TITLE: Computational estimation of the distribution of L-type Ca\(^{2+}\) channels in motoneurons based on variable threshold of activation of persistent inward currents

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ABSTRACT

In the presence of neuromodulators such as serotonin and noradrenaline, motoneurons exhibit persistent inward currents (PICs) that serve to amplify synaptic inputs. A major component of these PICs is mediated by L-type Ca\textsuperscript{2+} channels. Estimates based on electrophysiological studies indicate that these channels are located on the dendrites, but immunohistochemical studies of their precise distribution have yielded different results. Our goal was to determine the distribution of these channels using computational methods. A theoretical analysis of the activation of PICs by a somatic current injection in the absence or presence of synaptic activity suggests that L-type Ca\textsuperscript{2+} channels may be segregated to discrete hot spots 25 to 200 µm long and centered 100 to 400 µm from the soma in the dendritic tree. Compartmental models based on detailed anatomical measurements of the structure of feline neck motoneurons with L-type Ca\textsuperscript{2+} channels incorporated in these regions produced plateau potentials resulting from PICs activation. Furthermore, we replicated the experimental observation that the somatic threshold at which PICs were activated was depolarized by tonic activation of inhibitory synapses and hyperpolarized by tonic activation of excitatory synapses. Models with L-type Ca\textsuperscript{2+} channels distributed uniformly were unable to replicate the change in somatic threshold of PICs activation. Therefore, we conclude that the set of L-type Ca\textsuperscript{2+} channels mediating plateau potentials is restricted to discrete regions in the dendritic tree. Furthermore, this distribution leads to the compartmentalization of the dendritic tree of motoneurons into subunits whose sequential activation lead to the graded amplification of synaptic inputs.
INTRODUCTION

Motoneurons are the direct intermediary between the central nervous system and the skeletomuscular system. As a consequence, the firing behaviour of motoneurons is a critical determinant in the control of motor actions by the nervous system. The interplay between two sets of factors regulates the firing behaviour of motoneurons: the nature (e.g. inhibitory versus excitatory) and activity of their synaptic inputs, and the intrinsic properties of motoneurons. The latter play a major role in shaping the response to synaptic inputs, and include passive membrane properties (Barrett and Crill 1974; Clements and Redman 1989; Fleshman et al. 1988; Perreault 2002; Rose and Vanner 1988; Segev et al. 1990; Ulrich et al. 1994), dendritic geometry (Bras et al. 1987; Bui et al. 2003; McDonagh et al. 2002; Rall 1959), and the type and location of voltage-gated channels (Berg et al. 2004; Campbell and Rose 1997; Carlin et al. 2000b; Lee and Heckman 2001; Lee et al. 2003; Li and Bennett 2003; Muennich and Fyffe 2004; Perrier and Tresch 2005; Powers and Binder 2003) and ligand-gated channels (Alvarez et al. 1997, 1999; Hornby et al. 2002; Perrier and Hounsgaard 2003).

In the presence of monoamines such as serotonin and noradrenaline, motoneurons exhibit persistent inward currents (PICs) that can lead to sustained depolarization (Schwindt and Crill 1980; Hornby et al. 2002; Hounsgaard et al. 1984, 1988; Lee and Heckman 1996, 1998, 2000; Svirskis and Hounsgaard 1998). These PICs are composed of a calcium current mediated by the low-threshold slowly deactivating L-type Ca^{2+} channels (Carlin et al. 2000a; Lipscombe et al. 2004; Perrier and Hounsgaard 2003) and a sodium current mediated by persistent sodium channels (Lee and Heckman 2001; Li and Bennett 2003;
Li et al. 2004). The activation of PICs by excitatory synaptic inputs, and deactivation by inhibitory synaptic inputs, represent a powerful means by which synaptic inputs can be amplified (Kuo et al. 2003; Lee and Heckman 2000; Lee et al. 2003; Hultborn et al. 2003).

To fully understand how PICs modulate the response of motoneurons to synaptic inputs requires an understanding of the spatial relationships between the channels responsible for PICs and the synaptic inputs to motoneurons. Delgado-Lezama et al. (1999) showed that by spatially restricting serotonergic activity, the synaptic amplification by PICs could be confined to inputs innervating specific regions of the dendritic tree. This clearly demonstrated that amplification of synaptic inputs by PICs could be compartmentalized in the dendritic trees of motoneurons. The extent to which motoneurons can be compartmentalized is dependent in part upon the location of the L-type Ca^{2+} channels. There is a wealth of electrophysiological data suggesting the presence of L-type calcium channels on dendrites of motoneurons (Hounsgaard and Kiehn 1993; Carlin et al. 2000b; Svirskis et al., 2001). However, immunohistochemical studies offer little consensus as to the precise location of these channels. Immunoreactivity for Ca_{v}1.3 channels, the member of the L-type Ca^{2+} channel family responsible for PICs in motoneurons (Heckman et al. 2003) has been observed at: the soma and proximal dendrites (Westenbroek et al. 1998), second and third order dendrites (Carlin et al. 2000b), large numbers of punctate patches over the entire somato-dendritic surface (Simon et al. 2003), and a small number of punctate beads at distances of up to 1300 µm from the soma (Ballou et al. 2003). Recent calcium imaging studies reinforce the evidence for dendritic
calcium channels, but do not resolve the question of their precise location (Davenport et al. 2003).

Considering the variability of the reported distribution of the L-type Ca\textsuperscript{2+} channels produced by immunohistochemical analysis, our goal was to estimate the distribution of L-type Ca\textsuperscript{2+} channels on the dendrites of motoneurons using a strategy different from immunohistochemical analysis. To estimate the location of these channels, we used a computational analysis based on experimental observations of the varying somatic thresholds for PICs activation in the absence or presence of tonic excitatory or inhibitory synaptic activity (Bennett et al. 1998). We estimated L-type Ca\textsuperscript{2+} channels were located in discrete ‘hot-spots’ that were centered on the sites that shared a common membrane potential. Simulations using models with L-type Ca\textsuperscript{2+} channels placed at those estimated locations replicated the experimental observations of Bennett et al. (1998). Conversely, simulations using models with channels distributed uniformly throughout the dendritic tree were unable to replicate the same experimental observations. The distribution of L-type Ca\textsuperscript{2+} channels in hot spots led to the compartmentalization of the dendritic tree into computational units that could be sequentially recruited with increasing synaptic activity. Portions of this work have been presented in abstract form (Rose et al. 2002, 2003).
METHODS

Compartmental modelling

The construction of the compartmental models of feline neck motoneurons based on anatomical measurements has been described in detail in Bui et al. (2003). The value of the specific resistivity of the cytoplasm ($R_i$) selected for this study, $70 \ \Omega \cdot cm$, is based upon calculations for motoneurons (Barrett and Crill 1974) and is close to the value for saline (Hille 2001). The value of the specific resistivity of the membrane ($R_m$) was $15,000 \ \Omega \cdot cm^2$.

Simulations were performed using Saber, a mixed-signal simulator software package (Synopsys Inc., Mountain View, CA) (Carnevale et al. 1990). To calculate the total synaptic current that reaches the soma, the membrane potential of the cell body was clamped to $-64 \ \text{mV}$ to simulate resting conditions and to $-55 \ \text{mV}$ to simulate suprathreshold conditions. This is analogous to the experimental technique developed by Heckman and Binder (1988) and subsequently employed by Binder and colleagues (e.g. Powers and Binder 1995, 2000; Lee and Heckman 1996, 1998, 2000) to calculate the effective synaptic current generated by tonic activation of synaptic inputs to motoneurons.

Modelling synaptic conductance changes

Current injected by synaptic activation ($i_{syn}$) is a product of the conductance generated by channel opening and the driving potential, such that:

$$i_{syn} = g(E_{rev} - V_m)$$  \hspace{1cm} (1)
where $g$ is the conductance change, $E_{\text{rev}}$ is the reversal potential and $V_m$ is the membrane potential. Under steady-state conditions, the synaptic conductance change can be time-averaged, $\bar{g}$, as described by Bernander et al. (1991). In this case, the synaptic conductance in each compartment can be calculated by the following equation:

$$\bar{g} = g_{\text{peak}} f P t_{\text{peak}} e$$

(2)

where $g_{\text{peak}}$ is the peak conductance change, $t_{\text{peak}}$ is the time-to-peak of the conductance change, $f$ is the frequency of synaptic activity and $P$ is the probability of neurotransmitter release. The parameters for excitatory synapses on motoneurons were based on the experimental findings of Finkel and Redman (1983); $E_{\text{rev}}, t_{\text{peak}}$ and $g_{\text{peak}}$ were assigned values of 0 mV, 0.2 ms and 5.0 nS, respectively. The characteristics of inhibitory synapses were based on the experimental findings of Stuart and Redman (1990); $E_{\text{rev}}, t_{\text{peak}}$ and $g_{\text{peak}}$ were assigned values of -81 mV, 0.65 ms and 9.0 nS respectively.

The distribution of excitatory synaptic inputs to neck motoneurons has been estimated to be approximately one synapse per 14.3 $\mu\text{m}^2$ of surface membrane area throughout the dendritic tree, corresponding to a uniform density of 7 synapses per 100 $\mu\text{m}^2$ (Rose and Neuber-Hess 1991). Assuming that the ratio of excitatory to inhibitory synapses is 1:1, we distributed excitatory and inhibitory synaptic inputs at a density of 3.5 synapses per 100 $\mu\text{m}^2$ respectively. This represents 15,322, 15,966 and 13,756 total synapses for model motoneurons LAD5-4, LVN2-1, LVN4-1 respectively.

**Modeling L-type Ca$^{2+}$ channels**

The conductance change of L-type Ca$^{2+}$ channels $g_{L,\text{Ca}}$ was modeled as:
Here $g_{L,Ca}$ represents the maximal conductance per surface area. The variable $m$ is a voltage- and time-dependent activation variable. In the case of the L-type calcium channel, there is very little voltage-dependent inactivation (Lipscombe et al. 2004). The activation variable is modelled by the differential equation:

$$\frac{dm}{dt} = \frac{m_{\infty} - m}{\tau_m}$$

(4)

where the time constant of activation, $\tau_m$, was assigned a value of 20 ms (Carlin et al. 2000b). The steady state activation level, $m_{\infty}$, is given by:

$$m_{\infty} = \frac{1}{1 + e^{(V_m - V_{1/2})/k}}$$

(5)

where the half-activation voltage, $V_{1/2}$, was -33 mV (in proximity to values used by Booth et al. 1997, Carlin et al. 2000b and Svirkis et al. 2001) and the activation sensitivity, $k$, was assigned a value of -6 mV (Carlin et al. 2000b). Based on these values, $m_{\infty}$ reached 0.1 at a membrane potential of -60.6 mV. $E_{rev}$ for the calcium current was set at 60 mV (Carlin et al. 2000b).
RESULTS

Bennett et al. (1998) used the feline decerebrate preparation to determine the voltage threshold at which a somatic current injection activated PICs in motoneurons during tonic activation of excitatory synapses by stretch of the Achilles tendon (excitatory state), during tonic activation of inhibitory synapses through activation of the common peroneal nerves (inhibitory state), and in the absence of induced synaptic activity (resting state). In the excitatory state, the threshold measured at the soma was hyperpolarized (average: -56.4 mV) in comparison to the resting state (average: -50.6 mV). In the inhibitory state, the threshold measured at the soma was depolarized (average: -43.0 mV) in comparison to the resting state. QX-314 was used to block all sodium currents (Lee and Heckman 1999), and thus the current underlying the observed PICs was mediated by L-type Ca\(^{2+}\) channels. In the absence of sodium currents, the activation of these L-type Ca\(^{2+}\) channels produces an all-or-none depolarization termed plateau potentials. Based on these measurements, we can estimate the location of the L-type Ca\(^{2+}\) channels underlying the plateau potentials that were activated by the somatic current injection. We will first describe the theory upon which our estimative methods are based.

**Determination of L-type Ca\(^{2+}\) channel location using measurements of membrane potential throughout the dendritic tree**

**Theory**  Figure 1a shows the membrane potential along one dendrite, from soma to terminal end, in a hypothetical resting state at various levels of current injected at the soma (black lines). Initially, the membrane potential throughout the dendrite is at resting membrane potential. The membrane potential at the soma is raised by a somatic current injection (the direction of membrane polarization is indicated by the black arrow) to the
threshold for activating PICs in the resting state (thick black line). In the presence of the somatic current injection, the membrane potential along the dendrite decays with distance away from the soma due to the cable properties of passive neurons (Rall 1977). In a hypothetical excitatory state, the membrane potential is raised throughout the dendrite by excitatory synaptic activity (top thin gray line). If the excitatory synaptic activity is sufficient to activate PICs, as observed in some instances by Bennett et al. (1998), then the soma is hyperpolarized by a current injection (the direction of membrane polarization is indicated by the gray open arrow) to inactivate PICs. Subsequently, increasingly depolarized current is injected to raise the membrane potential at the soma (the direction of membrane polarization is indicated by the black arrow) to a threshold for activating PICs (thick gray line). At this threshold, the soma membrane potential is more hyperpolarized than in the resting state, in accordance with the observations made by Bennett et al. (1998) (Fig. 1b). The membrane potential in the more distal regions of the dendrites is more depolarized in the excitatory state than in the resting state due to the activation of excitatory synapses. Conversely, in a hypothetical inhibitory state, the membrane potential at the soma is raised by a somatic current injection (the direction of membrane polarization is indicated by the black arrow) to a threshold for activating PICs (thick black dashed line) that is more depolarized than in the resting state, again in accordance with the observations made by Bennett et al. (1998) (Fig. 1c). As the current injection becomes increasingly depolarized, the membrane potential at more distal regions of the dendrite is more hyperpolarized than in the resting state due to the activation of the inhibitory synapses. The membrane potential profiles at the threshold for activating PICs in the three states (thick lines in Fig 1a, b, c), represent three different
snapshots of the same event, the activation of PICs by a somatic current injection. The common element between these three snapshots is the presence of a region in the dendrites where the membrane potential has been raised sufficiently to reach a threshold ($\theta_{\text{PIC}}$) to initiate the activation of persistent inward currents.

For the sake of brevity, we will herein refer to the membrane potential profiles at the threshold for activating PICs in the three states simply as the membrane potential profile for that respective state. By superimposing these membrane potential profiles, we can estimate the regions of the dendritic tree where the channels underlying PICs may be located. Let us consider the superimposition of the membrane potential profile of a hypothetical dendrite in the resting state and in the excitatory state (Fig. 1d). Assuming that $\theta_{\text{PIC}}$ is constant throughout the dendrite, three possibilities are illustrated: $\theta_{\text{PIC}}$ is higher than the membrane potential at the intersection point between the two profiles ($V_{\text{int}}$), $\theta_{\text{PIC}}$ is equal to $V_{\text{int}}$, and $\theta_{\text{PIC}}$ is lower than $V_{\text{int}}$. Recall that these profiles depict the membrane potential in the dendrite just subthreshold to activation of the PICs. As the amplitude of a somatic current injection increases, the membrane potential throughout the dendrite moves upwards, approaching the membrane potential depicted in the profiles. In the case where $\theta_{\text{PIC}}$ is greater than $V_{\text{int}}$, the channels can only be in the region between the two curves (marked by thick black double-arrowhead line). Indeed, if the channels were located more proximal to this region, they should have been activated by a smaller somatic current injection in the resting state and the somatic threshold in the resting state should have been lower (less depolarized). Any channels located more distal to this region should have been activated by a smaller somatic current in the excitatory state and
the somatic threshold in the excitatory state should have been lower. In the case where $\theta_{\text{PIC}}$ is equal to $V_{\text{int}}$, the channels can only be located in a discrete point, which is the intersection point of the two profiles. Again, any channels proximal to this point would have been activated by a smaller current injection in the resting state, while any channel distal to this point would have been activated by a smaller current injection in the excitatory state. The case where $\theta_{\text{PIC}}$ is smaller than $V_{\text{int}}$ cannot exist because there is no region where the channels should not have been activated by a smaller current injection in one of the two states. Thus the membrane potential profiles in the resting state and in the excitatory state demarcate the region in which the L-type Ca$^{2+}$ channels are located, and their intersection point lies near the middle of this region.

The superposition of the membrane potential profiles for the resting state and for the inhibitory state (Fig. 1e) yields complementary, but slightly different interpretations. Assuming that $\theta_{\text{PIC}}$ is constant throughout the dendrite, three possibilities are illustrated: $\theta_{\text{PIC}}$ is higher than the membrane potential at the intersection point between the two profiles ($V_{\text{int}}$), $\theta_{\text{PIC}}$ is equal to $V_{\text{int}}$, and $\theta_{\text{PIC}}$ is lower than $V_{\text{int}}$. The cases where $\theta_{\text{PIC}}$ is greater than $V_{\text{int}}$, or where $\theta_{\text{PIC}}$ is lower than $V_{\text{int}}$ cannot exist. In these respective cases, the channels proximal to the left curves should have been activated in both states by lower current injections, the channels between the two curves should have been activated by lower current injections in the state represented by the right curve, and the channels more distal than the right curve should not have been activated in the state represented by the left curve. In the case where $\theta_{\text{PIC}}$ is equal to $V_{\text{int}}$, the intersection point of the resting state and the inhibitory state provides an indication of the most proximal point of the
region where the channels could be located (marked by thick black double-arrowhead line). Channels that are located more proximally than this point should have been activated by a smaller current injection in both states. If the membrane potential profile of all three states intersect at the same point (Fig. 1f), then the only region where channels could be located is the intersection point.

**Implementation of the theory** For three compartmental models of feline neck motoneurons, we simulated three states of synaptic activity. In the feline decerebrate preparation employed by Bennett et al. (1998), background synaptic activity is present throughout the experimental procedure. Thus, in the resting state, we simulated background synaptic activity corresponding to the tonic activation of 8% of all excitatory synapses (firing at 100 Hz with a $P$ of 0.50) and 10% of all inhibitory synapses (firing at 50 Hz with a $P$ of 0.50). At -64 mV, the net current reaching the cell body produced by this synaptic activity in the absence of voltage-gated channels was negligible for all three motoneurons. In the excitatory state, we simulated synaptic activity corresponding to the tonic activation of 24% of all excitatory synapses (firing at 100 Hz with a $P$ of 0.50) and 10% of all inhibitory synapses (firing at 50 Hz with a $P$ of 0.50). The current reaching the cell body produced by this synaptic activity in the absence of voltage-gated channels was 9.4 nA (LAD5-4), 7.9 nA (LVN2-1), and 5.8 nA (LVN4-1), which is slightly higher than the amount of current produced by the coactivation of Ia afferents from lateral gastrocnemius, medial gastrocnemius and soleus nerves (Lee and Heckman 2000). In the inhibitory state, we simulated synaptic activity corresponding to the tonic activation of 4% of all excitatory synapses (firing at 100 Hz with a $P$ of 0.50) and 20% of all
inhibitory synapses (firing at 50 Hz with a $P$ of 0.50). The net current reaching the cell body produced by this synaptic activity in the absence of voltage-gated channels was -6.7 nA (LAD5-4), -5.5 nA (LVN2-1), and -4.1 nA (LVN4-1), similar to the observed currents produced by activation of the common peroneal nerve (Powers and Binder 2000).

For every dendrite, we calculated the intersection point of the membrane potential profiles in the excitatory state and the resting state (Fig. 2). For the three motoneurons, the intersection points of the membrane potential profiles in the excitatory state and the resting state were distributed between 108 to 398 µm away from the soma. The median intersection points were respectively 253.6 µm (LAD5-4), 212.9 µm (LVN2-1), and 181.2 µm (LVN4-1) away from the soma (Fig. 2b, gray boxes). In comparison, the median lengths of the dendrites, from soma to terminal, were 1085.7 µm (LAD5-4), 1147.9 µm (LVN2-1), and 1094.5 (LVN4-1) (Fig. 2b, white boxes). In terms of branching order, the intersection points were located on dendritic branches whose branching orders were between 1 and 6, typically on second- to fourth-order dendrites (Fig. 2c, gray boxes). In comparison, the highest branching order of the terminal branches of motoneurons was 11 (Fig. 2c, white boxes). The intersection points were also calculated with an $R_m$ value of 5000 Ω·cm$^2$. The median intersection points were shifted distally 30 µm at most (data not shown).

The intersection points of the membrane potential profiles in the inhibitory state and the resting state were generally 50 to 100 µm more distal to the intersection points of the
membrane potential profiles in the excitatory state and the resting state (data not shown). The median intersection points were respectively 344.1 µm (LAD5-4), 297.6 µm (LVN2-1), and 262.2 µm (LVN4-1). The location of the intersection points of the membrane potential profiles in the inhibitory state and the resting state were also at membrane potentials that were more hyperpolarized than the intersection points of the membrane potential profiles in the excitatory state and the resting state (where \( \theta_{PIC} \) is lower than \( V_{int} \)). Recall that in our analysis, this case cannot exist. This contradiction could be due to either our selection of the level of excitatory and inhibitory synaptic activity or to the distribution of the excitatory and inhibitory synapses in our definition of the excitatory state and inhibitory state, or to our assumption of a constant \( \theta_{PIC} \). In light of the conflict between these two sets of data, we chose to model the distribution of L-type Ca\(^{2+} \) channels in a region bounded by the membrane potential profiles in the resting state and the excitatory state as described in Fig. 1D for the case where \( \theta_{PIC} \) is more depolarized than \( V_{int} \).

L-type Ca\(^{2+} \) channels were incorporated into the three motoneuron models as discrete regions (hot spots). The centre of these hot spots was selected to be the intersection point of the membrane potential profiles in the excitatory and resting state for each dendrite. If the intersection points of the membrane potential profiles for two soma-to-terminal trajectories are located on the same branch segment (between two branch points), a single hot spot was inserted in that branch segment and centered at the most proximal of the intersection points. Hot spots were extended into daughter branches or into parent branches whenever these cases arose. The length of the hot spots was arbitrarily set at
100 µm long. Triangular current ramps were injected at the soma at a speed of 5 nA/s. The threshold of PICs activation was measured as the membrane potential at which the derivative of the somatic membrane potential with respect to time was 0.1 V/s on the rising slope of the highest peak (Fig. 3). While this value may be arbitrary, visual inspection of the responses to the triangular current ramps verified that it corresponded approximately to the membrane potential at which a sharp inflection led to a plateau potential.

For each motoneuron, the density of L-type Ca\(^{2+}\) channels was adjusted until the somatic thresholds of plateau initiation in the resting condition was within 1 mV of the average value reported by Bennett et al. (1998). The changes in somatic thresholds for PICs activation in different states were qualitatively consistent with the experimental results (Table 1). Between the three motoneurons, the somatic thresholds in the excitatory state were on average 5.2 ± 1.7 mV lower than the somatic thresholds in the resting state. Conversely, the somatic thresholds for PICs activation in the inhibitory state were on average 7.2 ± 2.9 mV higher than in the resting state. The peak conductance density of L-type Ca\(^{2+}\) channels for LAD5-4, LVN2-1 and LVN4-1 was 30, 50, and 30 pS/µm\(^2\), respectively.

**Sensitivity of hot spot models to different parameters**

We assessed the sensitivity of the LVN4-1 model to different parameters such as the length of the L-type Ca\(^{2+}\) hot spots, their location and their distribution in discrete regions. For each change in the value of a particular parameter, we adjusted the
conductance density in order to produce a plateau in the resting state with approximately the same (+/- 1 mV) somatic threshold as calculated by Bennett et al. (1998), and then calculated the somatic threshold in the excitatory and in the inhibitory state (Table 2).

**Shifting the location of the L-type Ca^{2+} channel hot spots** We shifted the location of the 100 µm long hot spots 100 µm proximally, 100 µm distally, and 200 µm distally from the intersection point of the membrane potential profiles in the resting state and in the excitatory state, and measured the subsequent shifts in somatic threshold (Fig. 4a). None of these shifts improved the ability of the models to replicate the shifts in somatic threshold observed by Bennett et al. (1998); indeed the models with the hot spots centered 100 µm proximally (Fig. 4a, square) or distally (Fig. 4a, inverted triangle) were worse at replicating the shifts in somatic threshold. The model with hot spots centered 200 µm away from the intersection points could not produce a plateau potential in the inhibitory state. Thus, centering the hot spots at the intersection points provided the best means of reproducing the changes in somatic threshold as observed by Bennett et al. (1998).

**Varying the size of the L-type Ca^{2+} channel hot spots** We varied the size of the L-type Ca^{2+} channel hot spots while centering them at the intersection points of the resting state and excitatory state (Fig. 4b). All the models with hot spots of size within the range of 25 to 200 µm adequately reproduced the shift in somatic threshold observed by Bennett et al. (1998). Only the expansion of the hot spots by 50 µm (Fig. 4b, triangle) slightly improved the models in terms of the shift in somatic thresholds. The other size
changes in that range slightly lessened the ability of the models to replicate the shifts in somatic threshold. Within this size range, the shift in somatic threshold from resting state to the inhibitory state was largely independent of the size of the hot spots. A model with 300 μm long hot spots could reproduce the shift in somatic threshold from resting state to the inhibitory state observed by Bennett et al. (1998) (-8.6 mV compared to -7.6 mV). However, the shift in somatic threshold from resting state to the excitatory state was nearly three times the magnitude observed by Bennett et al. (1998) (-17.5 mV compared to -5.8 mV).

We also constructed a model of LVN4-1 where the L-type Ca\(^{2+}\) channels were distributed uniformly across the soma and the dendritic tree at a constant conductance density. The conductance density was adjusted to produce a plateau potential with a threshold of -50.0 mV in the resting state (Fig. 5a). The somatic threshold in the inhibitory state and in the excitatory state was -30.9 mV and -83.9 mV, respectively. However, inspection of the somatic membrane potential in response to the triangular current ramp in the excitatory state reveals no discernible plateau potential. Indeed, the presence of many small spikes in the derivative of the somatic membrane potential with respect to time (Fig. 5b) indicates the presence of numerous partial plateaus resulting from the activation of separate groups of L-type Ca\(^{2+}\) channels at membrane potentials as low as -150 mV. Similarly, a model of LVN4-1 where the L-type Ca\(^{2+}\) channels were distributed from the intersection point of the resting state and the inhibitory state to the terminal end of the dendrites could not produce plateaus in the inhibitory state due to the presence of many
partial plateaus. In the excitatory state, the somatic threshold in the excitatory state was -107.5 mV.

In another model, the L-type Ca$^{2+}$ channels were distributed at the soma only. A slight depolarizing shift (<2.0 mV) in the somatic threshold of plateau initiation was observed in both the inhibitory state and in the excitatory state. The depolarizing nature of the shift in the somatic threshold of plateau initiation in the excitatory state is clearly in disagreement with the experimental observations of Bennett et al. (1998).

**Variation in intensity of excitatory synaptic activity** To assess the sensitivity of the somatic threshold of PICs activation to the particular level of excitatory synaptic activity that we selected to recreate an excitatory state, we reduced the level of excitatory synaptic activity in LVN4-1 by activating 16% rather than 24% of all available excitatory synapses. This decrease in excitatory activity in the excitatory state decreased the net current arriving at the cell body in the absence of any voltage-gated channels from 5.8 nA to 3.1 nA. The membrane potential along every dendrite during the resting state and during the excitatory state was measured along with the intersection points of these two membrane potential profiles. The decrease in excitatory synaptic activity shifted these intersection points 70 µm distally (median: 251 µm). The L-type channel hot spots were centered at the new intersection points and the somatic thresholds for PICs activation were reassessed (Fig. 4c, square). In the excitatory state, the shift in somatic threshold for PICs activation was increased from -3.7 mV to -6.8 mV, closer to the experimental value measured by Bennett et al. (1998). In the inhibitory state, the shift in somatic
threshold for PICs activation was increased from 9.6 mV to 10.9 mV, further from the experimental value measured by Bennett et al. (1998).

**Variation in channel properties** We tested the sensitivity of the models to changes in the half-activation voltage \(V_{1/2}\) of the L-type \(Ca^{2+}\) channels. \(V_{1/2}\) was increased or decreased by 5 mV and we assessed the shifts in somatic thresholds in models with the different hot spot sizes and locations listed above (data not shown). When \(V_{1/2}\) was set to -28 mV, models with hot spots from 25 µm to 200 µm were able to replicate the shifts in somatic thresholds reported by Bennett et al. (1998). Similar to when \(V_{1/2}\) was set to -33 mV, the model with 50 µm long hot spots could best replicate the shifts in somatic thresholds. The model with hot spots based at the intersection points of the membrane potential profile of the resting state and the excitatory state could best replicate the shifts in somatic thresholds compared to models with hot spots located elsewhere.

When \(V_{1/2}\) was set to -38 mV, only a few of the models could produce a clear all-or-none plateau potential (50 µm and 150 µm long hot spots centered at the intersection points of the membrane potential profile of the resting state and the excitatory state; And 100 µm long hot spots centered 100 µm proximal to the intersection points) in the inhibitory state. The other models exhibited the presence of many partial plateaus.

Moreover, for both values of \(V_{1/2}\), models with uniformly distributed L-type \(Ca^{2+}\) channels were unable to exhibit shifts in somatic thresholds from the resting state to the excitatory state that were within 100 mV of the value reported by Bennett et al. (1998).
Therefore, while the exact size and location of the L-type Ca\(^{2+}\) hot spots seems to depend on the activation properties of the channels, our findings still support the conclusion that the channels are distributed in spatially restricted regions.

**Input-output properties of motoneurons in the presence of L-type Ca\(^{2+}\) channel hot spots**

The firing frequency of motoneurons is predicted by the current arriving at the cell body \((I_N)\) generated by synaptic activity (Powers and Binder 2000). To quantify the effect of distributing L-type Ca\(^{2+}\) channels in 100 µm hot spots centered at the intersection points of the membrane potential profiles in the resting state and the excitatory state, we measured the relationship between synaptic activity and current arriving at the cell body with and without these channels. Excitatory synapses were tonically activated (firing at 100 Hz with a \(P\) of 0.50) and the current arriving at the cell body was measured using a somatic voltage-clamp of -64 mV (Fig. 6a). With passive dendrites, the relationship between synaptic activity and current arriving at the cell body in motoneurons is sublinear due to the loss of synaptic current by saturation of excitatory driving potential (Rose and Cushing 1999). In the presence of L-type Ca\(^{2+}\) channel hot spots, the relationship between synaptic activity and current arriving at the cell body was amplified. For two of the cells, LAD5-4 and LVN4-1, the relationship between synaptic activity and current arriving at the cell body consisted of two nearly linear phases. The initial phase was much steeper than that seen in the models without L-type Ca\(^{2+}\) channels. This phase was a direct consequence of a sequential recruitment of hot-spots (Fig. 6b). The slope of the second phase was less than that seen for the first phase. Over this range of excitatory
synaptic activity, few if any additional hot spots were activated. The input-output properties of LVN2-1 were more complex. Most of the hot spots were activated over a narrow range of excitatory synaptic activity. As a consequence, the current reaching the soma increased abruptly as the percentage of active synapses increased from 16% to 32%.

**Clamping the somatic membrane potential to -55 mV**

During the repetitive firing of action potentials, the membrane potential at the soma enters a quasi voltage-clamp state set by the time-averaged membrane potential (Koch et al. 1995). Therefore, we reassessed the current arriving at the cell body with a somatic voltage-clamp of -55 mV to mimic these conditions (Fig. 7). Under these conditions, all three cells displayed the same input-output properties. There was an initial steep phase corresponding to the activation of L-type Ca\(^{2+}\) channels, and a flatter phase representing the saturation of the activation of L-type Ca\(^{2+}\) channels. Compared to the models clamped at -64 mV, the more depolarized somatic voltage-clamp reduced the level of synaptic activity required to activate the L-type Ca\(^{2+}\) channel hot spots and activation occurred over a narrower range of synaptic activity.

**Varying the degree of inhibition**

We repeated the simulations after adding tonically active inhibitory synaptic activity. 5% or 10% of all inhibitory synapses were activated at 50 Hz with a probability of release of 0.5. The measured current reaching the cell body was smaller in the presence of inhibition (Fig. 8a). The input-output properties were approximately linear for all three
cells at both levels of inhibitory synaptic activity. At intermediate levels of excitatory synaptic activity, the synaptic inhibition reduced the number of L-type Ca\textsuperscript{2+} channel hot spots activated, thereby reducing the magnitude of the PICs. This reduction in PICs serves as a means to amplify the effective inhibition produced by inhibitory synaptic activity as observed experimentally by Hultborn et al. (2003) and Kuo et al. (2003).
DISCUSSION

Based on the different somatic thresholds at which a somatic current injection activates PICs in the presence or absence of synaptic excitation or inhibition (Bennett et al. 1998), we designed an analytical procedure to estimate the distribution of L-type Ca\(^{2+}\) channels on motoneurons. This procedure suggests that L-type Ca\(^{2+}\) channels are located in discrete patches, or hot spots, spanning 25 to 200 µm, and centered 100 to 400 µm away from the cell body. Models with channels distributed within these ranges of location and size were able to reproduce the shifts in somatic threshold of PICs activation observed by Bennett et al. (1998). Models with channels distributed further from the soma could not replicate the shifts in somatic threshold of PICs activation. Specific models replicated the amplification of excitatory and inhibitory synaptic currents described by several investigators (Kuo et al. 2003; Lee and Heckman 2000; Lee et al. 2003; Hultborn et al. 2003).

Methodological considerations

**Intersection points of membrane potential profiles** The distribution of L-type Ca\(^{2+}\) channels in hot spots was based on the changes in membrane potential along the dendrites of motoneurons when PICs are activated in the presence of different levels of synaptic activity (Bennett et al. 1998). The superimposition of the membrane potential profiles in different states was used to estimate the location of L-type Ca\(^{2+}\) channels that produced PICs in the particular experimental preparation of Bennett et al. (1998). In this analysis, we assumed that the cells were essentially passive before PICs were activated. This is likely an over-simplification given the presence of several types of Ca\(^{2+}\) channels.
and delayed rectifier K⁺ channels on motoneuron dendrites (Ballou et al. 2003; Carlin et al. 2000a; Muennich and Fyffe 2004; Powers and Binder 2003; Simon et al. 2003; Westenbroek et al. 1998) and the absence of studies of the distribution of other types of channels. As well, we assumed that the resting membrane potential was uniform throughout the dendrites. While deviations from any of these assumptions will change the shape of the membrane potential profiles, as long as the overall shape of the membrane potential along a dendrite in the resting state is essentially monotonically decreasing with increasing distance away from the cell body, and the membrane potential along a dendrite in the excitatory state is essentially monotonically increasing with increasing distance away from the cell body, then the analysis of the superimposition of the membrane potential profiles will still restrict the region in which the channels underlying the PICs observed by Bennett et al. (1998) were located.

The membrane potential profiles, and subsequently, the exact location of the intersection point of the membrane potential profiles depended on the level of excitatory and inhibitory synaptic activity that we simulated to represent the three different states of synaptic activity. Therefore, we tested the sensitivity of the models to variations in the size and location of the hot spots. Within a certain range, the models showed shifts in somatic threshold for PICs activation that were within 5 mV of the average values calculated by Bennett et al. (1998). The model with L-type Ca²⁺ channels distributed uniformly across the soma and the dendrites exhibited shifts in somatic thresholds much larger than the values reported by Bennett et al. (1998), despite having adjusted the densities of the channel to produce a plateau in the resting state. This supports the
conclusion that in the preparation studied by Bennett et al. (1998), the distribution of L-type Ca$^{2+}$ channels underlying PICs must be restricted to discrete regions located a finite distance from the cell body.

**Constant threshold of PICs activation** In our theoretical analysis, the assumption that the local threshold of PICs activation, $\theta_{\text{PIC}}$, was constant throughout the dendrite (gray dashed straight lines in Fig. 1d-f) led to the conclusion that L-type Ca$^{2+}$ channels underlying plateau potentials were distributed in discrete regions within the dendritic tree. In a physiological setting, $\theta_{\text{PIC}}$ could be variable depending on the local densities and properties of various channels as well as the local electrotonic environment. A variable $\theta_{\text{PIC}}$ (leading to gray dashed curves rather than straight lines in Fig. 1d-f) could lead to the theoretical conclusion that the L-type Ca$^{2+}$ channels are distributed in a uniform manner or in multiple hot spots within the same dendrite.

However, it is critical to note that $\theta_{\text{PIC}}$ is not necessarily constant in our models; only the channel properties are set to be constant. $\theta_{\text{PIC}}$ in our models may well be variable as a result of heterogeneities in the local electrotonic environment set by local differences in cell geometry. While we have not considered the possibility of different hot spots within the same dendrite in our models, our simulations show that a uniform distribution of L-type Ca$^{2+}$ does not reproduce the changing threshold of PICs activation with synaptic activity.
Lack of persistent sodium current and further effects of QX-314

Two currents are known to underlie PICs in motoneurons (Heckman et al. 2003): a calcium current mediated by L-type Ca\(^{2+}\) channels, and a persistent sodium current whose source has not been resolved as of yet. We did not include this current in our models since Bennett et al. (1998) used the sodium channel blocker QX314 in their assessment of the somatic threshold of PICs activation. The sodium current plays a major role in spike initiation (Lee and Heckman 2001; Li and Bennett 2003; Li et al. 2004) and contributes 1/3 of the magnitude of sustained PICs (Li and Bennett 2003). The inclusion of such a current could increase the amount of current delivered by excitatory synaptic activity. Lee and Heckman (1999) showed that the use of QX314 depolarizes the threshold of plateau initiation in low conductance cells but hyperpolarizes the threshold for high conductance cells so that on average the threshold of plateau initiation across all motoneurons. Thus, assuming that Bennett et al. (1998) sampled motoneurons of various input conductance, the shift in PICs activation threshold should not change in the presence or absence of QX314 on average. Therefore, the shift in somatic threshold of PICs activation observed by Bennett et al. (1998) upon which this study is founded should not be significantly altered by the fact that QX-314 was used in their experimental procedures.

Magnitude of Ca\(^{2+}\) current

The size of the plateau potential and the magnitude of the persistent inward currents are larger than previously reported values (Bennett et al. 1998; Lee et al. 2003). Our primary objective in this study was to replicate the shifts in somatic threshold of PICs activation observed by Bennett et al. (1998) and not the exact size
characteristics of PICs and plateau potentials. These latter characteristics could be influenced by the presence of potassium currents and the number of activated synapses. Furthermore, the compound QX-314 that was used by Bennett et al. (1998) to prevent the firing of action potentials has been shown to reduce the magnitude of the PICs (Lee and Heckman 1999). This may have reduced the size of the plateau potential recorded by Bennett et al. (1998).

**L-type Ca\(^{2+}\) Channel Properties** Our models behaved similarly in terms of ability to replicate the shifts in somatic thresholds of PICs activation reported by Bennett et al. (1998) with L-type Ca\(^{2+}\) channels whose half-activation voltage was -28 mV or -35 mV. These values are within the -30 mV to -35 mV ranges used in other models of L-type Ca\(^{2+}\) channel models in motoneurons (Carlin et al. 2000b; Svirkis et al. 2001). However, with a half-activation voltage value of -38 mV (closer to the value of -40 mV used by Booth et al. 1997), our models could not produce an all-or-none plateau potential in the inhibitory state for many of the models tested with various size of hot spots and locations. To our knowledge, the channel properties of L-type Ca\(^{2+}\) channels in motoneurons have not been characterized and may well depend on serotonin which seems to facilitate the activation of plateau potentials (Perrier and Hounsgaard 2003). Our study suggests that if the activation properties of L-type Ca\(^{2+}\) channels are more hyperpolarized than previously thought, the presence of other voltage-gated channels may be essential to the generation of plateau potentials.
Suitability of motoneuron model  Our study is based on three neck motoneurons. As discussed previously (Bui et al. 2003), these motoneurons are morphologically similar in many respects to hindlimb motoneurons studied by Bennett et al. (1998). Furthermore, the variability in the dendritic tree structure of these three motoneurons is representative of the variability seen within motoneurons innervating the same muscle (Rose 1982; Cameron et al., 1983; Cullheim et al. 1987; Kernell and Zwaagstra 1989; Moritani et al. 2003). The synaptic innervation that was modeled was constrained by known anatomical observations of the innervation of neck motoneurons (Rose and Neuber-Hess 1991). However, inhibitory inputs to motoneurons from Ia inhibitory interneurons and Renshaw cells are distributed proximally (Burke et al. 1971; Fyffe 1991). Stimulation of the common peroneal nerves, as employed by Bennett et al. (1998) to define their inhibitory state, activates these inputs. Thus, it may be more appropriate to model the inhibitory state using a proximal bias for inhibitory inputs. However, stimulation of common peroneal nerves also excites group II afferents (Levin and Chapman 1987) and the distribution of synapses activated by these afferents is not known. Thus, the best means of replicating the inhibitory state remains unclear, but further studies of the effects of activating predominantly proximal inhibitory inputs on the shift in somatic threshold of PICs activation and the input-output properties of motoneurons have merit.

Anatomical versus Functional Distribution of L-type Ca$^{2+}$ channels

It was first suggested that L-type Ca$^{2+}$ channels on motoneurons were distributed in dendritic regions in light of electrophysiological evidence that persistent inward currents were more efficiently activated by synaptic activity rather than somatic current injections.
Several anatomical studies, based on the distribution of immunoreactivity for CaV1.3, the primary alpha subunit of the L-type Ca\textsuperscript{2+} channel responsible for the Ca\textsuperscript{2+} current underlying PICs, have confirmed the dendritic location of these channels (Ballou et al. 2003; Carlin et al. 2000; Simon et al. 2003; Westenbroek et al. 1998). However, due to methodological differences (e.g. species, scope of immunohistochemical analysis), the sum of these studies does not provide a definite description as to the specific distribution of these channels.

Several studies have described motoneuron models with plateau-like behaviours. A two-compartment model of motoneurons with L-type Ca\textsuperscript{2+} channels in the dendritic compartment (Booth et al. 1997) was able to produce the bistable firing patterns and the hysteresis in the relation between firing frequency and current injected that has been observed in the presence of serotonin (Lee and Heckman 1998; Schwindt and Crill 1980). Such a model presumes that L-type Ca\textsuperscript{2+} channels are distributed throughout the dendritic tree of motoneurons. A morphologically more accurate model with uniformly distributed L-like and N-like Ca\textsuperscript{2+} conductances was also able to replicate the hysteretic I-V relation (Carlin et al. 2000b). Svirskis et al. (2001) were also able to reproduce the hysteresis in the current-voltage curves of motoneurons by using models of turtle motoneuron with L-type Ca\textsuperscript{2+} channels distributed uniformly throughout the dendritic tree, or restricted to some dendritic branches only. Taylor and Enoka (2004) were able to produce bistable firing patterns in motoneuron models with L-type Ca\textsuperscript{2+} channels segregated to the proximal portions of the dendrites.
Thus, immunohistochemical and modelling studies have essentially estimated a wide range of possible locations or distributions of L-type Ca\(^{2+}\) channels in spinal motoneurons. Variations in the species and/or age of the animals studied may well have contributed to the observed variability in distribution. However, all of the distributions described need not be mutually exclusive. Distributions of L-type Ca\(^{2+}\) channels estimated using immunohistochemical approaches describe the anatomical distribution of L-type Ca\(^{2+}\) channels. Estimations of the distribution of these channels using an approach based on the analysis of electrophysiological data estimate the distribution of the L-type Ca\(^{2+}\) channels that mediate plateau potentials through the activation of PICs. This distribution is what we would call their functional distribution. The set of channels that comprise the functional distribution of L-type Ca\(^{2+}\) channels may consist of only a subset of all L-type Ca\(^{2+}\) channels. In other words, we are proposing that the L-type Ca\(^{2+}\) channels that were available for activation in the system studied by Bennett et al. (1998) were located in the hot spots described in our study, even though there may be L-type Ca\(^{2+}\) channels in different locations as well. The channels that are present in the anatomical distribution but not in the functional distribution could be excluded from the latter due to the lack of neuromodulatory coactivation, a localized reduced membrane excitability or a density too low to engage in the self-catalytic process that results in a persistent inward current. It is entirely possible that in another set of circumstances, motoneurons may possess another functional distribution of L-type Ca\(^{2+}\) channels that may or may not contain the channels distributed in the hot spots that we estimated, leading to different electrical behaviours. From a single anatomical distribution of
channels, different functional distributions may be produced through several mechanisms:

**Mechanisms responsible for different functional distributions of L-type Ca\(^{2+}\) channels in motoneurons**

**Organization of neuromodulatory synapses.** Synapses that release neuromodulatory neurotransmitters such as serotonin, noradrenaline, acetylcholine, GABA or glutamate may be distributed in an organized fashion (Fyffe 2001; Heckman et al. 2003). In the turtle, serotonergic axons that contact motoneurons are located mainly in the dorsolateral funiculus (Kiehn et al. 1992) such that serotonin modulates the activation of channels found mostly on the lateral dendrites of motoneurons (Delgado-Lezama et al. 1999). In fact, stimulation of the dorsolateral funiculus facilitates the activation of PICs by depolarization applied to the lateral side of the dendritic tree of motoneurons but not by depolarization applied to the medial side (Delgado-Lezama et al. 1999). Conversely, activity in the medial funiculus facilitates the activation of PICs (supposedly through the neuromodulatory action of a neuromodulator different than serotonin) by depolarization applied to the medial side of the dendritic tree of motoneurons but not by depolarization applied to the lateral side. Thus two different functional distributions of L-type Ca\(^{2+}\) channels were created by the activation of two distinct sets of neuromodulatory inputs. In cat hindlimb motoneurons, serotonergic synapses are distributed uniformly throughout the dendritic tree (Alvarez et al. 1999). Nonetheless, various functional distributions could be produced by the selective
activation of subsets of axons belonging to a single neuromodulatory system or different neuromodulatory systems that innervate different regions of the dendritic tree.

**Organization of neuromodulatory receptors** A second way to create different functional distributions of L-type Ca\(^{2+}\) channels in motoneurons is through an organized distribution of neuromodulatory receptors, rather than neuromodulatory synapses. In this manner, a uniform set of neuromodulatory inputs may still facilitate only a subset of all available L-type Ca\(^{2+}\) channels. Serotonergic receptors have different receptor subtypes producing different actions depending on the activated biochemical pathway (Hochman et al. 2001; Perrier and Hounsgaard 2003; Perrier et al. 2003; Rekling et al. 2000). Localized application of serotonin to the cell body, proximal dendrites or distal dendrites of isolated turtle motoneuron can either facilitate persistent inward currents or inhibit the firing of action potentials (Perrier and Hounsgaard 2003). Facilitation of PICs through the activation of 5-HT\(_2\) in turtle motoneurons was observed following application of serotonin to all areas of the neurons. Inhibition of spike generation was observed for application of serotonin to the cell body only. The mechanism for this inhibition is unknown but may involve 5-HT1A (Wang and Dun 1990) or the differential distribution of serotonin receptor subtypes in motoneurons.

**Organization of ionotropic synapses** The distribution of some ionotropic inputs to motoneurons may be sufficiently localized as to activate only a subset of L-type Ca\(^{2+}\) channels, thereby producing a functional distribution of these channels that is smaller than their anatomical distribution. In motoneurons, inputs from Renshaw cells, Ia
inhibitory neurons and the C-terminals from local cholinergic interneurons are distributed mainly on the soma or on proximal dendrites (Burke et al., 1971; Fyffe 1991; Hellström et al. 2003; Muennich and Fyffe 2004; Wilson et al. 2004). Synaptic inputs from contralateral vestibulospinal neurons are distributed preferentially on the medial dendrites of motoneurons (Grande et al. 2005).

**Organization of different voltage-gated channels** Finally, by modulating the local electrical environment of different sets of L-type Ca\(^{2+}\) channels, non-uniform distribution of voltage-gated channels, especially K\(^+\) channels, may lead to different functional distributions of L-type Ca\(^{2+}\) channels.

**Compartmentalization within the dendritic tree of motoneurons**

The dendritic trees of neurons can be compartmentalized into independent computational units (Koch 1999). In our models, the dendritic tree of motoneurons was segmented into a number of compartments as a consequence of the functional distribution of L-type Ca\(^{2+}\) channels as hot spots. The dendritic tree of motoneurons is characterized by dendrites extending up to 2000 µm away from the cell body and up to 11 orders of branching (Bui et al. 2003; Cullheim et al. 1987; Kernell and Zwaagstra 1989; Rose et al. 1985; Ulfhake and Kellerth 1981). As a reflection of its size, the electrotonic structure of motoneurons was also observed to be expansive (Bras et al. 1989; Bui et al. 2003; Clements and Redman 1989; Fleshman et al. 1988; Segev et al. 1990; Svirskis et al. 2001). Thus, the dendritic tree of motoneurons presents an excellent substrate for local interactions between synaptic inputs and voltage-gated channels. In our models, each computational
compartment is roughly defined by the location of the hot spots. The graded amplification of synaptic current reaching the soma was a result of the sequential and cumulative recruitment of hot spots. A similar compartmentalization was proposed by Svirskis et al. (2001) in their turtle motoneuron models with uniformly distributed L-type Ca$^{2+}$ channels, where different dendritic regions can become independently bistable. The summation of independently bistable compartments into a smooth linear relationship for the neuron as a whole has also been suggested for oculomotor neural integrators (Goldman et al. 2003). The graded activation of calcium currents by synaptic activity has been observed notably in neurons of the entorhinal cortex (Egorov et al. 2002), lamina I of the spinal dorsal horn (Prescott and De Koninck 2005) and in layer V pyramidal cells (Oakley et al. 2001; Reuveni et al. 1993). In the latter class of cells, evidence from simulations suggested that the Ca$^{2+}$ channels underlying the Ca$^{2+}$ plateaus were distributed in hot spots in the dendrites (Reuveni et al. 1993). Thus, the functional compartmentalization of motoneuron dendritic trees by Ca$^{2+}$ channels may be a general means of transforming an inherently all-or-none process (i.e. the activation of voltage-dependent channels) into a quasi-linear means of increasing or decreasing the net current reaching the soma during synaptic activity.
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REFERENCES


Prescott SA, De Koninck Y. Integration time in a subset of spinal lamina I neurons is lengthened by sodium and calcium currents acting synergistically to prolong subthreshold depolarization. *J Neurosci* 25: 4743-4754, 2005.


TABLE 1. Somatic threshold of PICs activation under different states of activity for motoneuron models with L-type Ca\(^{2+}\) channels distributed as 100 µm hot spots centered at the intersection points of the membrane potential profiles in the resting state and the excitatory state

<table>
<thead>
<tr>
<th></th>
<th>LAD5-4</th>
<th>LVN2-1</th>
<th>LVN4-1</th>
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<tr>
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<td>-49.9</td>
<td>-50.6</td>
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<tr>
<td>Excitatory state</td>
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<td>-56.9</td>
<td>-53.6</td>
<td>-56.4</td>
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<tr>
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<td>-40.3</td>
<td>-43.0</td>
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</table>

* Taken from Bennett et al. (1998)
† All values are in mV
TABLE 2. Somatic threshold of PICs activation under different states of activity for different models of a motoneuron, LVN4-1, incorporating L-type Ca\(^{2+}\) channels as hot spots of different sizes or at different locations

<table>
<thead>
<tr>
<th>State</th>
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<th>Hotspots at crossover points</th>
<th>Hotspots 100 µm proximal</th>
<th>Hotspots 100 µm distal</th>
<th>Hotspots 200 µm distal</th>
<th>Hotspots 25 µm long</th>
<th>Hotspots 50 µm long</th>
<th>Hotspots 150 µm long</th>
<th>Hotspots 200 µm long</th>
<th>Hotspots 300 µm long</th>
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</thead>
<tbody>
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<td>-50.9</td>
<td>-51.5</td>
<td>-51.2</td>
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<td>-50.5</td>
<td>-50.6</td>
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</tr>
<tr>
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<tr>
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<td>-39.9</td>
<td>-40.0</td>
<td>-38.5</td>
<td>-39.9</td>
<td>-42.1</td>
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</tr>
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</table>
FIGURE LEGENDS

Figure 1: Hypothetical profiles of membrane potentials along a dendrite just subthreshold to activation of persistent inward currents (PICs) by somatic current injection. A: Resting state (background synaptic activity). The thick black solid line ($V_{\text{threshold, resting state}}$) represents the membrane potential profile of the dendrite just before the activation of PICs. The thin black solid lines represent the membrane potential profile of the dendrite at various levels of current injection prior to the activation of PICs. The arrow indicates the direction of increasing depolarizing current. B: Excitatory state (elicited excitatory synaptic activity). The thick gray solid line ($V_{\text{threshold, excitatory state}}$) represents the membrane potential profile of the dendrite just before the activation of PICs. The thin gray solid lines represent the membrane potential profile of the dendrite at various levels of current injection prior to the activation of PICs. The lowest thin gray solid line represents the membrane potential profile of the dendrite with an initial hyperpolarizing current injection. The gray arrow indicates the direction of the initial hyperpolarizing current injection. The black arrow indicates the direction of increasing depolarizing current. C: Inhibitory state (elicited inhibitory synaptic activity). The thick black dashed line ($V_{\text{threshold, inhibitory state}}$) represents the membrane potential profile of the dendrite just before the activation of PICs. The thin black dashed lines represent the membrane potential profile of the dendrite at various levels of current injection prior to the activation of PICs. The arrow indicates the direction of increasing depolarizing current. D: Superimposition of membrane potential profile in the resting state (solid black line) and in the excitatory state (solid gray line). $V_{\text{int}}$ is the membrane potential at the intersection point between the two profiles. The thin gray long-dashed lines represent
possible values of $\theta_{PIC}$, the threshold of PICs activation. The thick black double-arrowhead line represents the possible locations of the channels underlying PICs. $E$: Superimposition of membrane potential profile in the resting state (solid black line) and in the inhibitory state (black short dashed line). The thin gray long-dashed line represents possible values of $\theta_{PIC}$, the threshold of PICs activation. The thick black double-arrowhead line to the right of the intersection of the membrane potential profiles in the resting state and in the inhibitory state represents the possible locations of the channels underlying PICs. $F$: Superimposition of membrane potential profile in the resting state, in the excitatory state and in the inhibitory state. The gray long-dashed line represents possible values of $\theta_{PIC}$, the threshold of PICs activation. The black circle represents the possible location of the channels underlying PICs.

**Figure 2:** Determination of the intersection points of membrane potential profiles for a dendrite of LVN2-1. $A$: Left, reconstruction of the dendritic tree of LVN2-1 with the examined dendrite highlighted. Right, membrane potential profile in the resting state (gray line) and in the excitatory state (black line) of highlighted dendrite shown in the reconstruction. $B$: Box plot of the intersection points for all dendrites of three motoneurons between the resting state and the excitatory state (grey boxes), and of the length of all dendrites (from soma to terminal, white boxes). $C$: Box plot of the branching order of the dendritic branches on which the intersection point lies (grey boxes), and of the terminal branches (white boxes). For LAD5-4, the 10th and 25th percentile intersection points are of branching order 2. For LVN2-1, the 50th and 75th percentile intersection points are of branching order 3.
Figure 3: Response to triangular current ramps (5 nA/s) of a motoneuron (LVN2-1) in the inhibitory state, resting state and excitatory state with L-type Ca$^{2+}$ channels distributed in 100 µm hot spots centered at the intersection points of the membrane potential profile of the resting state and the excitatory state. A: Reconstruction of the dendritic tree of LVN2-1 with Ca$^{2+}$ channels distributed in 100 µm long hot spots (red). The thicknesses of the dendrites where the hot spots are located have been increased for illustrative purposes. B: Somatic membrane potential. The somatic threshold of PICs activation was defined as the membrane potential at which the derivative of the somatic membrane potential with respect to time was equal to 0.1 V/s on the rising slope of the highest peak. Arrows mark the measured somatic threshold of PICs activation. C: Derivative of the somatic membrane potential with respect to time. The dotted line marks a value of the derivative of 0.1 V/s.

Figure 4: Comparison of the shift in somatic threshold of PICs activation from the resting state to the excitatory state, and from the resting state to the inhibitory state, for motoneuron LVN4-1. A: With various locations of 100 µm long hot spots. A plateau potential could not be elicited in the inhibitory state with 100 µm long hot spots centered 200 µm away from the intersection points. B: With various sizes of hot spots centered at intersection points of the membrane potential profiles of the resting state and the excitatory state. C: With a decreased level of excitatory activity in the excitatory state. Intersection points were recalculated prior to adding L-type Ca$^{2+}$ channels.
**Figure 5**: Response of a motoneuron (LVN4-1) to triangular current ramps in the inhibitory state, resting state and excitatory state with L-type Ca\(^{2+}\) channels distributed uniformly throughout the soma and the dendrite at a constant density (conductance/surface area). *A*: Somatic membrane potential. The somatic threshold of PICs activation was the membrane potential at which the derivative of the somatic membrane potential with respect to time was equal to 0.1 V/s on the rising slope of the highest peak. Arrows mark the measured somatic threshold of PICs activation. *B*: Derivative of the somatic membrane potential with respect to time.

**Figure 6**: *A*: Current reaching the soma following the tonic activation of excitatory synapses in the presence and in the absence of L-type Ca\(^{2+}\) channels distributed in 100 \(\mu\)m long hot spots centered at the intersection points. The membrane potential at the soma was clamped at -64 mV. *B*: Number of active L-type Ca\(^{2+}\) channel hot spots in response to the tonic activation of excitatory synapses. The maximum number of hot spots is marked by the dotted line.

**Figure 7**: Current reaching the soma following the tonic activation of excitatory synapses in the presence and in the absence of L-type Ca\(^{2+}\) channels distributed in 100 \(\mu\)m long hot spots centered at the intersection points. The membrane potential at the soma was clamped at -55 mV.

**Figure 8**: *A*: Current reaching the cell body following the tonic activation of excitatory synapses and the tonic activation of 5% or 10% of all inhibitory synapses. The L-type
Ca$^{2+}$ channels were distributed in 100 µm long hot spots centered at the intersection point. The membrane potential at the soma was clamped at -64 mV. $B$: Number of active L-type Ca$^{2+}$ channel hot spots in response to the tonic activation of excitatory synapses.
Distance from the cell body

A

Membrane potential

Distance from the cell body

B

Distance from the cell body

C

Membrane potential

Distance from the cell body

D

Membrane potential

Distance from the cell body

E

Membrane potential

Distance from the cell body

F

Membrane potential

Distance from the cell body

FIGURE 1

\[ \theta_{PIC} \]

\[ \theta_{PIC} > V_{int} \]

\[ \theta_{PIC} = V_{int} \]

\[ \theta_{PIC} < V_{int} \]
FIGURE 2
FIGURE 3
**FIGURE 4**

A

Bennett et al. (1998)

- Intersection point
- Shifted 100 µm proximal
- Shifted 100 µm distal

B

- Bennett et al. (1998)
- 100 µm long hot spots
- 25 µm long hot spots
- 50 µm long hot spots
- 150 µm long hot spots
- 200 µm long hot spots

C

- Bennett et al. (1998)
- 'Standard' excitatory synaptic activity
- Decreased excitatory synaptic activity

Shift in somatic threshold from resting state to excitatory state (mV)

Shift in somatic threshold from resting state to inhibitory state (mV)
FIGURE 5

A

Somatic membrane potential (mV)

Time (s)

-160
-140
-120
-100
-80
-60
-40
-20

resting
inhibitory
excitatory

B

\( \frac{dt}{dt} (\text{V/s}) \)

Time (s)

0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0

FIGURE 5
FIGURE 6
FIGURE 7
FIGURE 8