Modulation of motoneuronal firing behavior after spinal cord injury using intraspinal microstimulation current pulses: a modeling study

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Submitted 22 October 2006; accepted in final form 12 January 2007

ElBasiouny SM, Mushahwar VK. Modulation of motoneuronal firing behavior after spinal cord injury using intraspinal microstimulation current pulses: a modeling study. J Appl Physiol 103: 276–286, 2007. First published 18 January 2007; doi:10.1152/japplphysiol.01222.2006.—We simulated the effects of delivering focal electrical stimuli to the central nervous system to modulate the firing rate of neurons and alleviate motor disorders. Application of these stimuli to the spinal cord to reduce the increased excitability of motoneurons and resulting spasticity after spinal cord injury (SCI) was examined by means of a morphologically detailed computer model of a spinal motoneuron. High-frequency sinusoidal and rectangular pulses as well as biphasic charge-balanced and charge-imbalanced pulses were examined. Our results suggest that suprathreshold high-frequency sinusoidal or rectangular current pulses could inactivate the Na⁺ channels in the soma and initial segment, and block action potentials from propagating through the axon. Subthreshold biphasic charge-imbalanced pulses reduced the motoneuronal firing rate significantly (up to ~25% reduction). The reduction in firing rate was achieved through stimulation-induced hyperpolarization generated in the first node of Ranvier. Because of their low net DC current, these pulses could be tolerated safely by the tissue. To deliver charge-imbalanced pulses with the lowest net DC current and induce the largest reduction in motoneuronal firing rate, we studied the effect of various charge-imbalanced pulse parameters. Short pulse durations were found to induce the largest reduction in firing rate for the same net DC level. Subthreshold high-frequency sinusoidal and rectangular current pulses and low-frequency biphasic charge-balanced pulses, on the other hand, were ineffective in reducing the motoneuronal firing rate. In conclusion, the proposed electrical stimulation paradigms could provide potential rehabilitation interventions for suppressing the excitability of neurons to reduce the severity of motor disorders after injury to the central nervous system.

Spasticity: persistent inward current; computer simulation; electrical stimulation

Spasticity is a neurological impairment that usually develops after an upper motor neuron lesion (e.g., stroke or spinal cord injury, SCI). It is characterized by velocity-dependent muscle hypertonus and exaggerated tendon jerks resulting from the hyperexcitability of the stretch reflex (41). Clinical consequences of spasticity involve general weakness and loss of dexterity (5). Spasticity also interferes with volitional muscle activation and reduces the functional outcome of the residual voluntary drive after SCI. Current treatments of spasticity involve oral or intrathecal administration of nonspecific pharmacological agents (e.g., baclofen and clonidine) which cause general suppression of the neuronal activity in a patient already suffering from a reduced voluntary drive (3, 32).

The mechanisms underlying spasticity remain unclear. It is currently thought that the increased excitability of spinal motoneurons during spasticity could be partly due to changes in the intrinsic membrane properties of α-motoneurons, causing full activation of voltage-dependent dendritic persistent inward currents (PICs) after the injury (for review see Refs. 17, 55). After SCI, the activation and deactivation of the dendritic PIC are no longer under the control of the descending drive (e.g., presynaptic inhibition), leading to increased motoneuronal excitability and firing rate, muscle spasms, and long-lasting reflexes in response to afferent inputs (44, 60).

In most of its applications, electrical stimulation is used to modulate the excitability of neuronal elements to generate functional body movements, alleviate motor disorders, and restore sensory function, e.g., intraspinal microstimulation (ISMS), deep brain stimulation (DBS), and visual and cochlear prostheses (15, 16, 47, 54). In the present study, we simulated the effects of applying focal electrical current pulses to the central nervous system to inhibit, rather than activate, neuronal elements and reduce their activity. This would be beneficial in situations where reduction of the neuronal excitability in general is desired to alleviate motor disorders. One of these applications is the reduction of the hyperexcitability of spinal motoneurons, which contributes to the emergence of spasticity after SCI (6, 17). Therefore, we investigated electrical stimulation in the spinal cord as our model and spasticity as the motor disorder to be alleviated. More specifically, our goal was to reduce the hyperexcitability and increased firing rate of spinal motoneurons following SCI without prohibiting muscle activation through the residual volitional drive. By means of intraspinal microwires implanted in the spinal cord after the injury to restore limb movements, the developed paradigms could be delivered to reduce the severity of spasticity as well. To achieve this goal, three waveforms of electrical current pulses were examined in a motoneuron computer model: 1) high-frequency sinusoidal and rectangular current pulses, 2) low-frequency biphasic charge-balanced rectangular pulses, and 3) biphasic charge-imbalanced pulses.

A morphologically-detailed computer model of a cat spinal α-motoneuron was developed to examine the effect of these pulses on the excitability and firing behavior of motoneurons. Our results suggest that suprathreshold high-frequency sinusoidal and rectangular current pulses [>7 × threshold (T), >5 kHz] could inactivate Na⁺ channels and prohibit the
propagation of action potentials through the axon. Subthreshold biphasic charge-imbalanced pulses (<0.4T, 1–3 kHz, net DC <5 μA) were able to reduce the axonal firing rate significantly (up to ~25% reduction). The motoneuronal firing rate was reduced by the charge-imbalanced pulses at all levels of synaptic input, resulting in a reduction in the slope of the frequency-current (F-I) relationship. Conversely, subthreshold high-frequency sinusoidal and rectangular current pulses and low-frequency biphasic charge-balanced (subthreshold and suprathreshold) pulses were ineffective in inducing reduction in the motoneuronal firing rate. The former pulses did not provide adequate levels of sustained depolarization to inactivate Na⁺ channels in the soma and initial segment of the motoneuron, whereas the latter did not induce adequate levels of hyperpolarization in the initial segment or first node of Ranvier to reduce the motoneuronal firing rate. Results of the present study provide potential electrical stimulation paradigms that could be used as clinical interventions after SCI. In addition to the use of ISMS for restoring limb movements after the injury, the paradigms discussed here could also be used to reduce spasticity. Our results and the discussed concepts could also be extended to other applications where focal electrical microstimulation in the central nervous system is used (e.g., DBS and the management of bladder dysfunction). Part of this work was previously presented in abstract form (20).

METHODS

A three-dimensional (3D), morphologically detailed computer model of a cat α-motoneuron was developed to examine the effect of different extracellularly injected current pulses on the excitability and firing behavior of motoneurons. This model incorporated motoneuronal morphology and dendritic distribution of synaptic input. The model also included a distribution of dendritic channels that allowed it to reproduce a wide variety of motoneuronal behaviors (19).

Model structure. Full description of the model morphology, biophysical properties, and verification is provided in ElBasiouny et al. (19). Briefly, a compartmental cable model of a cat α-motoneuron was developed that had full representation of the motoneuronal structure, consisting of soma, initial segment, axon hillock, myelinated axon, and a dendritic tree (Fig. 1A). The dendritic tree was based on the 3D detailed morphology for type-identified triceps surae motoneurons [fatigue-resistant (FR) type, medial gastrocnemius motoneuron, identified as cell 43/5 in Culhime et al. (12)]. The model was developed with the NEURON simulation environment (28). The model passive properties were set based on previous studies for the same 43/5 FR motoneuron (12, 21). Voltage-gated ion channels at the soma, initial segment, and axon hillock determining the motoneuronal active properties included fast and persistent Na⁺, delayed rectifier K⁺, Ca²⁺-activated K⁺, and N-type Ca²⁺ channels. Ion channels at nodes of Ranvier included fast and persistent Na⁺ and slow K⁺ 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 afterhyperpolarizations with characteristics similar to experimental measurements (19).

The myelinated axon was based on the model developed by McIntyre and Grill (49), 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 conduction velocity of 83 m/s, which is comparable to that of FR motoneurons (29). The instantaneous motoneuronal firing rate was measured from the interspike intervals of the last node of Ranvier of the myelinated axon (~10 mm from the soma), which represents the firing rate seen by the muscle fiber of that motor unit.

Dendritic channels and synaptic input. The dendritic low-voltage-activated L-type calcium (CaL1.3 type) channels that mediate Ca²⁺ PIC were distributed over an intermediate region between 300 and 850 μm from the soma. With this dendritic distribution, the model behavior matched multiple sets of experimental measurements from cat motoneurons innervating the medial gastrocnemius muscle (19).

Given that involuntary muscle spasms and exaggerated tendon reflexes associated with spasticity 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 input 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 (9, 10, 22). For synaptic activation of the motoneuron, tendon vibration was simulated by activating the Ia-afferent synapses asynchronously at 180 Hz while adjusting the synaptic conductances to give Ia effective synaptic current (Ia IN) equal to that measured experimentally at resting potential (19). This is the current that effectively reaches the soma due to synaptic activation (27). To allow for systematic gradation of the synaptic input, the total number of Ia synapses was divided into groups and randomly distributed on the dendrites, which were then activated sequentially (18). This activation pattern resulted in a linear increase in the Ia IN 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 ElBasiouny et al., Ref. 19), synaptic input from systems other than the Ia-afferents was simulated by increasing the synapse conductances to generate higher synaptic currents (18). This allowed for the examination of the motoneuronal firing behavior in
response to different levels of synaptic input, and the F-I relationship was constructed (e.g., Fig. 5, closed circles).

Hyperexcitability of motoneurons during spasticity. The goal of the present study was to examine the efficacy of various electrical current pulses in suppressing the motoneuronal firing activity; thereby, the level of spasticity after SCI. During spasticity, spinal motoneurons become hyperexcitable and their dendritic PIC, which is responsible for the generation of high firing rates, long-lasting reflexes, and muscle spasms, becomes fully activated by synaptic input (23, 44, 45, 60). To simulate this condition, the model motoneuron was synaptically activated at 300% of the strength of the Ia-afferent system (Ia \( I_{IN} \approx 14.6 \text{nA} \)). The generated motoneuronal firing rate was described using the classical nomenclature that depicts the different linear ranges (primary, secondary, and tertiary ranges) of the F-I relationship (37, 38). The delivered level of synaptic input resulted in full activation of the Ca\(^{2+}\) PIC and generated a high firing rate in the tertiary range of the F-I relationship (Fig. 5, closed circles). Synaptic input that caused partial activation of the Ca\(^{2+}\) PIC in the secondary range of the F-I relationship were also investigated (Fig. 5).

To assess the level of activation of the dendritic Ca,1.3 channels and quantify the magnitude of the mediated current, we measured the average dendritic Ca\(^{2+}\) PIC (\( I_{Ca} \); e.g., Fig. 2, fifth trace). This average Ca\(^{2+}\) PIC was computed as follows:

\[
I_{Ca} = \frac{1}{n} \sum_{i=1}^{n} I_{Ca_i}
\]

where \( n \) is the number of compartments that have the Ca,1.3 channels, and \( I_{Ca_i} \) is the calcium current mediated by the Ca,1.3 channels located in compartment number \( i \).

Simulation of electrical stimulation through extracellular current pulses. The model motoneuron was stimulated with a monopolar extracellular point source electrode (similar to intraspinal microwires implanted in the spinal cord to restore limb movements after SCI; see Refs. 53, 57) positioned at \( X_e, Y_e, Z_e \) (Fig. 1A). The gray matter was represented as a 3D ohmic isotropic medium (30). The extracellular resistivity (\( \rho \)) of the gray matter was adjusted to replicate the current-distance relationship from Gustafsson and Jankowska (26; see Fig. 1B), and was set to 2,000 \( \Omega \text{cm} \). The dendritic morphology of the motoneuron was described in 3D coordinate space centered at the soma and parallel to the rostrocaudal (X), dorsoventral (Y), and mediolateral (Z) axes of the spinal cord (12). The representation of
the motoneuronal morphology relative to the source electrode and the principal axes of the spinal cord allowed for the computation of the extracellular field potential at each compartment in the model resulting from current injection at the electrode site using the following equation (48):

\[ V_e = \frac{1}{4 \pi \rho} \sum_{r} \sqrt{(x-x_i)^2 + (y-y_i)^2 + (z-z_i)^2} \]

where \( V_e \) is the extracellular field potential at each compartment, \( \rho \) is the extracellular electrical resistivity of the gray matter, and \( r \) is the radial distance between each compartment (located at \( x, y, z \)) and the source electrode.

**Verification of motoneuronal activation through extracellular current pulses.** Activation of the model motoneuron by extracellular injection of current pulses was compared with experimental data from Gustafsson and Jankowska (26). The model had current-distance relationship that matched experimental data (Fig. 1B). Similar to the experimental conditions, threshold currents were measured by means of an extracellular cathodic current pulse, 200 \( \mu \)s in duration, that caused activation of the cell. Threshold currents were measured along a track 50 \( \mu \)m lateral to the initial segment, and along the dorsoventral direction of the electrode position that provided the lowest threshold. Simulations were conducted with passive dendritic tree, i.e., no dendritic voltage-gated channels were present, as experimental measurements were obtained from anesthetized cats (26). Anesthesia suppresses the L-type calcium channels mediating the PIC in spinal motoneurons (25) and significantly reduces the availability of monoaminergic inputs to motoneurons (33). Therefore, the behavior of the motoneuronal dendrites in anesthetized preparations is dominated by their passive properties (55).

**Electrical stimulation paradigms.** The effect of three different waveforms of electrical current pulses on the firing rate of a spinal motoneuron was examined.

1) **High-frequency sinusoidal and rectangular current pulses.** Suprathreshold high-frequency sinusoidal and rectangular current pulses were tested in the model (Fig. 2, bottom trace). The pulse amplitude ranged between 50 (1.1T) and 500 \( \mu \)A (11T), and stimulation frequency ranged between 5 and 20 kHz.

2) **Biphasic charge-balanced rectangular pulses.** Symmetrical and asymmetrical biphasic charge-balanced rectangular pulses were examined in the model (Fig. 3A). The pulse amplitude ranged between 0.1T and 10T; pulse width ranged from 100 to 500 \( \mu \)s (for the short phase) and 1,000 to 1,500 \( \mu \)s (for the long phase); stimulation frequency ranged between 50 and 1,000 Hz.

3) **Biphasic charge-imbalanced pulses.** Subthreshold biphasic charge-imbalanced current pulses with a 4:1 ratio of the cathodic to anodic phases were tested in the model (Fig. 4, bottom trace). The cathodic pulse amplitude ranged between 0.1T and 1T; cathodic pulse width ranged between 50 and 500 \( \mu \)s; stimulation frequency ranged between 1 kHz and the highest stimulation frequency possible (when no delay was present between the pulses); net DC current ranged between 0.5 and 6 \( \mu \)A.

**RESULTS**

**Extracellular electrical stimulation of high-frequency sinusoidal and rectangular current pulses.** The effect of extracellular injection of high-frequency sinusoidal and rectangular current pulses on the firing behavior of motoneurons was simulated in Fig. 2. High-frequency sinusoidal and rectangular current pulses were examined because they are safe (i.e.,...
charge-balanced pulses) to apply in chronic applications. The extracellular stimulating electrode was moved freely in 3D space around the model motoneuron to investigate the effect of electrode location on the motoneuronal firing behavior. The stimulating electrode was initially placed relatively distal from the soma and initial segment (within the dendritic tree), at which location the motoneuronal firing threshold was 45 μA. The model motoneuron was activated in response to a synaptic input of 14.6 nA (nearly three times that of the Ia-afferent system) that caused full activation of the dendritic Ca²⁺ PIC (Fig. 2, fourth trace). After steady-state motoneuronal firing was reached, extracellular injection of suprathreshold high-frequency (5 kHz, 300 μA, ~7T) sinusoidal pulses was applied (Fig. 2, bottom trace). The delivered pulses produced a state of sustained depolarization primarily in the first node of Ranvier as it is the nearest structure to the stimulating electrode with the highest density of Na⁺ channels, and caused inactivation of the Na⁺ channels. The sustained depolarization of the first node of Ranvier was illustrated by the profile of the activation gating particle (m) of the Na⁺ channels in response to the delivered pulses in which a high and sustained activation level was achieved (Fig. 2, second trace). The sustained depolarization at the first node of Ranvier, in turn, resulted in inactivation of the action potentials propagating through the axon and hyperpolarization of the soma, initial segment, and dendrites (Fig. 2, third trace). The change in axonal membrane potential was reduced from 110 mV (height of the axonal action potentials) before stimulation to less than 30 mV after stimulation. The motoneuronal firing rate in response to the delivered pulses was then reduced to zero (Fig. 2, first trace). The delivered pulses also resulted in reduction of the dendritic Ca²⁺ PIC (indicated by I_{Ca} in Fig. 2, fourth trace) due to the hyperpolarization induced in the dendrites. Similar results were obtained when the extracellular stimulating electrode was placed at locations relatively near to the soma and initial segment structures (activation thresholds as low as 5 μA), or when suprathreshold rectangular current pulses were simulated. However, at those near locations, inactivation of Na⁺ channels was achieved in the initial segment and the first node of Ranvier (structures with the highest density of Na⁺ channels). Generally, axonal action potentials were inactivated in response to high-frequency sinusoidal or rectangular current pulses of amplitude seven times the motoneuronal activation threshold and larger (≥7T). Conversely, subthreshold high-frequency or suprathreshold low-frequency (<3 kHz) sinusoidal and rectangular current pulses were unable to provide adequate levels of sustained depolarization to inactivate Na⁺

Fig. 4. Effect of biphasic charge-imbalanced current pulses on the firing behavior of spinal motoneurons. A: Membrane potential of different elements of the model motoneuron in response to extracellular electrical stimulation of subthreshold imbalanced pulses and 300% of synaptic activation. B: Motoneuronal firing rate measured from the last node of Ranvier in response to charge-imbalanced pulses of net DC current 2.5 μA (trace 1) and 1.0 μA (trace 2) and dendritic I_{Ca} (bottom trace). C: Membrane potential of the initial segment and last node of Ranvier (top) and dendritic I_{Ca} (second trace) during blockade of Na⁺ channels in the model motoneuron. Data in B and C were measured during synaptic activation at 300% of Ia-synaptic input and extracellular electrical stimulation of subthreshold biphasic charge-imbalanced pulses of net DC current 2.5 μA (C, bottom traces). Pulse parameters were 5 μA and 200 μs for the cathodic phase; 2.5 μA and 100 μs for the anodic phase; net DC = 2.5 μA; 3.333.3 Hz (i.e., no delay between pulses). Motoneuronal activation threshold was 19 μA. Steady-state firing rate and dendritic PIC are indicated by the dotted lines in B. Cell firing rate was reduced by ~11 impulses (22%). The amount of reduction in firing rate and the dendritic PIC are indicated by the double-headed arrows in B. An initial rise in firing rate in response to electrical stimulation is indicated by the left arrow (B, first trace). Charge-imbalanced pulses of smaller net DC current (1 μA) caused less initial rise in firing rate (B, trace 2). During blockade of Na⁺ channels in the model motoneuron, membrane potential of the initial segment showed depolarization, whereas that of the last node of Ranvier showed hyperpolarization in response to the electrical stimulation (C, top). The resting membrane potential (V_m) is indicated by the dotted line in the top panel of C. The gradual reduction in the membrane potential of the initial segment is due to the reduction in magnitude of the PIC reaching the soma (double-headed arrow in C, second trace).
channels in the soma and initial segment; hence, firing rate was increased rather than reduced (not illustrated).

**Extracellular electrical stimulation of biphasic charge-balanced current pulses.** The application of low-frequency biphasic charge-balanced pulses to reduce the motoneuronal firing rate was also examined in the model. Biphasic charge-balanced pulses were chosen because they do not cause tissue damage and electrode corrosion (52). Different pulse parameters such as pulse shape (symmetrical and asymmetrical), amplitude (subthreshold and suprathreshold), pulse width (100–500 μs and 1,000–1,500 μs for the short and long phases, respectively), and stimulation frequency (50–1,000 Hz) were tested in the model (Fig. 3A). Biphasic charge-balanced pulses were ineffective in inducing reductions in the firing rate of the model motoneuron (Fig. 3B). Because of their relative low frequency, these pulses were incapable of maintaining an adequate level of hyperpolarization in the membrane potential of the initial segment or first node of Ranvier. This was especially difficult to achieve during the strong depolarization of the motoneuronal membrane by the full activation of the dendritic PIC and enhanced synaptic input, as is expected during spasticity.

**Extracellular electrical stimulation of biphasic charge-imbalanced current pulses.** Given that biphasic charge-balanced pulses could not reduce firing rate, and high-frequency sinusoidal and rectangular current pulses completely inactivated the axonal action potentials, which would result in a relaxed (i.e., paralysed) muscle, we investigated the application of biphasic charge-imbalanced pulses to reduce, rather than block, the motoneuronal firing rate reaching the muscle (Fig. 4). These pulses have been shown to have higher safe limits than those for monophasic pulses with equivalent net DC current (58). Biphasic charge-imbalanced pulses of 4:1 (cathodic to anodic phase proportion) were used (Fig. 4C, bottom trace, *inset*). This proportion allowed for the injection of charge-imbalanced pulses with a wide range of net DC current and pulse amplitudes within the subthreshold range for motoneuronal firing (<1T). The short duration of the anodic phase (half that of the cathodic phase) was chosen to allow for fast partial recovery of the injected charge in the tissue, which minimizes irreversible electrochemical reactions at the electrode-tissue interface (52). Extracellular injection of high-frequency, subthreshold, biphasic charge-imbalanced pulses (5 μA and 200 μs for the cathodic phase, 2.5 μA and 100 μs for the anodic phase, stimulation frequency of 3,333 Hz, net DC = 2.5 μA) resulted in an initial rise followed by a drop in the motoneuronal firing rate (~20%, Fig. 4B, first trace). The effective low DC current generated by the charge-imbalanced pulses produced subthreshold and sustained depolarization of the soma and initial segment, which caused the initial increase in firing rate (Fig. 4A, yellow color). Conversely, a sustained hyperpolarization was generated in the proximal dendrites and nodes of Ranvier in response to the subthreshold depolarization of the soma and initial segment by the imbalanced pulses (Fig. 4A, purple color). The induced axonal hyperpolarization reduced the resultant firing rate that propagated through the axon (Fig. 4B, *trace 1*), whereas the dendritic hyperpolarization reduced the dendritic PIC (Fig. 4B, second trace). The stimulating electrode was moved at various locations relative to the model motoneuron (i.e., near to and far from the initial segment) to examine the effectiveness of charge-imbalanced pulse stimulation in the reduction of the motoneuronal firing rate at different electrode locations (Table 1). Generally, pulse amplitudes lower than 40% of the motoneuronal activation threshold (<0.4T) were efficient in inducing reduction in axonal firing rate. Higher pulse amplitudes (>0.4T) caused direct activation of the initial segment and/or first node of Ranvier, and resulted in higher axonal firing rate.

To examine the effect of the charge-imbalanced pulse amplitude on the extent of the initial rise in the motoneuronal firing rate, we simulated the delivery of charge-imbalanced pulses of lower net DC current (2 μA and 200 μs for the cathodic phase, 1 μA and 100 μs for the anodic phase, stimulation frequency of 3,333 Hz, net DC = 1 μA). The initial rise in firing rate at the onset of stimulation was reduced significantly (Fig. 4B, *trace 2*). This demonstrates that the initial rise in firing rate is an instantaneous response to the direct stimulation of the initial segment, and is dependent on the level of stimulation (i.e., net DC current).

Because action potentials could obscure observing steady-state alterations in the membrane potential, we verified the effect of high-frequency, subthreshold, biphasic charge-imbalanced pulses on the membrane potential during the blockade of Na⁺ channels in the model motoneuron (Fig. 4C). This is similar to the effect of adding sodium channel blockers tetrodotoxin or lidocaine derivative QX-314 to motoneurons to block the sodium spikes (31, 42, 43). In response to synaptic activation, the membrane potential of the initial segment showed strong membrane depolarization, whereas that of the last node of Ranvier showed weak membrane depolarization due to its distal location (~10 mm) from the dendrites (Fig. 4C, *top*). Upon the delivery of the subthreshold biphasic charge-imbalanced pulses, differential polarization of the motoneuronal membrane potential was induced. The membrane potential of the initial segment was further depolarized, whereas those of the nodes of Ranvier and the dendrites were hyperpolarized by the effect of the charge-imbalanced pulses (Fig. 4C, *top*). Hyperpolarization of the dendritic membrane potential is illustrated by the reduction in the magnitude of the dendritic PIC reaching the soma (Fig. 4C, bottom trace), which resulted in subsequent gradual decrease in the membrane potential of the initial segment (Fig. 4C, *top*). Therefore, measurements from simulations in the presence and absence of motoneuronal firing confirmed the differential polarization effect induced in the motoneuronal membrane by the charge-imbalanced pulses and the hyperpolarization of the axonal membrane.

The effect of extracellular stimulation with biphasic charge-imbalanced current pulses on the F-I relationship of spinal
motoneurons was investigated (Fig. 5). The F-I relationship was constructed by plotting the steady-state firing rate evoked in response to different levels of synaptic input versus the effective synaptic current reaching the soma at each level of synaptic activation. The injection of biphasic charge-imbalanced current pulses resulted in a linear F-I relationship (Fig. 5, slope after stimulation = 3.1 impulse·s⁻¹·nA⁻¹; r² = 0.99). There was a reduction in the firing rate at all levels of synaptic input, indicating an overall reduction in the motoneuronal excitability even when the dendritic Ca²⁺ PIC was fully activated (during the tertiary range of cell firing).

**Effect of change in pulse parameters.** Because of the non-linear time- and voltage-dependent properties of the motoneuronal membrane potential and channel dynamics, the amount of reduction in firing rate by charge-imbalanced pulses is sensitive to variations in pulse parameters. To obtain charge-imbalanced pulses (of 4:1 cathodic to anodic phase ratio) of the lowest net DC current and largest induced reduction in firing rate, the effect of pulse parameters on the firing behavior of the model motoneuron was investigated (Fig. 6). The pulse shape (ratio between the anodic and cathodic phases) was not changed during these simulations. The net DC current of the charge-imbalanced pulses was varied through changing either the pulse amplitude (amplitude modulation) or the pulse frequency (frequency modulation) when other pulse parameters were kept constant (Fig. 6A). Reduction in the motoneuronal firing rate was obtained with net DC current as low as 0.5 μA (~5%). The increase in the net DC current (0.5–3 μA) resulted in larger reductions in firing rate (5–22%). Further increase in the net DC current (>3 μA) caused an increase in the firing rate due to direct activation of the initial segment and/or the first node of Ranvier. Interestingly, net DC current produced through amplitude modulation showed higher efficacy in reducing firing rate than that produced through frequency modulation (at 1 μA, compare open squares to closed circles in Fig. 6A).

To obtain the largest reduction in firing rate for the same net DC level, we studied the effect of pulse width on the firing behavior of the model motoneuron (Fig. 6B). The charge-imbalanced pulses were delivered at the highest frequency (i.e., no delay between pulses). Short pulse widths (<100 μs) were more efficient in reducing the motoneuronal firing rate, whereas longer pulse widths (~200 μs) resulted in lower reduction in firing rate (Fig. 6B).

**Alteration in electrical properties after SCI.** The effect of changes in the motoneuronal electrical properties after SCI on the reduction in the motoneuronal firing rate achieved through the delivery of the proposed electrical current pulses was also investigated. Changes in electrical properties after SCI such as the reduction in the motoneuronal firing threshold and the depolarization of the resting membrane potential were simulated. Each of these changes caused an increase in the excitability of the model motoneuron. Blockage of motoneuronal firing was achieved through the delivery of high-frequency sinusoidal pulses even after the alteration of these electrical properties (Fig. 7, B and C, gray bars). The effect of Na⁺ channel inactivation properties on the efficacy of the high-frequency current pulses in blocking motoneuronal firing was also examined. We found that blockade of motoneuronal firing
was still achieved even after increasing and decreasing the Na\(^+\) channel inactivation time constant by a factor of 2 (Fig. 7, D and E, black bars).

The effect of changes in the electrical properties of the motoneuron after SCI and the Na\(^+\) channel inactivation properties on the efficacy of biphasic charge-imbalanced pulses was also investigated. Reduction in the motoneuronal firing rate was still achieved by the delivery of charge-imbalanced pulses even after the alteration of these properties (Fig. 7, B–E, black bars).

**DISCUSSION**

The main goal of the present study was to develop extracellular electrical current pulses that could be delivered focally through microwires implanted in the central nervous system to reduce the excitability of neurons. The particular example we focused on was reducing the increased motoneuronal excitability and firing activity; thereby, level of spasticity, through electrical stimulation of the spinal cord using microwires implanted for restoring limb movements after SCI. Results of the present study propose various electrical current pulses for reducing the motoneuronal firing behavior that range from graded reduction (through subthreshold charge-imbalanced pulses) to full blockade (through suprathreshold high-frequency sinusoidal or rectangular current pulses) of motoneuronal firing rate. Subthreshold biphasic charge-imbalanced pulses reduced the motoneuronal firing rate through deactivation of Na\(^+\) channels in the first node of Ranvier, whereas suprathreshold high-frequency sinusoidal or rectangular current pulses blocked motoneuronal firing rate through inactivation of Na\(^+\) channels in the soma, initial segment, and first node of Ranvier. Our results also demonstrated that high-frequency sinusoidal pulses and biphasic charge-imbalanced pulses were still effective even after changing the motoneuronal electrical properties (which increased the motoneuronal excitability) and after changing the inactivation properties of the Na\(^+\) channels (Fig. 7). Suppression of the motoneuronal firing rate was maintained throughout the delivery of the charge-imbalanced pulses or high-frequency pulses. It is expected that the dendritic distribution of Na\(^+\) channels, which is still unknown, would not affect our results as the differential polarization induced by the high-frequency stimulation or the charge-imbalanced pulses is generated primarily in the first node of Ranvier and initial segment, which are the structures with the highest density of Na\(^+\) channels.

Electrical stimulation, both in the peripheral and central nervous system, has been previously used for the reduction of spasticity. This included stimulation of the spastic muscles (61), muscles antagonistic to the spastic ones (2), and stimulation of the dorsal columns of the spinal cord through epidurally placed electrodes (36). In the first case, reduction of spasticity could be due to the electrical stimulation of the cutaneous afferents (4, 14), which is thought to induce synaptic plasticity (e.g., long-term potentiation and depression) in the spinal circuitry (13, 14). The reduction in spasticity persisted for at least 30 min after stimulation (14). In the second case, reduction of spasticity could be attributed to the reduction in the motoneuronal PIC by the effect of Ia-reciprocal inhibition (40), whereas in the third case reduction of spasticity could be due to the increase in presynaptic inhibition by the effect of electrical stimulation (34). Conduction block of unwanted motor nerve activity was examined as well. This block was achieved using either DC stimulation (8) or high-frequency sinusoidal stimulation (7, 39, 62). In the present study, we investigated various electrical stimulation pulses that could be delivered through microwires implanted in the spinal cord to restore limb movements after SCI. These pulses would reduce the level of spasticity through the suppression of the increased motoneuronal excitability and firing behavior after the injury. This reduction was achieved either through the inactivation of Na\(^+\) channels in the initial segment by suprathreshold high-frequency sinusoidal or rectangular current pulses or through the deactivation of Na\(^+\) channels in the initial nodes of Ranvier by subthreshold charge-imbalanced pulses.

**High-frequency sinusoidal and rectangular current pulses.** High-frequency sinusoidal and rectangular current pulses were examined because they are charge-balanced, and their charge density and charge per phase are within the safe limits (710 \(\mu C/cm^2\) and 0.02 \(\mu C\)/phase for pulse parameters described in Fig. 2; see Ref. 1) for stimulating tissue in the central nervous system. These pulses were previously investigated in the peripheral nervous system to induce a conduction block of motor nerves (7, 39, 62), or to obtain normal recruitment order through activation of small to large nerve fibers (59; for a detailed review see Kilgore and Bhadra, Ref. 39). It has been suggested that high-frequency sinusoidal stimulation blocks nerve conduction by producing a region of sustained depolarization of the nerve fiber membrane potential, despite there being no net charge injected into the tissue (39, 62). To the best of our knowledge, the present study is the first to investigate injection of high-frequency sinusoidal or rectangular current pulses in the central nervous system. Our results also confirm effective depolarization blockade as the mechanism of action of high-frequency sinusoidal current pulses. This is supported by the behavior of the activation gating particle (m) during high-frequency sinusoidal current pulses (Fig. 2, second trace) and the reduction in the dendritic PIC by stimulation-induced hyperpolarization generated in structures distal to the stimulating electrode (Fig. 7).
2, second trace). Noteworthy, the slow recovery of Na⁺ channels from their inactivation after the cessation of high-frequency stimulation would make the cell refractory for some time following the cessation of stimulation.

**Biphasic charge-imbalanced pulses.** The design of stimulating protocols is usually challenged by the compromise between the efficacy of the stimulation paradigm, the safety of the tissue, and the integrity of the stimulating electrode (51). A monophasic stimulating waveform is the most efficacious, but the most unsafe paradigm with regards to tissue damage, whereas a charge-balanced biphasic stimulating waveform with very short interpulse intervals is the safest, but the least efficacious paradigm (51). Charge-imbalanced biphasic waveforms are considered the best in terms of the balance between efficiency, safety of the tissue, and maintenance of the integrity of the stimulating electrode (51). It has experimentally been shown that DC current stimulation (i.e., not through monophasic or charge-imbalanced pulses) up to 3 μA in the rat spinal cord can be safe (35). Furthermore, charge-imbalanced biphasic current pulses were found to have higher safe limits for tissue damage than those for monophasic (3.5 times higher safe limits than monophasic pulses with equivalent net DC current) or charge-balanced biphasic current pulses (58). Therefore, application of biphasic charge-imbalanced pulses is a safer alternative than DC or monophasic stimulation of an equivalent DC current. It also implies that biphasic charge-imbalanced pulses of net DC up to 10 μA could be tolerated safely by the spinal cord. In the present study, the efficacy of the stimulation paradigm was assessed by the amount of induced reduction in firing rate in response to the injected current pulses. Charge-imbalanced pulses as low as 0.5 μA and up to 3 μA were shown to reduce the motoneuronal firing rate (Fig. 6A). Given that the amplitude of the proposed pulses is small, the charge density, charge per phase, and current density per phase (35.3 μC/cm², 1 nC/phase, and 0.18 A/cm², respectively, for pulse parameters described in Fig. 4, and assuming an electrode stimulating surface area of 2,830 μm²) are far lower than the safety limits for electrical stimulation in the central nervous system (1). Because very little information is available in the literature on biphasic charge-imbalanced pulses, we investigated the effect of different pulse parameters on the evoked behavior of motoneurons. We noted that due to the time- and voltage-dependent intrinsic membrane properties, we could enhance the efficacy of the charge-imbalanced pulses (i.e., increase the amount of reduction in firing rate), or reduce the net DC current while achieving the same reduction in firing rate, by varying the pulse parameters (e.g., short pulse widths, amplitude modulation).

The net DC current injected into the tissue by the charge-imbalanced current pulses could affect the integrity of the stimulating electrodes (51). To overcome this problem, several approaches could be employed. First, electrode materials that have high resistivity to corrosion (e.g., platinum-iridium alloys) would be used (51, 52). Second, the delivery of the charge-imbalanced pulses could also be limited to the time of the spastic syndrome. That is, the charge-imbalanced pulses would be delivered only when the uncontrolled muscle spasms and long-lasting reflexes are activated. This strategy has been experimentally shown to minimize effectively microstimulation-induced tissue damage (46). Third, electrical stimulation could be rotated among the intraspinal microwires (electrode rotation; in which current pulses are delivered separately and consecutively through each electrode). The aforementioned techniques for minimizing electrode corrosion would also be beneficial in reducing any potential tissue damage that could result from the delivered pulses.

Our results showed that biphasic charge-imbalanced pulses with low net DC current were able to reduce the motoneuronal firing rate; however, an initial rise in firing rate was encountered at the onset of stimulation (Fig. 4B, trace 1). This initial increase in firing rate could then generate a muscle spasm or increase the level of an existing spasm. The initial increase in firing rate was shown to be an instantaneous response to the direct stimulation of the initial segment by the delivered pulses. Increasing the amplitude of the delivered pulses gradually (i.e., ramp) would reduce the initial rise in the motoneuronal firing rate and would prevent the generation of a muscle spasm at the onset of stimulation.

For the same net DC level, we found that charge-imbalanced pulses with short pulse durations (≤100 μs) induced greater reduction in firing rate than those with long pulse durations (≥200 μs; Fig. 6B). This dissimilarity in effect could be explained by the effect of the pulse duration on the dynamics of Na⁺ channels at the first node of Ranvier. The conductance of Na⁺ channels (G_{Na}; where G_{Na} = G_{Na0} \cdot m^3 \cdot h; G_{Na0} is the maximum Na⁺ channel conductance) depends primarily on a fast activation gating particle (m), and to a lesser extent on a relatively slow inactivation gating particle (h). That is, the m particle has a short time constant (τ\_m) relative to that of the h particle (τ\_h differs by two orders of magnitude from τ\_m), making m more sensitive to rapid variation in membrane potential than h (24). Therefore, current pulses with short durations that correspond to τ\_m and much shorter than τ\_h (≪ τ\_h ≈ 600 μs) would have their primary effect exerted on the m particle while minimally affecting the h particle, and would have greater influence on the conductance of Na⁺ channels. Conversely, current pulses with long durations would affect both the m and h particles of Na⁺ channels (i.e., long hyperpolarization of the membrane potential would reduce m, which reduces G_{Na0} and increase h, which increases G_{Na}). Therefore, the counteracting effects of m and h by the induced hyperpolarization at the first node of Ranvier by long-imbalanced pulses would reduce the efficiency of the delivered pulses in suppressing the conductance of axonal Na⁺ channels.

**General considerations.** The present model considered only the effect of the proposed current pulses on the firing behavior of a single motoneuron; however, the effect on other neuronal elements could be predicted. Suprathreshold high-frequency sinusoidal and rectangular current waveforms, which have pulses with large amplitudes (≥7T), would cause inactivation of action potentials in sensory and motor axons. Therefore, relaxation in muscle activity would result in response to this stimulation. This is similar to the conduction block of action potentials in motor nerves produced experimentally by high-frequency sinusoidal stimulation in the peripheral nervous system (7, 39, 62). However, high-frequency sinusoidal or rectangular current pulses would also prevent the residual voluntary drive after incomplete SCI from activating the muscle. Subthreshold biphasic charge-imbalanced pulses would have their effects primarily on the initial segment and nodes of Ranvier (structures with the lowest activation threshold). Given the low amplitude of these pulses (<0.4T), the
affected structures would be depolarized but not activated, and the induced hyperpolarization in adjacent nodes of Ranvier would reduce the axonal firing rate. Sensory axon terminals in the gray matter may be activated by the delivered pulses. However, stimulation of those axons would minimally affect the motoneuronal firing through their subsequent excitatory synaptic actions on the motoneurons. This is due to the large reduction in the dendritic PIC induced by the pulses.

The effectiveness of charge-imbalanced pulses in reducing the motoneuronal firing rate was found to be sensitive to the net DC current (i.e., amplitude of stimulation) and the stimulating electrode location relative to the motoneuron. That is, pulses that would be subthreshold to some motoneurons (those far from the stimulating electrode) might be suprathreshold to other motoneurons (those closer to the stimulating electrode). The amplitude of the delivered pulses should then be adjusted to avoid activation of a large number of motoneurons, which could generate a muscle spasm. Therefore, the amplitude of stimulation, number of intraspinal microwires, and degree of electrode rotation required to obtain a functional reduction in the overall muscle activity would need to be determined experimentally. Our results could also be beneficial in situations where suppression of the motoneuronal excitability in general is desired to provoke muscle relaxation. For instance, the proposed paradigms could be effective in the management of bladder dysfunction after SCI by reducing the hyperexcitability of external urethral sphincter motoneurons in Onuf’s nucleus during micturition and preventing the concomitant contraction of the sphincter and the detrusor muscle of the bladder.

Although extracellular electrical stimulation in the spinal cord was simulated in the present study, our results and the discussed concepts could also be extended to electrical stimulation in the brain. For instance, our results could be beneficial in understanding the mechanisms underlying deep brain stimulation. Electrical stimulation causes differential polarization of the stimulated neuronal elements and results in regions of depolarization and hyperpolarization. The spatial distribution and magnitude of these regions depend on the electrode geometry, orientation relative to the stimulating electrode, and morphology of the neuronal elements (11, 56). It has been recently shown that DBS produces complex patterns of excitation and inhibition in neurons in the vicinity of the electrode, and that the somatic activity of these neurons could be entirely different from their axonal activity, which represents the functional output of the stimulated nuclei (50). Understanding this decoupling of the somatic and axonal activity during DBS could resolve the discrepancy in neural recordings regarding the mechanism(s) of action of DBS (50). Therefore, comprehending the effects of extracellular electrical microstimulation on the firing behavior of axons and cell bodies is crucial in uncovering the mechanisms underlying the therapeutic effects of DBS. The present study discusses the same concepts of stimulation-induced differential polarization in response to electrical microstimulation in the spinal cord. We took advantage of the induced differential polarization to reduce the axonal activity despite the depolarization of the somatic membrane (Fig. 4).

In summary, extracellular electrical stimulation of suprathreshold high-frequency sinusoidal or rectangular current pulses, as well as subthreshold biphasic charge-imbalanced pulses through intraspinal microwires, were shown to reduce the increased excitability and firing activity of spinal motoneurons after SCI. The proposed stimulation paradigms applied through ISMS microwires used for restoring standing and walking after SCI could also provide a potential clinical therapy for reducing the severity of spasticity, in addition to the restoration of limb movements, following the injury.

GRANTS

This work was funded by the National Institute of Neurological Disorders and Stroke. V. K. Mushahwar is an Alberta Heritage Foundation for Medical Research (AHFMR) Scholar, and S. M. ElBasiouny was supported by an AHFMR Doctoral Studentship.

REFERENCES

MODULATION OF MOTONEURONAL FIRING USING INTRASPINAL MICROSTIMULATION


