Introduction

Microstimulation in the CNS can activate populations of neurons with greater specificity than is possible with larger electrodes on the surface of the spinal cord or brain (Gustafsson and Jankowska 1976; Ranck 1975). The potential thus arises for electrical activation of intact neuronal circuitry and, in turn, generation of distributed and controlled motor outputs for the study of the neural control of movement (Giszter et al. 1993) or for application in neural prostheses (Barbeau et al. 1999). Knowing what neural elements are activated by the stimulus is of fundamental importance in understanding the behavioral response, and in the case of neural prostheses, selective activation of targeted populations is required for device efficacy. However, in many regions of the CNS, local cells and fibers of passage are intermingled in close proximity to the electrode, and their thresholds are similar with conventional stimuli (McIntyre and Grill 1999, 2000; Ranck 1975).

We have previously developed asymmetric biphasic charge-balanced stimuli that increased the threshold difference between neurons with their cell bodies near the electrode (local cells) and fibers of passage (McIntyre and Grill 2000). However, this analysis was limited to idealized neural orientations and single stimuli. The first goal of the present study was to determine if asymmetric biphasic charge-balanced stimulus waveforms are effective in increasing the selectivity between cells and fibers in a specific instance of intraspinal microstimulation. The second goal was to determine if the waveforms are effective under repetitive activation with trains of stimuli. Our hypothesis was that stimulus trains would provide enhanced selectivity because of differences in the post-action potential excitability of cells and fibers of passage. In addition to the direct influence of the stimulus, an indirect influence can also affect the activation of local cells. This indirect influence arises from the excitation of presynaptic neural elements by the stimulus pulse, and their subsequent postsynaptic effects on local cells that can influence neural output (Baldissera et al. 1972; Gustafsson and Jankowska 1976). Therefore the third goal of this study was to quantify the effects of stimulation induced trans-synaptic inputs on extracellular activation of local cells.

We developed a computer-based integrated field-neuron model to study the influence of stimulus waveform and frequency on selectivity between cells and fibers of passage. The field-neuron model consisted of the extracellular electric field computed using a finite element model of microstimulation of the spinal cord, coupled to multi-compartment neuron models to determine the effects of extracellular stimulation on neural output. We modeled stimulation near Onuf's nucleus in the sacral region of the cat spinal cord as this provides a system amenable to experimental testing of the model predictions and...
is an area targeted for stimulation for restoration of bladder emptying (Grill et al. 1999; Prochazka et al. 2001). The axons of the preganglionic parasympathetic innervation of the bladder run in close proximity to the cell bodies of the somatic motoneurons innervating the external urethral sphincter (Nadelhaft et al. 1980; Thor et al. 1989; Vanderhorst and Holstege 1997). Contraction of the bladder and external urethral sphincter can be measured to determine activation of fibers and local cells, respectively. Therefore building our model around this physiological system will enable experimental testing of the model-designed stimulus parameters.

The influence of extracellular electric fields on neurons is related to the second difference of the extracellular potential along the extent of the individual neurons and will cause both regions of depolarization and regions of hyperpolarization in the same neuron (Basser and Roth 2000). Cathodic or anodic regions of depolarization and regions of hyperpolarization will cause both related to the second difference of the extracellular potential field-neuron model was developed to study neural activation by extracellular stimulation with microelectrodes within the spinal cord. A three-dimensional finite-element model of the spinal cord was used to solve for the potentials generated in the tissue by microstimulation. The resulting extracellular potentials were applied to detailed multi-compartment neural models used to represent the geometrical and electrical properties of both myelinated fibers of passage and motoneurons (including a branching dendritic tree, soma, initial segment and myelinated axon) to create an integrated field-neuron model.

**METHODS**

An integrated field-neuron model was developed to study neural activation by extracellular stimulation with microelectrodes within the spinal cord. A three-dimensional finite-element model of the spinal cord was used to solve for the potentials generated in the tissue by microstimulation. The resulting extracellular potentials were applied to detailed multi-compartment neural models used to represent the geometrical and electrical properties of both myelinated fibers of passage and motoneurons (including a branching dendritic tree, soma, initial segment and myelinated axon) to create an integrated field-neuron model.

**Fiber model**

The multi-compartment cable model of the myelinated axon, described in detail in McIntyre et al. (2002), contained 10 segments between successive nodes with an explicit representation of the myelin attachment segment (MYSA), paranode main segment (FLUT), and internode segment (STIN) regions of the fiber (Fig. 1; Tables 1 and 2; APPENDIX). The double-cable structure incorporated both linear and nonlinear membrane dynamics to represent the electrical behavior of the fiber. The nodes consisted of the parallel combination of nonlinear fast Na\(^+\), persistent Na\(^+\), and slow K\(^+\) conductances, a linear leakage conductance, and the membrane capacitance. The paranodal and internodal compartments included two concentric layers, each including a linear conductance in parallel with the membrane capacitance, to represent the myelin sheath and underlying axolemma. The myelinated axon model reproduced a wide range of experimental data including the strength-duration relationship, current-distance relationship, conduction velocity, afterpotential shape, and changes in excitability after a single or train of stimuli (McIntyre et al. 2002).

**Motoneuron model**

The cell body, consisting of the soma and axon initial segment, was modeled as a three-dimensional structure using nine compartments with geometries based on morphological data (Cullheim and Kellert 1978; Cullheim et al. 1987; Sasaki 1994). Motoneuron models traditionally represent the soma as one spherical compartment; however, the soma is a geometrically complex structure with tapering attachments to its processes. Therefore we developed a distributed soma model (McIntyre and Grill 2000) with six tapering cylinders where the large end of each tapered compartment connected to the other soma compartments and the small end of each tapered compartment connected to one of the five dendrites or the initial segment (Fig. 1; Table 1). The soma had a total membrane surface area of 4.920 \(\mu\)m\(^2\). The initial segment of the axon was modeled with three cylindrical compartments connected in series.

The soma included conductances representing nonlinear fast Na\(^+\),...
TABLE 1. Model geometric parameters (μm)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell body</td>
<td></td>
</tr>
<tr>
<td>Soma compartment length</td>
<td>15</td>
</tr>
<tr>
<td>Soma compartment diameter</td>
<td>7.35</td>
</tr>
<tr>
<td>Soma compartment diameter</td>
<td>4.35</td>
</tr>
<tr>
<td>Initial segment compartment length</td>
<td>10</td>
</tr>
<tr>
<td>Initial segment diameter</td>
<td>4</td>
</tr>
<tr>
<td>Myelinated axon</td>
<td></td>
</tr>
<tr>
<td>Fiber diameter</td>
<td>11.5</td>
</tr>
<tr>
<td>Number of myelin lamella</td>
<td>130</td>
</tr>
<tr>
<td>Node length</td>
<td>1</td>
</tr>
<tr>
<td>Node diameter</td>
<td>3.7</td>
</tr>
<tr>
<td>MYSA length</td>
<td>3</td>
</tr>
<tr>
<td>MYSA diameter</td>
<td>3.7</td>
</tr>
<tr>
<td>MYSA periaxonal space width</td>
<td>0.002</td>
</tr>
<tr>
<td>FLUT length</td>
<td>50</td>
</tr>
<tr>
<td>FLUT diameter</td>
<td>8.1</td>
</tr>
<tr>
<td>FLUT periaxonal space width</td>
<td>0.004</td>
</tr>
<tr>
<td>STIN length (first 4 internodes)</td>
<td>63.5</td>
</tr>
<tr>
<td>STIN length (last 6 internodes)</td>
<td>190.5</td>
</tr>
<tr>
<td>STIN diameter</td>
<td>8.1</td>
</tr>
<tr>
<td>STIN periaxonal space width</td>
<td>0.004</td>
</tr>
</tbody>
</table>

N- and L-type Ca$^{2+}$, delayed rectifier K$^+$, and Ca$^{2+}$-activated K$^+$ channels as well as a linear leakage conductance in parallel with the membrane capacitance (Fig. 1; Tables 3 and 4; Appendix). The initial segment included conductances representing linear increase from initial diameter to final diameter; first linear segment attached to initial segment; i linear increase from initial diameter to final diameter; i compartment attached to initial segment; j total initial segment compartments; k Myelinated axon references and notes in McIntyre et al. (2002); l Fabricius et al. (1994).

MYSA, myelin attachment segment. * Six total soma compartments; Cullheim et al. (1987); * compartments attached to dendrites; i linear increase from initial diameter to final diameter; i compartment attached to initial segment; j total initial segment compartments; Cullheim and Keller (1978); m myelinated axon references and notes in McIntyre et al. (2002); q Fabricius et al. (1994).

TABLE 3. General model electrical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuron rest potential (E_{rest})</td>
<td>−70.0</td>
</tr>
<tr>
<td>Membrane capacitance (C_{m}, μF/cm$^2$)</td>
<td>2</td>
</tr>
<tr>
<td>Cell body intracellular resistivity (ρ_{i}, Ω cm)</td>
<td>200</td>
</tr>
<tr>
<td>Dendrite intracellular resistivity (ρ_{d}, Ω cm)</td>
<td>200</td>
</tr>
<tr>
<td>Axon intracellular resistivity (ρ_{a}, Ω cm)</td>
<td>70</td>
</tr>
<tr>
<td>Periaxonal resistivity (ρ_{p}, Ω cm)</td>
<td>70</td>
</tr>
</tbody>
</table>

that originated at the soma. Each root dendrite had an arbor based on the results presented in Fig. 1 of Cullheim et al. (1987), with a root diameter of 7 μm and 25 terminations. The total dendritic arbor had 125 terminations and a surface area of 269,980 μm$^2$.

Spinal cord model

A three-dimensional finite-element model (FEM) was used to create an anatomically and electrically accurate volume conductor model of the cat sacral spinal cord (Miller and Henriquez 1990) (Fig. 2). The model geometry was derived from the histological data of Vanderhorst and Holstege (1997), who described the gray-and-white matter geometry as well as the anatomical location of the motoneurons innervating each of the muscle groups originating in the lumbar sacral spinal cord. The white matter was modeled as two-dimensionally anisotropic with a longitudinal conductivity of 0.0033 S/cm and a transverse conductivity of 0.00083 S/cm based on previous measurements in the dorsal columns (Ranck and BeMent 1965). The gray matter was modeled as isotropic with a conductivity of 0.002 S/cm as measured for cortical gray matter (Li et al. 1968; Ranck 1963). Surrounding the spinal cord was a layer of saline (0.02 S/cm).

The FEM represented the sacral spinal cord from the S₁ through the S₈ segments and consisted of 91,840 elements. The FEM was implemented in a commercially available finite-element software program, ANSYS 5.7 (ANSYS, Houston, PA) that used a frontal solution method (direct elimination solver) (Irons 1970) of the Laplace equation

$$ \nabla \cdot \sigma \Phi = 0 $$

where the output of the FEM model was the potential (Φ) at each node of the finite-element mesh (Fig. 2). Within 500 μm of the electrode, separation between the nodes of the finite element mesh was <50 μm, and at regions farther from the electrode, progressively larger element sizes were used (Fig. 2D). The potential at the boundary of the model was set to zero and was far enough from the central region of the model that the potentials in the gray-and-white matter differed by <1% when the distance from the center of the model to the boundary was 4 cm.

TABLE 4. Cell body and dendrite electrical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$ Nernst potential (E_{Na})</td>
<td>50.0</td>
</tr>
<tr>
<td>K$^+$ Nernst potential (E_{K})</td>
<td>−80.0</td>
</tr>
<tr>
<td>Leakage reversal potential (E_{Lk})</td>
<td>−70.0</td>
</tr>
<tr>
<td>Soma parameters</td>
<td></td>
</tr>
<tr>
<td>Maximum Na$^+$ conductance (g_{Na}), S/cm²</td>
<td>0.05</td>
</tr>
<tr>
<td>Maximum delayed rectifier K$^+$ conductance (g_{KDR}), S/cm²</td>
<td>0.3</td>
</tr>
<tr>
<td>Maximum N-type Ca$^{2+}$ conductance (g_{Ca}), S/cm²</td>
<td>0.05</td>
</tr>
<tr>
<td>Maximum L-type Ca$^{2+}$ conductance (g_{CaL}), S/cm²</td>
<td>0.0001</td>
</tr>
<tr>
<td>Maximum Ca$^{2+}$-activated K$^+$ conductance (g_{KCa}), S/cm²</td>
<td>0.3</td>
</tr>
<tr>
<td>Leakage conductance (g_{Lk}), S/cm²</td>
<td>0.0002</td>
</tr>
<tr>
<td>Initial segment parameters</td>
<td></td>
</tr>
<tr>
<td>Maximum Na$^+$ conductance (g_{Na}), S/cm²</td>
<td>0.5</td>
</tr>
<tr>
<td>Maximum K$^+$ conductance (g_{K}), S/cm²</td>
<td>0.1</td>
</tr>
<tr>
<td>Leakage conductance (g_{Lk}), S/cm²</td>
<td>0.01</td>
</tr>
<tr>
<td>Dendrite parameters</td>
<td></td>
</tr>
<tr>
<td>Leakage conductance (g_{Lk}), S/cm²</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

$^a$ Safonov et al. (1997); $^b$ Catterall (1981); $^c$ Traub and Llinas (1977); $^d$ Clements and Redman (1989); $^e$ Fleshman et al. (1988).
was doubled. Point-source electrodes were implemented by assigning currents to the appropriate nodes in the finite-element mesh. The simplification of using point-source electrodes to represent the potential distribution generated by metal microelectrodes is justified from our previous work demonstrating that neural activation produced by a point source was indistinguishable from that produced by sharp tipped microelectrodes (McIntyre and Grill 2001). The FEM was validated by comparing the model solution with homogeneous isotropic conductivities to that of a theoretical point source with <1% error. Because the bulk conductivity of the cord is linear, the potential distributions for different stimulus current magnitudes were scaled versions of the potential distribution generated by a unitary current stimulus (Hulbert and Tator 1993; Nicholson and Freeman 1975).

Simulations with the integrated spinal cord and neuron models

The neuron models were positioned within the volume conductor model of the spinal cord at locations based to experimental tracing

FIG. 2. Integrated model of the spinal cord and spinal neurons. The neuron models were used to represent a motoneuron innervating the external urethral sphincter (A), a preganglionic parasympathetic neuron innervating the bladder (B), and a fiber of passage in the white matter (C). D: a finite-element model, incorporating the inhomogenous and anisotropic electrical properties of the spinal cord, was used to solve for the potential distribution generated by microstimulation in the ventral horn. A nonuniform mesh, both transversely and longitudinally, was used to improve the accuracy near the electrode where the gradient of the electric field was the greatest. E: potential distribution generated in the finite-element model of the spinal cord by a point source of current (1 μA). The standard electrode location was in the ventral horn, marked by the point of maximum potential on the “plane of the electrode” potential distribution map. A potential distribution map is also shown for a plane displaced 500 μm from the electrode to illustrate the decay of the potential in the longitudinal direction.

study (Nadelhaft et al. 1980; Thor et al. 1989; Vanderhorst and Holstege 1997) (Fig. 2, A–C). The output of the FEM was the potential at each node of the finite-element mesh; however, the location of the nodes of the mesh did not necessarily correspond to the location of a given neuronal compartment. Therefore three-dimensional tessellation-based linear interpolation was used to determine the potential between points in the finite-element mesh (Watson 1999). The extracellular potentials generated by the stimulus were applied to the neuron models, and an equivalent set of distributed intracellular injected currents was calculated and used to stimulate the neurons (McIntyre and Grill 2000, 2001; Richardson et al. 2000; Warman et al. 1992).

All simulations (except for data presented in Fig. 3) used a standard electrode location in the ventral horn of the spinal cord (Fig. 2, D and E). This electrode location enabled comparison among the outputs of three populations of neurons activated by extracellular sources: neurons with their cell bodies near the electrode representing the somatic motoneurons controlling the external urethral sphincter (EUS) (Fig. 2A), neurons with their axons passing by the electrode but with their cell bodies within ~1,000 μm of the electrode representing the parasympathetic preganglionic neurons controlling the bladder (BLA) (Fig. 2B), and neurons with their axons passing by the electrode but with their cell bodies far from the electrode representing fibers of passage in the white matter (FOP) (Fig. 2C). The threshold for excitation with a single symmetrical, charge-balanced, cathodic first, biphasic stimulus 0.1 ms in duration (each phase) was 34 μA for all three models at the standard ventral horn electrode position (Fig. 2, D and E). This enabled comparison of changes in the neuronal output with alteration in the stimulus frequency and stimulus waveform from a reference point that represented the most commonly used stimulus waveform in neuroprosthetic applications.

RESULTS

We used an integrated model of the electric field generated by intraspinal microstimulation coupled to multi-compartment cable models of spinal neurons to study activation of CNS neurons by extracellular stimulation. The multi-compartment cable models were able to replicate a wide range of experimental data on the excitation properties of mammalian neurons, and the output of the integrated field-neuron model agreed qualitatively with responses generated by microstimulation of the sacral spinal cord. Asymmetric biphasic stimulus waveforms enabled selective activation of either cells near the electrode or fibers of passage during repetitive activation. Alterations in stimulus frequency, which exploited differences in the post-action potential excitabilities of the neuronal cell bodies and the fibers of passage, could also increase selectivity. In addition, the results indicate that stimulation induced trans-synaptic excitation/inhibition of local cells influenced the neuronal output during repetitive extracellular stimulation.

Dynamic firing properties of the neuron model

The motoneuron model was able to reproduce several independent sets of experimental data from mammalian motoneurons (Fig. 3). The action potential recorded in the soma had a magnitude and shape that matched well with experimental data (Barrett et al. 1980), and the depolarizing (DAP) and hyperpolarizing (AHP) afterpotentials had amplitudes and durations similar to in vivo recordings (Zengel et al. 1985) (Fig. 3A). The dynamic firing properties of the neuron model matched well with experimental recordings. When a constant current intracellular stimulus was applied
to the soma, the model exhibited spike-frequency adaptation that had a time course similar to experimental data (Sawczuk et al. 1995) (Fig. 3B). The steady-state firing rate of the motoneuron model as a function of the amplitude of the intracellular stimulus exhibited primary and secondary firing ranges that also matched well with experimental data (Schwindt and Crill 1982) (Fig. 3C).

Model comparison to spinal cord microstimulation experiments

The threshold current as a function of the electrode-to-neuron distance for the model motoneuron innervating the EUS was compared with the experimental measurements from Gustafsson and Jankowska (1976) (Fig. 4A). The data are plotted for an electrode trajectory that was 50 μm lateral to the cell body, and the penetration moved from dorsal to ventral from the lowest threshold stimulation site (electrode depth = 0 μm) for cathodic stimuli 200 μs in duration. The results show that the model thresholds matched well with the experimental thresholds under conditions that mimicked the experimental setup (Gustafsson and Jankowska 1976) (Fig. 4A).

The model neuron innervating the bladder (BLA) in the integrated field-neuron model exhibited changes in threshold relative to electrode position that corresponded with bladder pressures evoked by microstimulation of the S2 segment (Grill et al. 1999). Both the model and the experiment used charge-balanced, cathodic first, biphasic stimuli 0.1 ms in duration, and the experimental results were obtained with a 100-μA stimulus amplitude (Grill et al. 1999). Pressures evoked in the bladder as a function of electrode depth compared qualitatively with the inverse of the threshold for activation of the BLA model neuron for electrode penetrations along a similar trajectory (Fig. 4B). The inverse of the model neuron threshold was used as a proxy for the number of neurons that would be activated (Fig. 4B).
excited by a particular stimulus amplitude, and it was assumed that the bladder pressure was correlated with the number of activated neurons. This assumption is supported by the experimental results demonstrating the ability to grade bladder pressure, at a particular electrode location, by alterations in the stimulus amplitude (Grill et al. 1999). Points of low threshold in the BLA model near the intermediolateral cell column and near the base of the ventral horn corresponded to regions of high pressures in the experiment (Fig. 4B). Direct quantitative comparisons cannot be made due to geometrical differences in the model and experiment as well as the fact that pressure measurements were the result of activation of many neurons distributed around the electrode. However, the model results do correspond qualitatively with the experimental results, and the stimulus amplitudes necessary for activation of the model neurons correspond with the stimulus amplitude used in the experiment.

Strength-duration relationship of cells and fibers of passage

We examined the effect of changing the stimulus pulse duration on extracellular activation of the EUS, BLA, and FOP neuron models with the standard electrode position in the ventral horn (Fig. 2, D and E). While each neuron had the same threshold for a stimulus pulse duration of 0.1 ms, the FOP had lower thresholds than BLA and EUS for shorter duration pulses and the EUS had lower thresholds than BLA which had lower thresholds than FOP for longer pulse durations (Fig. 5). In turn, the chronaxie time ($\tau_{CH}$) was different for the different neurons. The local cell (EUS) had the longest $\tau_{CH}$ of 408 $\mu$s, the fiber of passage with its cell body near the electrode (BLA) had a $\tau_{CH}$ of 222 $\mu$s, and the fiber of passage with its cell body far from the electrode (FOP) had the shortest $\tau_{CH}$ of 118 $\mu$s. These values correspond well with previous experimental measurements of extracellular $\tau_{CH}$ for local cells (200–700 $\mu$s) and fibers of passage (50–200 $\mu$s) (Nowak and Bullier 1998a; Ranck 1975).

**FIG. 5.** Strength-duration relationship of local cells and fibers of passage. Threshold for extracellular stimulation of the external urethral sphincter motoneuron (EUS), the neuron innervating the bladder (BLA), and the fiber of passage in the white matter (FOP) in the integrated field-neuron model as a function of stimulus pulse duration of a symmetric charge-balanced biphasic stimulus. The chronaxie time ($\tau_{CH}$) was calculated using a least squares log-log fit to $I_{TH}(PD) = I_{TH} \ast [1 + (\tau_{CH}/PD)]$, where $I_{TH}$ is the threshold current, $I_{CH}$ is the rheobase current, and PD is the pulse duration (McIntyre and Grill 1998).

Frequency-dependent neuronal output of cells and fibers of passage

Mammalian neurons exhibit both DAPs and AHPs. The time course of these afterpotentials is different in the cell body and axon (Barrett et al. 1980; David et al. 1995; Zengel et al. 1985), and these afterpotentials affect the threshold for generation of subsequent impulses (McIntyre et al. 2002). We hypothesized that differences in the post-action potential excitability of cells and axons could be exploited to enhance selectivity by the appropriate choice of stimulus frequency. Figure 6 shows the response of the EUS neuron (local cell) compared with the response of the FOP to 50- and 125-Hz symmetrical biphasic stimulus trains at 35 $\mu$A. Both the local cell and fiber of passage fired in response to the first stimulus in both trains. Subsequent stimuli in the 50-Hz train fell within the AHP (period of decreased the excitability) of the local cell and fiber of passage. As a result, the neurons were unable to follow the stimuli in a 1:1 ratio, and the local cell and fiber of passage generated propagating action potentials in response to 33% and 43% of the stimuli, respectively. When the stimulus frequency was increased to 125 Hz once again, both the cell and fiber of passage fired in response to the first stimulus. Subsequent stimuli fell within the DAP (period of increased excitability) of the fiber, and it followed the stimulus frequency at 100%. However, the stimuli fell within a period of decreased excitability of the cell, and it generated action potentials is response to only 14% of the stimuli. These results demonstrate that modulation of the frequency of the stimulus train can enhance selectivity between activation of cells and fibers of passage within the CNS.

Influence of stimulus waveform and frequency on selectivity

Our previous work showed that alterations in the stimulus waveform could enhance selectivity between neuronal populations (McIntyre and Grill 2000). We measured the output of three different neurons in the vicinity of the electrode in response to trains of 25- to 150-Hz stimuli for three different stimulus waveforms (Fig. 7). Maps of the percent of stimuli that generated propagating action potentials during the stimulus train as a function of stimulus amplitude and frequency were generated for the EUS, BLA, and FOP neurons. At high stimulation frequencies, symmetrical charge-balanced biphasic cathodic phase first stimuli generated preferential activation of the FOP compared with the other two neurons (BLA, EUS), while their outputs were similar at low frequencies (Fig. 7A).

Selectivity between the local cell (EUS) and the passing fibers (BLA, FOP) was improved using asymmetrical charge-balanced biphasic stimuli. When an asymmetrical charge-balanced biphasic cathodic phase first stimulus waveform was used, there was a decrease in the threshold for activation of the local cell and an increase in the threshold to activate either passing fiber. For stimulus amplitudes between 35 and 50 $\mu$A, the local cell (EUS) fired at 100%, and there was no activation of either the local (BLA) or passing (FOP) fibers (Fig. 7B). When an asymmetrical charge-balanced biphasic anodic phase first stimulus waveform was used, there was a decrease in the threshold to activate both the local (BLA) and passing (FOP) fibers and an increase in the threshold to activate the local cell (Fig. 6C). For stimulus amplitudes between 38 and 50 $\mu$A,
both the local (BLA) and passing (FOP) axons fired at 100%, and there was no activation of the local cell (EUS). As with conventional biphasic pulses (Figs. 6 and 7A), selectivity between local (BLA) and passing (FOP) fibers could be enhanced using changes in stimulus frequency. At lower frequencies, both BLA and FOP had similar thresholds; however, at frequencies >50 Hz, near-threshold stimulus amplitudes resulted in 100% output from the FOP and <20% output from the BLA. Instances of selective activation of local fibers (BLA) over both local cells (EUS) and fibers of passage (FOP) were not observed.

Synaptic influence on extracellular activation of local cells

When using extracellular stimulation to activate local cells, it is possible to excite them directly with the stimulus and/or indirectly alter their excitability via excitation (by the stimulus) of synaptic terminals that make connections on the dendritic arbors of the local cells. Previous experimental results have shown that the thresholds for direct or indirect (trans-synaptically evoked) action potential generation in local cells are similar with extracellular sources (Baldissera et al. 1972; Gustafsson and Jankowska 1976). To determine the effects of indirect activation of local cells during extracellular stimulus trains, we developed a model of Ia excitatory input to the motoneuron model using detailed morphological data from the literature (Brown and Fyffe 1978; Burke and Glenn 1996) (Fig. 8). The Ia input model consisted of a 6-μm-diam myelinated fiber running in the medial dorsal column with a 4-μm-diam collateral projecting into the dorsal gray matter. The collateral branched into two 3-μm-diam fibers that each branched into two 2.5-μm-diam fibers, and each of these branched into two 2-μm-diam fibers that ended with a 1-μm-diam stem and a 3-μm-diam bouton that contacted a dendritic branch of the motoneuron. Alpha functions \[ \frac{I_{\text{syn}}}{t} = \frac{\beta_{\text{syn}}}{\tau} * e^{(-t/\tau)} * (V_m - E_{\text{syn}}) \] were used to describe the postsynaptic current at each of the eight contact sites on the dendritic arbor of the EUS motoneuron model. The parameters of the alpha functions were set such that a composite excitatory postsynaptic potential (EPSP) at the soma matched the sub-maximal Ia EPSP measured experimentally \( g_{\text{syn}} = 0.05 \mu S; \tau = 0.5 \text{ ms}; E_{\text{syn}} = 0 \text{ mV} \) (Burke 1968; Segev et al. 1990) (Fig. 8A).

During extracellular stimulation with the standard ventral horn electrode location (Fig. 2, D and E), action potentials were evoked at all eight boutons of the Ia input for extracellular stimulus amplitudes >7 μA (symmetrical charge-balanced biphasic stimulus), and the presynaptic fibers followed the stimulus frequency 100% for all the stimulus amplitudes examined. After the onset of extracellular stimulus pulse, there was a 1-ms delay before the onset of the postsynaptic current in the motoneuron. The millisecond delay represented activation of the presynaptic fiber, synaptic vesicle activation, transmitter release, diffusion and binding, and postsynaptic channel activation (Baldissera et al. 1972; Gustafsson and Jankowska 1976; Miles and Wong 1984). The output map of the motoneuron near the electrode with the Ia excitatory input (Fig. 8A) was only slightly different from the map without the synaptic input (Fig. 8D). At a stimulus amplitude of 35 μA, excitatory synaptic input resulted in an average increase in neural output of 4% for stimulus frequencies ranging from 75 to 150 Hz. The
The work of Gustafsson and Jankowska (1976) showed that, for dendritic electrode locations, indirect activation of motoneurons could occur at lower stimulus amplitudes than direct activation. Therefore we examined the effect of stimulus frequency on the neuronal output when the trans-synaptic influence (EPSP) from the stimulus was near-threshold ($g_{\text{syn}} = 0.2 \mu S$; Fig. 8B) and suprathreshold ($g_{\text{syn}} = 0.5 \mu S$) (Fig. 8C) for indirect trans-synaptic activation of the motoneuron with single stimuli. With stimulus amplitudes below the threshold for direct excitation (34 \mu A), the EUS neuron generated action potentials in response to the indirect activation that followed low-frequency stimulus trains in a one-to-one fashion; however, the indirect activation was unable to follow high-frequency stimulation and neuronal output decreased as the stimulus frequency was increased (Fig. 8, B and C). At stimulus amplitudes above the threshold for direct excitation, the EUS neuron with the stimulation induced excitatory synaptic inputs was able to follow higher stimulus frequencies at lower stimulus amplitudes than the EUS neuron without any synaptic input (Fig. 8D).

We also examined the effect of stimulation-induced inhibitory synaptic inputs on the output of the EUS neuron. Inhibitory postsynaptic potentials (IPSPs) traditionally have a longer time course than EPSPs. We implemented IPSPs with alpha functions ($g_{\text{syn}} = 0.2 \mu S; \tau = 3 \text{ ms}; E_{\text{syn}} = -80 \text{ mV}$) that produced a peak composite IPSP amplitude of $-3 \text{ mV}$ in the soma 5 ms after onset and lasted 30 ms (Miles and Wong 1984) (Fig. 8E). The output of the neuron with the stimulation-induced inhibitory synaptic input was reduced in comparison to the output of the neuron without any synaptic input but only during near threshold stimulation with frequencies $\geq 50 \text{ Hz}$ (Fig. 8, D and E). At a stimulus amplitude of 35 \mu A, the inhibitory synaptic input resulted in an average decrease in neural output of 16\% for stimulus frequencies ranging from 75 to 150 Hz. The minor effect of the inhibitory synaptic input on neuronal output was not dependent on the IPSP synaptic conductance, as a fivefold increase in the conductance ($g_{\text{syn}} = 1.0 \mu S$) resulted in little change in the neuronal output (Fig. 8F).

**DISCUSSION**

The objectives of the present study were to develop a quantitative biophysical understanding of the effects of extracellular stimulation within the CNS and to develop and test methods that enabled selective activation of targeted neuronal populations. We used a model of the electric field generated by intraspinal microstimulation coupled to multi-compartment cable models of spinal neurons (field-neuron model) to study activation of CNS neurons with trains of both conventional stimuli and asymmetric stimuli previously developed for selective activation of local cells or fibers of passage (McIntyre and Grill 2000). The results support four main conclusions: asymmetrical stimulus waveform enabled selective activation of either cells near the electrode or fibers of passage, using realistic neuronal orientations and volume conductor fields; the asymmetrical waveforms were at least as effective when used in a stimulus train as they were when delivered as single stimuli; alterations in stimulus frequency altered selectivity because of differences in the afterpotentials and post-action potential excitability between local cells and the fibers of passage; and the threshold for axon terminals in the vicinity of the electrode was lower than the threshold for direct activation of local cells, and thus stimulation induced trans-synaptic...
inputs influenced the neural response of local cells to extracellular stimuli.

Model limitations

The results obtained with the integrated field-neuron model of intraspinal microstimulation matched well with experimental data but are limited by three general sources. The first set of limitations is related to the neuron models. The models were parameterized based on results from cat lumbar motoneurons as these cells are the most completely characterized cells within the spinal cord. However, the specific properties of the EUS and BLA neurons may not be well represented by lumbar motoneurons. The morphology of EUS motoneurons (Sasaki 1994) is comparable to that of lumbar motoneurons, while preganglionic parasympathetic neurons of the bladder (BLA) have smaller somas (3,009 vs. 4,920 μm² for our model) and less extensive dendritic trees (39,138 vs. 269,80 μm² for our model) than lumbar motoneurons (Morgan and Ohara 2001). Available electrophysiological data on EUS neurons show they are very similar to the S-type lumbar motoneurons that were used to characterize our models (Hochman et al. 1991; Sasaki 1991). In vivo measurements on cat BLA neurons revealed action potential durations (mean = 5.7 ms) substantially longer than lumbosacral motoneurons, but similar afterhyperpolarization durations (60 ± 12 ms) (de Groat et al. 1982), and neonatal BLA neurons have lower intracellular current thresholds and lower firing rates under tonic depolarization (10–20 imp/s) than lumbar motoneurons (Miura et al. 2000). Thus there are morphological and electrophysiological differences between our model motoneuron and BLA neurons. However, quite different neurons respond very similarly to extracellular stimulation, and changes in excitation patterns with different stimulus parameters were qualitatively similar (Grill and McIntyre 2001; McIntyre and Grill 1999, 2000). Therefore we feel the use of a lumbar motoneuron model was justified as it allowed us to parameterize accurately and test the excitation properties of the model neurons.

While both the motoneuron and myelinated axon models accurately captured the dynamic firing properties recorded experimentally, there are two factors that may limit their ability to reproduce the dynamic firing properties of the neuron at frequencies >100 Hz. The first factor is K⁺ accumulation in the periaxonal space of the myelinated axon. During high-frequency activation (300 Hz) of myelinated axons, K⁺ concentration in the periaxonal space can increase to the point of reversing the concentration gradient driving internodal K⁺ currents. The normally outward fast and slow K⁺ currents of the internode reverse to inward currents as a result of changes in the K⁺ Nernst potential and generate increased excitability and ectopic discharge (David et al. 1993; Kapoor et al. 1993). This increase in excitability of the myelinated axon during and after very high-frequency stimulation was not included in the present model. However, if included, K⁺ accumulation in the periaxonal space would most likely act to enhance the selectivity of fibers of passage over cells seen in Figs. 6 and 7A with high-frequency stimulation.

Another factor that may limit the ability of the model neurons to represent accurately high-frequency dynamic firing is the absence of nonlinear conductances on the modeled dendritic arbor. There are several different types of nonlinear Na⁺, K⁺, and Ca²⁺ ion channels on the dendrites of motoneurons (Caldwell et al. 2000; Campbell and Rose 1997; Carlin et al. 2000). The roles of these channels remain unclear, but high-frequency stimulation (200–300 Hz) in combination with serotonin activates a persistent inward current mediated by Ca²⁺ channels on the dendrites that increases the excitability of the motoneuron and in some cases leads to bistability (Hounsgaard and Kiehn 1993; Svirskis et al. 2001). In a previous study, we found that inclusion of nonlinear conductances in the dendrites did not produce substantial differences between the thresholds of cells and fibers of passage for single extracellular stimuli (McIntyre and Grill 2000). However, at higher stimulus frequencies, extracellular stimulation could activate Na⁺ and Ca²⁺ conductances on the dendrites acting to enhance the excitability of local cells and altering the selectivity that can be achieved by alterations in the stimulus waveform.

The second set of limitations in this study was related to the calculation of the extracellular electric field produced by the microelectrode. We assumed that the electric field was imposed instantaneously within the medium (i.e., quasi-static conditions) (Plonsey and Heppner 1967), and we used low-frequency values of the conductivity of the tissue medium in the finite-element model. In general, biological conductivities have a small reactive component (Ackman and Seitz 1984; Eisenberg and Mathias 1980). A small increase in conductivity has been observed at higher frequencies (Nicholson 1965; Ranck 1963; Ranck and BeMent 1965); however, >90% of the power in a 100-Hz train of rectangular 100-μs pulses is contained at frequencies <400 Hz. We therefore expect that the assumption of quasi-stationary was justifiable.

A second assumption made when calculating the extracellular field was to ignore the impact of the presence of the neuron. If the electrode is in close proximity to the neuron, then the neural structure may distort the electric field within the medium and thus alter the effect of the stimulus on the neuron (Lee and Grill 2001). To account for differences in the extracellular potential across the neuron, the soma was divided into six individual elements rather than treating it as a single lumped element. For the neuron with its cell body close to the electrode (EUS), the difference in the extracellular potential from one side of the soma to the other was 13 mV for a 35-μA stimulus with the standard electrode location used in Figs. 6–8. The use of a multi-compartment soma accounted for the differences in the extracellular potential around the neuron; however, it did not account for the distortion of the extracellular field resulting from the presence of the neuron. We expect this assumption to have introduced only minimal errors in the prediction of excitation for the comparatively large electrode-to-neuron distances considered in this study.

The third set of limitations was related to the representation of the synaptic input on the motoneuron. There exist a total of ~2,500 synaptic contacts on the cell body and dendritic tree of motoneurons (Barnstrom 1993). Our synaptic input model represented only a very small fraction of the total synaptic input to motoneuron (Fig. 7). Consistent with experimental results (Baldissera et al. 1972; Gustafsson and Jankowska 1976), modeled pre-synaptic fibers were excited by stimulus amplitudes below the threshold for direct activation of the local cell. Thus it is likely that a large number of presynaptic fibers will be activated with stimulus amplitudes needed for direct activation, and the effect on the postsynaptic neuron membrane
potential (via postsynaptic potentials) and membrane resistance (via the opening of ion channels) could be substantial. The majority of boutons (∼60%) making contact with the cell body and proximal dendrites of the motoneuron are inhibitory, whereas the boutons contacting distal dendrites are split ∼50% excitatory, ∼50% inhibitory (Barnstrom 1993; Holstege and Calkoen 1990). Therefore during high-frequency extracellular stimulation the overall effect on the cell body may be inhibitory. These effective IPSPs could summate at high frequencies and decrease the excitability of the motoneuron by hyperpolarizing the membrane potential and decreasing the membrane resistance. The effect of this indirect influence of the extracellular stimulus on the postsynaptic neuron could further enhance the selectivity of fibers of passage over cells near the electrode when stimulating at high frequencies but could also limit the ability to activate selectively local cells even with the appropriate stimulus waveform.

However, our results suggest that even large-conductance inhibitory influences on the neuron do not generate substantial changes in the neuronal output (Fig. 8, E and F). This is because when stimulating local cells with extracellular sources, the site of action potential initiation is in one of the first few nodes of Ranvier (McIntyre and Grill 1999; Nowak and Bullier 1998a,b). As a result, the change in excitability of the cell body and dendrites from the synaptic inhibition is of little importance ∼1 mm down the axon. Thus the axonal (and by definition neuronal) output from the stimulus was relatively unaffected by the synaptic inhibition. In addition, during high-frequency stimulation with stimulus amplitudes between 100 and 125% of the threshold for direct activation, and high-conductance synaptic inhibition (Fig. 8F), the transmembrane potential recorded at the soma showed little and sometimes no firing while the axon was able to follow the stimulus frequency with much greater efficiency.

Effects of waveform and stimulus frequency on selectivity

Our results indicate that alterations in both the stimulus waveform and stimulus frequency enable selective activation of targeted neuronal populations (Figs. 6 and 7). When high-frequency stimulus trains were used, fibers of passage had greater neuronal output than local cells because of differences in post-action potential excitability. This effect was not seen with the local axon with its cell body relatively close to the electrode (BLA) because the afterpotentials of the cell body were propagated electrotonically to the first few nodes of the myelinated axon. As a result, the afterpotentials of the axon near the cell body were similar to those of the cell body. Therefore our results predict that the increase in selectivity at high frequencies is limited to fibers with their cell bodies far from the electrode (Fig. 7). However, it should be noted that while the selectivity of fibers of passage can be increased with high-frequency stimulation, local cells could still respond to the stimulus, albeit a lower general output (Figs. 6 and 7). The limited output of the local cells from high-frequency stimulation could still be great enough to generate functional activation of their efferent target, and conversely, driving fibers of passage >100 Hz may exceed the physiological limits for those neurons and result in unexpected or unwanted effects. In addition, the firing of the local cell at high stimulus frequencies was not only dependent on its direct excitation characteristics but also on indirect trans-synaptic influences (Fig. 8). In turn, the ability to activate selectively fibers of passage over local cells with high-frequency stimulation is dependent on the net indirect influences on the local cells being inhibitory as predominantly excitatory inputs could enhance activation of the local cells.

Our results demonstrate that a much more robust technique for achieving selective activation of local cells or fibers of passage is alteration of the stimulus waveform. When an asymmetrical charge-balanced biphasic cathodic phase first stimulus waveform designed to activate selectively the cells near the electrode was used (McIntyre and Grill 2000), we saw strong selective activation of the local cell (Fig. 7B). Conversely, when an asymmetrical charge-balanced biphasic anodic phase first stimulus waveform designed to activate selectively fibers of passage near the electrode was used (McIntyre and Grill 2000), we saw strong selective activation of fibers of passage (Fig. 7C). The long-duration prepulse of these waveforms alters the level of sodium channel inactivation, thereby increasing the excitability in elements hyperpolarized by the prepulse and decreasing the excitability of elements depolarized by the prepulse (Grill and Mortimer 1995; McIntyre and Grill 2000). Antidromic activation of neurons projecting to the region near the electrode (via activation of their axon terminals) also occurred, independent of the stimulus waveform or frequency used, and thus selective activation of either local cells or fibers of passage will be effected by the stimulation induced trans-synaptic effects on the local cells (Fig. 8).

Implications for deep brain stimulation

The results of this study, although directed toward intraspinal microstimulation, also provide insight into the effect of stimulus frequency on the arrest of tremor by high-frequency extracellular stimulation of deep brain structures [deep brain stimulation (DBS)]. At higher frequencies (>100 Hz), tremor is suppressed with most patients finding the best results with stimulation frequencies of ∼150 Hz (Benabid et al. 1996; Obeso et al. 2001). It has been hypothesized that high-frequency stimulation inhibits tremor by trans-synaptic inhibition of local cells via IPSP summation and decreases in the membrane resistance (Benazzouz et al. 1995; Boraud et al. 1996; Dostrovsky et al. 2000). This hypothesis is supported by the fact that GABAergic IPSPs have a time course that matches well with maximal summation occurring at stimulus frequencies where DBS is most effective (Fig. 8, E and F).

While the mechanisms regulating the therapeutic effects of DBS are not clear, the fact that cathodic stimuli are more effective than anodic stimuli (Benabid et al. 1996), combined with strength-duration results suggest that the targeted neuronal elements are axonal in nature (Ashby et al. 1999; Holsheimer et al. 2000; McIntyre and Grill 2000). The results of this study show that high-frequency stimulation enhances the selective activation of fibers of passage and that axon terminals have lower stimulation thresholds than local cells. However, our results show that the local cell was able to follow high-frequency stimulus trains with 100% output even if the stimulation induced trans-synaptic effects were inhibitory (Fig. 8, E and F). Therefore the results of this study suggest that effects of high-frequency extracellular stimulation within the CNS are activation of fibers of passage, axon terminals, and local cells.
near the electrode. If the stimulation-induced trans-synaptic effects on the local cells are predominately inhibitory (as is the case in the target nuclei for DBS), then there should be a suppression of underlying activity in the local cells during the inter-stimulus interval as a result of the hyperpolarization of the dendrites and cell body impeding normal synaptic integration. However, the interpretation of suppression of activity during the interstimulus interval to represent block of activity in that nucleus, as seen in experimental recordings (Benazzouz et al. 1995; Boraud et al. 1996; Dostrovsky et al. 2000), does not account for the effects of direct excitation of local cells resulting from the stimulus train.

**APPENDIX**

The ionic currents of the neural models can be written in the general form of

\[
I_{\text{ion}} = g_{\text{ion}}(V_m - E_{\text{ion}})
\]

where \( g_{\text{ion}} \) is the maximum conductance for the individual ion channel (Tables 3 and 4) multiplied by gating variables that range from 0 to 1 (Hodgkin and Huxley 1952). The time and voltage dependence of each gating parameter \( (\omega) \) is given by

\[
\tau_{\omega} = 1/[(\omega + \beta_\omega)]
\]

\[
d\omega/dt = \alpha_\omega(1 - \omega) - \beta_\omega\omega = (\omega - \omega)\tau_{\omega}
\]

The time course and magnitude of the activation and inactivation parameters used in the simulations are given in the following text. The membrane dynamics were derived to be representative of neural excitation at 36°C and based on the experimental references given in METHODS and the previous motoneuron modeling work of Booth et al. (1997) and Jones and Bawa (1997). The models were implemented using NEURON v4.3.1 (Hines and Carnevale 1997) and a time step of 0.005 ms. Individuals interested in reproducing the results of this study or using these models in their own work are encouraged to contact us for the appropriate NEURON files and instruction on their use. Times are in milliseconds, voltages are in millivolts, currents are in milliamperes per square centimeter, and concentrations are in millimolar.

**Nodal fast sodium current**

\[
I_{Na} = g_{Na} * m^3 * h * (V_m - E_{Na})
\]

\[
\alpha_m = [0.4 * ((V_m + 60))] / (1 - e^{-(V_m + 10)/10})
\]

\[
\beta_m = [0.34 * ((V_m + 104))] / (1 - e^{-(V_m + 64)/10})
\]

\[
\tau_h = 16 \times (1 + e^{-(V_m + 13)/10})
\]

**Nodal persistent sodium current**

\[
I_{Na} = g_{Na} * h^2 * (V_m - E_{Na})
\]

\[
\alpha_h = [0.125 * ((V_m + 17))] / (1 - e^{-(V_m + 17)/10})
\]

\[
\beta_h = [0.000853 * ((V_m + 24))] / (1 - e^{-(V_m + 24)/10})
\]

**Nodal slow potassium current**

\[
I_k = g_k * s * (V_m - E_k)
\]

\[
\alpha_s = 0.39 * (1 + e^{-(V_m + 80)/10})
\]

\[
\beta_s = 0.039 * (1 + e^{-(V_m + 80)/10})
\]

**Initial segment fast sodium current**

\[
I_{Nad} = g_{Nad} * m^3 * h * (V_m - E_{Nad})
\]

\[
\alpha_n = [0.4 * ((V_m + 60))] / (1 - e^{-(V_m + 10)/10})
\]

\[
\beta_n = [0.4 * ((V_m + 40))] / (1 - e^{-(V_m + 40)/10})
\]

\[
\tau_a = 30 \times (1 + e^{-(V_m + 80)/10})
\]

\[
h_a = 1 / (1 + e^{-(V_m + 65)/10})
\]

**Initial segment persistent sodium current**

\[
I_{NaP} = g_{NaP} * m^4 * h * (V_m - E_{NaP})
\]

\[
\alpha_p = [0.0353 * ((V_m + 28.4))] / (1 - e^{-(V_m + 28.4)/10})
\]

\[
\beta_p = [0.000083 * ((V_m + 33))] / (1 - e^{-(V_m + 33)/10})
\]

**Initial segment delayed rectifier potassium current**

\[
I_{Kd} = g_{Kd} * n^4 * s * (V_m - E_{Kd})
\]

**Soma fast sodium current**

\[
I_{NaS} = g_{NaS} * m^3 * h * (V_m - E_{NaS})
\]

\[
\alpha_n = [0.4 * ((V_m + 60))] / (1 - e^{-(V_m + 10)/10})
\]

\[
\beta_n = [0.4 * ((V_m + 32))] / (1 - e^{-(V_m + 32)/10})
\]

\[
\tau_n = 5 \times (1 + e^{-(V_m + 50)/10})
\]

\[
n_s = 1 / (1 + e^{-(V_m + 30)/10})
\]

**Soma delayed rectifier potassium current**

\[
I_{KdS} = g_{KdS} * n^4 * s * (V_m - E_{KdS})
\]

**Soma calcium dynamics**

\[
[Ca]_s = 2
\]

\[
[Ca]_s(t = 0) = 0.0001
\]

\[
d[Ca]_s / dt = 0.01 \times ((E_{Ca} - E_{Ca}) / (4 \times [Ca]_s))
\]

**Soma N-type calcium current**

\[
I_{CaN} = g_{CaN} * m^2 * h * (V_m - E_{CaN})
\]

\[
\alpha_m = 15
\]

\[
\beta_m = 50
\]

\[
h_m = 1 / (1 + e^{-(V_m + 50)/10})
\]

**Soma L-type calcium current**

\[
I_{CaL} = g_{CaL} * p * (V_m - E_{CaL})
\]

\[
\tau_p = 400
\]
Soma calcium-activated current density

\[ I_{\text{calc}} = \frac{g_{\text{calc}}}{g_{\text{calc}}_{\infty}} \left[ \left( \frac{[Ca^{2+}]_i}{[Ca^{2+}]_{inf}} \right) \left( [Ca^{2+}]_{inf} + 0.141 \right) \right] \times (V_m - E_{\text{calc}}) \]

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