Simulation Of Dendritic L-Type Ca Channels' Warm-Up Phenomenon In Spinal Motoneurons

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***Abstract*— In this study a computer-based model was developed to simulate the warm-up property of the low-voltage activated (LVA) L-type calcium channels. The LVA L-type calcium channel is one of two channels that mediate persistent inward currents in spinal alpha (α) motoneurons and plays an important role in integrating & modulating motor firing behaviors. This study was prompted by experimental observations that showed nonlinear and hysteretic behaviors of the LVA L-type calcium channel in a membrane patch, indicating that these nonlinear properties are part of the channel behavior. In this work, we introduce a new model for the LVA L-type calcium channel that incorporated the warm-up property. The new model has successfully reproduced experimental recordings from the membrane patch and from the whole cell. Our work provides a more accurate model of the LVA L-type calcium channel that could be used to reexamine the dendritic distribution of these channels on α-motoneurons.**

Keywords*— *L-type calcium channel, Facilitation, Warm-up, Motoneurons.

I. Introduction

Motor neurons (motoneurons) innervate skeletal muscles and, therefore play an essential role in shaping the motor output of the nervous system. Motoneurons integrate the sensory and motor inputs to generate firing rates that control a wide range of motor activities, such as standing, walking and running. One of the main intrinsic features that enable the motoneurons to play this role is that they have ion channels that exhibit persistent inward currents (PICs). These PICs maintain the cell's state of firing for long periods of time even in absence of excitatory synaptic inputs [1]–[5]. PICs are activated by excitatory synaptic inputs and deactivated by inhibitory synaptic inputs providing a powerful means to amplify both types of synaptic inputs in the motoneurons [4]. They enhance and modulate the input-output gain of the whole motor pool. PICs are important for normal motor function, but the lack of their inhibitory control after spinal cord injury could result in abnormal motor behaviors such as spasticity. The lost inhibitory control over these channels result in prolonged PIC activation that cause sustained cell firing and uncontrolled long-lasting muscle contraction.

PICs in motoneurons are mediated by persistent sodium channels [6], [7] and low-voltage–activated L-type calcium channels [8], [9]. The sodium channels are characterized by partial inactivation and fast deactivation characteristics, whereas the L-type calcium channels are characterized by low-voltage-activation threshold, high conductances, no inactivation and slow deactivation characteristics [10]. These characteristics make the L-type calcium channels the main mediator of PICs. The precise spatial distribution of the L-type calcium channels within the motoneurons is still unclear. Computer simulation studies [11], [12] suggested a dendritic location of such channels on a mid-band region on the dendritic tree. However, immunohistochemical studies for the L-type calcium channels shows that these channel do exist on the cell soma and proximal dendrites as well [9], [13]–[16].

It has been recently shown that L-type calcium channels do exist on the soma, and that they exhibit nonlinear hysteretic behaviors [17]. Isolated cell membrane patches were shown to exhibit very slow deactivation for the L-type calcium channels, which results in a prolonged Ca^{+2} currents (tail currents) even after the termination of excitatory inputs. Amplitude and duration of these tail currents depend on the prepulse voltage that was applied to the L-type calcium channel [17]. That study revealed that the L-type calcium channels undergo facilitation/warm-up property, such that the amplitude and the duration of the tail currents are a function of the prepulse voltage as well as the pulse width. Moreover, a large variability in the channel response was observed, such that the tail currents did not always have the same amplitude and duration, but they all exhibit the same qualitative shape. The L-type calcium channels warm-up property may potentially account for many non-linear hysteretic cell behaviors, that were thought to rely only on the dendritic distribution of the channel.

The goal of this study is to develop a new L-type calcium channel model that includes the warm-up property. The new channel model revises the channel model developed in [11] to reexamine the L-type calcium channel dendritic distribution in spinal α-motoneurons.

This work was funded by US NIH-NINDS, Grant no. NS091836, to S.M.E

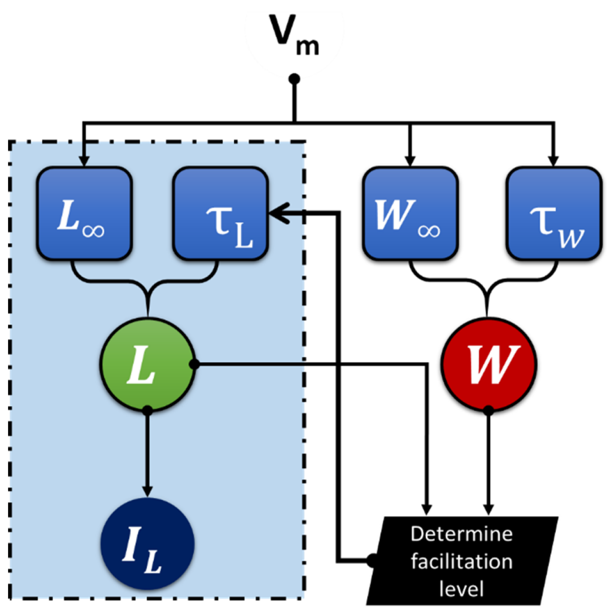

Fig. 1. Showing the original channel model on the left with light blue background, and on the right, the new introduced "W" gating variable to represent the graded facilitation property. Both gating variables are processed to determine the facilitation state.

II. Methodology

A. Model Morphology & Biophysical Properties

The modeling in this work was developed using the NEURON simulation environment (version 7.4) [18]. The cell model was based on that published in [11], [19], which is for a fast fatigue resistant (FR) α-motoneuron of an adult cat. The cell model consisted of 731 compartment which represents the three-dimensional (3D) structure of the FR-motoneuron. The FR-motoneuron model consisted of cell soma, axon hillock (AH), initial segment (IS), and the dendritic tree.

The passive biophysical properties were set according to published values [19]–[21] for the same cell type. The specific membrane resistance (R_m) where set to 225 Ω for the soma, AH, and IS and 11,000 Ω for the dendrites. The specific membrane capacitance (C_m) was set to 1µF/cm^2 and the specific axial resistance (R_a) to 70 Ω.cm for all the cell model compartments.

The active properties were simulated by adding voltage gated ion channels to the soma, including fast sodium channels (Naf), delayed rectifier potassium channels (Kdr), calcium-activated potassium channels [K(Ca)], and N-type calcium channels (CaN, $Ca_V2.2$). These active channels are responsible for generating action potentials. The channels kinetics parameters and conductances were verified by matching published data [1], [22], [23].

B. L-type Calcuim Channel Basic Model

Based on the observations published in [17], a new L-type calcium channel model was developed to simulate the warm-up property for the L-type calcium channels. First we introduce the basic model developed in [11]. This model did not include the warm-up property. Second, we show the modifications made to the basic model in order to simulated the warm-up property.

The current equation for the basic channel model is shown in (1)

$$I_L = g_{max} * L * (V_m - E_L) \qquad (1)$$

Where I_L is the resulting ion current, g_{max} is the maximum conductance of the channel, L is the channel activation gating variable that represents the activation level of channel, V_m is the membrane potential, and E_L is the reversal potential for calcium. The activation gating variable L is calculated through the first order differential equation shown in (2).

$$dL/dt = (L_\infty - L)/\tau_L \qquad (2)$$

Where L_∞ is a voltage dependent variable that represents the steady state activated channels percentage at a given membrane voltage, and τ_L is the channel time constant which represents how fast a given channel could activate or deactivate.

The blue box in Fig. 1 represents the basic channel model published in [11].

C. L-type Calcium Channel Warm-Up Model

The L-type calcium channel warm-up property was described as a kinetic function of the channel history. The L-type calcium channels exhibit tail currents (i.e.: lag in channel deactivation) based on the history of the voltage, that was applied to that channel. For example, a one second prepulse voltage-step would cause the channel to exhibit a tail current after a voltage-step pulse, which the channel would not exhibit in case of no prepulse voltage step. These tail currents are variable in amplitude and duration based on the channel voltage history applied to it.

A new gating variable (state) W was included to represent the degree of facilitation for the L-type calcium channels (i.e., the warm-up state). The W state varies between 0 and 1 under certain conditions, such as prolonged channel activation or prolonged depolarization, to represents how much a channel is facilitated. The facilitation level for a channel determines the duration of the tail current the channel would exhibit.

The gating variable W is calculated through the first order differential equation (3).

$$dW/dt = (W_\infty - W)/\tau_W \qquad (3)$$

Where W_∞ is a voltage dependent variable that determines the steady state warm-up level. τ_W is a voltage dependent time constant that determines the duration required for a channel to reach a facilitated state. Both W_∞ & τ_W are function of the cell membrane voltages. τ_W voltage activation curve was designed to give fast facilitation times at high voltage potentials.

The complete channel model is shown in Fig. 1, where both gating variables L & W are calculated simultaneously. The values of both gating variables L & W are then used to determine the facilitation level of the channel. At high levels of facilitation, the channel time constant τ_L is altered to slow down the channel deactivation process; keeping the channel open to generate tail currents. The duration of tail currents is dependent on the facilitation level of the L-type calcium channel.

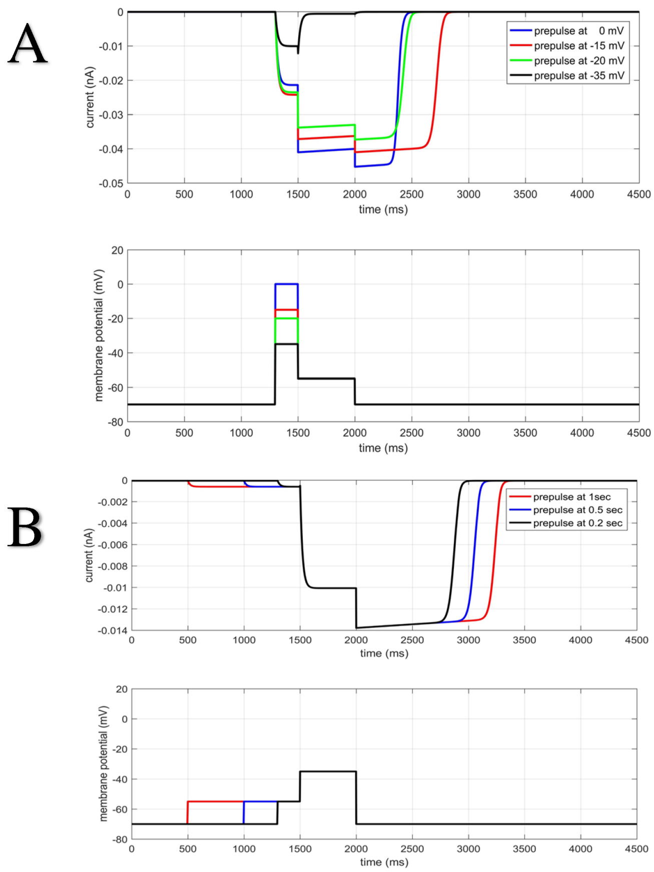

Fig 2. Shows the introduced L-type calcium channel model with the facilitation property under a membrane patch voltage clamp simulation, the simulation show that tail current duration is dependent on the prepulse voltage and pulse width. A: shows tail currents that are evoked by a long prepulse. B: shows tail currents evoked by short high voltage prepulse.

All channel kinetic parameters (τ_L , L_∞ , τ_W, W_∞), tail current min-max length and activation curves were constrained based on experimental values in literature [8], [17], [23], [24].

D. *L-Type Calcium Channel Variable Response.*

It has been observed experimentally that the L-type calcium channels have high variability in tail currents amplitude and duration across trials [17]. To model such channel variability two independent uniform random number generators were used. The first random number generator controlled the tail current amplitude, whereas the second controlled the tail current duration. The parameters of the two random generators were adjusted to produce tail currents with amplitudes and durations within the experimental range [17].

III. Results and Discussion

Our work was verified in a two-step testing strategy. First, the new channel model was tested in a simulation of membrane patch to simulate the behaviors shown at the channel level. Second, the new channel model was verified in simulations at the cell level. Using our new channel model, we were able to generate tail currents.

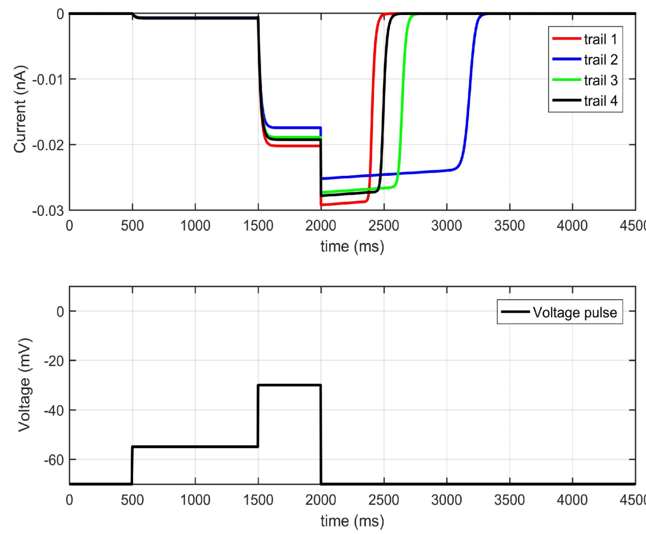

Fig 3. Shows the variability in the simulation results when a cell membrane patch model is tested with the same voltage pulse multiple times. The test shows variability in the channel amplitude and tail current length.

A. *Patch Simulations*

In a computer model, we simulated a cell membrane patch, and a single electrode voltage clamp test was conducted with 500 ms voltage step pulse, and four different voltage prepulses. This simulation tested the effect of varying the prepulse amplitude on the tail current amplitude and duration generated from the new channel model (Fig. 2A). The model reproduced successfully the experimental data recorded in Moritz et al. [17] Fig. 4A.

We also tested the effect of the prepulse duration on the new channel model. Three voltage step prepulses of different pulse width were tested (Fig. 2B). The model produced the experimental behaviors recorded in Moritz et al [17]. (compare

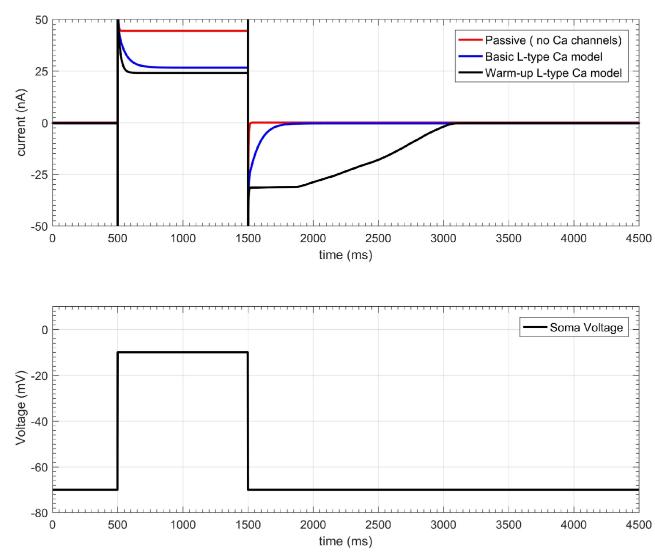

Fig 4. Shows the whole FR-motoneuron single electrode voltage clamp current response when applied with a voltage step clamp for 1S. the trace in blue shows the basic channel model cell response, the red trace shows the cell current response with no channels, and black trace shows the cell response with the new channel model that include warm-up property.

Fig. 2B with [17] Fig. 4B). A longer prepulse was able to delay the channel deactivation for longer durations.

To constrain the channel variability properties, the new channel model was tested with the same voltage pulse while keeping the random number generators activated (Fig 3). The new channel model once again reproduced variability in the tail current amplitude and duration within the same range of experimental data [17].

B. *Cell Simulations*

In this section, we verified the new channel model in the FR-motoneuron with a whole uniform distribution (L-type calcium channels covered all dendrites). A single electrode voltage clamp experiment was simulated, where a voltage step pulse was applied to the cell soma, and the current driven by the voltage clamp was measured. The results were compared to the basic channel model with the same simulation parameters. Different voltage levels were applied on the soma of the FR-cell, and current was recorded as shown in Fig. 4. Unlike the basic channel model that was used before, the new channel model simulation replicated experimental data in [17] (compare [17] Fig. 2 with Fig. 4).

The channel variability was also found not to alter the whole cell response as shown experimentally, unlike cell membrane patches, the whole motoneuron response is shown to be consistent. The results of FR-motoneuron with a variable channel response showed that random variability in tail current amplitude and duration cancel out each other, leading to a consistent cell response between trails.

IV. CONCLUSION AND FUTURE WORK

In this work, we introduced a new L-type calcium channel model that was able to simulate different experimental results with many nonlinear properties. The new channel model reproduced the variability in tail currents observed in experimental data with both variable amplitude and duration voltage prepulse.

The L-type calcium channel property was shown to affect the whole cell model behavior, indicating that the dendritic location of these channels is not only the main cause of the nonlinear cell behaviors.

Based on these results the next step is to reexamine the dendritic distribution of the L-type calcium channel in motoneurons. Using a more accurate model of the channel.

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