Chapter 6

CONCLUSIONS

Epidural electrical stimulation has been shown to facilitate the recovery of motor function and voluntary movement in humans (Harkema et al., 2011) and rats (Urban, 2018). Computational studies have aimed to better understand the mechanism underlying this motor recovery. Early work focused on stimulation of the dorsal fibers and roots (J Ladenbauer et al., 2010). More recently, studies (Capogrosso et al., 2013; Moraud et al., 2016) have emphasized the role of feedback pathways driven by the muscle spindle feedback. In this thesis, I have shown in simulation that facilitation of synaptic input to interneurons in the rat spinal cord is possible with both biphasic and monophasic epidural stimulation, using voltage magnitudes consistent with those used in biological experiments.

A 3D volume conductor model of the rat spinal cord and a 3-column by 7-row electrode array was constructed based on a transverse slice of an MRI image of a rat spinal cord. Biphasic and monophasic stimulation pulses were analyzed for frequency content. Material conductivity and permittivity values (anisotropic for white matter and muscle, isotropic for CSF, platinum, parylene C, gray matter, and bone) were found as close as possible to the dominant frequencies of Gaussian biphasic and monophasic stimulation pulses. A simple 3D model of an interneuron in the rat spinal cord was constructed based on data from (Ostroumov, 2007; Thurbon et al., 1998; Santos et al., 2009). Based on (Destexhe, Mainen, and Sejnowski, 1994), a threshold of -10 mV for the membrane voltage at the axon tip was chosen to indicate that a neuron had activated (released neurotransmitters from the tip of the axon). The synapse weight necessary for a single presynaptic event to generate an EPSP large enough to achieve neurotransmitter release from the axon tip was

determined for synapse locations along the length of each dendrite (see Fig. 3.6). Synapse weights less than this amount were used in Chapter 5 to allow for the possibility of facilitation rather than causing neurotransmitter release directly.

Static and time-domain solutions to the volume conductor models were found for biphasic and monophasic stimulation of 18 characteristic (unique under translation and mirroring across the x=0 and z=0 planes) bipolar combinations (Section 2.2.1). Static voltages and voltage time-series were extracted from these simulations at locations corresponding to neuron locations under and between each row of electrodes at 3 different depths (see Figs. 4.1 and 4.2) and used with different voltage scaling factors to obtain the extracellular voltages used in the NEURON simulations.

NEURON simulations of neurons exposed to a single stimulation pulse without any synaptic activity (for each type of stimulation (biphasic or monophasic) and all 18 characteristic combinations) were conducted. The minimum amount of stimulation to activate a neuron was 2.75 V for monophasic stimulation and 3.75 V for biphasic stimulation. This is within range of stimulation voltages used in actual experiments (i.e. 1 V to 8 V from Parag Gad, Roy, Choe, Zhong, et al., 2015), so this implies that direct stimulation of at least some of the interneurons in the spinal cord should be expected. For monophasic stimulation, the stimulation pulse causes activation of the neuron and also results in an action potential which spreads throughout the neuron (by orthodromic and antidromic propagation). Biphasic stimulation, on the other hand, only causes an action potential in some neurons at or above 8 V of stimulation. Activation of some neurons may occur with stimulation magnitudes less than 8 V without generating a traditional action potential because the membrane voltage at the axon tip exceeds -10 mV, resulting in at least some neurotransmitter release. The locations of neurons electrically stimulated sufficiently to release neurotransmitters using an amplitude of less than 5 V of stimulation were presented in Tables 4.2 and 4.3. Results for neurotransmitter release using an amplitude of less

than 10 V were presented graphically (see Sections 4.A, 4.3 and 4.4).

Axon tip membrane voltage for all the NEURON simulations without synaptic input were plotted against the voltage from static simulations at the axon tip ($V_{static}^{\text{AxonTip}}$) and the second derivative of the static voltage at the axon tip along the axon. Activated neurons were found to be scattered across a wide range of ($V_{static}^{\text{AxonTip}}$) and the second derivative of the static voltage without any obvious clustering. However, plotting the difference in the static voltage between the axon tip and the soma ($V_{static}^{\text{AxonTip}} - V_{static}^{\text{soma}}$) vs axon tip membrane voltage resulted in some interesting relationships (see Fig. 4.22) which would be useful for predicting neuron activation. While the exact behavior seen in Fig. 4.22 is likely dependent on the neuron parameters and geometry defined in Chapter 3, based on the large number of neuron locations and electrode configurations, it seems likely that similar behavior could be found for other neuron parameters.

Modeling facilitation was done with NEURON simulations that included a single sub-threshold synaptic input arriving at times before, during, and after a stimulation pulse. Synaptic input was modeled at the distal tip and middle of each dendrite. A significant amount of facilitation of neuron activation occurred when the synapse weight and/or the stimulation pulse magnitude was sufficiently large. This facilitation was dependent on both the orientation of the axon and the dendrite on which the synaptic input was located.

A significant contribution of this thesis is the discovery of an interval which I call the facilitation window. The stimulus pulse and the synaptic input need not occur simultaneously for facilitation to occur, instead the onset of the synaptic input may occur at any time inside the facilitation window and still experience a facilitation effect. This window is a function of synaptic weight, the stimulating field strength, which is itself a function of electrode positions and neuron geometry. As either the

stimulation magnitude or the synapse weight decreased, the size of the facilitation windows reduced and the number of facilitated neurons also reduced. For the parameters studied in this thesis, the facilitation windows can be as large as 115 ms wide. Some neurons were facilitated at the lowest stimulation voltage magnitude tested (0.5 V) when tested with some of the largest synapse weights. With stimulation magnitudes of 5 V or less, monophasic stimulation produced more facilitation compared with biphasic stimulation with the exception of synapses on the distal tips of dendrites and the largest synapse weight (4.783 nS).

Examples of facilitation using biphasic and monophasic stimulation were shown, including the facilitation windows. Each of these examples showed facilitation windows with the synapse triggered both before and after the stimulation pulse. However, for all the examples, there exists an optimal time delay between the synaptic input and the stimulation pulse which results in the "least effort" facilitation (lowest magnitude stimulation and lowest synapse weight). For the biphasic examples, the "least effort" timing occurs if the synaptic input occurs before the stimulation pulse and the stimulation pulse occurs when the ion channel variable m_{IKdrSM} is at a maximum for ion channels near the synapse and h_{INaSM} is at a minimum near the synapse. For some of the monophasic examples, the "least effort" facilitation timing is such that the synaptic input and the stimulation pulse occur at the same time. For the rest of the monophasic examples, the "least effort" facilitation occurs when the stimulation pulse occurs after the synaptic input and when V_m is at a maximum at the synapse location, m_{IKdrSM} is approaching maximum, m_{IKaSM} is close to maximum, m_{INaSM} is near maximum, and h_{INaSM} is approaching minimum. A more comprehensive study of the facilitation windows and "least effort" facilitation timing is an important future issue to be considered.

A search for features which separate simulations of neurons resulting in facilitation from simulations that did not result in facilitation was conducted. I found that the

features ($V_{static}^{\text{Synapse}} - V_{static}^{\text{Soma}}$, $V_{static}^{\text{IS}} - V_{static}^{\text{Soma}}$) based on the static volume conductor simulations were able to separate many of the facilitated (and activated by stimulation only) neurons from non-activated neurons. These static voltage features are much more readily computed than the time-domain volume conductor simulations and NEURON simulations, so a machine learning algorithm could likely be built around these or similar features to find optimal facilitation configurations while reducing computation time.

6.1 Discussion

This is the first large scale computational study of facilitation of synaptic input with electrical stimulation. Direct activation and robust facilitation of interneurons in the spinal cord were found to occur at biologically relevant stimulation thresholds. The facilitation occurs as a result of the temporal interaction between the synapse conductance, the stimulation pulse, and the ion channel dynamics (particularly the ion channels close to the synaptic input). After a stimulation pulse or synaptic input, the ion channel dynamics are slower to return to a resting state than the membrane voltage. These dynamics lead to facilitation window(s) (or periods of time) before and/or after a stimulation pulse during which a sub-threshold synaptic input is able to control the output of a neuron. This means that there is no strict requirement that the synapse input occur exactly at the same time as the stimulation pulse. The size of these facilitation windows depends on the stimulation voltage, synapse weight, geometry and orientation of the neuron, stimulation geometry, and synapse location. The maximum width of these facilitation windows is ~115 ms for the models that were studied and a significant amount of the facilitation window(s) are at least 25 ms wide. A facilitation window with a width of 25 ms means that if the sub-threshold stimulation pulses occur with a frequency of 40 Hz, some of the neurons in the spinal cord will be almost continuously facilitated. Current rodent model

stimulation experiments have found that stepping is best recovered with stimulation pulse frequencies of 40-60Hz (Parag Gad, Roy, Choe, Creagmile, et al., 2015). One hypothesis for the narrow frequency tuning of good motor recovery is that this is due to a network effect (Jilge et al., 2004). The discovery of the facilitation window in this thesis suggests another hypothesis: the optimum stimulation frequency is the one that results in near constant facilitation of synaptic input without causing too much direct activation of neurons. Further in vivo or slice experiments with single neuron recording could test this hypothesis.

This thesis is also the first large scale comparison of monophasic vs biphasic electrical stimulation of interneurons in the spinal cord. Monophasic stimulation resulted in more interneuron activation and facilitation compared with the same magnitude of biphasic stimulation. However, the decreased amount of direct activation of interneurons from biphasic stimulation may actually allow for a wider range of stimulation voltages to be used for facilitation without causing direct interneuron activation. There are also some differences in the timing of the facilitation windows for biphasic and monophasic stimulation (summarized earlier in this chapter). It remains to be seen whether this effect could be used intelligently to modulate response to sensory input as a function of step cycle or some other function. Phase dependent modulation of spinal neurons has been found to improve balance and gait in spinal rodent models (Moraud et al., 2016). This study also suggests an additional control parameter which could be used for precise modulation of key spinal neurons during a gait cycle.

For this application, the activating function (Rattay, 1999) is not as useful a predictor of facilitation or activation compared with other features such as gradients of voltage along different parts of the neuron's geometry. The activating function is still used as a standard predictor of neuron activation in the field, but others have noticed that the activation function was less predictive (Zierhofer, Feb./2001).

6.2 Comparing with the literature

There are very few studies that use comprehensive computational models to study epidural stimulation in spinal cord injury. None of these studies have considered the facilitation effect. Previous studies used direct activation (as measured by action potential generation) of a neuron without synaptic input as the criteria for neuron recruitment. Previous models have used computational models to support specific hypotheses about the primary neural mechanisms of epidurally stimulated recovery. The discovery of the facilitation mechanism supports alternative explanations for the roles of key neural populations in spinally stimulated recovery.

As mentioned in Section 1.1, Capogrosso et al., 2013 concluded that activation of interneurons in the spinal cord was not possible in commonly used stimulation ranges. There are significant differences in our models (most of which are described in Section 1.1). In particular, their model uses a neuron with a larger dendritic arbor, larger diameter axon, and larger soma, all of which would make direct activation harder. The size of the neuron that I have modeled is in the distribution of neurons presented in Thurbon et al., 1998 Table 3 (see Section 3.2 for more details).

An important difference is their use of passive dendrites with limited support of synaptic input. My results indicate that the behavior of the ion channels in the dendrites is critical to the facilitation effect. I propose that the facilitation effect is an important consideration for determining the activity, the role, and the relative importance of particular spinal neural populations.

The computational work in this thesis suggests that facilitation of interneurons in the postural control circuitry may be an important, if not critical, part of motor recovery. This implies that multi-electrode epidural stimulating arrays should be designed to facilitate the functions of interneurons in the gray matter of the spinal cord. The absence of this facilitation may hamper the neural pathways that transmit

critical information from muscle spindle feedback, which has been proposed as a key mechanism for epidural stimulation in SCI (Moraud et al., 2016). This observation also suggests that future epidural stimulating arrays should be designed to provide adequate facilitation of these critical pathways.

6.3 Future work

While multi-electrode epidural stimulating arrays were originally designed for reducing and/or blocking pain, there has been little study of electrode design that is specialized for spinal cord injury recovery. With current array designs, it is likely that facilitation of interneurons plays a role in recovery of motor function. As researchers design new stimulation arrays, it may be important to design them to optimize the facilitation of interneurons rather than just focusing on the dorsal roots. Otherwise, the new designs may make it harder to facilitate interneurons. The work in this thesis can provide a starting point for the computational design of new electrode arrays. A simple, but computationally intensive, approach could use the following cycle. First, propose a multi-electrode geometry, the methods introduced in this thesis can then be used to determine the degree of facilitation in a spinal region of interest. The gradient of a function which measures the quality of the facilitation is then used to adjust the array design parameters. The updated array is then used to restart the cycle.

There may also be ways to tune the stimulation waveform to optimize the effect on the ion channels or perhaps precondition the ion channel states so that they are more responsive to future stimuli. One approach to study this problem would be to use linear quasi-active approximations to the ion channels similar to that found in Remme and Rinzel, 2011. Remme and Rinzel, 2011 studied the role of active ion channels found that each ion channel conductance propagation and summation of excitatory postsynaptic potentials (EPSPs) without external stimulation. They

found that ion channels can contribute to either a regenerative membrane current which amplifies the effect of the EPSP, or a restorative current, which accelerates the decay of the EPSP. The extension of their model to include external stimulation could provide a starting point for the analysis of the optimal stimulating waveform shape. This linearized model would have to be augmented with numerical simulations to obtain the truly optimal waveform. The detailed time domain simulations in this thesis would support the computational study of new waveform shapes. An optimization cycle analogous to the one described above could be used. Another way to alter ion channel states would be with pharmacological methods.