Simulation of Dendritic Ca\textsubscript{v}1.3 Channels in Cat Lumbar Motoneurons: Spatial Distribution

Sherif M. ElBasiouny,\textsuperscript{1,2} David J. Bennett,\textsuperscript{1,2,3} and Vivian K. Mushahwar\textsuperscript{1,2}

\textsuperscript{1}Department of Biomedical Engineering, and \textsuperscript{2}Centre for Neuroscience, Faculty of Medicine and Dentistry; and \textsuperscript{3}Faculty of Rehabilitation Medicine, University of Alberta, Edmonton, Alberta, Canada

Submitted 18 April 2005; accepted in final form 19 August 2005

ElBasiouny, Sherif M., David J. Bennett, and Vivian K. Mushahwar. Simulation of dendritic Ca\textsubscript{v}1.3 channels in cat lumbar motoneurons: spatial distribution. J Neurophysiol 94: 3961–3974, 2005. First published August 24, 2005; doi:10.1152/jn.00391.2005. We used computer simulations to study the dendritic spatial distribution of low voltage-activated L-type calcium (Ca\textsubscript{v}1.3 type) channels, which mediate hysteretic persistent inward current (PIC) in spinal motoneurons. This study was prompted by the growing experimental evidence of the functional interactions between synaptic inputs and active conductances over the motoneuron dendritic tree. A compartmental cable model of an adult cat α-motoneuron was developed in NEURON simulation environment constituting the detailed morphology of type-identified triceps surae α-motoneuron and realistic distribution of group Ia afferent-to-motoneuron contacts. Simulations of different distributions of Ca\textsubscript{v}1.3 channels were conducted and the resultant behavior was compared to experimental data. Our results suggest that Ca\textsubscript{v}1.3 channels do not uniformly cover the whole motoneuron dendritic tree. Instead, their distribution is similar to that of synaptic contacts. We found that Ca\textsubscript{v}1.3 channels are primarily localized to a wide intermediate band overlapping with the dendritic Ia-synaptic territory at dendritic distances of 300 to 850 μm (0.62 ± 0.21A) from the soma in triceps surae α-motoneurons. These findings explain the functional interaction between synaptic inputs and the Ca\textsubscript{v}1.3 channels over the motoneuron dendritic tree.

INTRODUCTION

Since their discovery by Schwindt and Crill in cat motoneurons, persistent inward currents (PICs) were suggested to be mediated by calcium ions (Schwindt and Crill 1980b). Recent evidence showed that PICs in spinal motoneurons of various species are mediated by two main classes of channels: low-voltage–activated L-type calcium (LVA L-type Ca\textsuperscript{2+}, specifically Ca\textsubscript{v}1.3; Hounsgaard and Kiehn 1989; Schwindt and Crill 1980b) and persistent sodium (Li and Bennett 2003) channels.

In their early work Schwindt and Crill (1980a) suggested that PICs were generated near or at the soma. However, different experimental protocols (Bennett et al. 1998; Hounsgaard and Kiehn 1993; Lee and Heckman 1996), anatomical localization of channels (Carlin et al. 2000a; Simon et al. 2003), and computer simulations (Booth et al. 1997; Carlin et al. 2000b; Gutman 1991) emphasized the dendritic origin of PICs. Furthermore, measurements of Ca\textsuperscript{2+} PIC under voltage-clamp conditions in spinal motoneurons showed that they exhibit hysteresis in the current–voltage (I–V) relationship. The hysteresis is ascribed to the distal location of the channels mediating this current relative to the soma, suggesting again a dendritic origin of the L-type Ca\textsuperscript{2+} channels where they cannot be fully voltage clamped (Carlin et al. 2000b; Lee and Heckman 1998b; Li and Bennett 2003). The factors influencing this hysteresis are mainly the dendritic location of the Ca\textsubscript{v}1.3 channels relative to the soma, and the intrinsic activation/inactivation properties of these channels.

Upon activation of Ca\textsubscript{v}1.3 channels, a strong PIC is triggered that overcomes potassium-mediated outward currents resulting in the formation of a negative-slope region in the steady-state I–V relationship. Sustained depolarization (plateau potentials) and long-lasting self-sustained firing (bistability) can therefore be seen if the cell is sufficiently depolarized by intracellular current injection or excitatory synaptic input (Conway et al. 1988; Crone et al. 1988; Hounsgaard et al. 1988; Lee and Heckman 1998a).

Schwindt and Crill (1980b) suggested that synaptic currents do not sustain spontaneous bursts of motoneuronal activity and postulated an interaction of synaptic and membrane mechanisms that allows PICs to dominate the neural behavior. This interaction was confirmed experimentally where it was observed that the somatic voltage threshold for generating plateau potentials can be altered by background synaptic activity (Bennett et al. 1998). Dendritic channels mediating PICs amplify the synaptic input and enhance the delivery of synaptic current to the soma (Binder et al. 1996; Johnston et al. 1996; Yuste and Tank 1996). These channels receive neuromodulatory monoaminergic inputs originating from the brainstem (Binder et al. 1996; Hounsgaard et al. 1988; Takahashi and Berger 1990; Wang and Dun 1990; White et al. 1991), thus information on the dendritic spatial distribution of these channels, which is currently sparse, is important to predict the effects of neuromodulators on dendritic processing. The goal of this study was to investigate the dendritic spatial distribution of Ca\textsubscript{v}1.3 channels in spinal motoneurons. Our hypothesis was that the dendritic spatial distribution of these channels overlaps with that of synaptic inputs from multiple origins, and this overlap results in the functional interactions seen experimentally between these two systems.

To test this hypothesis we developed a compartmental cable model of a spinal motoneuron based on the detailed morphology of fatigue-resistant (FR) cat α-motoneuron, and included realistic distribution of Ia-afferent synapses (Braunstrom 1993; Burke and Glenn 1996; Cullheim et al. 1987; Segev et al. 1990). The model was tuned to match the intrinsic properties of

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
cat motoneurons with passive dendrites, and it was then used to predict the most likely dendritic spatial distribution of CaV1.3 channels that would match the firing behavior of motoneurons with active dendrites. Different spatial distributions of the CaV1.3 channels over the dendritic tree were tested, and the resulting model behavior was compared to experimental data. Our results suggest that the dendritic spatial distribution of CaV1.3 channels is similar to that of Ia-synaptic contacts, and that these channels are primarily localized within the synaptic territory region (dendritic region with highest Ia-synaptic population). Part of this work was previously presented in abstract form (ElBasiouny and Mushahwar 2004).

Model morphology, biophysical properties, and verification

We developed a computer-based cable model of an adult cat α-motoneuron using NEURON simulation environment (Hines and Carnevale 1997). The model had full representation of the α-motoneuron structure and consisted of soma, axon hillock (AH), initial segment (IS), and dendritic tree. The dendritic tree was based on the three-dimensional (3D) detailed morphology for type-identified triceps surae α-motoneuron [FR type, medial gastrocnemius (MG) motoneuron, identified as cell 43/5], labeled intracellularly with horseradish peroxidase (HRP). The total dendritic arbor was incorporated into the program and was modeled with 731 compartments representing 11 root dendrites and 165 terminations (Fig. 1A; Cullheim et al. 1987).

The soma was modeled as a single spherical compartment of diameter 48.8 μm (Cullheim et al. 1987). The AH was represented as a series of 11 tapered cylindrical compartments of total length 20 μm (Kellerth et al. 1979), with a base diameter at the soma side of 13 μm and diameter at the IS side of 3 μm (Conradi 1969; Kellerth et al. 1979). The IS was represented as a series of three cylindrical compartments of total length 30 μm and a constant diameter of 3.3 μm (Kellerth et al. 1979).

The passive parameters determining the cable properties of the motoneuron were set according to the values reported by Fleshman et al. (1988) for the same 43/5 FR motoneuron. The specific membrane resistance ($R_m$) was set to 225 Ω · cm$^2$ for the soma, AH, and IS, and 11,000 Ω · cm$^2$ for the dendrites. The values of $R_m$ for the soma, AH, and IS took into consideration the leak current induced by electrode penetration into the soma. The specific membrane capacitance ($C_m$) was set to 1 μF/cm$^2$ and the specific axial resistance ($R_a$) to 70 Ω · cm for all the compartments in the model (Clements and Redman 1989; Fleshman et al. 1988; Segev et al. 1990).

The model included voltage-dependent ion channels previously identified and described experimentally in cat α-motoneurons (Barrett and Crill 1980; Barrett et al. 1980; Schwindt and Crill 1977), as well as N-type calcium channels described in turtle spinal motoneurons (Hounsgaard and Mintz 1988). The soma included conductances representing nonlinear fast sodium channels (Naf), delayed rectifier potassium channels (Kdr), calcium-activated potassium channels [K(Ca)], and N-type calcium channels (CaN, Ca$_{\alpha_2}$2.2). The AH and IS included conductances representing persistent sodium (Nap), Naf, and Kdr channels (Table 1). The parameters describing the kinetics of these channels were adapted from McIntyre and Grill (2002) and are shown in the APPENDIX.

The Naf and Kdr channels are responsible for the generation of action potential (AP) spikes. The voltage-dependent activation curves of the Naf channels in the AH and IS are more hyperpolarized than those in the soma. This makes the IS the site of initiation of the AP in the cell. Nap channels in the AH and IS are responsible for regulation of the cell rhythmic firing (Lee and Heckman 2001). Their density is <1% of the Naf channels and their activation curves are more hyperpolarized than those of the Naf channels (Crill 1996). The CaN channels allow for calcium influx during AP and also regulate the K(Ca) conductances, which are responsible for shaping the membrane.
trajecotory during the afterhyperpolarization (AHP) following the AP spike (Booth et al. 1997).

The model was verified by comparing its properties to experimental data from in vivo and in vitro measurements. Because experimental measurements were obtained from anesthetized cats (Fleschman et al. 1988; Hochman and McCrea 1994a,b), the model properties were measured with a passive dendritic tree. The model variables were tuned to have properties within the 95% confidence range of experimental data. The cell input resistance ($R_i$) and membrane time constant ($\tau$) were adjusted to experimental values for the 43/5 FR motoneuron (Cullheim et al. 1987). The model excitability was assessed by measuring the rheobase current ($I_{\text{rheo}}$), short-pulse current threshold (SPCT), and the firing voltage threshold ($V_{\text{th}}$). The model generated AP and AHP with properties very close to experimental recordings. Table 2 shows values of the different model properties compared to experimental data. All experimental data are presented as mean ± SD.

### CaV1.3 channels

Experimental data demonstrated that PICs in spinal motoneurons are evenly mediated by Nap and nifedipine-sensitive LVA L-type CaV$^{2+}$ (CaV1.3 type) channels (Li and Bennett 2003). Nap PIC was found to activate and deactivate rapidly with apparent somatic activation potential of 7 mV below the spike threshold and show significant inactivation (Li and Bennett 2003). On the other hand, CaV$^{2+}$ PIC was found to activate and deactivate slowly with apparent somatic activation potential of ±5 mV of the spike threshold and show no inactivation (Li and Bennett 2003). In the present study, we focused on the CaV1.3 channels because our interest was in determining their most likely spatial distribution over the dendritic tree.

After matching the intrinsic properties of cat motoneurons under anesthesia (with passive dendrites), CaV1.3 channels were added to the dendritic tree to simulate the PICs and firing behavior of a motoneuron with active dendrites. The parameters describing the kinetics of the CaV1.3 channels were adapted from Carlin et al. (2000b) and based on experimental recordings in mice and rat (Carlin et al. 2000a; Xu and Lipscombe 2001). The activation time constant of the channels was adapted to fit tail currents that are seen experimentally at the termination of somatic long voltage pulses (Li and Bennett 2003). Because the apparent somatic activation potential of PICs in cat spinal motoneurons is about 10–30 mV positive to the resting membrane potential (Schwindt and Crill 1980b), the half-activation potential was adjusted to activate the CaV1.3 channels within this range as seen from the soma (half-activation potential = −43 mV) and allow plateau activation within the observed experimental values.

The experimental measurements of CaV$^{2+}$ PIC were based on recordings done by Lee and Heckman (1999) in cat motoneurons after injection of the lidocaine derivative QX-314, and Li and Bennett (2003) in rat motoneurons after injection of tetrodotoxin (TTX), to block Na$^+$ channels. Measurements of CaV$^{2+}$ PIC in the model were done under voltage-clamp conditions after leak current subtraction. Leak current was estimated from the subthreshold region by fitting a regression line (Fig. 2B). A slow triangular voltage command of slope 4 mV/s was used. The soma was perfectly clamped over the voltage range for activation of the CaV$^{2+}$ PIC (from −70 to −30 mV).

In the model, we included CaV1.3 channels only over the dendritic tree. However, there are undoubtedly other dendritic active conductances some of which might mediate outward currents that counteract the CaV$^{2+}$ PIC (Powers and Binder 2000). Given that CaV$^{2+}$ PIC measurements from the model were compared to experimental data in which no channel blockers (except for Na$^+$) were used, the inward current in this study represents the “effective” CaV$^{2+}$ PIC, which includes the contribution of outward conductances.

### Dendritic synaptic inputs

The monosynaptic Ia-afferent system was represented in the model (Fig. 1B). The dendritic spatial distribution of Ia-afferent synapses was based on the realistic distribution of group IA afferent-to-motoneuron contacts labeled intracellularly with HRP from cat FR motoneurons (Burke and Glenn 1996; Burke et al. 1979; Glenn et al. 1982). The total number of Ia-afferent boutons was 300 based on the data from Segev et al. (1990). Two patterns of Ia-afferent activation were simulated in this study: sustained muscle stretch, resulting in tonic input, and tendon vibration. For tonic input, the synapses were activated at a high frequency that resulted in a smooth somatic membrane potential. The synapse conductances were set such that the somatic membrane potential was subthreshold for firing (Bennett et al. 1998). To simulate the effects of tendon vibration, the Ia afferents were activated at 180 Hz and the synapse conductances were set such that the Ia effective synaptic current (I$_{\text{IA}}$), which is the amount of Ia current reaching the soma, was approximately 4.8 nA with the soma clamped at the resting potential. This is the value of I$_{\text{IA}}$ seen experimentally in MG motoneurons in the decerebrate cat that had received the noradrenergic alpha agonist methoxamine (Lee and Heckman 2000). Given the variation in conduction velocity in Ia-afferent fibers (80–110 m/s in cat), synchronous activation of their axons through vibration in the periphery leads to asynchronous activation of their synaptic terminations (Segev et al. 1990). We simulated this temporal dispersion among the Ia synapses by dividing them in four groups that were activated asynchronously. Synapses were randomly assigned to a group and the synaptic distribution in each group was similar to that of Fig. 1B. All groups were activated at the vibration frequency but with a 25% phase shift. The parameters of the Ia-afferent synapses (reversal potential and time to peak) were chosen based on previous experimental and modeling studies (Jones and Bawa 1997; Segev et al. 1990).

### Distributions of the CaV1.3 channels

Four different spatial distributions of the CaV1.3 channels over the dendritic tree were tested in the model. For each distribution, the density of channels was uniform with respect to the membrane area and was adjusted such that the magnitudes of CaV$^{2+}$ PIC (depth of initial peak $I_{\text{peak}}$ and sustained peak $I_{\text{peak}}$; Fig. 2B) were within the experimental range (Table 3). To verify our results, simulations were run under different model conditions, and the model behavior was compared to different experimental measurements from cat motoneurons. The $F$–$I$ relationship was obtained with the use of all channels in the model, whereas Ia $I_{\text{IA}}$ enhancement and V–I relationships were obtained with only the CaV1.3 channels active. Moreover, an independent method based on a technique used by Rose et al. (2002, 2003)
 evaluation of CaV1.3 channel distributions

The criterion used for evaluating the various distributions was to match three different and independent sets of experimental measurements from cat motoneurons innervating the MG muscle. These measurements were: 1) properties of Ca\textsuperscript{2+} PIC and F–I relationship, 2) changes in the somatic plateau threshold and F–I relationship arising from background synaptic activity, and 3) enhancement of Ia synaptic current.

PROPERTIES OF CA\textsuperscript{2+} PIC AND F–I RELATIONSHIP. Properties of the Ca\textsuperscript{2+} PIC obtained from the model were compared to those obtained experimentally from decerebrate cats that had received QX-314 and methoxamine (Lee and Heckman 1999). Because QX-314 reduces Ca\textsuperscript{2+} currents in addition to blocking Na\textsuperscript{+} currents (Lee and Heckman 1999; Talbot and Sayer 1996) the amplitude of experimentally measured Ca\textsuperscript{2+} PIC from motoneurons may be underestimated. However, the magnitude of the Ca\textsuperscript{2+} PIC measured by Lee and Heckman (1999) was found to form nearly 50% of I\textsubscript{th} and about 61% of I\textsubscript{th} of the total PICs. This contribution of Ca\textsuperscript{2+} PIC is similar to that observed experimentally in rat spinal motoneurons in the presence of TTX, a selective blocker of Na\textsuperscript{+} channels, in which Ca\textsuperscript{2+} PIC contributed approximately 50% of the initial peak (I\textsubscript{th}) and 67% of the sustained peak (I\textsubscript{th}) of the total PICs (Li and Bennett 2003).

The F–I relationship was obtained when no channels were blocked and measuring the instantaneous firing rate at the soma during intracellular injection of slow triangular current ramps of slope 4 nA/s (Fig. 2C). This was compared to the results of Bennett et al. (1998) on medial and lateral gastrocnemius–soleus motoneurons in the decerebrate cat.

CHANGES IN THE SOMATIC PLATEAU THRESHOLD AND F–I RELATIONSHIP ARISING FROM BACKGROUND SYNAPTIC ACTIVITY. To study the effect of tonic synaptic activation on the somatic threshold of plateau activation and on the properties of the F–I relationship, simulations were done with and without synaptic activation and responses were compared to each other (Fig. 6, A and B). Synaptic excitation was produced by tonic activation of the Ia-afferent synapses (Bennett et al. 1998). The V–I relationship was obtained with only CaV1.3 channels present, by measuring the somatic membrane potential while injecting a slow, increasing current ramp of 4 nA/s at the soma. The injected current ranged from 0 to 20 nA. Table 3 shows the experimental values for both F–I and V–I relationship properties.

ENHANCEMENT OF IA SYNAPTIC CURRENT. The magnitude of Ca\textsuperscript{2+} PIC was adjusted to be within the experimental range for the decerebrate cat that had received methoxamine (Lee and Heckman 2000), and properties of Ia synaptic current enhancement (peak Ia I\textsubscript{sp} and hyperpolarized Ia I\textsubscript{sp}, which is the effective synaptic current measured at the resting potential) were compared to those from the same preparation (Table 3). The Ia I\textsubscript{sp} was computed as the difference between the F–I relationships obtained during no synaptic activity (at rest) and during tendon vibration at 180 Hz (Lee and Heckman 2000).

RESULTS

The primary goal of this study was to determine the most likely dendritic distribution of the CaV1.3 channels mediating Ca\textsuperscript{2+} PIC in cat spinal motoneurons. We used the amount of hysteresis, which is represented by the difference between the Ca\textsuperscript{2+} PIC activation and deactivation potentials seen at the soma, seen experimentally in Ca\textsuperscript{2+} PIC to predict the dendritic distribution of these channels. Channel properties were maintained constant in all the tested distributions.

One of the advantages for using computer simulations is that they can overcome some limitations that face experimental techniques. One of these limitations when using the voltage-
clamp technique is the knowledge of which parts of the cell are well clamped and which parts are not. Moreover, it is difficult to know experimentally which portions of the dendritic tree are active and contributing to the obtained measurements. The present model provided a unique opportunity to predict the spatial distribution of the CaV1.3 channels over the motoneuron dendritic tree by combining realistic 3D morphology of the motoneuron dendritic tree, realistic dendritic distribution of the Ia afferents, and experimental electrophysiological recordings from the same motoneurons after blocking Na⁺ PIC. Simulation results were compared to different and independent sets of experimental electrophysiological measurements (properties of Ca²⁺ PIC and F–I relationship, changes in the somatic plateau threshold and F–I relationship arising from background synaptic activity, and voltage-dependent enhancement of the synaptic current). Four different distributions were tested and their results were compared to experimental data and validated by the use of an independent technique described by Rose et al. (2002, 2003).

Whole uniform density distribution

In this distribution, the density of the CaV1.3 channels was uniform throughout the dendritic tree (0.78 ± 0.49 Å; median: 0.72Å; Fig. 2A). The density of channels (0.06 mS/cm²) was adjusted such that the amplitude of Ca²⁺ PIC was within the experimental range. Figure 2A shows the number of the CaV1.3 channels at various distances from the soma normalized to the maximum number of channels over the dendritic tree, and was calculated from the number of channels and membrane surface area at various distances from the soma. The relative number of CaV1.3 channels was compared to the number of synaptic contacts over the dendritic tree. The measured Ca²⁺ PIC showed very little hysteresis (V_on – V_off = 2.3 mV; Fig. 2B). This was also reflected in the F–I relationship by a complete overlap of the F–I segments during the ascending and descending phases of the injected ramp current (i.e., no bistability behavior; Fig. 2C).

These results could be explained by the strong influence of the proximal dendrites on the soma. In this distribution, the CaV1.3 channels cover the whole dendritic tree (both proximal and distal dendritic branches). Ca²⁺ PIC from proximal dendrites is not hysteretic because these dendrites are too close to the soma and are well voltage clamped. Ca²⁺ PIC from the distal dendrites is hysteretic as a consequence of the distal location of these dendrites relative to the soma and thus are imperfectly voltage clamped. Therefore, the proximal dendrites, which are electrotonically close to the soma, dominate the cell behavior causing the nonhysteretic Ca²⁺ PIC to dominate the total PIC seen at the soma. This proposed dominance of proximal dendrites was tested in the model by placing the CaV1.3 channels only on the proximal dendrites (from the soma up to a dendritic path distance of 500 μm). We found that there was no bistability in the F–I relationship and there was a complete overlap of the upward and downward segments of the F–I relationship (data not shown), confirming that proximal dendrites were dominating the behavior seen from the whole uniform density distribution. Therefore, these results suggest that the CaV1.3 channels should be located more distally to produce the proper amount of hysteresis in the measured Ca²⁺ PIC.
**Middle uniform density distribution**

In this distribution, the density of the $\text{CaV}1.3$ channels was uniform in the region of the dendritic tree starting from 500 $\mu$m dendritic path distance from the soma ($1.0 \pm 0.41\lambda$; median: 0.93$\lambda$; Fig. 3A). The channel density (0.125 mS/cm$^2$) was adjusted such that the amplitude of $\text{Ca}^{2+}$ PIC was within the experimental range. The measured $\text{Ca}^{2+}$ PIC showed an appropriate amount of hysteresis ($V_{\text{on}} - V_{\text{off}} = 14.7$ mV; Fig. 3B). However, the effect of $\text{Ca}^{2+}$ PIC was too strong on the soma firing behavior, causing the cell to fire at very high nonphysiological rates (Fig. 3C). Bistability was also seen in the $F-I$ relationship, but the difference between firing rates at the same injected current was larger (about 50 imp/s) than that seen experimentally (usually from 5 to 15 imp/s). Despite our best efforts to match the physiological firing rates by reducing channel density to reduce $\text{Ca}^{2+}$ PIC, we could not achieve physiological firing rates while maintaining the magnitude of $\text{Ca}^{2+}$ PIC within the experimental range.

To further assess the effect of $\text{Ca}^{2+}$ PIC on the cell behavior, we measured the $\text{Ia}_A$ enhancing the soma at different membrane potentials (Fig. 3D). The $\text{Ia}_A$ enhancement profile was similar to that seen experimentally, but the $\text{Ia}_A$ peak value (about 18.5 nA) was high, indicating a strong effect of $\text{Ca}^{2+}$ PIC (compare Fig. 3D to Fig. 3A in Lee and Heckman 2000). This peak $\text{Ia}_A$ is considered high, even though its absolute value is within the acceptable confidence range of experimental data. This is because the measured $\text{Ia}_A$ enhancement in the model arises only from the contribution of $\text{Ca}^{2+}$ PIC, whereas the experimental $\text{Ia}_A$ enhancement is attributed to the contribution of both $\text{Na}^+$ and $\text{Ca}^{2+}$ PICs.

The proper amount of hysteresis in $\text{Ca}^{2+}$ PIC in this distribution of the $\text{CaV}1.3$ channels indicates that the channels are placed approximately around the correct location. However, the strong effect of $\text{Ca}^{2+}$ PIC on the cell firing behavior indicates that the PIC reaching the soma should be lowered by either localizing the channels over a limited middle region or placing the channels more distally on the dendritic tree.

**Distal uniform density distribution**

In this distribution, the density of the $\text{CaV}1.3$ channels was uniform (0.4 mS/cm$^2$) in the region of the dendritic tree starting from 1-mm dendritic path distance ($1.47 \pm 0.33\lambda$; median: 1.4$\lambda$; Fig. 4A) from the soma. The measured $\text{Ca}^{2+}$ PIC ($I_{\text{in}}$ and $I_{\text{sus}}$) was small in magnitude and very hysteretic (Fig. 4B). $\text{CaV}1.3$ channels were activated at low hyperpolarized somatic potentials (at $-68.5$ mV, indicated by the dotted line in Fig. 4B), and deactivated at much lower hyperpolarized somatic potentials ($-127$ mV, indicated by the arrow in Fig. 4B) relative to experimental values (Table 3). With this distribution, we could not achieve $\text{Ca}^{2+}$ PIC with features similar to those of experimental measurements regardless of our best attempts to modify the $\text{CaV}1.3$ channel density. Activation of $\text{Ca}^{2+}$ PIC at a potential lower than the recruitment threshold ($-55.5$ mV) caused the cell to start firing at relatively high rates, which normally correspond to the secondary segment of the $F-I$ relationship, and the cell could not produce bistability (Fig. 4C). The peak $\text{Ia}_A$ was also found to be shifted closer to the resting potential and occurred at a potential corresponding to that at which $\text{Ca}^{2+}$ PIC was fully activated (Fig. 4D; compare to Fig. 3A in Lee and Heckman 2000). These results suggested that the majority of the $\text{CaV}1.3$ channels cannot be located at such distal locations from the soma and should be located more proximally.

**Wide band distribution**

Results from the middle uniform density distribution suggested that the $\text{CaV}1.3$ channels may be localized over a limited middle region or be placed more distally. However, results from the distal uniform density distribution showed that
the Ca\textsubscript{v}1.3 channels cannot be located too distally from the soma. Our hypothesis was that there is an overlap between the dendritic spatial distribution of the Ca\textsubscript{v}1.3 channels and that of the Ia-afferent synapses, based on the functional interaction seen experimentally between the dendritic active conductances and synaptic inputs from the Ia afferents (Bennett et al. 1998; Lee and Heckman 2000). The Ca\textsubscript{v}1.3 channels were therefore placed over the dendritic region covered most by the Ia-afferent synapses at dendritic path distance between 300 and 850 μm (0.62 ± 0.21μ; median: 0.61μ; Fig. 5A) from the soma. The density of the channels was uniform (0.14 mS/cm\textsuperscript{2}) and was adjusted to give Ca\textsuperscript{2+} PIC of magnitude within the experimental range (Table 3). The measured Ca\textsuperscript{2+} PIC matched all the features seen experimentally and showed a proper amount of hysteresis (Fig. 5B; Table 3). The F–I relationship also had properties that agreed with experimental recordings (Fig. 5C; Table 3). Furthermore, the effect of Ca\textsuperscript{2+} PIC on the cell firing behavior was appropriate as the maximum firing rate and the difference in firing rates at the same injected current levels (bistability range) were within physiological ranges. Figure 5D shows the Ia \textsubscript{IN} enhancement profile. The hyperpolarized Ia \textsubscript{IN} was within the experimental range.
whereas the peak Ia $I_N$ was within the expected range of synaptic current that is enhanced only by Ca$^{++}$ PIC (Table 3). Figure 6, A and B, respectively, illustrates the changes in the properties of $F$–$I$ and $V$–$I$ relationships attributed to tonic synaptic activation of Ia-afferent synapses. Tonic excitation of the Ia-afferent synapses lowered the transition frequency by 8 Hz, and the activation threshold of the plateau potential at the soma by 3.4 mV relative to the rest condition, in accordance with experimental findings (Bennett et al. 1998).

Moreover, Ca$^{++}$ PIC measured under voltage-clamp conditions using long voltage pulses showed an activation profile very similar to that of experimental data (Fig. 6C). Ca$^{++}$ PIC was activated slowly during the pulse onset with half-activation time of 271 ms (experimental data range 300 ± 120 ms at 50 mV; Fig. 7D in Li and Bennett 2003) and full-activation time of 1 s (Li and Bennett 2003). It was deactivated slowly as well at the termination of the pulse-producing tail current that lasted for 1 s (Fig. 6C, compare to Fig. 7C in Li and Bennett 2003). The tail current, as seen from the soma, results from both the distal location of the Ca$_V$1.3 channels over the dendritic tree relative to the soma, and is considered to be a significant indicator of the location of these channels relative to the soma. We also investigated the changes in the shape of AHP seen after the activation of plateau potentials (Bennett et al. 1998). Figure 6D shows the shape of AHP before (1) and after (2) activation of plateau potentials by intracellular current injection obtained from the model. Comparison of interspike intervals of equal duration showed that there was a reduction in the size of AHP and the appearance of afterdepolarization (indicated by the arrow) in the same manner reported from in vivo measurements (Bennett et al. 1998). The results from this distribution were consistent with the three different and independent sets of experimental measurements, suggesting that the Ca$_V$1.3 channels are distributed over that middle range of the motoneuron dendritic tree where the Ia afferents form the majority of their contacts.

**Customized distribution**

To verify that the Ca$_V$1.3 channels are located at the wide band distribution, we reversed the question by obtaining the channel locations while clamping the soma at the observed experimental values for both the “rest” and “synaptic excitation” conditions as described by Rose et al. (2002, 2003). The membrane potential was directly measured from each compartment in the model, and the relationship between the membrane potential and the distance along each root dendrite was plotted for both the “rest” and “synaptic excitation” conditions, and the intersection of the two plots represented the location of channels that satisfied both experimental conditions. The channels were then placed with uniform density in a region centered around the average intersection site on each root dendrite.

Investigation of 98 terminal dendrites with maximum dendritic lengths varying from 400 to 1,800 μm was completed, and the intersection of their plots was obtained (only 73 showed intersections). The location of the channels was found to be at dendritic path distance of 551.9 ± 159.7 μm from the soma. This location was common on most of the root dendrites because the median was 510.6 μm, showing little scatter in the data from all terminal dendrites. This is the center of the region covered by the majority of Ia-afferent synapses and is the same region that was previously described in the wide band distribution.

Terminal dendrites that did not show intersection of plots were characterized by having the Ia synapses located primarily proximal to the soma (up to 500 μm from the soma). This localization of the Ia synapses caused the membrane potential trace along the dendritic length during the Excitation condition to decay before crossing the trace during the Rest condition. Conversely, Fig. 7A shows the intersection sites of the membrane potential profiles along the dendritic length for two different dendritic segments that showed intersection of their plots. The distribution of synapses along the dendritic segment is indicated by the black dots at the top of each graph. The
intersection site for each dendrite was dependent on the synaptic distribution on that dendrite. Figure 7B compares the distribution of Ia-afferent synapses (same data in Fig. 1B) and the distribution of the CaV1.3 channel locations obtained from the plot-intersection method. The distribution of the CaV1.3 channels corresponds to the synaptic distribution on the dendritic tree and is primarily localized to an intermediate wide band at dendritic path distances from 300 to 900 μm from the soma. The normal distribution of the channel locations (indicated by the thin line in Fig. 7B) is similar to that of the Ia-synapse locations (indicated by the thick black line in Fig. 7B). Both distributions peak at a dendritic distance of 500 μm from the soma.

The spatial width of the CaV1.3 channel regions was varied around the intersection site on each root dendrite to obtain proper amplitude of Ca\(^{2+}\) PIC. It was possible to get acceptable magnitudes of Ca\(^{2+}\) PIC only when the width of the region containing the CaV1.3 channels was at least 300 μm centered around the average intersection site for each root dendrite (0.235 mS/cm\(^2\)) in the model of our MG motoneuron. With this distribution, the measured Ca\(^{2+}\) PIC showed a proper amount of hysteresis (Fig. 7C) and was very similar to that measured from the wide band distribution (Fig. 5B).

Spatiotemporal measurements of Ca\(^{2+}\) current

The wide band distribution was then used to study the spatiotemporal characteristics of the membrane potential (Fig. 8B, top graph) and Ca\(^{2+}\) current (Fig. 8B, bottom graph) along the length of a dendritic branch and at different points in time during repetitive cell firing in response to excitation of the Ia-afferent synapses (Fig. 8A). Ca\(^{2+}\) current had its maximum amplitude during the repolarization phase of the AP (Fig. 8B, bottom graph, trace c), and not the peak of AP at which maximum depolarization of the soma membrane takes place. This could explain the appearance of afterdepolarization and reduction of the AHP seen after the activation of plateau potentials (Fig. 6D2; Bennett et al. 1998).

**DISCUSSION**

Distribution of CaV1.3 channels over the dendritic tree

Our results suggest that the CaV1.3 channels located on the dendrites of spinal motoneurons do not uniformly cover the whole dendritic tree. Rather, they are preferentially localized within the Ia-synaptic territory, a region that lies approximately 300 to 850 μm (about 0.6A) from the somata of MG motoneurons. This finding provides a very likely explanation for the functional interaction seen experimentally between the dendritic active conductances and synaptic inputs. Bennett and colleagues (1998) showed that motoneuron excitation through synaptic activation, as opposed to intracellular current injection, alters the somatic threshold of plateau potentials. Measurements of Ia \(I_N\) in the decerebrate preparation, which has tonic activity in the monoaminergic axons originating from the brain stem (Baldissera et al. 1981; Lee and Heckman 1998a), revealed strong enhancement of the synaptic current by PICs (Kuo et al. 2003; Lee and Heckman 2000; Powers and Binder 2000). The strong influence of synaptic inputs on the activation threshold of PICs as seen from the soma supports the spatial overlap between the dendritic distributions of the PIC-mediating channels and the synaptic inputs suggested by the present study.

Different dendritic distributions of CaV1.3 channels were tested in the model. For each distribution, the model behavior...
location of the dendritic wide band (nearly 0.6λ) from the soma. Thus, the soma will not be greatly affected by the channel distribution within that band, but will be affected primarily by the total current reaching it. Accordingly, the channel density will be the only variable affected by the type of distribution because it determines the proper magnitude of the Ca$^{2+}$ PIC that is obtained. Moreover, our results do not reject the presence of the CaV1.3 channels in both proximal and distal dendrites, but confirm that their highest density appears to be within the Ia-synaptic territory region. Thus our main finding is that the distribution of the CaV1.3 channels is similar to that of Ia-synaptic inputs and these channels are primarily localized within the Ia-synaptic territory.

Simulations done by Rose et al. (2002, 2003) on reconstructed spinal motoneurons innervating the neck extensor muscles of the adult cat suggested that CaV1.3 channels are localized in a single 100-μm aggregation, termed “hot spot,” on each dendritic branch. These “hot spots” were found to start at dendritic distances between 150 to 400 μm from the soma. The CaV1.3 channels at these locations mimicked the change in threshold for plateau potentials caused by tonic synaptic excitation. This localization has recently been implemented in a model by Taylor and Enoka (2004a,b) focused on assessing the patterns of motor unit discharge. The technique described by Rose et al. (2002, 2003) was used as an independent validation tool for the findings of the present model. However, a number of differences between the present model and that of Rose et al. (2002, 2003) should be noted. Only CaV1.3 channels were used in the aforementioned model, and the distribution of Ia synapses was uniform throughout the dendritic tree. In the current study we extended the investigation by: 1) incorporating other channels to examine the model firing behavior, 2) using realistic distribution of Ia-afferent synapses, and 3) comparing the results to several different and independent sets of experimental data. Our results showed that the technique of using intersections of the membrane potential profiles along the length of dendritic segments during “rest” and “synaptic excitation” for localizing the CaV1.3 channels is inadequate for determining the location of these channels and is contingent on the pattern of synaptic distribution over the dendritic tree. Some dendritic segments did not show intersection of their plots because of the distribution of the Ia synapses on their segments. The membrane potential profile along the dendritic length under the “rest” condition was in general similar in all the dendrites, although the dendritic depolarization under the “synaptic excitation” condition was dependent on the distribution of synapses over that dendrite (see Fig. 7A). Having a realistic distribution of synapses over the motoneuron dendritic tree is essential for obtaining correct results using this technique.

Our results are also in agreement with previous modeling and experimental studies, which suggested that CaV1.3 channels should be included distally on the dendritic tree to reproduce experimental observations. In a model with only L-type Ca$^{2+}$ channels and no synapses, Carlin et al. (2006b) were unable to achieve late-onset and hysteretic Ca$^{2+}$ currents close to what is seen experimentally unless the CaV1.3 channels were placed distally starting from the third branch point (which varies between 15 and 1,600 μm from the soma). Bennett et al. (1998) predicted that inward currents underlying the plateau potentials in MG motoneurons should arise at locations around 0.5A from the soma to be consistent with their measurements.
The average location of Ia-afferent synapses is $0.57 \pm 0.36 \mu m$, whereas the suggested wide band of the CaV1.3 channels in this study is located at $0.62 \pm 0.21 \mu m$. Finally, using antibody labeling in turtle motoneurons Simon et al. (2003) observed that CaV1.3 channels were always located at synaptic sites, but not all synapses were associated with these channels. However, with immunohistochemical labeling techniques, it is usually difficult to differentiate between channels related to the motoneuronal structure and those located around them in presynaptic terminals.

Distribution of synaptic inputs over the dendritic tree

One could argue that the model represents only synaptic input from the monosynaptic Ia-afferent system and that the dendritic tree may be widely covered by synaptic contacts from other systems resulting in a uniform distribution of synaptic input. The overall distribution of group Ia-boutons does not cover the entire dendritic tree of motoneurons but is confined to a roughly rectangular 3D region (the Ia-synaptic territory). This region represents the intersection of Ia-collateral trajectories and the radially organized motoneuron dendrites, and contains 95% of the identified Ia-contacts (Burke and Glenn 1996; Segev et al. 1990). However, multiple studies suggest that synaptic contacts, in general, do follow the profile formed by the Ia inputs. The overall spatial distribution of the inhibitory synapses from Renshaw cells on the dendritic tree of cat lumbar motoneurons was found to be between 65 and 706 $\mu m$ from the soma, which closely resembles the distribution of excitatory Ia-afferent synapses (Fyffe 1991). Furthermore, results from ultrastructural studies on quantitative synaptology in different types of cat MG motoneurons showed that the total number of synaptic boutons from all different inputs is larger at 300 $\mu m$ than at 100 and 700 $\mu m$ from the soma in all motoneuron types (Brannstrom 1993).

More strikingly, serotonergic contacts on the dendrites of spinal motoneurons have their maximum number in the same region as that suggested for the CaV1.3 channels in the present study (see Fig. 4B in Alvarez et al. 1998), which is consistent with their modulatory effects on these channels (Berger and Takahashi 1990; Hounsgaard and Kiehn 1989). This shows that the spatial distribution of all synaptic contacts mimics that of the Ia afferents’ and peaks within the suggested location for the CaV1.3 channels. Therefore, $Ca^{++2}$ PIC would equally enhance synaptic inputs from various systems resulting in near-linear summation, as has been observed experimentally (Powers and Binder 2000; Prather et al. 2001). Distal synaptic inputs, which appear to be much fewer in number, would get “boosted” by the Ca$^{++2}$ PIC once they reach the location of the CaV1.3 channels.

Model considerations

One could contend that the modeling parameters have been adjusted so that a reference set of experimental data was mimicked. Thus the suggested distribution of the CaV1.3 channels in this study is not the only solution because other parameters may equally mimic experimental data. However, the technique used in the customized distribution to predict the location of the CaV1.3 channels provided an independent validation method of the predicted results. Single intersection sites of the membrane potential profiles were found on each dendritic branch indicating only one location of those channels on that branch that could satisfy experimental data. Moreover, the model behavior was compared to multiple independent sets of experimental measurements. Therefore, we believe that the suggested distribution of CaV1.3 channels is reasonably accurate, but has to be directly confirmed by appropriate labeling of these channels.

In summary, computer simulations in the present study allowed testing various dendritic spatial distributions of the CaV1.3 channels in spinal motoneurons. Our results point out the strong correlation between the spatial distribution of CaV1.3 channels and synaptic inputs over the dendritic tree of motoneurons. This overlap may result in the functional interactions seen experimentally between these two systems. The detailed role of CaV1.3 channels in generating plateau potentials and in enhancing and integrating synaptic inputs is currently under investigation.
CALCIUM DYNAMICS. The intracellular Ca\textsuperscript{2+} concentration (measured in mM) in the soma depends on the total compartmental Ca\textsuperscript{2+} current $I_{Ca}$ according to the following balance equation (Booth et al. 1997; McIntyre and Grill 2002).

$$d[Ca]/dt = f [-\alpha \times I_{Ca} - k_c \times [Ca]]$$  
(A15)

$$f = 0.01 \quad \alpha = 1 \text{ mol/mC/cm}^2 \quad k_c = 8 \text{ ms}^{-1}$$  
(A16)

$[Ca^{2+}] = 2 \text{ mM}$  
(A17)

where $f$ is the percentage of free to bound Ca\textsuperscript{2+} (set to 0.01 based on report from Helmcen et al. 1996). The parameter $\alpha$ converts the total $I_{Ca}$ to Ca\textsuperscript{2+} concentration. $k_c$ is the Ca\textsuperscript{2+} removal rate, where Ca\textsuperscript{2+} is removed by uptake into internal stores or by pump extrusion (Booth et al. 1997). In the soma, the total Ca\textsuperscript{2+} current is mediated by the N-type Ca\textsuperscript{2+} channels. $[Ca^{2+}]$, is the extracellular Ca\textsuperscript{2+} concentration, and $[Ca^{2+}]_{in \alpha}$ is the intracellular Ca\textsuperscript{2+} concentration at time $= 0$.

The reversal potential for Ca\textsuperscript{2+} was calculated from Nerst equation as follows

$$E_{Ca} = [(R \times (I / [Z / F])) / \ln ([Ca^{2+}] / [Ca^{2+}])]$$  
(A18)

$$R = 8.31441 \text{ V} \cdot \text{mol}^{-1} \cdot \text{K}^{-1} \quad T = 36^\circ \text{C} + 273.15$$  
(A19)

$Z = 2 \quad F = 96,485.309 \text{ Cmol}^{-1}$  
(A20)

where $R$ is the gas constant, $T$ is the absolute temperature on the Kelvin scale, $Z$ is the valence for Ca\textsuperscript{2+}, and $F$ is Faraday's constant. The factor 1,000 is used to convert $E_{Ca}$ to mV.

CA\textsuperscript{2+}-DEPENDENT K\textsuperscript{+} CHANNELS. The calcium-dependent potassium channel [K(Ca\textsuperscript{2+})] is activated according to the following Hill expression (Booth et al. 1997).

$$I_{KCa2+/3} = \tilde{g}_{KCa2+/3} \times ([Ca^{2+}] / [Ca^{2+}]) \times (V_m - E_k)$$  
(A21)

$$\tilde{g}_{KCa2+/3} = 0.02 \text{ Scm}^{-2} \quad K_d = 0.0005 \text{ mM} \quad E_k = -80 \text{ mV}$$  
(A22)

where $[Ca^{2+}]$ is the intracellular calcium concentration, and $K_d$ is the half-saturation level.

N-TYPE CA\textsuperscript{2+} CHANNELS.

$$I_{CaN} = \tilde{g}_{CaN} \times m^h \times h \times (V_m - E_{Ca})$$  
(A23)

$$\tilde{g}_{CaN} = 0.01 \text{ S/cm}^2$$  
(A24)

$$\tau_m = 15 \text{ ms}$$  
(A25)

$$m_i = 1/\left[e^{(V_m + 55)/8} + 1\right]$$  
(A26)

$$\tau_h = 50 \text{ ms}$$  
(A27)

$$h_i = 1/\left[e^{(V_m + 38)/9} + 1\right]$$  
(A28)

Initial segment and axon hillock channels

FAST NA\textsuperscript{+} CHANNELS.

$$I_{Na} = \tilde{g}_{Na} \times m^h \times h \times (V_m - E_{Na})$$  
(A29)

$$\tilde{g}_{Na} = 1.34 \text{ Scm}^{-2} \quad E_{Na} = 50 \text{ mV}$$  
(A30)

$$\alpha = \left[-0.4 \times (V_m + 55)\right] / \left[e^{(V_m + 55)/9} + 1\right]$$  
(A31)

$$\beta = \left[0.4 \times (V_m + 33)\right] / \left[e^{(V_m + 33)/9} + 1\right]$$  
(A32)

$$\tau_m = 30\left[e^{(V_m + 60)/15} + e^{(V_m + 80)/15}\right]$$  
(A33)

$$h_i = 1/\left[e^{(V_m + 55)/9} + 1\right]$$  
(A34)

PERSISTENT NA\textsuperscript{+} CHANNELS.

$$I_{NaP} = \tilde{g}_{NaP} \times m^h \times (V_m - E_{Na})$$  
(A35)

$$\tilde{g}_{NaP} = 0.033 \text{ Scm}^{-2} \quad E_{Na} = 50 \text{ mV}$$  
(A36)

$$\alpha = \left[-0.353 \times (V_m + 21.4)\right] / \left[e^{(V_m + 21.4)/9} + 1\right]$$  
(A37)

$$\beta = 0.000883 \times (V_m + 25.7) \left[e^{(V_m + 29.7)/9} + 1\right]$$  
(A38)

DELAYED RECTIFIER K\textsuperscript{+} CHANNELS.

$$I_{K} = \tilde{g}_{K} \times n^h \times (V_m - E_{K})$$  
(A39)

$$\tilde{g}_{K} = 0.17 \text{ Scm}^{-2} \quad E_{K} = -80 \text{ mV}$$  
(A40)

$$\tau_n = 5/\left[e^{(V_m + 55)/9} + 1\right]$$  
(A41)

$$n_i = 1/\left[e^{(V_m + 38)/9} + 1\right]$$  
(A42)

Dendritic channels

LVA L-TYPE CA\textsuperscript{2+} (Ca\textsubscript{V,1.3}) CHANNELS.

$$I_{CaV} = \tilde{g}_{CaV} \times l \times (V_m - E_{Ca})$$  
(A43)

$$\tilde{g}_{CaV} = \text{ see results for values of each distribution} \quad E_{Ca} = 60 \text{ mV}$$  
(A44)

$$\tau_i = 60 \text{ ms}$$  
(A45)

$$I_c = 1/\left[e^{(V_m + 45)/9} + 1\right]$$  
(A46)

Dendritic synaptic inputs

The synaptic current $I_{syn}$ resulting from the activation of individual synapses of different systems, was modeled as a battery $E_{syn}$ representing the equilibrium potential of the synapse, in series with a time-varying conductance $g_{syn}$. This conductance was described by an alpha function (Jones and Bawa 1997; Rall 1967; Segev et al. 1990) with time to peak ($\tau_{syn}$) as follows

$$I_{syn}(t) = \tilde{g}_{syn}(t) \times (V_m - E_{syn})$$  
(A47)

$$\tilde{g}_{syn}(t) = \tilde{g}_{syn} \times [\tau_{syn} \times e^{-(t-t_{mech})}]$$  
(A48)

$$\tau_{syn} = 0.2 \text{ ms} \quad \tilde{g}_{syn} = 0.0033 \text{ Scm}$$  
(A49)

ACKNOWLEDGMENTS

We thank Dr. Ted N. Carnevale for technical assistance with NEURON code and Drs. Marc D. Binder, C. J. Heckman, and Randall K. Powers for valuable discussions and reading an early version of this manuscript.

GRANTS

Funding for this work was provided by Alberta Heritage Foundation for Medical Research, Canadian Institutes of Health Research, and National Institutes of Health to V. Mushahwar.

REFERENCES


J Neurophysiol • VOL 94 • DECEMBER 2005 • www.jn.org


