Suppressing the excitability of spinal motoneurons by extracellularly applied electrical fields: insights from computer simulations

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ElBasiouny SM, Mushahwar VK. Suppressing the excitability of spinal motoneurons by extracellularly applied electrical fields: insights from computer simulations. J Appl Physiol 103: 1824–1836, 2007. First published August 16, 2007; doi:10.1152/japplphysiol.00362.2007.—The effect of extracellularly applied electrical fields on neuronal excitability and firing behavior is attributed to the interaction between neuronal morphology and the spatial distribution and level of differential polarization induced by the applied field in different elements of the neuron. The presence of voltage-gated ion channels that mediate persistent inward currents (PICs) on the dendrites of spinal motoneurons enhances the influence of electrical fields on the motoneuronal firing behavior. The goal of the present study was to investigate, with a realistic motoneuron computer model, the effects of extracellularly applied electrical fields on the excitability of spinal motoneurons with the aim of reducing the increased motoneuronal excitability after spinal cord injury (SCI). Our results suggest that electrical fields could suppress the excitability of motoneurons and reduce their firing rate significantly by modulating the magnitude of their dendritic PIC. This effect was achieved at different field directions, intensities, and polarities. The reduction in motoneuronal firing rate resulted from the reduction in the magnitude of the dendritic PIC reaching the soma by the effect of the applied electrical field. This reduction in PIC was attributed to the dendritic field-induced differential polarization and the nonlinear current-voltage relationship of the dendritic PIC-mediating channels. Because of the location of the motoneuronal somata and initial segment with respect to the dendrites, these structures were minimally polarized by the applied field compared with the extended dendrites. In conclusion, electrical fields could be used for suppressing the hyperexcitability of spinal motoneurons after SCI and reducing the level of spasticity.

spasticity; persistent inward current; polarization

Over the past two decades experimental recordings have revealed the presence of voltage-gated ion channels over the dendrites of spinal motoneurons (10, 34, 35, 44, 50). Some of these dendritic channels mediate a persistent inward current (PIC), which is a depolarizing current that does not inactivate with prolonged membrane depolarization. This dendritic PIC plays a fundamental role in determining the response of the motoneuron to synaptic inputs and in shaping the motor output. After spinal cord injury (SCI), activation of PICs in motoneurons is no longer under the control of descending drive (e.g., lack of inhibition), leading to increased motoneuronal excitability and firing rate, muscle spasms, and long-lasting reflexes in response to afferent inputs (45, 54).

Electrical fields have been used previously to study the electrical properties and firing behavior of neurons in the brain (11, 12) and spinal cord (SC) (3, 14, 15, 35, 52, 53). In the present study, we investigated the application of electrical fields to reduce spasticity after SCI. The degree of differential polarization induced in different elements of a neuron exposed to an electrical field is determined by the relationship between the applied field properties (e.g., intensity, polarity, and direction) and the dendritic morphology and orientation relative to the imposed field, the internal resistance of the cell, and the spatial distribution, density, and type of dendritic voltage-gated ion channels (35). The net effect of field-induced polarization on the firing behavior of the neuron is critically dependent on the position of the soma and initial segment (sites of spike initiation) with respect to the dendrites, and hence their location relative to the neutral point (where no polarization takes place). Actively firing pyramidal or Purkinje cells, with somata positioned asymmetrically relative to their dendrites, experience an increased firing rate during a soma-depolarizing field and a reduced firing rate during a soma-hyperpolarizing field (4, 11). Spinal motoneurons, on the other hand, have dendrites distributed around a central soma, and their spike initiation structures experience little polarization compared with that of their dendrites. This suggests that their excitability could not be modulated effectively by electrical fields similarly to brain cells. However, modulation of the activation level of their dendritic voltage-gated conductances by electrical fields could provide an alternative means for regulating their excitability.

The goal of the present study was to investigate, with computer simulations, the effect of applied direct current (DC) and alternating current (AC) electrical fields on the magnitude of the dendritic PICs and the resulting excitability of spinal motoneurons. This would be beneficial in suppressing the increased excitability of motoneurons following SCI and the emergence of spasticity during which the PIC is fully activated. Our results demonstrate that application of weak DC or AC electrical fields could suppress the excitability of motoneurons and reduce their firing rate. The reduction in firing rate was due to the induced reduction in the magnitude of the dendritic PIC reaching the soma by the applied field. The reduction in PIC was achieved in both the depolarized and hyperpolarized segments of the dendrites because of the nonlinear current-voltage (I-V) relationship of the dendritic PIC-mediating channels. The results of the present study propose application of electrical fields as an effective clinical therapy to alleviate spasticity after SCI. Part of this work was previously presented in abstract form (19).
METHODS

A morphologically detailed computer model of a spinal α-motoneuron was developed to investigate the effect of electrical fields on the excitability and firing behavior of motoneurons. This model incorporated realistic motoneuron morphology, realistic dendritic distribution of synaptic inputs, and a dendritic distribution of ionic channels that closely reproduced several electrophysiological recordings from spinal motoneurons (17).

Model structure. A full description of the model motoneuron morphology, biophysical properties, and verification is provided by ElBasyouny et al. (17). Briefly, a compartmental cable model of a cat motoneuron was developed that had full representation of the α-motoneuron structure, consisting of soma, initial segment, axon hillock, dendritic tree, and a myelinated axon. The model dendritic tree was based on the three-dimensional (3D) detailed morphology for type-identified triceps surae motoneurons [fatigue-resistant (FR) type, medial gastrocnemius (MG) motoneuron, identified as cell 43/5 by Cullhlem et al. (13)]. The model was developed with a NEURON simulation environment (30). The passive parameters determining the cable properties of the motoneuron were set based on previous studies for the same 43/5 FR motoneuron (13, 20). Somatic and initial segment voltage-gated ion channels determining the motoneuron active properties included fast and persistent Na+, delayed rectifier K+, Ca2+-activated K+, and N-type Ca2+ channels. Channel densities were set such that the model active properties were within the 95% confidence range of experimental measurements from cat α-motoneurons, and the model generated action potentials and afterhyperpolarization potentials with characteristics similar to empirical recordings (17).

The myelinated axon was based on the model developed by McIntyre and Grill (47) in which nodes of Ranvier and myelin were electrically represented. The myelinated axon had a fiber diameter of 14 μm, 10 nodes of Ranvier, and a conduction velocity of 83 m/s, which is comparable to that of FR motoneurons (33).

Dendritic channels and synaptic inputs. The dendritic low-voltage-activated L-type calcium (CaV1.3 type) channels that mediate Ca2+ PIC were distributed over a region between 300 and 850 μm reaching the soma as more synapses were activated. Given that involuntary muscle spasms and exaggerated tendon reflexes are usually triggered by mild sensory stimulation (e.g., rubbing or cooling of the skin, limb movements, or muscle contraction), activation of the motoneuron by synaptic inputs was simulated in the present model. The synapses were distributed over the dendritic tree based on the realistic distribution of Ia afferent-to-motoneuron contacts from cat FR motoneurons (8, 9, 24). Activation of the Ia afferent system through tendon vibration was simulated by activating the Ia-afferent synapses asynchronously at 180 Hz while adjusting the synapse conductances to give Ia effective synaptic current (Ia Is) of 4.8 nA at resting potential (17). To allow for systematic gradation of the Ia synaptic input, the total number of Ia synapses was divided into eight nearly equal groups, randomly distributed on the dendrites, that were then activated sequentially (16). This activation pattern resulted in a linear increase in the Ia Is reaching the soma as more synapses were activated. Given that the distribution of different synaptic inputs appears to be effectively similar to that of the Ia afferents [for discussion see ElBasyouny et al. (17)], synaptic input from systems other than the Ia afferents was simulated by increasing the synapse conductances to generate higher synaptic currents (16). This allowed for the examination of the motoneuronal firing behavior in response to different levels of synaptic inputs and the frequency-current (F-I) relationship was constructed (e.g., Fig. 6A, top).

Hyperexcitability of motoneurons during spasticity. The goal of the present study was to examine the application of electrical fields for reducing the level of spasticity resulting from the increased excitability of spinal motoneurons after SCI. During spasticity, spinal motoneurons become hyperexcitable and their PIC, which is responsible for the generation of high and sustained motoneuronal firing, long-lasting reflexes, and muscle spasms, becomes fully activated by synaptic inputs (26–29, 45, 46, 54). To simulate this condition, the model motoneuron was synaptically activated at 300% of the strength of the Ia-afferent system (Ia Is = 14.6 nA). This level of synaptic input resulted in full activation of the Ca2+ PIC and generated a high firing rate in the tertiary range of the F-I relationship (see Fig. 6A, top). We also modified the model electrical and PIC-mediating channel (persistent Na+ and CaV1.3 channels) properties to replicate those changes seen after SCI (see Figs. 7, A and B). These changes enhance the excitability of motoneurons and contribute to the physiological manifestation of spasticity after injury. Under these conditions, the level of synaptic input required to activate fully the dendritic Ca2+ PIC decreased to 100% of the strength of the Ia-afferent system (Ia Is = 5 nA). This allowed us to investigate the efficacy of using electrical fields in reducing the excitability of spinal motoneurons after SCI and the emergence of spasticity. Synaptic inputs that caused partial activation of the Ca2+ PIC were also investigated (see Fig. 6A).

To assess the level of activation of the dendritic CaV1.3 channels and quantify the magnitude of the mediated current, we measured the average dendritic Ca2+ PIC (ICa2+; see, e.g., Fig. 4A, 2nd trace). This average Ca2+ PIC was computed as follows:

$$I_{Ca2+} = I/n \times \sum_{i=1}^{n} I_{Ca2+}$$

where $n$ is the number of compartments that have the CaV1.3 channels, and $I_{Ca2+}$ is the Ca2+ current mediated by the CaV1.3 channels located in compartment $i$.

Simulation of extracellular stimulation through electrical fields. Electrical fields resulting from current flow between two parallel plate electrodes and applied in 3D space parallel to the rostrocaudal, dorsoventral, or mediolateral axes of the SC were simulated. The gray matter was represented as a 3D uniform ohmic, i.e., isotropic, medium (35). The distance between the two plate electrodes was 8 mm. The model motoneuron was placed between the plate electrodes in which the surrounding electrical field was uniform. This was indicated by the linear relationship of the extracellular voltage gradient along the cell axis between the electrode plates, whereas that parallel to the plates was zero (see Fig. 3B, left). The dendritic morphology of the cell was described in 3D coordinate space centered at the soma and parallel to the rostrocaudal, dorsoventral, and mediolateral axes of the SC (13). The representation of the motoneuron morphology relative to the plate electrodes and the principal axes of the SC allowed for the computation of the extracellular field potential for each compartment using the following equation:

$$V_e = I \times \rho \times (x - 0.5D)$$

where $V_e$ is the extracellular field potential for each compartment, $I$ is the current, $\rho$ is the extracellular electrical resistivity of the gray matter, $x$ is the radial distance along the field axis between each compartment and the positive plate electrode, and $D$ is the distance between the two parallel plate electrodes. DC and AC electrical fields of different waveforms (e.g., sinusoidal, rectangular, and trapezoidal) were simulated by varying the shape of the applied current.

In the present study, we placed the myelinated axon, initial segment, and axon hillock of the model motoneuron in the dorsoventral direction of the SC such that electrical fields applied along that axis induced the greatest polarization of their membrane potential and significantly affected the spiking mechanism of the model motoneuron (Fig. 1A). Electrical fields applied along the rostrocaudal or mediolateral axes of the SC, i.e., orthogonal to the orientation of the myelinated axon, initial segment, and axon hillock, polarized the dendrites and had no effect on the membrane potential of the myelinated axon, initial segment, and axon hillock of the model motoneuron.
Verification of motoneuronal activation through electrical fields.

To verify the effect of electrical fields on the behavior of the model motoneuron, we simulated the experiments conducted by Hounsgaard and Kiehn (35) on spinal motoneurons of the turtle during the application of an electrical field in the presence of different channel blockers [tetrodotoxin (TTX) and apamin]. The effect of TTX and apamin was simulated in the model by blocking the Na1 and Ca2+ activated K+ channels, respectively. As demonstrated experimentally, a sustained depolarization in the somatic membrane (plateau potential) of the model motoneuron was evoked during application of both soma-depolarizing and soma-hyperpolarizing electrical fields because of the activation of the dendritic Ca2+ persistent inward current (PIC) (I\textsubscript{Ca}; 2nd trace) measured simultaneously during the application of a DC electrical field along the dorsoventral axis of the SC (3rd trace) and activation of synaptic inputs (bottom trace). Dotted lines in the 1st, 2nd, 4th, and 5th traces show the magnitude of the somatic transmembrane potential and I\textsubscript{Ca}, in response to synaptic activation only. During the application of an E+ DC field, the soma and initial segment were hyperpolarized, whereas they were depolarized during the application of an E− field. C: cell firing evoked by field stimulation along the dorsoventral axis of the SC when no synaptic input was activated. A sinusoidal electrical field (bottom trace) was applied as shown in A that evoked cell firing during the cathodic phase of the field when the soma was depolarized (trace 1). Higher firing rate and activation of the dendritic Ca2+ PIC were evoked when the field intensity was increased (trace 2). Arrow in trace 2 shows the flat baseline of the somatic transmembrane during activation of the plateau potential [compare to Fig. 3A1 in Chan et al. (12)]. Cell firing was interrupted and the somatic membrane potential was lowered when an intracellular hyperpolarizing current pulse (~4.5 nA, 4,500 ms) was injected at the soma during the activation of the dendritic plateau potential [trace 3; compare with Fig. 3A2 in Chan et al. (12)].

**Verification of motoneuronal activation through electrical fields.**

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The firing behavior of the model motoneuron evoked by the application of an electrical field, and in the absence of synaptic activation, was simulated in Fig. 1C. A sinusoidal electrical field was applied along the dorsoventral axis of the SC and parallel to the orientation of the axon, initial segment, and axon hillock of the motoneuron (Fig. 1A). This field caused depolarization of the somatic transmembrane potential during the cathodic phase of the field and its hyperpolarization during the anodic phase of the field. Cell firing was evoked during the cathodic phase of the imposed field when the field intensity was increased (Fig. 1C, trace 1). Further increase in the intensity of the applied field resulted in a high motoneuronal firing rate, and the baseline transmembrane potential became flattened during the high spiking period (Fig. 1C, trace 2) as seen experimentally (12, 14, 35). When an intracellular hyperpolarizing current pulse was injected at the soma during the high spiking phase, cell firing was reduced and terminated early (Fig. 1C, trace 3) and the somatic membrane potential was more hyperpolarized. This demonstrated qualitatively the independent and additive interaction between the polarization imposed by the
electrical field and that induced by the intracellularly injected current pulse (12, 14).

RESULTS

Persistent inward currents. Activation of low-threshold PICs, primarily those mediated by dendritic Cav1.3 channels, in motoneurons below the injury contributes to the activation of long-lasting reflexes and muscle spasms (26, 45). Dendritic Cav1.3 channels were included in the present model based on a distribution that matched several electrophysiological recordings obtained from motoneurons (17). Activation of the Ca\(^{2+}\) PIC resulted in the nonlinear behaviors described in motoneurons [i.e., negative slope region in the steady-state I-V relationship (Fig. 2A) and generation of plateau potentials when spikes were blocked (Fig. 2B)] (23).

Effect of application of DC electrical fields. The mechanism by which electrical fields affect neuronal activity is shown in Fig. 3. The application of a DC electrical field along the mediolateral axis of the SC (Fig. 3A, left) resulted in the formation of an extracellular voltage gradient along the direction of the field axis (Fig. 3B, left, blue trace). This induced extracellular voltage gradient altered the transmembrane potential of the neuronal elements along the field axis and caused current to flow inward, effectively depolarizing one half of the cell, and outward, effectively hyperpolarizing the other half of the cell (Fig. 3B, right, blue trace). The transmembrane potential of neuronal elements oriented orthogonally to the field direction was not affected by the imposed field (Fig. 3B, right, red trace). The spatial distribution of the dendritic transmembrane potential resulting from the imposed electrical field is shown in Fig. 3A, right. The induced change in transmembrane potential varied linearly with distance along the axis of the imposed field, indicating that long dendritic extensions will be polarized mostly by the effect of the field (Fig. 3B, left). This is in agreement with experiments conducted on spinal motoneurons (35). Because of the anatomic location of the soma and initial segment being relatively in the center of the motoneuron (i.e., near the neutral point where no field-induced polarization takes place), these structures experienced the lowest degree of polarization (Fig. 3A, right).

Neurons are normally activated through their synaptic inputs. After SCI, brief sensory stimulation causes full activation of the motoneuronal PIC, which is implicated in the production of involuntary muscle spasms and exaggerated tendon reflexes associated with spasticity (45). Therefore, to simulate this condition, the model motoneuron was activated synaptically with Ia \(I_N\) of 14.6 nA (nearly 3 times that of the Ia-afferent system) that caused full activation of the dendritic Ca\(^{2+}\) PIC (16). The effect of DC electrical fields on the behavior of the actively firing motoneuron was then examined (Fig. 4). When the DC field was applied along the mediolateral axis of the SC, a reduction in firing rate was observed (Fig. 4A, top trace). Reduction in firing rate was also associated with a reduction in the magnitude of the dendritic Ca\(^{2+}\) PIC (see \(I_{Ca}\) in Fig. 4A). The amount of reduction in \(I_{Ca}\) was comparable to that of the firing rate, indicating that the drop in \(I_{Ca}\) was the primary factor in reducing the firing rate and not the direct polarization of the soma and initial segment by the imposed field.

The reduction in firing rate observed on application of the DC field could be explained as follows. Before application of the field, the dendritic Ca\(^{2+}\) PIC was fully activated by the effect of the synaptic input. When the DC field was applied, half of the dendritic tree was hyperpolarized whereas the other half was depolarized (similar to Fig. 3A, right). Dendritic Cav1.3 channels of the hyperpolarized part of the dendritic tree were deactivated, resulting in a reduced Ca\(^{2+}\) PIC (i.e., less negative inward current). In contrast, dendritic Cav1.3 channels of the depolarized part of the dendritic tree were more activated; however, the magnitude of their Ca\(^{2+}\) PIC depended on the dendritic membrane potential because of the nonlinear I-V relationship of the Cav1.3 channels (Fig. 4B). Depolarized dendritic segments whose membrane potential was lower than the potential of the turning point (at \(-15\) mV in Fig. 4B) experienced an increase in the magnitude of the mediated Ca\(^{2+}\) PIC (i.e., more negative inward current), whereas depolarized dendritic segments whose membrane potential exceeded the potential of the turning point experienced a reduction in the mediated Ca\(^{2+}\) PIC (i.e., less negative inward current). Given that the extended dendritic segments experience higher levels of polarization relative to the soma and initial segment, the net effect of the depolarizing DC field was a reduction in the mediated Ca\(^{2+}\) PIC, with little effect of direct polarization on the somatic spiking mechanism by the imposed field.

The effect of the applied electrical field on the amplitude of the dendritic Ca\(^{2+}\) PIC was confirmed by direct measurements of the PIC (Fig. 4D) from dendritic branches aligned parallel to the field (blue dendritic pathway shown in Fig. 4C, bottom). Before application of the electrical field, the Ca\(^{2+}\) PIC was fully activated along these dendritic branches around the soma (Fig. 4D, dashed trace). After the application of the electrical field, the Ca\(^{2+}\) PIC decreased, indicating a reduction in the magnitude of the dendritic Ca\(^{2+}\) PIC (Fig. 4D, top trace).
field, the magnitude of the Ca\(^{2+}\) PIC was significantly suppressed on the hyperpolarized part of the dendrites, whereas it did not change much on the depolarized half of the dendrites (Fig. 4D, solid trace). Because of the nonlinear properties of the I-V relationship of the CaV1.3 channels, increased membrane depolarization in the depolarized half of the dendrites did not compensate for the reduction of the Ca\(^{2+}\) PIC on the hyperpolarized part of the dendrites. In fact, suppression of the Ca\(^{2+}\) PIC by the effect of the electrical field was more evident at dendritic compartments distal from the soma because of the strong depolarization experienced by those compartments. The spatial distribution of the Ca\(^{2+}\) PIC was also illustrated pictorially during the presence and absence of the electrical field (Fig. 4C). Further support for the aforementioned mechanism of the effect of electrical fields on motoneuronal firing was provided by the profile of the activated plateau potential by the applied field in the presence of TTX and apamin (Fig. 1B). The measured \(I_{Ca}\) during soma-depolarizing and soma-hyperpolarizing DC fields (1st and 3rd phases in Fig. 1B) indicated a reduction in the magnitude of the Ca\(^{2+}\) PIC relative to that during the period in which the field was removed (2nd phase in Fig. 1B).

When the DC field applied along the mediolateral axis was reversed in polarity, reduction in firing rate was achieved as well, but to a lesser extent (compare conditions \(E^+\) and \(E^-\) in top trace of Fig. 4A). This variation in the amount of reduction in firing rate based on field polarity could be explained by the asymmetry of the dendritic projections around the soma along the mediolateral axis. That is, more CaV1.3 channels might be (de)activated in one half of the dendritic tree than in the other half because of the asymmetry of the motoneuronal dendritic projections around the soma, their geometry and orientation relative to the field, and the distribution of the CaV1.3 channels. Similar results were obtained when a reversed-polarity DC field was applied along the rostrocaudal axis of the SC (not illustrated).

To study the effect of electrical fields on the spiking mechanism of spinal motoneurons, a DC field applied along the
The dorsoventral axis of the SC and parallel to the orientation of the axon, initial segment, and axon hillock of the model motoneuron was simulated (Fig. 5). When the DC field was applied such that the axon, initial segment, and axon hillock were extracellularly hyperpolarized (condition $E^+/H_{11001}$ in Fig. 5), two active processes occurred that simultaneously reduced the motoneuronal firing rate. 1) The initial segment was directly inhibited by the effect of the applied field, causing less activation of the $\text{Na}^+$ channels and generation of action potentials. 2) The net dendritic $\text{Ca}^{2+}$ PIC, indicated by $I_{\text{Ca}}$, reaching the soma and initial segment was reduced at a slower rate by the effect of the applied field on the dendrites as explained in Fig. 4A. The net effect of these two processes was a rapid and significant reduction in the motoneuronal firing rate (Fig. 5, top trace, $E^+/H_{11001}$). Conversely, when the DC field was applied such that the axon, initial segment, and axon hillock were extracellularly depolarized (condition $E^-/H_{11002}$ in Fig. 5), two counteracting processes occurred simultaneously that affected the firing rate. 1) The initial segment was directly stimulated by the effect of the applied field, causing more activation of the $\text{Na}^+$ channels and generation of action potentials. 2) The net dendritic $\text{Ca}^{2+}$ PIC, indicated by $I_{\text{Ca}}$, reaching the soma and initial segment was reduced at a slower rate by the effect of the applied field on the dendrites as explained in Fig. 4A. The net effect of these two processes was a rapid and significant reduction in the motoneuronal firing rate (Fig. 5, top trace, $E^+/H_{11001}$). Conversely, when the DC field was applied such that the axon, initial segment, and axon hillock were extracellularly depolarized (condition $E^-/H_{11002}$ in Fig. 5), two counteracting processes occurred simultaneously that affected the firing rate.

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**Fig. 4.** Effect of DC electrical fields on the firing behavior of the model motoneuron. **A:** somatic firing rate (top trace) and $I_{\text{Ca}}$ (2nd trace) during activation of synaptic inputs (300% that of the Ia-afferent system, 3rd trace). After steady-state firing was reached, a DC field (bottom trace) of either polarity ($E^+$ or $E^-$) was applied along the mediolateral axis of the SC (as shown in Fig. 3). Arrow shows the instantaneous increase in firing rate at the onset of the electrical field. Dotted lines in the top and 2nd traces illustrate the steady-state level of firing rate and $I_{\text{Ca}}$, respectively. The amount of reduction in $I_{\text{Ca}}$ is also indicated by the arrow in the 2nd trace. **B:** I-V relationship of the dendritic CaV1.3 channels. Vertical dotted line illustrates the membrane potential at which the inward current starts to decrease (turning point). Vertical arrows show the direction of the mediated current through the CaV1.3 channels. **C:** spatial distribution of $I_{\text{Ca}}$ mediated through the dendritic CaV1.3 channels before (top) and after (bottom) application of the electrical field. Current flowing axially through the model dendritic compartments toward the soma is not shown. **D:** amplitude of $I_{\text{Ca}}$ flowing through the dendritic segments aligned parallel to the imposed field (shown in blue in C) before (dashed line) and after (solid line) the application of the electrical field in A. Distribution of the CaV1.3 channels along the dendritic (blue) branches is indicated by the horizontal blue bars.
of the applied DC field on the dendrites is evident in the reduction relative strength of the aforementioned processes. The effect of the steady-state firing rate was determined mainly by the activation level of the dendritic CaV1.3 channels, as shown by A (Fig. 6). During the secondary range of the net result of these two processes was an initial rise in firing rate followed by a slow decline (Fig. 5, top trace, condition E−). The steady-state firing rate was determined mainly by the relative strength of the aforementioned processes. The effect of the applied DC field on the dendrites is evident in the reduction of I\textsubscript{Ca}. Despite the reduction in I\textsubscript{Ca} during condition E− in Fig. 5, there was little change in firing rate due to the direct stimulation of the axon, initial segment, and axon hillock by the imposed field.

**Effect of DC electrical fields on excitability of spinal motoneurons.** The effect of electrical fields on the F-I relationship of spinal motoneurons was investigated as well. Application of weak DC electrical fields of intensity 8.5 and 30 mV/mm along the mediolateral axis of the SC in response to different levels of synaptic input resulted in a linear F-I relationship over the full range of the effective synaptic current reaching the soma (Fig. 6A, top). This effect was achieved by modulating the activation level of the dendritic CaV1.3 channels, as shown by I\textsubscript{Ca} in Fig. 6A, bottom, which resulted in a shallower graded activation of the Ca\textsuperscript{2+} PIC in response to an increasing level of synaptic input. This is also supported by the shallower slope of the linear F-I relationship compared with that of the secondary range before the application of the electrical field. The intensity of the applied field modulated the slope of the linear F-I relationship. Furthermore, application of the DC field suppressed the excitability of spinal motoneurons even when synaptic inputs caused partial activation of the Ca\textsuperscript{2+} PIC. During the secondary range of the F-I relationship, the afterhyperpolarization regulates the activation level of the CaV1.3 channels, resulting in a graded activation of the Ca\textsuperscript{2+} PIC. Therefore, application of DC electrical fields acted in tandem with the afterhyperpolarization and resulted in a more potent regulating effect on the magnitude of the Ca\textsuperscript{2+} PIC. In sum, application of DC electrical fields resulted in a reduction in the excitability of spinal motoneurons, and the magnitude of this reduction could be modulated by varying the intensity of the applied field.

Application of a DC electrical field of intensity 30 mV/mm resulted in suppression of the overall excitability of the model motoneuron; however, it facilitated its recruitment (horizontal arrow in Fig. 6A, top) and caused an increase, rather than a reduction, in the motoneuronal firing rate in response to low levels of synaptic input (vertical arrow in Fig. 6A, top). That is, at low levels of synaptic input the Ca\textsuperscript{2+} PIC that was activated by the application of the DC field was higher in magnitude than that originally activated by the synaptic input alone (Fig. 6B; I\textsubscript{a} I\textsubscript{N} = 6 nA). This increase in magnitude of the Ca\textsuperscript{2+} PIC at low levels of synaptic input facilitated the recruitment of the motoneuron. Nevertheless, application of a weaker DC electrical field of intensity 8.5 mV/mm avoided the facilitation of motoneuronal recruitment but reduced the overall motoneuronal excitability to a lesser extent (Fig. 6A).

The effect of DC field intensity applied along the principal axes of the SC (rostrocaudal, dorsoventral, and mediolateral) on the motoneuronal firing rate in response to a synaptic input of 14.6 nA (3 times that of the Ia-afferent system) is shown in Fig. 6C. Generally, there was a linear relationship between the intensity of the applied field and the change in firing rate. DC fields applied in some directions were found to be more effective in reducing, or even turning off, cell firing. For instance, DC fields applied along the rostrocaudal axis of the SC resulted in a linear reduction of firing rate but could not turn off firing completely (Fig. 6C). On the other hand, DC fields applied along the mediolateral axis of the SC were more effective in reducing firing rate (note higher slope in Fig. 6C) and were able to turn off firing completely. This dissimilarity in effect could be explained by the number of dendritic projections (and dendritic CaV1.3 channels) of the model motoneuron that were polarized by the imposed field along that axis.

DC fields that directly polarized the axon and initial segment as well as the dendritic tree (along the dorsoventral axis) had the strongest impact on firing rate. DC fields that hyperpolarized the axon and initial segment resulted in the steepest reduction of firing rate until firing was totally blocked at high field intensities (Fig. 6C). Conversely, DC fields that depolarized the axon and initial segment resulted in an increase in firing rate (maximum increase was nearly 20%) until inactivation of the Na\textsuperscript{+} channels was encountered and firing was blocked (Fig. 6C).

**Alteration in electrical properties after SCI.** The effect of changes in motoneuronal electrical properties after SCI on the reduction in firing rate of spinal motoneurons during the application of electrical fields was also investigated. These changes contribute to the increased motoneuronal excitability following SCI. Changes in motoneuronal active [e.g., firing threshold (V\textsubscript{th})] and passive [e.g., resting membrane potential (V\textsubscript{rest}); specific membrane resistance (R\textsubscript{m})] properties (31–33) were simulated, and the Ia afferent synapses were activated at

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**Fig. 5.** Effect of DC electrical fields that caused direct polarization of the axon, initial segment, and axon hillock. Motoneuronal firing rate (top trace) and I\textsubscript{Ca} (2nd trace) during activation of synaptic inputs (300% that of the Ia-afferent system, 3rd trace) are shown. After steady-state firing was reached, a DC field (bottom trace) of either polarity (E+ or E−) was applied along the dorsoventral axis of the SC (as shown in Fig. 1A). Dotted lines in the top and 2nd traces illustrate the steady-state level of firing rate and I\textsubscript{Ca}, respectively. The amount of reduction in I\textsubscript{Ca} is also indicated by the arrow in the 2nd trace.
300% to cause full activation of the dendritic PIC. Reduction in the motoneuronal firing rate by the application of a DC electrical field along the mediolateral axis of the SC was achieved after the alteration of these electrical properties independently (Fig. 7A; conditions B–I in Fig. 7B). Moreover, reduction in firing rate was still achieved (≈32% reduction; condition J in Fig. 7, A and B) even when a combination of changes in electrical properties of the model motoneuron was concurrently implemented to further increase its hyperexcitability ($V_{th}$ was reduced by 5 mV, $V_{rest}$ was depolarized by 5 mV, and $R_m$ at the soma and dendrites was increased by 33%). Importantly, when the combined changes in electrical properties were included, the level of synaptic input required to activate the dendritic PIC fully decreased and full activation of the dendritic PIC was achieved at 100% of the strength of the Ia-afferent system.

Given that the percent change in the PIC amplitude after SCI is not yet determined experimentally, we examined the likelihood of the change in PIC amplitude being a result of the upregulation of the Na$^+$ and Ca$^{2+}$ PICs in chronic injury. The density of the persistent Na$^+$ and CaV1.3 channels was increased such that the amplitude of the Na$^+$ and Ca$^{2+}$ PICs was increased to 150%. This is the ratio by which the PIC amplitude was increased in response to the administration of the noradrenergic $\alpha_1$-agonist methoxamine to the decerebrate cat preparation (43). Under this condition, application of a DC electrical field along the mediolateral axis of the SC caused significant reduction in the motoneuronal firing rate (≈47% reduction; condition K in Fig. 7B). When the combined changes in electrical properties were included in conjunction with the increase in the PIC amplitude, substantial reduction in firing rate was still achieved (≈67% reduction; condition L in Fig. 7B). Given that a recent immunohistochemical study reported no change in the density of the CaV1.3 channels in spinal motoneurons of spastic rats after chronic SCI (1), we also examined the possibility of the enhancement in PIC amplitude due to changes in channel properties (e.g., hyperpolarization of the half-activation potential of CaV1.3 channels) following injury. The half-activation potential of the CaV1.3 channels was hyperpolarized by 5 mV in conjunction with the combined changes in electrical properties. Under these conditions, application of electrical fields resulted in a significant reduction in firing rate (≈55% reduction; condition M in Fig. 7, A and B).

Again, a low level of synaptic input (100% of Ia $I_{th}$) was required to activate the dendritic PIC fully in conditions K–M in Fig. 7B. Thus our results demonstrate that the reduction in firing rate due to the application of electrical fields is still viable after SCI when changes in the electrical and PIC-mediating channel properties of spinal motoneurons take place.

We also investigated the effect of changes in the distribution or activation pattern of synaptic inputs after SCI on the reduction in firing rate of spinal motoneurons during the application of electrical fields (Fig. 7C). This was achieved by simulating...
the experiments conducted by Delgado-Lezama et al. (14) in which preferential activation of different parts of the dendritic tree (medial vs. lateral) was obtained through the activation of different descending tracts (medial vs. dorsolateral funiculus).

In the present study, the medial synapses were preferentially activated at 500%, whereas the lateral synapses were activated at 100% during the application of a DC electrical field along the mediolateral axis of the SC (condition B in Fig. 7C). Similarly, preferential activation of lateral, rostral, and caudal synapses were simulated during the application of the same field along the mediolateral axis of the SC (conditions C–E in Fig. 7C, respectively). These simulations were compared with the homogeneous activation of all synapses during the application of the same field (condition A in Fig. 7C). Our results show that reduction in the motoneuronal firing rate was still achieved during preferential activation of synaptic contacts that caused full activation of the dendritic PIC.

**Effect of AC electrical fields on firing behavior of spinal motoneurons.** Because long-duration DC electrical fields could potentially cause tissue damage and induce long-term changes in the excitability of neurons (11) that in some cases may not be desirable, we investigated the effect of low-frequency AC electrical fields (10.5 Hz) of different waveforms on the firing behavior of motoneurons (Fig. 8). An AC electrical field of square waveform was applied along the mediolateral axis, and the motoneuronal firing rate was measured (Fig. 8A). Reduction in the motoneuronal firing rate was achieved during the different phases of the applied AC field (Fig. 8A, top trace) and was also accompanied by a comparable reduction in I<sub>Ca</sub> (Fig. 8A, middle trace). However, the amount of reduction in both the firing rate and I<sub>Ca</sub> was variable during the different phases of the applied AC field because of the asymmetry of the model motoneuronal dendritic projections around the soma along the mediolateral axis. Peaks in firing rate were seen during the abrupt transitions between the different phases of the AC field due to the capacitive property of the membrane (Fig. 8A). To minimize these transitional peaks in firing rate, a trapezoidal-waveform field of the same intensity was examined to allow for a relatively smooth transition between the different phases of the AC field. Peaks in firing rate were minimized, and a relatively smoother I<sub>Ca</sub> was achieved (Fig. 8B). The effect of sinusoidal electrical fields on the firing behavior of spinal motoneurons was also simulated as these fields are widely used in experimental setups (11, 12, 35). During a sinusoidal field the intensity of the field continuously varies with time and so does the change in field polarity during the different phases of the field. Thus a variable reduction in firing rate and I<sub>Ca</sub> was achieved with no sudden peaks because of the smooth variation.
in the field intensity (Fig. 8C, black trace). Sinusoidal electrical fields of higher frequencies (100 Hz) resulted in a steady, but lower, reduction in firing rate (Fig. 8C, gray trace).

**DISCUSSION**

The goal of the present study was to provide a “proof of principle” that extracellularly applied electrical fields can modulate the excitability of spinal motoneurons through regulating the magnitude of their dendritic PIC. Our results demonstrate, for the first time, that DC and AC electrical fields can suppress the excitability of active motoneurons and induce reduction in their firing rate. This reduction is modulated by the intensity of the imposed field. The suppression of motoneuronal excitability suggested by the present study results from the interaction between the field-induced polarization, the position of the soma with respect to the dendrites, and the activation level of the dendritic voltage-gated channels. The reduction in firing rate was not instantaneous and took hundreds of milliseconds to reach steady state (∼200–300 ms). This is due to the distal location of the dendritic CaV1.3 channels, their slow kinetics, and the capacitive property of the cell membrane (36).

**Comparison with experimental results.** Our simulation results are in agreement with previous experimental studies in which the effects of electrical fields were investigated. Application of electrical fields to spinal motoneurons was examined experimentally to demonstrate the dendritic origin of the Ca²⁺ channels responsible for the Ca²⁺ plateau (35). It is evident from the illustrations in the report by Hounsgaard and Kiehn (35) that there was a reduction in the motoneuronal firing activity when a soma-depolarizing DC field was applied compared with that when the DC field was removed (see Fig. 6A, b and c, in Ref. 35). Mid-molecular-layer stellate cells of the turtle cerebellum, which have dendrites oriented in all directions (similar to motoneurons), were found to be modulated by reversed electrical fields (11). Our results are also in concurrence with the study of Delgado-Lezama et al. (14), in which the effect of facilitation of local synaptic inputs on the motoneuronal firing rate and generation of plateau potentials during the application of DC electrical fields was studied. In the study of Delgado-Lezama et al. (14), applied electrical fields facilitated the generation of plateau potentials and increased the firing rate when synaptic inputs were activated. The discrepancy between the effect of applied electrical fields on motoneuronal firing rate described in the present study and that seen in the study of Delgado-Lezama et al. (14) is probably due to the difference in the activity of motoneurons before the application of the field. In the study of Delgado-Lezama et al. (14), the examined motoneurons were inactive before the application of the field.
application of the electrical field (see Figs. 1 and 2 in Ref. 14). Application of an electrical field facilitated the activation of plateau potentials in response to the activation of synaptic inputs. This observation was confirmed in our study by the early recruitment of an inactive motoneuron due to the application of an electrical field (Fig. 6A). However, when the electrical field was applied during motoneuronal firing activity, the firing rate was reduced. This condition was not examined experimentally in the study of Delgado-Lezama et al. (14).

Therefore, the present study demonstrated that electrical fields can facilitate the recruitment of inactive motoneurons but suppress the excitability of those already active (particularly motoneurons with fully activated PIC). Weak electrical fields can avoid the facilitation of motoneuronal recruitment but are less effective in suppressing the overall motoneuronal excitability. During spasticity, motoneurons are actively firing with their PIC fully activated (45, 46). Application of electrical fields would then effectively suppress the excitability of those motoneurons, thereby reducing the severity of spasticity.

**Spatial activation of dendritic PIC.** One could contend that the effect of electrical fields in reducing the motoneuronal firing rate is contingent on the condition of having the entire dendritic tree strongly depolarized by synaptic inputs, causing full activation of the PIC in all dendritic regions. Thus motoneurons whose dendrites are partially depolarized by synaptic inputs would experience an increase in their firing activity by the applied field. In the present study, we examined various patterns of synaptic activation that caused either strong depolarization of the whole dendrites or depolarization that was confined to parts of the dendritic tree (Fig. 7C). In the latter, synaptic activation resulted in full activation of the dendritic PIC only in the depolarized portions of the dendrites and partial activation of the PIC in the other portions. Under these conditions, the electrical field was still effective in reducing the motoneuronal firing rate. Therefore, the dendritic tree does not need to be entirely depolarized for the suppression of firing rate by electrical fields to be effective. In other words, the effect of the electrical field in suppressing the motoneuronal excitability is independent of the pattern of synaptic input that activates the dendritic PIC.

**Changes in motoneuronal properties following SCI.** It has been shown that spinal motoneurons undergo changes in their electrical and morphological properties and the number and distribution of synaptic inputs after SCI (5, 31–33, 38–40). Morphologically, Kitzman (38) showed that after SCI and the full development of spasticity there was a reduction in the size of the soma and the number of the primary, secondary, and tertiary dendrites of motoneurons innervating the spastic muscles. The motoneuronal electrical properties, on the other hand, showed a different trend. Some electrical properties were reduced (e.g., time constant, afterhyperpolarization duration), some were increased (e.g., rheobase, voltage threshold), and others did not change (e.g., resting membrane potential, action potential height, input resistance) (32). For the synaptic inputs, Kitzman (39, 40) showed that after the development of spasticity there was a significant increase in the ratio of excitatory to inhibitory inputs; however, the increase appeared to originate primarily from interneuronal inputs. In the present study, we examined the effects of the change in motoneuronal electrical properties and distribution of synaptic activation on the reduction of firing rate induced by application of electrical fields. Our results demonstrated that application of electrical fields for the suppression of the motoneuronal excitability is still effective after SCI when the motoneuronal electrical properties and the level and distribution of synaptic activation are altered (Fig. 7). The change in morphological properties was not examined in the present study because it is not clear from published records whether the loss of the dendritic branches was equal in all directions (i.e., equal loss of the dendritic branches along the mediolateral, dorsoventral, and rostrocaudal axes of the SC) or biased toward some directions. Motoneurons that exhibit no directional bias in the loss of their dendritic segments would have their soma near the neutral point of the imposed field and would experience reduction in their firing rate during fields of reversed polarity (similar to Fig. 4, A and B). On the other hand, motoneurons that exhibit directional bias in the loss of their dendritic segments would have their soma shifted relative to the neutral point and would experience a net increase or reduction in their firing rate according to the field polarity. Application of AC fields of high intensity in this case would minimize the effects of the motoneuronal morphological changes after SCI or even of motoneurons with highly asymmetric dendrites relative to the soma (e.g., motoneurons on the border of the gray and white matter have dendrites that trail that border (6)). The high field intensity would create a steep change in the extracellular voltage gradient along the cell and would inactivate the Na+ channels during the soma depolarization phase of those motoneurons, which would cease their firing (similar to the effect of condition $E^-$ along the dorsoventral axis in Fig. 6C). The AC field would also minimize the directional bias in the loss of the dendritic segments by reversing the field polarity across the cell during the alternating phases of the field.

In the present study, we activated the dendritic PIC fully by activating the synaptic inputs at 300% of the strength of the Ia-afferent system. One could argue that during spasticity muscle spasms are triggered by moderate synaptic inputs that activate the dendritic PIC. Thus activating the motoneuron with a high level of synaptic input does not simulate the activation of the PIC during spasticity. When the combined changes in electrical and/or PIC properties were included in the model to simulate spastic motoneurons after SCI more realistically, full activation of the dendritic PIC was achieved at a lower level of synaptic input (100%, $I_{As} \approx 5$ nA), a level that was barely adequate to recruit the motoneuron before these changes. Under these conditions, reduction in the motoneuronal firing rate was still obtained through the application of electrical fields (Fig. 7). Hence, suppression of the motoneuronal excitability by the applied electrical field is obtained regardless of the level of synaptic input required to cause full activation of the dendritic PIC. One could also argue that the densities of the PIC-mediating channels (persistent Na+ and CAV1.3 channels) in the present model were based on measurements obtained from motoneurons of animals with no injury (17). Consequently, these densities do not reflect the change in PIC amplitude after injury. It has been shown experimentally that motoneurons in chronic spinal rats have enhanced (nearly 2-fold increase) PICs relative to acute spinal animals (29); however, the percent change in the PIC amplitude relative to that with no injury is yet undetermined. Recently, an immunohistochemical report showed no change in the density of CAV1.3 channels mediating the dendritic PIC in motoneurons.
of spastic rats after chronic SCI (1). Furthermore, indirect assessment of the motoneuronal PIC amplitude obtained from the firing profile of motor units in human volunteers with a paired motor unit analysis technique, in which the difference in firing rate at recruitment and derecruitment (Fr - d) is presumed to be proportional to the amplitude of the PIC (26), showed similar values of Fr - d in healthy volunteers and individuals with chronic SCI, indicating similar PIC amplitudes in both populations (25, 26). Altogether, these observations indicate that recovery of near-normal levels of PIC amplitude takes place after chronic injury, and that the lack of control of this PIC through the neuromodulatory drive from the brain stem leads to the muscle spasms seen after injury. Nevertheless, in the present model we examined various conditions in which the motoneuronal PICs had similar or upregulated (150%) amplitudes (through a change in either channel densities or channel properties) compared with those measured from uninjured animals (Fig. 7). Under these conditions, suppression of motoneuronal excitability was still achieved by the application of electrical fields.

Motoneuronal firing behavior during spasticity. The firing behavior of spinal motoneurons during spasticity could be inferred from that of human motor units after SCI (54). During muscle spasms, the firing behavior of human motor units was found to be variable. Thomas and Ross (54) found that the firing rate of the majority of motor units was proportional to the intensity of muscle spasms; other motor units fired in doublets only near the peak intensity of the spasm, whereas the rest of the motor units had a relatively constant firing rate. For the second and third groups, muscle spasms may be generated because of synchronous activation of their motoneurons. Reduction of the firing rate as proposed in the present study may suppress muscle spasms by reducing the motoneuronal firing rate of the first group and desynchronizing the motoneuronal spiking of the second and third groups. Application of electrical fields of moderate intensity could facilitate the recruitment of inactive motoneurons, if present, during muscle spasms. However, the firing rate of those newly recruited motoneurons will be constrained by the applied field. Consequently, small synaptic inputs, which could otherwise trigger muscle spasms, would not cause the motoneurons to fire at high rates because of the suppression of the dendritic PIC by the applied electrical field. Weak electrical fields would avoid recruiting inactive motoneurons but would be less effective in suppressing the motoneuronal activity (Fig. 6A).

Effect of electrical fields on the various neuronal elements in the SC. Applied electrical fields would result in polarizing not only motoneurons in the SC but also other neuronal elements (e.g., sensory and motor axons, descending fibers, and interneurons). For sensory axons, primary afferents will be the most sensitive structures to the imposed field because of their large diameter and low threshold for polarization. Application of an electrical field would modulate the level of synaptic input reaching the cells (some sensory afferents would be depolarized, whereas others would be hyperpolarized). Our results showed that reduction of firing rate was achieved at most levels of synaptic inputs (Fig. 6A) and under different synaptic activation patterns (Fig. 7C). Similarly, motor axons could be polarized by the applied field. Depolarization of motor axons, which would increase muscle activity, could be modulated by using high-intensity electrical fields that would inactivate the Na⁺ channels at nodes of Ranvier and prevent axonal firing (similar to the effect of condition E− along the dorsoventral axis in Fig. 6C). Descending axons could also be activated by the applied electrical field. The resultant effect of their stimulation would be a preferential activation of synaptic inputs to motoneurons. The simulations in Fig. 7C illustrate that suppression of motoneuronal excitability through application of electrical fields could be achieved even during nonhomogeneous activation of dendritic synaptic inputs. Interneurons would be similarly polarized by the applied field. Analogous to their proposed effect on motoneurons with highly asymmetric dendrites relative to the soma, high-intensity electrical fields would suppress the excitability of asymmetric interneurons by inactivating their Na⁺ channels.

Open questions. Although the present study addressed a number of issues related to the effects of electrical fields on motoneuronal excitability as a means for reducing spasticity, other factors were not studied and warrant further investigation. Among these factors are the effects of electrical fields on ionic conductances not included in the model (e.g., Ih, the hyperpolarization-activated current). Second, the gray matter in the present study was represented as an isotropic medium (35). A more accurate electrical representation would be as an anisotropic medium (47). Third, the trajectory of the motor axon in the present study was straight. Realistically, motor axons follow variable 3D trajectories in the SC. However, we compensated for that by examining the effect of electrical fields applied along the three different axes of the cord (Fig. 6C). Finally, the shape of the dendritic trees of motoneurons exhibits large variations, with some having highly asymmetric trees. Therefore, the morphology of the model motoneuron, although realistic, cannot account for all the motoneurons in the SC.

In summary, the present study demonstrates the feasibility of using electrical fields to suppress motoneuronal excitability after SCI and provides specifications for new experiments to verify the effect of electrical fields on the activity of motoneuronal pools. If proven to be effective, electrical fields could provide a novel rehabilitation therapy for suppressing the increased excitability of spinal motoneurons and the severity of spasticity following SCI.

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