch between firing modes.

**Methods**

Computational experiments were performed on a single-compartment model of the myenteric neuron with the membrane populated by ion channels conducting 8 types of currents. These were sodium currents (TTX-sensitive, INa-TTX, and TTX-resistant, INav1.5 and INav1.9), N-type calcium current (ICaN), potassium currents (delayed rectifier, IKd and M-type, IKm), and non-specific currents through hyperpolarization activated channels (Ih) and passive leak Ileak. The membrane potential was described by the equation

\[
C_m \cdot \frac{dE}{dt} = -I_{\text{leak}} - I_{\text{Na}} - I_{\text{TTX}} - I_{\text{Nav1.5}} - I_{\text{Nav1.9}} - I_{\text{CaN}} - I_{\text{Kd}} - I_{\text{KM}} - I_h - I_{\text{stim}},
\]

where \(C_m = 1 \mu F/cm^2\) is the specific membrane capacitance and \(I_{\text{stim}}\) is the stimulating current applied from external source. The Hodgkin-Huxley type equations and parameters of all ion currents but \(I_h\) were described in detail in our previous work.\(^3\) The \(I_h\) current in this model was described similarly:

\[
I_h = G_h \cdot n \cdot (E - E_h),
\]

where \(G_h = 0.04 \text{ mS/cm}^2\) is the maximum conductivity of \(h\)-channels, \(n\) is the activation variable obeying the equations:

\[
dn/dt = (n_{\infty} - n)/\tau_n; \quad \tau_n = Q_10^{593} \cdot \exp(0.4 \cdot (E + 73)/9)/[1 + \exp((E + 73)/9.0)], \quad n_\infty = 1.0/[1 + \exp((E + 78)/11.0)].
\]

The reversal potentials for the leakage, \(Na^+\), \(Ca^{2+}\), and \(K^+\) currents, and non-specific cationic h-current were, respectively, \(E_L = -75 \text{ mV}\), \(E_{Na} = +62 \text{ mV}\), \(E_{Ca} = +132 \text{ mV}\), \(E_K = -89 \text{ mV}\), and \(E_h = -28 \text{ mV}\).

In simulations aiming at investigating the impact of partial currents on the cell electroresponsiveness, the maximum conductances, but not kinetic parameters, of the currents were changed compared to earlier model\(^3\) (see Results section).

**Simulations were performed using NEURON software\(^6\)**

Standard current-clamp and voltage-clamp stimulation protocols were employed. The step-wise voltage-clamp\(^4,5\) was used to get the time-varying instantaneous current-voltage relation (I-V) for the total membrane current and its components. The I-V represented a set of current values measured at the same moment of time \(t_k\) after the voltage step onset for clamped voltages ranging from \(-80 \text{ mV}\) to \(+70 \text{ mV}\) (or to \(+140 \text{ mV}\) in case of ICaN). A family of instantaneous I-Vs for different moments \(t_k\) \((k = 1..N)\) composed the dynamic I-V, which converged to the steady-state I-V with increasing time after the onset. The dynamic I-Vs were further analyzed by analogy with the steady-state I-Vs, of which the zero-current points characterize the number and stability of steady states of the membrane potential.\(^10,15,17,18\) Such points of the steady-state I-V, if located on the limb of, respectively, negative or positive slope, indicate, respectively, the unstable or stable electrical steady-states; and the latter are further specified as ‘up-states’ or ‘down-states’ if correspond to high or low depolarization membrane potentials. Unlike the steady-state I-V, the instantaneous I-V evolves in time and so does zero-current points. To characterize this evolution quantitatively the membrane potentials corresponding to zero-current points of k-th I-V from the given family were plotted vs. time-moments \(t_k.\)\(^15\) The resulting diagram displaying existence and life-times of the membrane depolarization up-states provides a novel tool to quantify the dynamics of electrical excitability of cells.

In the dynamic voltage-clamp mode, the total and component currents were computed in response to the command voltage of complex waveforms. The latter were single or multiple action potentials experimentally pre-recorded from myenteric neurons in intact mouse ganglia\(^3\) stimulated by a depolarizing pulse or step in the current-clamp mode. Similar stimuli in the current-clamp mode were applied to the model neuron and the evoked membrane potentials with accompanying ion currents were computed for comparison with the prototype. In this mode, the maximum partial conductances were adjusted to approach the model response to that of the prototype pre-recorded in the same mode\(^3\) and used here as a voltage command in the dynamic clamp mode.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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