Chapter 1

INTRODUCTION

A survey conducted by the Christopher & Dana Reeve Foundation in 2008 (summarized in (Cahill, Fredine, and Zilberman, 2009)) revealed that approximately 1.9% of the population of the United States (5,596,000 people) reported some form of paralysis of their arms and legs. Stroke is the leading cause of paralysis (29% or 1,608,000 people) followed by spinal cord injury (SCI) (23% or 1,275,000 people). Work accidents (28%) and motor vehicle accidents (24%) are the leading causes of spinal cord injury followed by sporting and recreational accidents (16%). The average person with a SCI has been living with it for 15.6 years. It has been estimated that about 300,000 people of this population are confined to wheel chairs. It is this population that motivates this thesis.

While physical therapy is helpful for people with SCI, it is often insufficient to recover useful motor and autonomic function. In such cases, alternative treatments are sought. Epidural stimulation has recently shown promise as a therapy for SCI (Harkema et al., 2011). In this therapeutic approach, a multi-electrode array is implanted in the epidural space over either the cervical enlargement for quadriplegia patients or over the lumbosacral enlargement for paraplegics. A series of pulse trains are applied to selected electrodes in order to excite and *facilitate* the operation of the sensory motor feedback circuits that control limb activity. See Fig. 2.8 for an example of electrode placement.

The analysis in this thesis is most relevant to the motor complete paraplegic subpopulation, but the results should also benefit the treatment of motor complete quadriplegia. From a clinical perspective, paralysis is defined to be motor complete when no voluntary control of muscle is possible for the muscles which are inner-

vated by the spinal cord below the level of the injury. While the work in this thesis is most motivated by efforts to use epidural stimulation to recover motor function in complete paralysis, the results should be useful in cases of incomplete or partial paralysis.

In the absence of supraspinal input (from the brain) there is an upregulation of inhibitory receptors in the lumbosacral spinal cord after an SCI. Combined with the loss of supraspinal input, the lower limb motor control circuitry enters an inactive state. Without supraspinal input, Edgerton and colleagues (Edgerton et al., 2008; Courtine et al., 2009) discussed how pharmacological mechanisms (such as quipazine (a serotonin agonist)) and/or epidural stimulation can be used to modulate the physiological state of the spinal cord to facilitate locomotion or standing in response to signals from afferent fibers (sensory input). For both pharamcological mechanisms and epidural stimulation, the key to facilitation appears to be an application of the right amount of sub-threshold drug intervention and/or electrical stimulation. Too much of either can cause direct neuron activation independent of the state of the afferent inputs.

Studies in humans (Harkema et al., 2011) have shown similar responses (standing, walking, improvements to autonomic function) to epidural stimulation. However, in humans most of the injuries leave some supraspinal input intact, just not enough (in the case of clinically complete spinal injuries) to activate the spinal cord without external help (from epidural, transcutaneous, or other form of stimulation). It is unclear how much the remaining supraspinal input is involved with the standing and walking behaviors in spinally stimulated humans with SCI, but epidural stimulation has also been shown to facilitate voluntary leg movement in clinically complete subjects. Thus epidural stimulation is able to facilitate the spinal cord response to both afferent and weak supraspinal input. These studies (Edgerton et al., 2008; Harkema et al., 2011) have also shown that the optimal selection of the stimulat-

ing parameters (for example, the amplitude on each electrode, frequency, polarity, and pulse width) varies significantly across subjects, making it difficult to find the optimal stimulation parameters. All of these studies appear to work best with stimulation that is best described as sub-threshold. In experiments with live animals, that threshold is usually based on the observed level of muscle activity. Stimulation with magnitudes above direct motor stimulation is usually counter productive. In this thesis the threshold is based on neurotransmitter release. The mechanisms underlying the facilitation of motor function using epidural stimulation are at present poorly known. A better understanding of how epidural stimulation facilitates spinal cord function may allow us to build better electrode arrays (design, number of electrodes, location in the spinal cord, etc.), shape stimulating waveforms, and perhaps find better targeted drug therapy.

This thesis uses computational methods to study the facilitation of interneurons inside the spinal cord with glutamate receptive synapses as suggested in (Edgerton et al., 2008). In this thesis, facilitation is defined as a process which allows sub-threshold synaptic inputs to control the output of the neuron (i.e. neurotransmitter release) in the presence of electrical stimulation. Table 1.1 summarizes the possible outcomes (neurotransmitter release, facilitated neurotransmitter release, or no neurotransmitter release) for varying amounts of stimulation and synaptic input. Existing computational studies have focused on direct activation of the dorsal afferent fibers as they enter the spinal cord (Capogrosso et al., 2013; J Ladenbauer et al., 2010), finding that the spinal interneurons are too difficult to activate. However, these simulations did not include synaptic input in a meaningful way or include active dendrites.

To study facilitation, I built a volume conductor model of the rat spinal cord, including an epidural electrode array and 3D models of interneurons with synaptic input, located at 66 locations throughout the cord in 6 different orientations. These

Table 1.1: This table summarizes the possible results of varying amounts of stimulation and synaptic input for a neuron in the spinal cord. Sub-threshold and super-threshold refer to the threshold of synaptic input or stimulation voltage necessary for a given neuron to release neurotransmitters. Neurotransmitter release in response to sub-threshold synaptic input can be facilitated by sub-threshold stimulation under certain conditions as seen in Chapter 5.

Synaptic input	Stimulation level	AP[*]	NT[†] release	Description
sub-threshold	None	no	no	sub-threshold synaptic input
None	sub-threshold	no	no	sub-threshold stimulation
super-threshold	None	yes	yes	neuron activated by synaptic input
None	super-threshold	maybe	yes	neuron activated by stimulation pulse
sub-threshold	sub-threshold	no	no	no facilitation
sub-threshold	sub-threshold	yes	yes	neuron activated by sub-threshold synaptic input facilitated by sub-threshold stimulation pulse

[*] Does an action potential (AP) occur in the neuron?
[†] Does the neuron release neurotransmitters (NT)?

neurons were exposed to 18 characteristic bipolar electrode combinations with both biphasic and monophasic stimulation waveforms to determine thresholds for neurotransmitter release with and without a varying amount of synaptic input. The timing between the synaptic input and the stimulation pulse was also varied to study the time sensitivity and optimality of the stimulation.

Stimulation thresholds for neurotransmitter release were found first in the case of epidural stimulation without synaptic input using stimulation voltage magnitudes of up to 10 V. After testing 28512 different neuron stimulation configurations (66 neuron locations, 6 axon orientations, 18 characteristic bipolar electrode pairs, 2 polarities, and 2 stimulation pulse shapes), the minimum amplitude of electrical stimulation required to raise the neuron's axon tip to above -10 mV and release neurotransmitters (based on (Destexhe, Mainen, and Sejnowski, 1994)) was found to be 2.75 V for monophasic stimulation and 3.25 V for biphasic stimulation. Plotting the maximum membrane voltage at the axon tip against $V_{static}^{AxonTip} - V_{static}^{Soma}$ (where $V_{static}^{AxonTip}$ and V_{static}^{Soma} are the extracellular voltages computed using a static volume conductor simulation at the axon tip and soma respectively) showed that this difference could be used to predict stimulation patterns that would cause neurotransmitter

release.

Next, simulations were conducted with a single sub-threshold synaptic input arriving at one of 10 locations on each neuron at times before, during, and after a sub-threshold stimulation pulse. Stimulation pulses of magnitude 5 V or less were tested with 8 sub-threshold synapse weights at locations in the middle of the distal dendrite and 3 synapse weights at the locations on the distal tips of the dendrites. Since both the synaptic input and the stimulation pulse are sub-threshold (i.e. do not cause the axon tip membrane voltage to go above -10 mV), the synaptic input is facilitated by the stimulation pulse if together they cause the axon tip membrane voltage to go above -10 mV. Windows of time in which facilitation occurs were found for many stimulation configurations and synapse weights. If the sub-threshold synaptic input is large enough, a 0.5 V stimulation pulse is enough to facilitate neurotransmitter release in some neurons. A greedy search for features which were able to identify stimulation configurations which cause facilitation found that $V_{static}^{Synapse} - V_{static}^{Soma}$ (the difference in the static voltage between the synapse location and the soma) and $V_{static}^{IS} - V_{static}^{Soma}$ (the difference in the static voltage between the initial segment of the axon and the soma) were able to separate 60-89% of the facilitated stimulation configurations from 21 out of 22 datasets (separated by synapse weight) and 42% in the remaining dataset.

This is the largest scale study of the facilitation effect. The facilitation effect is a function of many variables (timing, synapse weight, ion channel densities, neuron geometries, etc.). A large-scale computational campaign was helpful to identify the various phenomena (some of which are non-intuitive) and to organize the results into a smaller set of rules that would otherwise be difficult without such a large-scale study.

1.1 Review of existing literature

Much of the research into electrical stimulation of the spinal cord was initially motivated by success in using electrical stimulation of the spinal cord to temporarily stop or reduce chronic pain (Shealy, Mortimer, and Reswick, 1967; Hosobuchi, Adams, and Linchitz, 1977; Aló and Jan Holsheimer, 2002). Electrical stimulation of the spinal cord for reducing the pain associated with multiple sclerosis (MS) showed improvement in mobility, sensory function, and bladder function (Cook and Weinstein, 1973; Illis, Sedgwick, and Tallis, 1980). Two dimensional (B. Coburn, 1980) and then three dimensional (Barry Coburn and Sin, 1985) finite element volume conductor models of electrical stimulation of the spinal cord were conducted. The results of the 3D volume conductor simulations were used in combination with compartmental models of myelinated fibers (i.e. without soma or dendrite compartments) in the white matter of the spinal cord to predict firing thresholds (Barry Coburn, 1985). Efforts to use electrical stimulation of the spinal cord to activate or inactivate certain neurons/fibers while not interfering with other neurons lead to increased computer modeling of axon fibers in the spinal cord. In the field of spinal cord stimulation, this problem lead to more models of external electric stimulation of axons (mostly myelinated axons of different diameters in the white matter and dorsal roots) in uniform materials (B. Coburn, 1988; Rubinstein and Spelman, 1988; Rubinstein, 1991; Richardson, C. C. McIntyre, and W. M. Grill, 2000) and 3D volume conductor models (Struijk, Holsheimer, van Veen, et al., Jan./1991; J. Holsheimer and J. J Struijk, 1992; Struijk, Holsheimer, van der Heide, et al., Sept./1992; Johannes J. Struijk, Jan Holsheimer, and Boom, 1993; J Ladenbauer et al., 2010; Capogrosso et al., 2013; Lempka et al., 2015).

Most of these authors consider a neuron or fiber to be activated or recruited if an action potential is generated or travels a certain distance in the neuron. This definition can be problematic if the stimulation also blocks the axon potential from reaching

the axon tip, or if the axon tip is stimulated sufficiently to release neurotransmitters without an action potential. This will be discussed more in Chapters 3 and 4.

(Rattay, 1999) derived and proposed the activation function (which is proportional to the second derivative of the extracellular voltage along the axon) to predict whether a uniform neuron fiber will be stimulated (depolarized) or hyperpolarized without actually simulating neurons. This turned out not to be useful for the type of neurons simulated in this thesis. There is more discussion of this in Chapters 4 and 5.

In the last decade, a small number of papers used computational means to study epidural stimulation for SCI therapy. (C. C. McIntyre and Warren M Grill, 2002; Capogrosso et al., 2013) also modeled activation of motorneurons and interneurons in the gray matter of the spinal cord. Capogrosso et al. (2013) also included some limited simulations of a sub-threshold synapse, but they did not appear to study how this relates to facilitation. Other researchers have modeled sub-threshold external electrical stimulation of whole neurons (Tranchina and Nicholson, 1986). Ephaptic interactions between neurons (Holt and Koch, 1999; Gold et al., 2009; Anastassiou et al., 2011) are another sub-threshold phenomena.

Remme and Rinzel, 2011 looked at the role of active dendritic conductances in the propagation and summation of excitatory postsynaptic potentials (EPSPs) using a linear quasi-active approximation to membrane and ion channel dynamics. Their analysis showed that EPSPs are attenuated and sharpened by restorative ion channel currents, and amplified and broadened by regenerative ion channel currents. This analysis suggests that modeling active ion channels in the dendrites is important when EPSPs are modeled. Their analysis methods may also be useful for future work on understanding the effect of electrical stimulation on ion channels.

Spinal stimulation has also been investigated for reduction of the spasticity of-

ten seen in SCI. (ElBasiouny and Mushahwar, 2007) model suppression of excess synaptic inputs to spinal motoneurons using extracellularly applied electric fields. This thesis simulates facilitating synaptic input rather than interfering with it.

The style of the volume conductor model used in this thesis is most similar to that of (J Ladenbauer et al., 2010; Capogrosso et al., 2013). (Capogrosso et al., 2013) has enough similarities to the approach taken in this thesis to warrant a comparison. (Capogrosso et al., 2013) models square current pulses using static voltage simulations. This thesis uses time domain volume conductor simulations of Gaussian monophasic and biphasic voltage stimulation. (Capogrosso et al., 2013) assumes purely resistive materials with frequency independent conductivity. In this thesis, I pick the conductivity and real valued permittivity based on the largest frequency component of the stimulation waveform. Based on the analysis in Chapter 2, the conductivity of the gray matter and white matter used in (Capogrosso et al., 2013) is too high. The conductivity of the cerebrospinal fluid differs (2 nS vs 1.7 nS). The interneuron geometry differs significantly (they use scaled cat interneurons, I use a constructed geometry), the soma diameter is similar, but the axon diameter differs. They use a value of 2.5 µm for the diameter of the axon which comes from cat studies (Saywell et al., 2011), while I use a value of 0.8 µm from rat studies (Ostroumov, 2007; Nunes et al., 2017; Saliani et al., 2017). (Capogrosso et al., 2013) uses passive dendrites while I use an active model based on (Ostroumov, 2007). Both studies use a similar synapse model, although they do not state the weight of the synapse and appear to trigger the synapse based on the stimulation of a dorsal root rather than the general model of facilitation (with multiple values of synapse weights and time offset between the stimulation pulse and the synaptic event) used in this thesis. The paper concludes that epidural stimulation of interneurons in the spinal cord is unlikely, while this thesis concludes that facilitation of interneurons is possible.

The stimulation voltage ranges used in this thesis (0.25 V to 10 V) have been se-
lected to cover the voltage range used experimentally by my colleagues at UCLA:
3 V (P. Gad et al., 2012), 5 V to 7 V (Desautels et al., 2015), 4 V (Parag Gad, Roy,
Choe, Creagmile, et al., 2015), and 1 V to 8 V (Parag Gad, Roy, Choe, Zhong, et al.,
2015).

1.2   Contributions of thesis

Rather than trying to solve the general problem of understanding how epidural
electrical stimulation interacts with the complex network activity of neurons in the
spinal cord, this thesis focuses on the problem of understanding through simulation
how the activation of a single neuron (in many locations/orientations) may be facil-
itated by spinal stimulation in combination with an excitatory postsynaptic poten-
tial (EPSP) (either naturally occurring background activity or voluntarily induced).
Hopefully these results will be useful in understanding how epidural stimulation
can be used to facilitate recovery of motor movement.

The problem of facilitation is broken down into an investigation of how a single
EPSP interacts with epidural stimulation. The EPSP in this example is assumed to
be triggered by the release of neurotransmitters from an action potential in a presy-
naptic neuron. The action potential in the presynaptic neuron could have many
possible sources, including: the brain, sensory neurons, baseline activity, and other
facilitated or stimulated neurons. Building this simulation requires building a finite
element volume conductor model of an epidural stimulation array in a rat spinal
cord along with a model for the neurons in the spinal cord. After building these
models, the two components of facilitation (synapse weight and stimulation volt-
age) must be understood separately before understanding the interaction.

- Chapter 2 develops a finite element geometrical and electrical model of a rat
  spinal cord based on MRI images of a rat spine. This chapter also includes a

discussion on choosing material properties based on the frequency spectrum of monophasic and biphasic stimulation waveforms.

- A model neuron is described in Chapter 3. The results of injecting current pulses into the model neuron are presented to allow comparison with other studies. The synapse weight necessary for a single presynaptic event to generate an EPSP event large enough to achieve neurotransmitter release from the axon tip was also determined. Synapse weights less than this amount allow for the possibility of facilitation rather than causing neurotransmitter release directly.

- Chapter 4 looks at the effect of epidural stimulation on neurons with no synaptic activity using the volume conductor simulations presented in Chapter 2 and the neuron models from Chapter 3. The locations of neurons electrically stimulated sufficiently to release neurotransmitters using an amplitude of less than 5 V of stimulation are presented in Tables 4.2 and 4.3. Results for neurotransmitter release using an amplitude of less than 10 V are presented graphically. Plots of the membrane voltage at the axon tip for all simulations vs the static extracellular voltage, the second derivative of the static extracellular voltage, and the difference in static extracellular voltage between the axon tip and the soma are presented. These show that the membrane voltage at the axon tip cannot be predicted from the second derivative of the static voltage or the static voltage at the axon tip directly. However, the difference in static extracellular voltage between the axon tip and the soma showed a clear relationship with neuron activation (neurotransmitter release).

- Chapter 5 pulls this all together with simulations of interneurons exposed to varying amounts of sub-threshold synaptic input at times before, during, and after a sub-threshold epidural stimulation pulse. Simulations included all

the stimulation configurations and neurons from Chapter 4 using 5 different magnitudes of stimulation voltage combined with 8 sub-threshold values of synapse weights for synapses located in the middle of each distal dendrite, and 3 sub-threshold values of synapse weights for synapses located at the distal tip of each dendrite.

- Examples of facilitation are shown for some of the neurons discussed in Chapter 4. These examples show facilitation can occur with synapse triggers both before and after the stimulation pulse, but there are time intervals during which facilitation takes less magnitude of stimulation and/or synapse weight. For the examples using biphasic stimulation, the least effort facilitation occurred if the synaptic input occurred 0 to 20 ms before the stimulation pulse. For the examples using monophasic stimulation, the least effort facilitation occurred either 0 to 20 ms before the stimulation pulse or 0 to about 20 ms after the stimulation pulse depending on the polarity of the stimulation. The particular timing of the least effort facilitation windows appears to depend on the value of some of the ion channel state variables near the synapse.

- Histograms and stacked bar charts are presented showing the number of neurons vs facilitation window size for each pairing of stimulation magnitude and synapse weight. These show a significant amount of facilitation at the largest magnitude (5 V) stimulation voltage and largest but sub-threshold synapse weight. There is a decrease in the size of the facilitation window, and number of neurons facilitated as either the magnitude of stimulation or synapse weight decreases. In general, monophasic stimulation causes more simulated facilitation than biphasic stimulation, with the exception of simulations with synapses located on the distal tips of the dendrites, 5 V of stimulation, and the largest

sub-threshold synapse weight (4.783 nS).

- A greedy search was conducted to find a series of features in either the static volume conductor simulations or the stimulation only (no synaptic input) simulations that would allow separation of simulations that showed facilitation from those that did not. As in Chapter 4, features based on the difference in static voltage between particular locations on the neuron and the soma were best at separating simulation parameters that would result in facilitation from the rest. In particular, the difference in static voltage between the synapse location and the soma and the difference in static voltage between the initial segment and the soma were able to separate the most facilitated neuron simulations from the rest.

- Chapter 6 summarizes the main contributions of the thesis and suggests future work.