Model-Based Optimization of Spinal Cord Stimulation

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biomedical Engineering in the Graduate School of Duke University

2015
ABSTRACT

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Abstract

Chronic pain is a distressing, prevalent, and expensive condition that is not well understood and is difficult to treat. Spinal cord stimulation (SCS) is an increasingly prevalent therapy for treating intractable chronic pain when conventional therapies are ineffective, but the efficacy of SCS has stagnated since its inception. The mechanisms underlying the neuronal effects of SCS are not well understood, and prior efforts to improve SCS focused on the hardware (e.g., implantable pulse generators, stimulation leads, programmer) used to deliver SCS, rather than determining and exploiting the underlying physiological mechanism(s) for the therapy. This dissertation addresses this unmet need by characterizing the responses of sensory neurons to SCS using computational modeling and in vivo recordings and then exploiting the mechanisms underlying the observed responses to improve SCS through the design of novel temporal patterns of stimulation.

A biophysically-based network model of the dorsal horn circuit was constructed using interconnected dorsal horn interneurons and a wide dynamic range (WDR) projection neuron with representations of both local and surround receptive field inhibition. Cellular and network responses relevant to pain processing were reproduced by the model including wind-up, A-fiber mediated inhibition, and inhibition due to activation of surround receptive fields. A single node of the model representing only
the local receptive field could not reproduce the magnitude or time course of SCS-mediated inhibition. However, inclusion of inhibition due to activation of primary afferent inputs from surrounding receptive fields was both necessary and sufficient to reproduce SCS-mediated inhibition. WDR neuron responses to SCS were dependent on stimulation frequency, as stimulation frequencies within the clinically employed range of 30-100 Hz maximally inhibited the model WDR neuron, while frequencies less than 30 Hz or greater than 100 Hz produced less inhibition. Finally, reducing the influence of GABAergic interneurons, by weakening their primary afferent inputs or connections to the model WDR neuron, shrunk the range of SCS frequencies that produced maximal inhibition and altered the frequency at which SCS had a maximally inhibitory effect.

The responses of antidromically identified sensory neurons in the lumbar spinal cord were recorded during 1-150 Hz SCS in both healthy rats and neuropathic rats following chronic constriction injury (CCI) to test the predictions of the model. Projection neuron responses to SCS in both healthy and CCI rats were dependent on stimulation frequency, and the response predicted by the Gate Control circuit described a subset of in vivo SCS-frequency dependent responses. However, computational models of spinal microcircuits representing distinct sensory computations beyond Gate Control were required to reproduce the full gamut of experimentally measured frequency response relationships. In a subset of rats, intrathecal administration of
bicuculline, a GABA$_A$ receptor antagonist, but not CGP 35348, a GABA$_B$ receptor antagonist, increased spontaneous and evoked activity in projection neurons, enhanced excitatory responses to SCS, and reduced inhibitory responses to SCS. The effects of GABA$_A$ antagonism on projection neuron activity were consistent with model predictions and were suggestive of a broader role of GABAergic inhibition in modulating sensory projection responses to peripheral afferent input and SCS.

A novel temporal pattern of SCS, termed dual frequency SCS, was designed using the computational model to improve SCS by exploiting the excitatory-inhibitory interactions underlying the effects of SCS. Dual frequency SCS was implemented by delivering two distinct frequencies simultaneously to distinct dorsal column fiber populations. Dual frequency SCS produced greater inhibition of model sensory neurons than constant frequency SCS, and the inhibitory effects were robust to stimulation conditions, such as imperfect selectivity of dorsal column fibers, local/surround receptive field bias, and the partial loss of GABAergic inhibition. Experimental measurements of the effect of dual frequency SCS on spinal sensory neurons in anesthetized rats confirmed model predictions of the greater efficacy and robustness of select pairs of dual frequency SCS over conventional SCS, suggesting that dual frequency SCS is a novel approach to improving the therapeutic efficacy of SCS.
The outcomes of this dissertation are an improved understanding of the mechanisms underlying SCS, computational and experimental tools with which to continue the development and improvement of SCS, and novel strategies for improving the therapeutic efficacy of SCS. The insights gained from this dissertation may result in improvements in clinical SCS that significantly improve the therapeutic outcomes of SCS and thereby the quality of life of individuals affected by chronic pain.
Dedication

To my loving parents—Weixing Zhang and Zhenmei Li—for their unconditional
support and love during my life, and to all my friends, fellow Blue Devil fans, and
Ragnar teammates, among others, for giving me the time of my life at Duke.
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1. Introduction

This chapter has been previously published and is used with permission (Zhang et al. 2014).

1.1. Spinal Cord Stimulation

Spinal cord stimulation is a treatment option for patients with refractory chronic pain including failed back surgery syndrome (FBSS), complex regional pain syndrome (CRPS), and idiopathic conditions such as fibromyalgia and irritable bowel syndrome. (Kumar et al., 2007, Wall and Melzack, 1996, Guan, 2012) Over 30,000 individuals receive SCS devices annually for chronic pain, and SCS is a growing industry with global annual sales exceeding $1.8 billion. In conventional SCS, short duration current or voltage pulses are delivered at a constant frequency through an epidural electrode to excite the axons in the dorsal columns that carry sensory non-nociceptive information from the source of pain (Oakley and Prager, 2002, Shealy et al., 1972). Stimulation parameters such as amplitude, pulse duration, pulse repetition frequency, and the configuration of active electrode contacts are selected based on a combination of paresthesia location, pain relief, and comfort and can have a significant impact on clinical outcomes (Table 1) (Aló and Holsheimer, 2002, Cameron, 2004, Turner et al., 2004). Patients undergoing SCS report higher quality of life, greater pain relief, and more frequent resumption of normal activities and employment relative to individuals
undergoing pharmacological treatment alone (Cameron, 2004, Kumar et al., 2007). For some indications, SCS along with conventional therapies (drugs, physical therapy) is both more efficacious and cost-effective than conventional therapies alone (Kumar et al., 2002, Kumar and Rizvi, 2013)

Despite the success of SCS, there remain significant opportunities to improve the clinical efficacy of SCS. Notably, SCS has a relatively low mean “success rate” for treatment and significant variation in efficacy (Figure 1.1): only 58% of patients experienced successful outcomes—defined as a 50% or greater improvement in self-reported pain—based on data from two reviews of clinical studies and case series encompassing 1972 through 2013 (North et al., 1993, Taylor et al., 2013). Furthermore, success rate does not correlate with study year (R = 0.09, p = 0.4 t-test), indicating that the therapy is not improving with innovation and experience. As well, an analysis of 74 studies originally intended to reveal prognostic factors for SCS efficacy identified only one statistically significant trend: a negative correlation between study quality as assessed by Jadad score and reported clinical success (Taylor et al., 2005, Taylor et al., 2013).

The lack in improvement in SCS efficacy over the years, the high variability of clinical success rates, and the apparent dependence of efficacy on pain etiology (Kumar
et al., 1998) suggest that the state of understanding of the neurophysiology underlying chronic pain and SCS are incomplete. Theoretical and computational models of the neural circuitry underlying the nociceptive system may provide insights regarding the mechanisms of action of SCS. However, existing theoretical frameworks are largely untested, and few existing computational models exist. In particular, the Gate Control Theory (Melzack and Wall, 1965) provided the initial mechanism of action for SCS (Shealy et al., 1972) and is still considered a plausible depiction of the mechanisms of SCS (Linderoth et al., 2009, Guan, 2012). Experimental models of SCS provided insights into the effects of SCS on both the activity of sensory dorsal horn neurons and the behaviors of animal models of neuropathic pain (Linderoth and Foreman, 2006). Concurrently, computational models of SCS provided insights into anatomical substrates and geometric aspects of the SCS electrode important to dorsal column activation and led to electrode designs more capable of targeting specific dermatomes (Holsheimer and Wesselink, 1997). However, both experimental and computational models assume that the Gate Control Theory is sufficient to describe the mechanisms underlying SCS, and efforts to model the neural circuitry associated with SCS and to test proposed networks experimentally are sparse.

To facilitate the continued development and optimization of this promising therapy, we discuss in this chapter prior theoretical, experimental, and computational
models that describe the mechanisms of SCS. We focus on the neural circuits related to neuropathic pain and SCS in the dorsal horn and supraspinal centers and identify clinically relevant gaps in our knowledge of these circuits that must be addressed. In addition, we review and critique existing experimental and computational models of SCS and discuss avenues for the development of novel network models of SCS that can improve our understanding of the mechanisms of SCS.

In this dissertation, we consider conventional clinical SCS for the treatment of refractory neuropathic pain syndromes. Recently, high frequency SCS using pulse repetition frequencies in the kilohertz ranges was reported to provide pain relief without concomitant paresthesia (Al-Kaisy et al., 2014). The ability of HFSCS to suppress pain in animal models appears comparable to that of conventional SCS (Song et al., 2014, Shechter et al., 2013), but the mechanisms of action have not been investigated and may be distinct from those of conventional SCS (Shechter et al., 2013), so we will not discuss this therapy in this review. SCS for peripheral vascular disease and angina pectoris may achieve therapeutic effects through mechanisms distinct from those related to neuropathic pain (Linderoth et al., 2009, Meyerson and Linderoth, 2003), and these applications for SCS are also not discussed in detail.
1.2. **Theoretical Models for SCS**

1.2.1. **The Gate Control Theory**

The Gate Control Theory proposed by Melzack and Wall (1965) presented a possible neuron network in the dorsal horn that could explain non-linearities in pain perception, and the theory provided possible mechanism by which pain could be relieved. The proposed network consists of a "transmission (T) cell" responsible for relaying pain signals to the body's "action system" that was modulated by excitatory inputs from peripheral A-fiber and C-fiber afferent inputs and inhibitory inputs from inhibitory interneurons ("SG Cells") in the substantia gelatinosa (Melzack and Wall, 1965). That the inhibitory interneuron could be activated by enhanced A-fiber activity via dorsal column stimulation and thereby suppress pain transmission (Woolf and Wall, 1982a) served as initial and continued inspiration for SCS (Shealy et al., 1972, Linderoth et al., 2009). However, for the network described by the Gate Control Theory to be a valid mechanistic depiction of SCS, two specific observations regarding the effects of SCS on the dorsal horn circuit must be made: SCS must suppress the activity of "wide dynamic range" (WDR) dorsal horn projection neurons (Willis et al., 1974a, Chung et al., 1979) (Simone et al., 1991) through A-fiber mediated mechanisms, and SCS-mediated inhibition must involve segmental inhibitory interneurons.
Extensive neurophysiological evidence exists to indicate that SCS suppresses WDR neuron activity, and recent studies have shown that this inhibition depends on A-fiber activity. Early single unit recording studies revealed that stimulation of the dorsal columns inhibits the activity of deep laminae (IV, V) WDR dorsal horn neurons (Foreman et al., 1976b, Lindblom and Meyerson, 1975, Hillman and Wall, 1969, Duggan and Foong, 1985), and these studies are more extensively reviewed elsewhere (Linderoth and Foreman, 1999, Linderoth et al., 2009). SCS may also prevent WDR neuron sensitization due to long-term potentiation (LTP) of C-fiber inputs (Wallin et al., 2003) and wind-up induction (Guan et al., 2010), and SCS also inhibits WDR neurons in animal models of neuropathic pain (Yakhnitsa et al., 1999). Finally, recent work has shown that the suppression of neuronal activity in WDR neurons and pain alleviation during SCS in animal models of neuropathic pain requires activation of dorsal column fibers corresponding to A-fibers originating from the site of pain (Yang et al., 2011, Guan, 2012), supporting this feature in the Gate Control Theory architecture as well as SCS as a means to modulate pain.

As predicted by the circuit underlying the Gate Control Theory, inhibition of dorsal horn neurons involves spinal inhibitory mechanisms. SCS-induced inhibition of dorsal horn neurons is disrupted by cooling or lesioning of the dorsal horn caudal but not rostral to the stimulation site (Foreman et al., 1976b, Hillman and Wall, 1969), and
inhibition occurs with greater strength when the SCS electrode is placed at a spinal level close to the affected dermatome(s) (Smits et al., 2012). Studies demonstrating that bicuculline, a GABA A antagonist, reduces SCS-mediated inhibition of dorsal horn projection neurons (Duggan and Foong, 1985) and induces hyperalgesia and allodynia in otherwise healthy rats (Sivilotti and Woolf, 1994) implicate GABAergic inhibition from local interneurons as the driver of A-fiber mediated inhibition. Supporting this observation, hyperalgesia and allodynia in animal models of neuropathic pain can be reversed by the administration of the GABA A and GABA B agonists muscimol and baclofen (Hwang and Yaksh, 1997). Immunohistochemical labeling and electron microscopy demonstrate that WDR projection neurons possess GABAergic synaptic boutons (Lekan and Carlton, 1995), and the presynaptic neurons of GABAergic synapses on Lamina I projection neurons originate from inhibitory interneurons in laminae I-III of the dorsal horn (Todd, 2010, Zeilhofer et al., 2012), thus confirming the neuronal origin of spinal segmental GABAergic inhibition. Studies on the relationship between GABA and SCS (reviewed in Linderoth and Foreman, 1999) confirmed this finding in neuropathic rats and posited GABAergic modulation as a way to enhance the efficacy of SCS (Cui et al., 1998, Cui et al., 1996, Linderoth and Foreman, 1999, Schechtmann et al., 2010). The discovery of the relationship between GABA and SCS had clinical implications as well: clinical trials of SCS paired with the GABA B agonist baclofen
demonstrated that the administration of baclofen during SCS enhanced the effect of SCS in 48 patients for which SCS alone was ineffective in relieving pain (Lind et. al. 2004, Lind et. al. 2007).

One aspect of the Gate Control Theory based explanation for the mechanisms of SCS that has not been investigated in detail is the occurrence of SCS-mediated excitation of dorsal horn neurons. Specifically, SCS has been also reported to excite dorsal horn WDR neurons, and in some cases, SCS excites and inhibits the same neuron (Foreman et al., 1976b, Dubuisson, 1989). The interaction between SCS-mediated excitation and inhibition appears on the circuit underlying the Gate Control Theory and may be of clinical importance, as this balance of excitation and may partially explain why SCS-mediated pain relief is dependent on stimulation frequency, with 50-80 Hz being the most common clinical range (Oakley and Prager, 2002, Guan, 2012). However, few explanations exist for why applying Aβ-fiber threshold tactile or electrical stimulation directly to the receptive field of a WDR neuron may excite the neuron while SCS may putatively inhibit the neuron, and the relationship between SCS-mediated excitation and inhibition at different frequencies has not been explored.
1.2.2. Beyond the Pain Gate

Although the Gate Control Theory explains a number of features of pain relief from SCS, the network proposed by the Gate Control Theory is insufficient to describe all of the features of pain and SCS (Figure 1.2), and clinical observations underscore some limitations of this theory. First, the Gate Control Theory alone cannot account for why SCS may produce pain relief over a receptive field in which allodynia, or pain from non-noxious stimulation of a local receptive field, also occurs (Campbell and Meyer, 2006). Second, SCS does not affect the perception of acute pain from the region of paresthesia, whereas the Gate Control Theory predicts that sustained activation of large myelinated fibers, such as is the case in clinical SCS, should mask all pain. Furthermore, the Gate Control Theory posits that suppression of spinal projection neurons is sufficient for all pain relief, but pain relief by SCS depends heavily on etiology. Specifically, SCS is approved by the United States Food and Drug Administration only for FBSS and complex regional pain syndrome, and trials of SCS for other indications such as phantom limb pain and spinal cord injury have yielded lower success rates (Kumar et al., 1998). Finally, pain relief provided by SCS can persist for up to 30 minutes after the cessation of stimulation (Lindblom and Meyerson, 1975), whereas the Gate Control Theory only predicts pain relief while large myelinated inputs (e.g. the dorsal columns) are activated preferentially over smaller unmyelinated fibers.
Inhibition from surrounding receptive beyond that hypothesized by the Gate Control Theory has been documented and may affect neuronal responses to SCS (Hillman and Wall, 1969, Menetrey et al., 1977). For example, mechanical and electrical stimulation of low-threshold afferents originating from receptive fields surrounding the primary excitatory receptive field of a neuron results in inhibition of that neuron (Hillman and Wall, 1969, Menetrey et al., 1977) and can be similar to inhibition induced by dorsal column stimulation (Foreman et al., 1976b). These observations were corroborated by a recent study demonstrating that SCS inhibited the C-fiber component of a WDR neuron’s response, while A-fiber stimulation of a peripheral nerve corresponding to the local receptive field of the WDR neuron at the same frequency could not (Yang et al., 2014). Further supporting the importance of surround inhibition to pain modulation is the observation that receptive fields of dorsal horn projection neurons enlarge following peripheral nerve injury (Woolf and Wall, 1982a), inflammation (Kawamata et al., 2005), or the intrathecal administration of bicuculline in the case of nociceptive-specific neurons (Kawamata et al., 2005). These observations, coupled with recent anatomical studies revealing GABAergic connections between dorsal horn neurons extending across several spinal levels (Todd, 2010, Szucs et al., 2013), suggest that a center-surround excitatory-inhibitory architecture may better represent the effects of peripheral afferent activity and SCS on dorsal horn neuron
activity. Further exploration of the contribution of surround inhibition to the inhibitory
effects of SCS and the design of SCS electrodes capable of exploiting surround inhibition
could produce improvements in clinical pain relief.

As well, significant controversy remains as to whether the "Transmission Cells"
in the Gate Control circuit refer to a generalized functional class or a specific
morphologically and anatomically localized population of neurons in the spinal cord.
Functionally, "Transmission Cells" in the Gate were theorized as being responsive to
both non-nociceptive myelinated and nociceptive unmyelinated afferent inputs
(Melzack and Wall, 1965). That WDR neurons respond to both non-nociceptive and
nociceptive stimulation and that some WDR neurons send projections to the sensory
thalamus implicated these neurons as playing that role (Willis et al., 1974a, Chung et al.,
1979). However, the original Gate Control theory did not explicitly define the
morphology or laminar locations of the projection neurons described as the
"Transmission Cells" responsible for relaying nociceptive signals to supraspinal centers.
Studies attempting to classify sensory neurons by physiological response (Chung et al.,
1986), and morphological characteristics (Woolf and King, 1987) did not initially reveal
strong relationships. In addition, specifically identified WDR neurons projecting to
sensory thalamus were located in both superficial (lamina I) and deep (laminae IV-VI)
dorsal horn (Willis et al., 1974b, Ferrington et al., 1987). Taken together, these data
suggested that functional rather than morphological characteristics or laminar location
defined the Transmission Cells of the Gate Control circuit. As a result, both classical and
recent studies on the effects of SCS on sensory neuron activity identified and recorded
from neurons based on their physiological responses to natural stimulation (e.g., WDR
neurons) rather than on specific anatomical or morphological characteristics
(Handwerker et al., 1975, Foreman et al., 1976a, Yakhnitsa et al., 1999, Guan et al., 2010).

More recent morphological studies have suggested that, at least in superficial
laminae, there may be a correlation between cell morphology and function (Todd, 2010).
For example, neurons in lamina II of the dorsal horn can be classified into islet, central,
radial, and vertical morphologies, with more radial and vertical cells being
glutamatergic and more islet and central cells being GABAergic (Yasaka et al., 2010,
Zheng et al., 2010). Dual simultaneous recordings from synaptically connected neurons
have also suggested that relationships exist between connectivity, morphology, and
behavior. In one proposed pathway that incorporates these relationships, transiently
firing central cells receive excitatory inputs from Aδ primary afferents and connect onto
delayed firing vertical cells via excitatory glutamatergic synapses. Vertical cells, in turn,
synapse onto tonic Lamina I projection neurons via glutamatergic synapses (Lu and Perl,
2005). Tonically firing islet and central cells receive Aδ-fiber and C-fiber primary
afferent inputs and may modulate signal flow along this excitatory pathway via
GABAergic (Lu and Perl, 2003) and glycinergic (Takazawa and Macdermott, 2010) synapses. However, these studies were conducted in patch clamp preparations that precluded antidromic identification of projection neurons or physiological response classification based on natural stimulation (brush, press, pinch, crush), preventing a strong link between morphology, location, and function. In addition, sensory neurons from deeper laminae (Lamina IV-VI) that may also be responsive to SCS were not recorded in these studies. As a result, neuronal responses from patch clamp stimulation in these morphological studies could not be easily reconciled with studies of neuronal or behavioral responses to SCS, hindering the development of a network model of SCS based on emerging schemes (Todd, 2010).

WDR neurons responsive to both A-fiber and C-fiber inputs are not the only class of neuron present in the dorsal horn; low-threshold (LT) neurons that are responsive primarily to light touch and nociceptive-specific (NS) neurons that respond only to noxious mechanical and thermal stimulation are also prevalent in the dorsal horn (Chung et al., 1979) and may contribute to the SCS response. NS and WDR neurons are both active during noxious mechanical and thermal stimuli (Simone et al., 1991, Coghill et al., 1993), and NS and WDR neurons may encode distinct aspects of pain (Blomqvist and Craig, 2000). It is also likely that the networks underlying NS and WDR neuron behavior are different, as NS neurons located in superficial laminae of the dorsal
horn are organized into modular networks (Zheng et al., 2010) but, unlike WDR neurons, do not appear to receive direct inputs from Aβ fibers (Todd, 2010, Torsney, 2011). In addition, NS neurons become sensitized after bicuculline administration (Torsney and Macdermott, 2006) and during the progression of neuropathic pain (Lavertu et al., 2013), suggesting that pathological changes involving these neurons contribute to chronic pain. In fact, a recently developed scheme states that rather than being defined by the output of one type of neuron from a single circuit, nociception is a population response comprising responses from distinct "microcircuits" that are each responsible for specific aspects of perception (Prescott and Ratté, 2012). This "microcircuit" hypothesis suggests that LT, WDR, and NS neurons are wired differently in the dorsal horn and by extension may respond differently to SCS; for example, NS neurons being active during noxious stimuli while unresponsive to SCS may explain why SCS does not inhibit acute pain. However, the responses of low-threshold and NS neurons to peripheral stimulation and SCS have not been extensively documented, and the connectivities depicted by the microcircuit theory have not been confirmed.

Along with these clinical observations, the Gate Control Theory does not account for progressive changes that accompany the transition between an acute injury and chronic pain (Woolf, 2011). Aberrant sprouting of myelinated fibers into laminae where
they typically do not enter occurs following a peripheral nerve injury (Woolf et al., 1992), resulting in the formation or unmasking of excitatory connections onto neurons in the superficial dorsal horn (Kohno et al., 2003) and the sensitization of NS neurons (Kawamata et al., 2005, Von hehn et al., 2012). The abnormal sprouting and unmasking of excitatory connections is correlated with the time course of hypersensitivity to mechanical and thermal stimuli in rats following nerve constriction or nerve crush (Woolf et al., 1995, Kim et al., 1997), suggesting a relationship between abnormal afferent sprouting and altered pain perception. Furthermore, the expression of synaptic receptors associated with excitation (AMPA, NMDA, NK1) increases following peripheral nerve injury over the same time frame as behavioral indications of pain (Von hehn et al., 2012, Goff et al., 1997, Bleakman et al., 2006); the levels of other markers associated with the dorsal horn pain network (bNOS, µ-opioid receptors) in neuropathic animals also deviate significantly from normal and fluctuate over time in a manner that differed between animal models(Goff et al., 1997). This latter finding underscores the need to understand how specific changes in the dorsal horn network may affect responses to SCS, as differences in the mechanisms underlying different neuropathic pain syndromes may explain differential outcomes to SCS by etiology.

Progressive loss of inhibitory mechanisms occurs in conjunction with alterations in dorsal horn network connectivity, contributes to the progression of neuropathic pain,
and is also not predicted by the Gate Control Theory. Loss of strong A-fiber mediated inhibition is evident in sensory dorsal horn neurons following a peripheral nerve lesion (Woolf and Wall, 1982a), and at least some of this loss can be explained by the reduction in GABA-mediated IPSCs in the dorsal horn due to the death of GABAergic interneurons (Moore et al., 2002b) or reductions in the amount of GABA released into the dorsal horn (von Hehn et al., 2012). In particular, disruption of the expression of the KCC2 transporter following pathological changes in glial cell activity results in neuronal excitation when normally inhibitory GABAergic synapses are activated (Coull et al., 2005). Furthermore, activation of GABAergic inputs to neurons in which the KCC2 transporter is disrupted results in markedly increased levels of spontaneous and evoked activity in dorsal horn neurons (Keller et al., 2007), and dorsal horn projection neurons sensitized following a neurogenic injury may be "rescued" through the administration of the KCC2 activator CLP 257 (Lavertu et al., 2013). The role of KCC2 function in SCS remains unclear, as levels of KCC2 apparently do not correlate with increases in paw withdrawal threshold in neuropathic rats during SCS (Janssen et al., 2012), but understanding this relationship may provide insights into the specific inhibitory mechanisms underlying SCS. Finally, many nociceptive-specific neurons in superficial laminae of the dorsal horn receive polysynaptic excitatory inputs from Aβ fibers that are unmasked following the administration of bicuculline, suggesting that network changes
combined with the loss of GABAergic inhibition both contribute to pathological nociception (Torsney and Macdermott, 2006, Torsney, 2011). The lack of accounting by clinical SCS treatment plans for pathological changes to the level of inhibition in the dorsal horn circuit may contribute to the degradation of SCS efficacy with continued disease progression (Kumar et al., 2007, Taylor et al., 2005), and understanding these changes may yield better treatment plans.

1.3. Experimental models of SCS

The state of knowledge of both the underlying mechanisms of SCS and the most efficient and effective methods to deliver SCS remains incomplete. The effects of SCS on dorsal horn neurons are not entirely known, and the optimal set of stimulation parameters (electrode configuration, pulse duration, pulse amplitude, pulse repetition frequency) has yet to be determined. The combination of experiments in preclinical animal models and computational modeling has advanced the state of knowledge regarding the mechanisms of SCS and novel electrode geometries designed to deliver more spatially selective stimulation. The remainder of this review describes these experimental and computational approaches and their contributions to advancing SCS.
1.3.1. Animal models of SCS

Single unit recordings in anesthetized cats and non-human primates enabled characterized the excitatory and inhibitory effects of single pulses of dorsal column stimulation during natural peripheral inputs (brush, press, pinch, crush) and electrical stimulation of peripheral nerves (Hillman and Wall, 1969, Foreman et al., 1976a, Lindblom et al., 1978). In some cases, neurons were specifically identified as projection neurons through stimulation of the contralateral spinothalamic tract (Hillman and Wall, 1969, Foreman et al., 1976a). These studies provided experimental support for the Gate Control Theory and suggested that SCS had a net inhibitory effect on the activity of dorsal horn neurons. However, as these studies were conducted in healthy, anesthetized animals (Duggan and Foong, 1985, Hillman and Wall, 1969, Foreman et al., 1976b), the relationship between SCS and behaviors associated with pathological pain could not be characterized, and the degree to which the results of these studies apply to neuropathic pain is unclear.

The development of rat models of chronic pain (Kim et al., 1997, Decosterd and Woolf, 2000) and protocols to assess awake animal behavior during SCS were vital to demonstrating that SCS can relieve pathological pain (Linderoth and Foreman, 2006). The first of these experiments demonstrated that decreases in mechanical withdrawal threshold following nerve injury were reversed by SCS (Meyerson et al., 1995). The role
of segmental GABAergic systems (Cui et al., 1996, Cui et al., 1998), as well as descending 5-HT pathways (Song et al., 2011) in modulating the effects of SCS were clarified through the use of these behavioral models and have even led to clinical trials investigating the efficacy of combining SCS with GABA\textsubscript{b} agonists (Lind et al., 2008). However, a limitation in these studies is that they did not relate improvements in pain-related behaviors to activity of dorsal horn neurons, so only correlative relationships between pharmacological interventions and behavioral responses can be drawn from these studies. In other studies, SCS was shown to suppress the activity of dorsal horn neurons in neuropathic animals in response to natural stimulation of the hindpaw ipsilateral to sciatic nerve injury (Yakhnitsa et al., 1999), but pharmacological manipulations were not applied. More recent studies using neuropathic rats showed that SCS-mediated suppression of wind-up in dorsal horn neurons (Guan et al., 2010) and associated increases in mechanical withdrawal thresholds (Yang et al., 2011) are dependent on the activation of A\textsubscript{\beta} afferents originating from the injured nerve, but these studies did not verify that recorded neurons were projection neurons whose activity is directly correlated to pain (Simone et al., 1991). Recordings of the responses of projection neurons to SCS during pharmacological interventions and in neuropathic pain models are necessary to provide insights into the direct effects of SCS on pain suppression.
1.3.2. Computational modeling of SCS

Experimental models have contributed to understanding the neurophysiological mechanisms underlying pain relief by SCS, while computational models of SCS have been used to inform the development of electrodes capable of delivering targeted SCS. Motivation for early models of SCS by Coburn and colleagues came from "chance observations" (Coburn and Sin, 1985) that SCS could elicit effects on pain, motor deficits, bladder dysfunction, and a range of other disorders with spinal pathologies (e.g., spinal cord injury, cerebral palsy, peripheral vascular disease) (Cook and Weinstein, 1973, Illis et al., 1978, Illis et al., 1980). Coburn and colleagues developed finite element models of the electrical environment of the spinal cord and coupled them to simple biophysical models of neural elements present in the cord (Coburn, 1985, Coburn and Sin, 1985). Extracellular voltages calculated using these models were comparable to values recorded from both primate and human cadaver spinal cords (Coburn and Sin, 1985), and model-generated strength-duration relationships between axon excitation thresholds and stimulation pulse durations matched clinically observed relationships between stimulation amplitude, stimulation pulse width, and paresthesia threshold (Coburn, 1985). These results suggested that computational models of spinal cord anatomy and neuronal biophysics could be used together to model the clinical effects of SCS.
Subsequent model based design of innovative SCS electrodes incorporated more biophysical realism and increased understanding of how electrode geometries and tissue properties affect SCS efficacy. Holsheimer and colleagues added anisotropy and inhomogeneity to tissue electrical properties and determined the electrical and geometric factors that contributed most to determining the neural elements activated by SCS (Holsheimer and Wesselink, 1997, Struijk et al., 1992, Struijk et al., 1993a, Struijk et al., 1993b, Struijk et al., 1991, Wesselink et al., 1998a, Wesselink et al., 1998b).

Furthermore, these anatomically-based models revealed that the medial-lateral position of the electrode (Struijk et al., 1991), the thickness of the cerebrospinal fluid layer (Struijk et al., 1991), the curvature of dorsal root fibers (Struijk et al., 1992, Struijk et al., 1993b), and the presence of axon collaterals (Struijk et al., 1992, Struijk et al., 1993b) all substantially affected thresholds of dorsal column fibers. Importantly, the modeling results were validated by clinical measurements of paresthesias (Struijk et al., 1993a).

Using these models, Holsheimer and colleagues developed a “guarded tripole” electrode geometry that preferentially depolarized dorsal column fibers over dorsal root fibers and exhibited the capability to activate more selectively the dorsal column fibers originating from the site of peripheral pain. This design was predicted to be an improvement over previous electrode configurations, as the increased dorsal column fiber selectivity was expected to provide better paresthesia coverage over the source of
pain, corresponding to better "gating" according to the Gate Control theory (Shealy et al., 1972) as well as reduced paresthesias from regions beyond the source of pain due to dorsal root activation (Holsheimer and Wesselink, 1997, Aló and Holsheimer, 2002). The design was used in a clinical study to estimate the diameters of activated dorsal column fibers at various points along the spinal cord (Holsheimer and Wesselink, 1997, Wesselink et al., 1998a, Wesselink et al., 1998b) The results of this experiment provided data on the realistic morphology and distribution of fibers in the dorsal column that can be used in future electrode designs, and recent SCS electrodes have employed the “guarded tripole” geometry with varying degrees of clinical success (Abejon et al., 2005, Alo et al., 2002, Butyen, 2003, Wesselink et al., 1998b). Further development and refinement of finite element models of spinal cord stimulation have allowed computational assessments of the neural elements activated by SCS (Aló and Holsheimer, 2002) and the development of electrode geometries capable of focusing stimulation to specific regions of the dorsal horn (Sankarasubramanian et al., 2011).

Implicit in finite element modeling studies of SCS is that the Gate Control Theory sufficiently describes the neural network of the dorsal horn and therefore the neurophysiological basis of pain relief due to SCS (Holsheimer, 2002). However, the effects of SCS on the dorsal horn pain circuit have not been explicitly modeled. Although biophysical models of many neuron types and neuronal systems are
numerous and frequently used (Hines and Carnevale, 1997b), very few models of dorsal horn neurons have been published, and to date, no network models are capable of reproducing the inhibitory effects of SCS. Existing models of dorsal horn neurons represent tonic (Melnick et al., 2004b, Prescott and De Koninck, 2005), phasic (Prescott et al., 2008), and single-spiking (Prescott et al., 2008) cells and reproduce observations made from isolated dorsal horn neurons, but these neuronal models work in isolation rather than within a functional dorsal horn network. The few network models of the dorsal horn that exist are either pure mathematical functions (Britton et al., 1996, Britton and Skevington, 1996), or biophysical models (Farajidavar et al., 2008, Aguiar et al., 2010) that do not reproduce A-fiber inhibition, thus limiting their utility in modeling the effects of SCS. The development of network models of pain circuits in the dorsal horn and supraspinal centers will enable the development of more effective SCS treatment strategies.

1.4. Specific Aims

SCS is a promising treatment for chronic pain refractory to conservative medical management. The plateau in clinical efficacy reflects an incomplete understanding of the systems underlying the neuronal effects of SCS and points to the need to consider the effects of SCS the neural circuits and systems underlying chronic pain. This dissertation describes a model-based design-driven study that combined computational
modeling and *in-vivo* electrophysiological experiments to investigate the neuronal effects of SCS. Insights gained from the development of computational and experimental tools to investigate SCS were then applied to the design of novel temporal patterns for improving SCS. The specific aims addressed by this dissertation are as follows:

**1.4.1. Develop a computational model of the dorsal horn pain processing circuit and use this model to simulate the effects of spinal cord stimulation.**

In Chapter 2, a network model of the dorsal horn pain processing circuit was constructed using existing biophysical models of single dorsal horn neurons connected in a manner consistent with the organization of neurons in the dorsal horn (Melzack and Wall, 1965, Britton et al., 1996, Aguiar et al., 2010). The model was validated against existing experimental data and simulated the effects of dorsal column stimulation on the activity of projection neurons during an on-going peripheral sensory input. The network model of the Gate Control circuit reproduced several key features observed in pain processing, notably wind-up and A-fiber mediated inhibition, but the addition of a surround receptive field inhibition was necessary to reproduce SCS-mediated inhibition. The model further predicted that neuronal responses to SCS exhibit a non-monotonic relationship with SCS frequency and that GABAergic inhibition modulates the SCS frequency response relationship.
1.4.2. Develop an animal model of SCS that can be used to test predictions generated from the computational model regarding the effects of SCS on dorsal horn projection neurons.

Chapter 3 describes experiments aimed at validating hypotheses from Aim 1 in which the responses of antidromically identified projection neurons in anesthetized rats were recorded during SCS and peripheral stimulation. The effects of GABAergic inhibition on experimentally observed SCS-frequency response relationships were assessed by measuring responses after intrathecal application of bicuculline methiodide, a GABA\textsubscript{A} receptor antagonist and/or CGP 35348, a GABA\textsubscript{B} receptor antagonist. The relationship between SCS frequency and projection neuron activity predicted by the Gate Control circuit was present in a subset of recorded neurons and sensitive to bicuculline methiodide. However, the Gate Control circuit was insufficient to account for the full gamut of observed heterogeneous frequency dependent responses. Computational models of spinal microcircuits, representing additional interactions between nociceptive and non-nociceptive inputs reproduced projection neuron responses to SCS, suggesting that circuits beyond the Gate Control circuit contribute to the overall SCS response.
1.4.3. Design more optimal strategies for SCS through temporal patterning of the stimulation train.

Finally, Chapter 4 and Appendix I describe model-based design and optimization of novel temporal patterns of stimulation intended to be more effective and efficient than conventional SCS. First, non-regular temporal patterns were optimized using a genetic algorithm to suppress the model output neuron more effectively than conventional SCS (Appendix I). Second, dual frequency SCS was implemented by concurrently delivering two frequencies of SCS to two distinct populations of dorsal column fiber inputs to the model (Chapter 4). Appropriately selected combinations of dual frequency SCS were more effective at suppressing model neuron activity than conventional SCS at the average of the constituent frequencies, were more efficient than clinical standard 50 Hz SCS, and were robust to stimulation conditions and the loss of GABAergic inhibition. Experimental measurements of spinal sensory neuron responses to dual frequency SCS verified model predictions of efficacy compared to conventional SCS, suggesting that dual frequency SCS is a valid strategy for improving SCS. The outcomes of this dissertation are an improved understanding of the mechanisms underlying SCS, computational and experimental tools with which to continue the development and improvement of SCS, and novel temporal patterns for improving the therapeutic efficacy of SCS.
Figure 1.1 Success rate of SCS as a function of study year.

A scatter plot showing reported mean success rates from clinical studies on SCS over the period of 1973-2013 where "success" is defined as 50% or greater subjective pain relief reported by the patient. Studies mentioned solely in one review and studies mentioned in both reviews are delineated by different markers. Adapted from (North et al., 1993, Taylor et al., 2013).
Figure 1.2: Spinal mechanisms underlying neuropathic pain and SCS.

A schematic showing changes that occur to the circuitry and neurochemistry of the pain processing network in the dorsal horn during the induction and maintenance of neuropathic pain during SCS. The progression of neuropathic pain may involve but is not limited to increases in the levels of excitatory neurotransmitters and decreases in the levels of inhibitory neurotransmitters, aberrant sprouting of primary afferent fibers, unmasking of excitatory connections in the dorsal horn (+), and the loss of inhibitory controls via altered synaptic connectivity (X), interneuronal death, or changes in the function of GABA receptors.
2. Modeling the effects of spinal cord stimulation on wide dynamic range dorsal horn neurons: influence of stimulation frequency and GABAergic inhibition

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2.1. Introduction

Spinal cord stimulation (SCS) is a therapy for chronic, neuropathic pain in which epidurally implanted electrodes stimulate primary afferent (Aβ) collaterals in the dorsal columns. Although more than 30,000 new patients receive SCS every year, the success rate, defined as the proportion of patients receiving 50% or greater pain relief, is on average 60% (North et al., 1993, Taylor et al., 2013), varies widely across studies and pain etiologies (18% to 100%), and declines over treatment time (Taylor et al., 2013, Kumar et al., 2007). Despite advances in technology and surgical techniques, success rates have plateaued, and significant opportunities remain to improve clinical efficacy (Taylor et al., 2013, Cameron, 2004, North et al., 1993).

An incomplete understanding of the mechanisms underlying pain relief by SCS contributes to the plateau in efficacy. The Gate Control Theory provides a putative mechanism of action for SCS: stimulating myelinated Aβ-fiber collaterals in the dorsal columns activates inhibitory interneurons in the dorsal horn that suppress the
transmission of nociceptive information from wide dynamic range (WDR) spinal projection neurons to the brain (Melzack and Wall, 1965). However, the Gate Control Theory does not account for disruption of the excitatory-inhibitory balance in the dorsal horn network due to disinhibition (Moore et al., 2002a) or central sensitization (Von hehn et al., 2012) that occur during the course of neuropathic pain. In addition, the single receptive field depicted by the Gate Control Theory does not explain why applying Aβ-fiber threshold tactile or electrical stimulation directly to the receptive field corresponding to the source of pain produces allodynia (Campbell and Meyer, 2006), while SCS that produces paresthesia over the region including and surrounding the site of pain may provide pain relief. This latter observation implies that SCS affects afferents from both local and surrounding receptive fields (Shealy et al., 1972), a prediction also supported by prior studies of the effect of SCS on dorsal horn neurons (Hillman and Wall, 1969, Foreman et al., 1976a), but more recent studies and models have not examined the role of surround inhibition in the efficacy of SCS.

Additionally, the Gate Control Theory does not explain the narrow range of stimulation frequencies (50-80 Hz) considered optimal for clinical SCS (Oakley and Prager, 2002), as the Gate Control Theory predicts that higher frequencies of A-fiber stimulation should result in greater inhibition of dorsal horn projection neurons (Chung et al., 1984a, Chung et al., 1984b, Duggan and Foong, 1985). Earlier acute recordings
from WDR projection neurons during SCS in animal models used frequencies (1-10 Hz) below the clinical range (Foreman et al., 1976a, Hillman and Wall, 1969), and later studies on the behavioral and neuronal effects of SCS in neuropathic animal models employed only the "clinical baseline" of 50 Hz (Cui et al., 1996, Yakhnitsa et al., 1999, Cui et al., 1998). An assessment of the relationship between SCS frequency and efficacy in neuropathic rats did show that SCS applied at 4 Hz and 60 Hz was more effective in alleviating hyperalgesia than SCS applied at 100 Hz and 250 Hz (Maeda et al., 2008), but this study did not link behavioral correlates of pain relief to neuronal activity. In contrast to these experiments, a large range of clinical stimulation frequencies between 2 and 200 Hz have been cited (North et al., 1993) with 50-80 Hz being the most commonly used but anecdotally reported range (Kumar et al., 2007) and a general minimum of 25 Hz (Oakley and Prager, 2002).

Most importantly, the network architecture proposed by the Gate Control Theory that putatively drives the effects of SCS is largely untested beyond phenomenological reports of potentiation to repetitive C-fiber stimulation ("wind-up") (Herrero et al., 2000) and A-fiber mediated inhibition (Woolf and Wall, 1982b) in dorsal horn neurons. Biophysical computational models of neural circuits can be used to test theories and validate networks, but prior models of the dorsal horn are limited to general frameworks for the possible arrangement of excitatory and inhibitory nodes in the
dorsal horn (Melzack and Wall, 1965, Hillman and Wall, 1969), mathematical functions relating dorsal horn output to afferent inputs (Britton et al., 1996, Britton and Skevington, 1996), or biophysical models of individual neurons. Recent network models (Aguiar et al., 2010, Le Franc and Le Masson, 2010) are capable of reproducing wind-up but do not include inhibitory influences on the output projection neuron as depicted in the Gate Control Theory and as required to model A-fiber mediated inhibition and SCS.

We developed a comprehensive biophysical network model to simulate the effects of SCS on WDR projection neuron activity. We compared the properties of our network model to data from independent cellular and network studies on dorsal horn neurons and studied the effects of SCS applied at different frequencies on model WDR projection neuron activity (Melnick et al., 2004b, Aguiar et al., 2010). Our results provide insight into the mechanisms underlying SCS and quantify the effects of changes in dorsal horn inhibitory mechanisms on SCS. Our model is limited to segmental spinal mechanisms of conventional SCS and does not account for supraspinal or descending mechanisms that contribute to SCS-mediated pain relief (Song et al., 2011) or novel kilohertz-frequency SCS (Al-Kaisy et al., 2014). Some preliminary results were reported in abstract form and in conference proceedings (Zhang et al., 2013).
2.2. Materials and methods

We developed a computational network model of the dorsal horn circuit by connecting biophysically-based compartmental models of dorsal horn neurons via representations of excitatory and inhibitory synapses using a network architecture based on existing schemes of dorsal horn nociceptive processing (Britton et al., 1996, Aguiar et al., 2010, Melzack and Wall, 1965) (Figure 2.1). Membrane dynamics of the individual neurons and synaptic properties were based on experimental measurements where available, and the strengths of synaptic connections were tuned to generate realistic electrophysiological responses to stimulation of primary afferent and dorsal column fibers. All simulations were conducted in the NEURON v. 7.2 simulation environment (Hines and Carnevale, 1997a) using a time step of 0.0125ms and 2nd order implicit Crank-Nicholson integration.

2.2.1. Model neuron geometry

The computational model comprised cable models of inhibitory interneurons (IN), an excitatory interneuron (EX), and a WDR projection neuron, each with electrical characteristics based on patch clamp recordings from substantia gelatinosa and deeper dorsal horn neurons (Aguiar et al., 2010, Melnick et al., 2004b, Prescott and De Koninck, 2005, Ruscheweyh and Sandkühler, 2002) (Figures 2.1-2.2). Each model neuron consisted of four inter-connected single compartments representing the dendrites, soma,
axon initial segment, and axon. Dimensions of the tonic and delayed firing EX and WDR interneurons were as follows: the dendrite was a cylinder 400 μm in length and 2.5 μm in diameter; the soma was a sphere 20 μm in diameter; the axon hillock was a cone that decreased in diameter from 2 to 1 μm over a length of 9 μm; the axon was a cylinder 1 μm in diameter and 1,000 μm long (Aguiar et al., 2010). Dimensions of the transient EX interneuron and the tonic IN interneuron were as follows: the dendrite was a cylinder 1371 μm in length and 1.4 μm long; the soma was a sphere 10 μm in diameter; the axon hillock was a cone that decreased in diameter from 1 to 0.5 μm over a length of 30 μm (Melnick et al., 2004a, Melnick et al., 2004b).

2.2.2. Neuron and synapse biophysics

Hodgkin-Huxley-like membrane models were implemented to represent ionic currents at each compartment in each neuron. For the tonic EX interneuron, IN interneuron and WDR projection neuron, parameters were set according to previously published values and such that neurons exhibited tonic firing during step depolarization (Figures 2.2b, 2.2c) (Aguiar et al., 2010, Melnick et al., 2004b, Prescott and De Koninck, 2005, Prescott and Koninck, 2002). Several additional changes were made to match more closely data from dorsal horn neurons. First, a delayed rectifier K⁺ current was added to the dendrites ($g_K = 0.036 \, S/cm^2$) and soma ($g_K = 0.0011 \, S/cm^2$) of the tonic EX and WDR neurons to reflect the locations of K⁺ channels on spinal neurons (Wolff et al., 1998), and
the length of the axon hillock of the WDR neuron was increased from 3μm to 9μm to facilitate action potential initiation at the axon hillock and to match model frequency-current (f-I) relationships to experimental data (Figure 2.2) (Wolff et al., 1998, Safronov et al., 1997). In addition, conductances corresponding to fast Na⁺ channels were removed from the soma of all neurons because this conductance is apparently not present in the cell bodies of neurons in the substantia gelatinosa; the increased total hillock Na⁺ conductance due to the increase in hillock size compensated for the removal of somatic Na⁺ conductance in the tonic EX and WDR neurons (Safronov et al., 1997). Finally, Ca²⁺-dependent ionic conductances required for "wind-up" were preserved from a previous model of the WDR projection neuron (Aguiar et al., 2010), but conductances for all Ca²⁺ and persistent Na⁺ currents were reduced by 5% to reproduce better the progression of wind-up during 1 Hz C-fiber threshold peripheral stimulation (Schouenborg and Sjolund, 1983, Herrero et al., 2000).

Dorsal horn interneurons, in particular lamina II excitatory interneurons that receive C-fiber inputs, exhibit transient and delayed firing characteristics as well as tonic firing (Ruscheweyh and Sandkühler, 2002, Yasaka et al., 2010). To implement a transiently firing interneuron, the hillock Na⁺ conductance of the tonic interneuron was decreased to 0.73 S/cm² (Melnick et al., 2004a). To implement a delayed firing interneuron, a voltage-dependent potassium A-current (I_{KA}), modified from a model of
In a spinal motoneuron (Safronov et al., 2000) was introduced to the dendrite ($g_{KA,dend} = 0.0025 \text{ S/cm}^2$), soma ($g_{KA,soma} = 0.015 \text{ S/cm}^2$) and axon hillock ($g_{KA,hillock} = 0.3 \text{ S/cm}^2$) of the tonic interneuron (Figure 2.2d). Activating and inactivating gating variables $n$ and $h$ and their respective rate constants ($\alpha_n$, $\beta_n$, $\alpha_h$, $\beta_h$) are described as functions of temperature (T) in degrees Celsius, membrane potential $V_m$ in mV, and the potassium reversal potential $E_k$ of -84 mV in equations 2.1-2.5 (Ruscheweyh and Sandkühler, 2002, Yasaka et al., 2010). In addition, the hillock fast Na$^+$ conductance was reduced to 1.73 S/cm$^2$ and the delayed rectifier K$^+$ conductances were increased to 0.144 S/cm$^2$ in the dendrite, 0.0043 S/cm$^2$ in the soma, and 0.304 S/cm$^2$ in the axon hillock to make the excitability consistent with experimental data. These changes were incorporated into the original tonic EX interneuron because blockade of $K_A$ using 4-aminopyridine in delayed firing neurons restores tonic firing behavior (Ruscheweyh and Sandkühler, 2002).

\[
I_{\text{tra}} = g_{\text{tra}} n^4 h (V_m - E_k) \quad (2.1)
\]

\[
\alpha_n = g^{(T-25)/10} \cdot \frac{0.062 \cdot \left(V_m - 15\right)}{\exp \left(-\frac{\left(V_m - 25\right)}{5}\right) - 1} \quad (2.2)
\]

\[
\beta_n = g^{(T-25)/10} \cdot \frac{0.007 \cdot \left(V_m - 22\right)}{\exp \left(-\frac{\left(V_m - 22\right)}{15}\right) + 1} \quad (2.3)
\]

\[
\alpha_h = g^{(T-25)/10} \cdot \frac{0.062}{\exp \left(-\frac{\left(V_m - 15\right) + 85}{14}\right) + 1} \quad (2.4)
\]
\[ \beta_t = 3^{(T-23)/19} \cdot \frac{D_02}{\exp \left( \frac{-\left( T - T_{\text{syn}} \right)}{14} \right) + 1} \] (2.5)

Postsynaptic currents generated by synaptic inputs (I_{syn}) were modeled as functions of synaptic conductance (g_{syn}), synaptic reversal potential (E_{syn}), and time (t) after a presynaptic event (t_{\text{spike}}) with rise (\tau_1) and decay (\tau_2) time constants from published values for AMPA, NMDA, GABAa, and glycine synapses (Destexhe et al., 1998, Aguiar et al., 2010) (Equations 2.6-2.8, Table 2.1):

\[ I_{syn} = g_{syn}(V_m - E_{syn}) \] (2.6)

\[ g_{syn} = g_{\text{tot}} \cdot \left( \frac{(T_{\text{spike}}/\tau_2) - (T_{\text{spike}}/\tau_1)^{1/2}}{(T_{\text{spike}}/\tau_2)^{1/2} - (T_{\text{spike}}/\tau_1)^{1/2}} \right) \cdot \left( \exp \left( -\frac{T_{\text{spike}}}{\tau_1} \right) - \exp \left( -\frac{T_{\text{spike}}}{\tau_2} \right) \right) \] (2.7)

\[ T_p = \frac{3\tau_1 - \tau_2}{\alpha - \tau_2} \cdot \ln \left( \frac{T_{\text{spike}}}{\tau_2} \right) \] (2.8)

Although the NK1 receptor is metabotropic, the response to NK1 receptor activation was modeled as a slow alpha-function to account for the net effects of NK1 receptor activation on intracellular Ca\(^{2+}\) concentration, consistent with an earlier model of wind-up (Aguiar et al., 2010). The properties of individual synapses were largely preserved from prior models (Destexhe et al., 1998, Aguiar et al., 2010), but the reversal potential of the GABAa synapse was changed from -80 mV to -70 mV except when noted to be...
consistent with patch clamp recordings of GABAergic responses in dorsal horn neurons (Lu and Perl, 2003). In addition, a glycinergic synapse was implemented and included in the connection from the local IN neuron to the WDR neuron, as GABAa and glycine receptors often co-localize in the superficial and deep dorsal horn (Todd, 2010).

2.2.3. Network architecture

The architecture of the model (Figure 2.1a) was based on the Gate Control Theory and an existing network model capable of reproducing wind-up (Aguiar et al., 2010, Melzack and Wall, 1965). Peripheral inputs from 15 Aβ-fibers, 15 Aδ-fibers, and 30 C-fibers represented spatiotemporal dispersion of the afferent inputs. A sensitivity analysis indicated that increasing the number of primary afferent inputs did not affect the A- and C-fiber evoked responses of the model neurons as long as the total input conductances were kept constant. The local IN interneuron received excitatory inputs from Aβ-fibers via AMPA synapses, the EX interneuron received excitatory inputs from C-fibers via AMPA, NMDA, and NK1 synapses, and the WDR neuron received convergent AMPA and NMDA inputs from Aβ-fibers and Aδ-fibers, NK1 inputs from C-fibers, and GABAergic and glycinergic inputs from the IN interneurons (Figure 2.1b) (Aguiar et al., 2010). A GABAergic connection between the local IN and EX interneurons represented indirect inhibition of the C-fiber input by A-fiber activity. In contrast to the Aguiar (2010) model, no GABAergic synapses were implemented in the
C-fiber to WDR connection, as direct inhibitory connections between C-fibers and projection neurons are rare (Alvarez et al., 1993). Inhibitory and excitatory synapses were not segregated, as immunohistochemical studies have shown that inhibitory and excitatory synapses are uniformly distributed across the soma and dendrites of dorsal horn neurons (Lekan and Carlton, 1995). Randomizing the locations of the A-fiber and C-fiber synaptic inputs onto the IN and WDR neurons as well as the synaptic connections between the IN and EX interneurons and the WDR neurons did not affect the input-output relationships of the network. Placing inhibitory synapses on different points of the dendrite of the WDR neuron also did not appreciably alter the inhibitory response of the WDR neuron relative to the case where the inhibitory synapses were placed at the same segment.

2.2.4. Inputs and simulations

The inputs to the model spanned the appropriate conduction velocities across a population of A-fiber and C-fiber peripheral afferents, as temporal, aspects of the input are critical to neuronal responses to stimulation (Herrero et al., 2000). Peripheral afferent stimulation was implemented by generating recurring events at the Aβ-, Aδ-, and C-fiber inputs with arrival latencies determined according to published conduction velocities (Aβ: 14-30 m/s; Aδ: 2.2-8 m/s; C: 0.6-1.5 m/s) (Harper and Lawson, 1985) assuming 100 mm between the site of stimulation and the dorsal horn network, as in
previous studies (Herrero et al., 2000, Woolf and Wall, 1982b). Increasing or decreasing the conduction distance between the peripheral source and the WDR neuron increased and decreased the latencies of the peripheral A- and C-fiber evoked responses in the WDR neuron but did not affect responses to SCS. In addition, randomizing the location of the synaptic inputs along the dendrites of the neurons had only a small effect on the WDR neuron's firing rate during simulated primary afferent stimulation as compared to all synapses placed on the middle segment of the dendrite of the receiving neuron.

The firing rate of the WDR neuron was the primary output of the model because WDR firing rate is correlated with the magnitude of pain following peripheral capsaicin injection (Simone et al., 1991) and because studies of SCS in healthy and neuropathic animal models use WDR neuron firing rate as a proxy for SCS efficacy (Foreman et al., 1976a, Linderoth et al., 2009, Guan, 2012). For model validation, the activity of the WDR neuron was compared to recordings from dorsal horn projection neurons during low-frequency (1-2 Hz) peripheral nerve electrical stimulation. For comparison with data in Foreman et. al. (1976), we applied primary afferent spike trains with individual inter-spike intervals drawn from a homogeneous Poisson process such that spike rates along individual afferent fibers (Aβ mean = 9 spikes/s, Aδ mean = 9 spikes/s, C mean = 2.5 spikes/s) had mean frequencies representative of peripheral afferent activity during steady-state natural "pinch" stimulation (Slugg et al., 2000). SCS was simulated by
applying a constant frequency train of events through separate inputs representing dorsal column collaterals of the Aβ-fibers. For simulations of SCS during neuropathic pain, we applied Poisson spike trains through peripheral afferents with means and variances consistent with those of spike trains recorded from dorsal root ganglia neurons and peripheral neuromas in rats with neuropathic pain (Aβ mean = 2.2 spikes/s, Aδ mean = 2.2 spikes/s, C mean = 1.5 spikes/s) (Wall and Gutnick, 1974, Liu et al., 2000), and we set 30% of the Aβ- and Aδ-fiber inputs to exhibit bursting behavior (Kajander and Bennett, 1992, Liu et al., 2000). To represent inhibition during SCS due to activation of Aβ-fibers from surrounding receptive fields, we implemented an additional IN interneuron, receiving a separate set of 15 Aβ-fiber inputs, that was connected to the EX and WDR neurons via GABAergic synapses (Figure 2.1b) (Hillman and Wall, 1969). Throughout all SCS simulations, we accounted for the possibility of collisions between orthodromic and SCS-triggered antidromic action potentials along each Aβ-fiber using a script written in MATLAB (MathWorks, Natick, MA) and transmitted the net spike train to the network model (Iggo, 1958).

2.3. Results

We implemented a computational network model of a dorsal horn circuit consisting of biophysical model neurons and realistic synaptic connections. We first demonstrate that the individual model neurons exhibit f-I relationships and
electrophysiological responses comparable to published data. We then validate the network model by comparing activity of the model WDR neuron during peripheral stimulation to corresponding data from several experimental studies. Subsequently, we use the model to reveal that inhibition of WDR projection neuron activity by SCS requires inhibition from surrounding receptive fields and depends strongly on the stimulation frequency. Finally, we predict that reduced inhibition in the dorsal horn, whether due to loss of GABAergic mechanisms or loss of KCC2 function, attenuates the suppression of WDR activity by SCS and alters the relationship between SCS frequency and WDR neuron inhibition.

2.3.1. Cellular biophysics

We compared the activity of model neurons during constant current injection with published f-I data from dorsal horn neurons (Figure 2.2). The f-I relationships of the tonic EX, tonic IN, and WDR neurons (T = 23° C) were all within experimental ranges derived from recordings of tonically firing neurons in the dorsal horn at 23° C (Ruscheweyh and Sandkühler, 2002, Melnick et al., 2004b). The IN interneuron exhibited slightly higher firing rates than experimental neurons at lower current stimulation amplitudes, and the firing threshold of the EX interneuron (25 pA) was slightly larger than that of experimental neurons (Figure 2.2a). At all amplitudes of current injection, the IN and tonic EX neurons exhibited constant frequency tonic firing
(Figure 2b, 2c), and the IN interneuron exhibited spike amplitude accommodation at higher amplitudes of stimulation, consistent with experimental data. Consistent with experimental traces, the delayed firing neuron exhibited delayed spikes at relatively high (>175 pA) amplitudes of current injection and transitioned to slow tonic firing with an initial period of interspike interval facilitation at even higher amplitudes of current injection (Figure 2.2d). The transient EX interneuron exhibited an initial burst of action potentials that fell within the range of experimentally observed spike counts followed by adaptation (Figure 2.2e). Finally, similar to isolated rat deep dorsal horn neurons, the model WDR projection neuron generally exhibited tonic behavior but continued to discharge action potentials after the cessation of current injection if held at a membrane potential more depolarized than -65 mV due to persistent Na⁺ and Ca²⁺ currents vital to wind-up during repetitive afferent stimulation (Figure 2.2f) (Morisset and Nagy, 1999, Morisset and Nagy, 2000, Aguiar et al., 2010).

2.3.2. Network response

We simulated 1 Hz stimulation of peripheral afferents, and the activity of the model WDR neuron was compared to representative cases or ranges from previous experimental studies (Herrero et al., 2000, Schouenborg and Sjolund, 1983, Foreman et al., 1976a, Woolf and Wall, 1982b, Woolf and King, 1987). These simulations did not include representations of surround inhibition, as C-fiber threshold stimulation of
afferents from surrounding receptive fields, as conducted in the corresponding experiments, causes excitation that overrides surround inhibition (Hillman and Wall, 1969, Kato et al., 2011, Menetrey et al., 1977). The model WDR neuron exhibited wind-up characterized by a response to C-fiber stimulation that potentiated with each stimulus; consistent with experimental data, the A-fiber evoked response did not potentiate (Figure 2.3a) (Herrero et al., 2000). Both the cumulative number of spikes and the distinct A- and C-fiber evoked responses of the WDR neuron during 15 s of stimulation fell within experimental ranges (Figure 2.3b, 2.3c) (Schouenborg and Sjolund, 1983, Woolf and King, 1987). Although the initial C-fiber evoked response of the model WDR neuron exhibited more spikes than experimental responses (Woolf and King, 1987, Handwerker et al., 1987), the ratio of the number of spikes fired by the WDR neuron after the last stimulus to the number of spikes fired after the first stimulus (253 %) was consistent with prior studies (Aguiar et al., 2010).

We assessed the sensitivity of the WDR response to peripheral afferent stimulation to the strengths of the A-fiber inputs onto the inhibitory interneuron, inhibitory connections within the model, and biophysics of the EX interneuron. The magnitude of the A-fiber response and the degree of wind-up to C-fiber stimulation were sensitive to the strength of direct inhibition between the IN and WDR neuron (Figure 2.4). Halving the strength of the Aβ-fiber inputs onto the IN neuron increased
the A-fiber evoked response in the WDR neuron by 1 spike initially and 3 spikes after 15 s of wind-up, increased the C-fiber evoked response by 1 spike initially and 8 spikes after 15 s, and increased the degree of wind-up due to a 1 Hz stimulus after 15 s from 230 % to 271 % of the initial response (Figure 2.4a). Halving the strength of the GABAa synapse from the IN to the WDR neuron increased the A-fiber evoked response by 1 spike initially and 3 spikes after 15 s, increased the C-fiber evoked response by 1 spike initially and 6 spikes after 15 s, and increased the degree of wind-up to 257 % of the initial response after 15 s. Doubling the strength of the IN to WDR GABAa synapse reduced the A-fiber evoked response by 3 spikes initially and after 15 s of stimulation and reduced the degree of wind-up by the WDR neuron to 200 %. However, doubling the strength of the A-fiber to IN synapse had only minor effects on the response, as this did not cause the IN neuron to fire more action potentials than in the default case (Figure 2.4b). Furthermore, replacing the tonic EX interneuron with transient and delayed firing EX interneurons did not affect the A-fiber evoked response in the model WDR neuron but completely eliminated the C-fiber evoked response, as the transient and delayed firing EX interneurons were considerably less excitable by peripheral afferent stimulation than the tonic EX interneuron.

Some WDR neurons in the dorsal horn are inhibited by stimulation of A-fiber afferents, and this inhibition typically follows a brief period of A-fiber mediated
excitation (Duggan and Foong, 1985, Foreman et al., 1976a, Hillman and Wall, 1969), suggesting a polysynaptic relay between A-fiber afferents and the WDR neuron. We delivered paired pulse stimulation to model afferents at 0.5 Hz for 160 s: within each pair, the first pulse activated Aβ-, Aδ, and C-fiber afferents, and the second pulse activated only Aβ- and Aδ-fiber afferents (Figure 2.5a). During the stimulus train, the delay between the C-fiber threshold and A-fiber threshold stimuli was decreased from 150 ms to 100 ms in 10 ms increments and from 100 ms to 20 ms in 20ms increments. Consistent with experimental data (Woolf and Wall, 1982b), if the second pulse was delivered 25 ms or later before the arrival of the C-fiber volley from the first pulse, then the second pulse generated a period of brief excitation followed by period of "strong" inhibition (Figure 2.5b). In addition, reducing only the strength of the GABAergic synapse between the IN and WDR neurons by 50% reproduced "weak" inhibition (Woolf and Wall, 1982b) of the C-fiber evoked response by A-fiber threshold stimulation (Figure 2.5c).

2.3.3. Spinal cord stimulation: surround receptive fields

We applied primary afferent inputs with statistical characteristics consistent with A- and C-fiber activity recorded during "pinch" stimulation (Slugg et al., 2000) while delivering 1 Hz SCS to dorsal column collaterals of Aβ-fiber afferents. We quantified the baseline activity of the model WDR neuron in response to the afferent input as well
as the magnitude and duration of inhibition following SCS. First, we applied SCS only to the Aβ afferent collaterals emerging from the local receptive field (Figure 2.6a-2.6b) consistent with the Gate Control Theory mechanism of SCS. Stimulation of local receptive fields alone did not replicate experimentally observed inhibition due to SCS. In contrast to experimental neurons, the model WDR neuron was suppressed to 47% of baseline, maximum inhibition occurred 20 ms after the SCS pulse, and inhibition lasted for only 50 ms (Figure 2.6c). We attempted to correct these discrepancies by altering the strengths of the inputs to the IN neuron, the connection between the IN and WDR neuron, and the connection between the IN and EX neuron. Doubling the strengths of the inputs to the IN neuron or the synapse between the IN and WDR neurons caused the WDR neuron to be completely inhibited by SCS and extended the duration of inhibition to 70 ms and 60 ms, respectively. However, these changes also reduced the average spontaneous activity of the model WDR neuron to far lower rates than observed experimentally (Figure 6e-6f) (Foreman et al., 1976a). Altering the connection from the IN to the EX interneuron had only a small effect on the firing of the model WDR neuron.

Since local receptive field inhibition alone could not reproduce the SCS-mediated inhibition observed experimentally, we introduced inhibition from surround receptive fields and replicated the experiment by delivering SCS through Aβ-fiber collaterals from both local and surround receptive fields (Figure 2.7). Aβ-fiber inputs from local
receptive fields synapsed onto both the local IN and the WDR neuron in the model, while Aβ-fiber inputs from surrounding receptive fields synapsed onto a distinct IN neuron that, in turn, formed an inhibitory synapse with the local EX and WDR neurons (i.e., a "center surround" scheme; Figure 2.7a-2.7b). The sole addition of the inputs from the surround IN neuron allowed the model to reproduce both the absolute and relative (vs. baseline activity) inhibition by SCS as well as the time course of the inhibition following the SCS pulse (Figure 2.7c) without affecting the spontaneous activity of the WDR neuron during pinch (Foreman et al., 1976a).

Dorsal horn projection neurons exhibit a wide range of responses to SCS, ranging from transient, incomplete inhibition to complete inhibition that lasts for over 70 ms (Foreman et al., 1976a). We hypothesized that the strength of direct and indirect inhibition of the WDR neuron from local interneurons as well as the extent of surround inhibition of the WDR neuron were responsible for this variation (Figure 2.8). We weakened and strengthened the inputs by 50 % to both the local and surround IN neurons, the strength of the GABAergic synapse between the local and surround IN neurons and the WDR neuron, and the strength of the GABAergic synapse between the IN neurons and the local EX neuron. Although a range of responses could be produced by altering any of these connections, altering the strength of the inputs to both the local and surround IN neurons had the greatest effect on the response of the WDR neuron to
SCS (Figure 2.8). Weakening the inputs to the IN neuron prevented the complete inhibition of the model WDR neuron, reduced the maximal degree of inhibition to 6% of baseline, and reduced the time required for the neuron to return to baseline by 20 ms (Figure 2.8b, 2.8e). Strengthening the inputs to the IN neurons prevented the model WDR neuron from either returning to baseline activity within 100 ms or exhibiting a post-inhibitory rebound (Figure 2.8b, e). In contrast, altering the connections from the IN to EX neurons had a small effect on the response of the model WDR neuron to simulated SCS (Figure 2.8c, 2.8f).

2.3.4. Spinal cord stimulation: frequency dependence

We predicted that the interaction between convergent excitatory and inhibitory inputs onto the WDR neuron underlies the commonly used clinical frequency range of 50-80 Hz. To test this hypothesis, we delivered a random spike train, with firing statistics consistent with a peripheral neuroma (Liu et al., 2000), to all C-fiber inputs and all local Aβ- and Aδ-fiber inputs while also delivering SCS through the local and surround dorsal column inputs. The effects of SCS on WDR neuron activity were strongly dependent on SCS frequency but not monotonic in nature (Figure 2.9). At SCS frequencies below 30 Hz, intervals between bursts of interneuron activity were sufficiently long such that IPSPs decayed before the next Aβ-fiber collateral activation from SCS excited the WDR neurons. This excitation, combined with ongoing excitation
from peripheral afferent inputs, resulted in irregular, bursting activity in the WDR neuron (Figure 2.9b). At SCS frequencies between 30 Hz and 100 Hz, Aβ-fiber stimulation caused tonic activation of the IN interneurons and thereby persistent inhibiton of the WDR neuron (Figure 2.9c). However, the firing rate of the interneuron did not increase linearly with the frequency of SCS, and at SCS frequencies above 100 Hz, the interneuron-mediated inhibition of the WDR neuron saturated. As a result, Aβ-fiber excitation of the WDR neuron due to SCS overcame maximal tonic inhibition from the IN interneurons, resulting in an increase in WDR firing rate at SCS frequencies greater than 100 Hz (Figure 2.9d). Replacing the tonic EX neuron with a transient or delayed firing EX neuron reduced baseline activity in the WDR neuron from 68 spikes/s to 55 spikes/s and 46 spikes/s, respectively, but neither change affected the relationship between WDR firing rate and SCS frequency, as the reduced excitability of the EX interneuron lessened its influence on the WDR neuron.

2.3.5. Spinal cord stimulation: loss of GABAergic inhibition

The efficacy of SCS decreases over time with the progression of neuropathic pain (Kumar et al., 2007, Taylor et al., 2013). We reduced the strength of the local and surround GABAergic inhibition in the model to simulate the loss of inhibition that occurs following an initial injury and during the progression of neuropathic pain (Figure 2.10) (Campbell and Meyer, 2006, Costigan et al., 2009, Woolf and Wall, 1982b). Altering
the strengths of the connections within the network affected the spontaneous activity of
the WDR neuron, so we show both the raw firing rate of the WDR neuron and the
activity of the WDR neuron normalized to activity when no SCS was applied. The
activity of the WDR neuron during SCS was either maintained or increased by all
reductions in GABAergic inhibition (Figure 2.10). The greatest changes in the
relationship between WDR neuron activity and SCS frequency occurred when the
strength of the GABAergic synapse between the IN and the WDR neurons was
attenuated and when both the local and surround inhibitory inputs were weakened.
When only the synapse from the local IN neuron was weakened, SCS failed to inhibit the
WDR neuron at frequencies greater than 80Hz (Figure 2.10b, 2.10c). However, when the
IN to WDR synapses from both the local and surround IN neurons were weakened by
50%, SCS no longer fully inhibited the WDR neuron, and the maximum frequency at
which SCS had a net inhibitory effect on WDR activity was reduced from 140 Hz to 75
Hz. In addition, the frequencies of SCS that produced the greatest inhibition of the
WDR neuron decreased from 40-80 Hz to 20 Hz (Figure 2.10c). Reducing the strength of
the Aβ inputs to the local and surround IN neurons prevented SCS from fully inhibiting
the WDR neuron, although SCS inhibited WDR neuron activity with SCS frequencies of
40-85 Hz under this condition (Figure 2.10c). Reducing GABAergic inhibition of the EX
interneuron had a negligible effect on SCS frequency dependence, as C-fiber afferent inputs could fire maximally at 2 Hz and were thus overridden by A-fiber activity.

The loss of function of the KCC2 Cl⁻ transporter and the resulting depolarizing shift in anionic reversal potentials contributes to the loss of GABAergic inhibition in dorsal horn neurons and has been implicated as an important mechanism in the generation and maintenance of neuropathic pain (2003, Coull et al., 2005). To simulate this process, we shifted the reversal potential of the GABAergic and glycinergic synapses on the EX and WDR neurons in the model from -70 mV to -54 mV (Coull et al., 2003, Boulenguez et al., 2010) in 4 mV increments, while maintaining the original synaptic conductances, and we quantified the effect on the relationship between SCS frequency and WDR activity (Figure 2.11a). Shifting the GABAergic and glycinergic receptor reversal potentials had a substantial effect on both the baseline activity of the WDR neuron prior to SCS (Figure 2.11b) and on the inhibition of the WDR neuron by SCS (Figure 2.11c). An initial depolarizing shift of only 4 mV increased spontaneous activity by 11 %, prevented full inhibition of the WDR by SCS, and reduced the maximum frequency at which SCS had an inhibitory effect from 140 Hz to 85 Hz. Further shifts of 4 mV and 8 mV in the anionic reversal potential increased baseline activity by 26 % and 45 %, reduced SCS-generated inhibition of the WDR neuron between 40 Hz and 85 Hz, and shifted the SCS frequency at which maximum inhibition
occurred from 60 Hz to 20 Hz. The final depolarizing shift to -54 mV prevented SCS from inhibiting the WDR neuron by more than 22% at any frequency tested (Figure 2.11c). No appreciable differences in WDR baseline activity or the effects of SCS were observed between shifting the anionic reversal potential in both the WDR and EX neuron and shifting the anionic reversal potential only in the WDR neuron (Figure 2.11c); shifting the anionic reversal potential in only the EX neuron produced negligible changes in the behavior of the WDR neuron.

2.4. Discussion

The objectives of this study were to quantify the response of dorsal horn WDR neurons to peripheral afferent activity and SCS and to characterize how these relationships changed with varying levels of synaptic inhibition. We developed a biophysically based network model of the dorsal horn that reproduced key non-linear behaviors associated with pain, including wind-up and A-fiber mediated inhibition (Mendell and Wall, 1965, Woolf and Wall, 1982b, Herrero et al., 2000). Using the validated model, we found that suppression of WDR projection neuron activity was strongly dependent on the frequency of SCS as well as the properties of local and surround dorsal horn inhibition, which change during the progression of neuropathic pain.
2.4.1. **Network model: excitatory-inhibitory balance**

The ability of the model to reproduce both excitatory and inhibitory phenomena using a single parameterization supports the network described by the Gate Control Theory. The responses of deep dorsal horn projection neurons to several types of electrical and natural stimuli showed that wind-up and A-fiber mediated inhibition may exist in tandem, as depicted in the model (Hillman and Wall, 1969, Mendell, 1966, Price et al., 1971). In addition, intracellular recordings from dorsal horn neurons during sural nerve stimulation indicated that some neurons that wind-up also exhibit IPSPs immediately following an excitatory A-fiber response (Price et al., 1971), and this finding is consistent with an EPSP-IPSP sequence following dorsal column stimulation (Baba et al., 1994, Foreman et al., 1976a). This pattern of excitation followed by inhibition in dorsal horn neurons was replicated in experiments that measured the responses in unidentified dorsal horn neurons evoked by A- and C-fiber threshold stimulation (Woolf and Wall, 1982b, Woolf and King, 1987). Finally, the dendrites of dorsal horn neurons receive both glutamatergic and GABAergic boutons (Alvarez et al., 1993, Carlton et al., 1992, Todd, 2010), and these synapses appear to be distributed uniformly across the dendrites (Lekan and Carlton, 1995). Taken together, these studies support the convergence of excitation and inhibition onto the model WDR neuron, and no prior
computational model has reproduced this wide range of both excitatory and inhibitory phenomena.

Differences in firing behavior between interneurons may contribute to the balance between the inhibitory influence of A-fiber afferent inputs and the excitatory influence of C-fiber afferent inputs (Ratté et al., 2013), and a diversity of firing behaviors including phasic, delayed firing, and transient firing patterns have been observed in dorsal horn neurons (Ruscheweyh and Sandkühler, 2002, Yasaka et al., 2010). Of particular relevance to our model, inhibitory interneurons appear to exhibit predominately tonic firing while excitatory interneurons appear to exhibit predominately phasic or delayed firing behavior (Yasaka et al., 2010), although tonically firing excitatory interneurons have also been reported (Santos et al., 2007). In contrast to the model incorporating the tonic EX interneuron, models including the transient or delayed firing EX interneuron showed no C-fiber evoked response in the WDR neuron during 1 Hz peripheral afferent stimulation, as decreased Na+ conductances in both neurons and the IKA conductance needed to generate delayed firing reduced the overall excitability of the EX interneuron (Todd, 2010, Yasaka et al., 2010); however, altering the properties of the EX interneuron did not affect the response of the WDR neuron to SCS. These observations indicate that while the firing properties of excitatory interneurons and inhibitory interneurons are critical to processing of primary afferent inputs during
natural stimuli, the spinal segmental effects of SCS on WDR neurons are primarily due to direct A-fiber mediated mechanisms. However, phasic or delayed firing interneurons may connect to other interneurons or lamina I projection neurons not depicted in our model or to circuits for receptive fields beyond those affected directly by pain, and they may serve additional roles through these connections (Lu and Perl, 2005, Zheng et al., 2010).

The interaction between excitatory primary afferent inputs and GABAergic inputs also explains neuronal responses to SCS. SCS generates both excitation and inhibition within a few milliseconds of the stimulus (Foreman et al., 1976a, Dubuisson, 1989), and the administration of bicuculline, a GABA$_{	ext{A}}$ receptor antagonist, eliminated the inhibitory effects of SCS and enhanced SCS-mediated excitation (Duggan and Foong, 1985). The reduction in the extent and duration of inhibition evoked in the model WDR neuron by single pulses of SCS after weakening of the GABAergic synapses onto the WDR neuron mirrored these findings. Furthermore, changing the weights of the GABAergic synapses onto the WDR neuron produced a range of responses to single pulses of SCS that paralleled those seen in experiments (Foreman et al., 1976a). This finding suggests that the ratio of excitatory inputs to inhibitory inputs varies across different dorsal horn neurons, and the aggregate output from different dorsal horn circuits governs the pain response (Prescott and Ratté, 2012). In contrast, changing the
strength of the GABAergic connection between the IN and EX interneuron did not affect the activity of the WDR neuron during SCS, suggesting that direct monosynaptic inhibition rather than indirect inhibition drives SCS mediated suppression of WDR neuron activity.

The model further predicted that GABAergic inhibition underlies the relationship between SCS-mediated inhibition of the WDR neuron and SCS frequency. Decreasing the weight of the synapses on the IN neuron or from the IN to the WDR neuron, intended to represent reductions in the level of dorsal horn GABA and a shift from GABAergic to glycinergic inhibition following peripheral nerve injury (Moore et al., 2002a), underscores the importance of direct GABAergic inhibition to the efficacy of SCS (Cui et al., 1996). The additional decline in SCS-mediated inhibition of WDR neuron activity when both local and surround inhibition were weakened suggests that more widespread loss of GABAergic inhibition beyond the point of injury contributes to the development and maintenance of chronic pain (Campbell and Meyer, 2006, Costigan et al., 2009, Moore et al., 2002a) and may contribute to the diminished efficacy of SCS over time (Kumar et al., 2007). The altered dependence of WDR neuron inhibition on SCS frequency in the modeled disease state also suggests that stimulation parameters that produce optimal pain relief may be different at different stages of neuropathic pain progression. In contrast, altering the properties of the inhibition between the IN and EX
neurons did not affect the relationship between the frequency of SCS and WDR firing rate, because the frequency of C-fiber primary afferent inputs onto the interneurons (mean = 2.3 spikes/s) is substantially lower than clinical frequencies of SCS (30-80 Hz). As a result, A-fiber mediated inhibition at lower frequencies of SCS and A-fiber mediated excitation of the WDR neuron overrides C-fiber mediated effects on the EX interneuron and the WDR neuron at higher frequencies of SCS.

Finally, the model predicted that the loss of GABAergic and glycinergic inhibition due to disruption of KCC2 transporter activity reduces SCS efficacy in a manner dependent on the extent of the resulting shift in the anionic reversal potential. The shifts in the anion reversal potential increased WDR neuron responses to peripheral activity and SCS similarly to those following loss of GABA in the dorsal horn, suggesting that these changes may occur concurrently during the progression of neuropathic pain. Supporting this prediction are the observations that blockade of GABAa receptors using bicuculline and antagonist block of KCC2 similarly and independently reduced response thresholds in rat dorsal horn projection neurons (Keller et al., 2007, Lavertu et al., 2013). Further, the model predicts that the loss of KCC2 must occur on projection neurons to have an influence on SCS efficacy, as depolarizing the anion reversal potential on the EX interneuron produced negligible changes in the relationship between WDR activity and SCS frequency. These findings may explain
why levels of KCC2 expression do not necessarily correlate with SCS-mediated
restoration of paw withdrawal thresholds in neuropathic rats, particularly if reductions
in KCC2 expression following injury occur primarily on interneurons (Janssen et al.,
2012).

2.4.2. SCS: surrounding receptive fields

Although tactile or electrical stimulation of surround receptive fields inhibits
dorsal horn neurons (Menetrey et al., 1977, Hillman and Wall, 1969), few studies have
considered the contributions of surrounding receptive field activation to the efficacy of
SCS. Early studies demonstrated that SCS produced inhibition in dorsal horn neurons
similar to that produced by stimulation of a peripheral nerve from receptive fields that
did not make excitatory connections with that same neuron (Hillman and Wall, 1969,
Foreman et al., 1976a). Furthermore, receptive fields of dorsal horn neurons expand
following some types of neurological injuries, and the administration of intrathecal
bicuculline reproduces pathological receptive field expansion, strongly suggesting that
the loss of surround inhibition contributes to the progression of neuropathic pain (Drew
et al., 2004). Finally, recent anatomical studies revealed GABAergic connections
between dorsal horn neurons that may extend across several spinal segments (Todd,
2010, Szucs et al., 2013), suggesting that inhibition from surround receptive fields could
indeed contribute to inhibition of WDR projection neurons by SCS. These data coupled
with our finding that inhibition due to SCS could be reproduced without affecting the spontaneous activity of the neuron only by including inhibition arising from surrounding receptive fields support the hypothesis that activation of dorsal column fibers from receptive fields surrounding the source of pain is necessary for SCS-mediated pain relief.

2.4.3. Model limitations

Although the model reproduced cellular and network properties and key behaviors related to pain, several limitations must be considered. Our model does not account for the full range of projection neuron responses to peripheral stimulation or to all modes of SCS. For example, our model WDR neuron exhibited slight deviations in response to peripheral stimulation, such as a stronger initial C-volley at the onset of 1 Hz stimulation than experimentally observed, and the lower experimental bound of the C-fiber response could not be attained even when the strength of the IN-WDR GABA synapse was doubled. Furthermore, the characteristics of A- and C-fiber evoked responses vary across neurons; both the number of spikes in the C-volley and the percentage increase in the C-fiber responses over the course of 1 Hz stimulation vary across studies (Schouenborg and Sjolund, 1983, Woolf and King, 1987, Mendell, 1966, Herrero et al., 2000). In addition, neurons exhibit varying degrees of inhibition following A-fiber stimulation, and the properties of A-fiber inhibition change during the
progression of neuropathic pain state (Woolf and Wall, 1982b). We did not consider the effects of kilohertz-frequency SCS, which may provide pain relief by blocking the conduction of action potentials along primary afferents or ascending projections rather than through modulation of projection neuron activity (Shechter et al., 2013), but the mechanisms underlying kilohertz-frequency SCS have not been studied in sufficient detail to include in our model. Finally, some dorsal horn neurons exhibit wind-down—successively decreasing activity during repetitive peripheral stimulation—a phenomenon that is not accounted for by our model and whose specific mechanisms remain unclear.(Mendell, 1966, Woolf and King, 1987) Whether these discrepancies are due to neuron biophysics, network connectivity, or a combination of these two factors remains to be clarified.

As well, our implementation of the inputs to and outputs from our network were simplified. Although representative of the appropriate spectrum of conduction velocities across a population of Aβ-, Aδ-, and C-fibers, all primary afferent inputs to the IN and WDR neurons were located at the same dendritic segment. In reality, excitatory and inhibitory synapses are distributed throughout the dendritic arbors of these neurons (Lekan and Carlton, 1995, Polgar et al., 2010). However, randomizing the location of synaptic inputs from either the afferent inputs or the interneurons onto the WDR neurons did not appreciably affect the input-output characteristics of the network.
Therefore, we placed all synapses onto the WDR neuron at the same location. Furthermore, the patterns of afferent inputs included constant low-frequency (0.5-1 Hz) peripheral stimulation and estimates of afferent activity during pinch (Slugg et al., 2000) and neuropathic pain (Wall and Gutnick, 1974), but our inputs were not representative of other natural noxious mechanical stimuli or thermal stimuli. However, few studies have quantified the inputs to the dorsal horn following natural peripheral stimulation (e.g. brush, press, pinch) or during neuropathic pain, and our inputs were sufficient for the generation of realistic activity in the model WDR projection neuron. Finally, the firing rate of the WDR neuron was the primary output of the network because activity of the WDR neuron correlates with the magnitude of pain following capsaicin injection and because prior studies used WDR activity as a proxy for SCS efficacy (Foreman et al., 1976a, Linderoth et al., 2009, Guan, 2012). However, our model does not take into account the activity of all types of projection neurons, such as low-threshold or nociceptive-specific neurons that receive sensory input and contribute to sensory coding (Blomqvist and Craig, 2000).

In particular, our model does not account for the role of superficial nociceptive specific projection neurons that play an important role in the sensory encoding of mechanical pain (Blomqvist and Craig, 2000). These neurons respond specifically to Aδ- and C-fiber threshold stimuli, are involved in the development of neuropathic pain, and
serve as the output node for complex superficial dorsal horn sensory processing networks (Todd, 2010). Recent work has shown that polysynaptic excitatory pathways in lamina I terminate onto NK1 expressing lamina I projection neurons (Lu and Perl, 2005, Zheng et al., 2010), and these excitatory pathways affect the activity of NK1 expressing lamina I NS neurons during A-fiber stimulation in rat models of neuropathic pain (Baba et al., 2003). In addition, administration of bicuculline and strychnine (Torsney and Macdermott, 2006) and the induction of inflammatory pain (Torsney, 2011) both unmask Aβ-fiber evoked responses in NK1+ lamina I NS neurons in rodent models. Although our model predicted that EX interneurons do not play a significant role in modulating the effects of SCS on WDR neurons, disinhibition of superficial excitatory interneurons whose axons terminate onto superficial nociceptive neurons may contribute to the development of allodynia and other pathological pain behaviors. Furthermore, SCS is effective in treating alldynia in rat models of neuropathic pain, and relief of alldynia by SCS involves GABAergic mechanisms (Cui et al., 1996, Stiller et al., 1996), suggesting that the restoration of inhibition of these superficial excitatory pathways may be an additional mechanism by which SCS relieves pain. In addition, the paucity of monosynaptic inhibitory connections from Aβ-fibers onto superficial neurons (Torsney, 2011) combined with pathological disinhibition may help to explain why clinical SCS provides only 50-70 % pain relief (Taylor et al., 2013) despite experimental
reports of total suppression of WDR neurons. However, modeling the superficial
pathway is currently not feasible, as depictions of this pathway typically involve at least
three relays between Aβ afferents and Lamina I neurons (Torsney and Macdermott,
2006, Torsney, 2011) that may all in turn receive inhibitory inputs. Neither the specific
properties of these neurons nor the properties of the relevant synaptic connections
required to build such a model are well known. When data are available, the
development and validation of a model of the superficial dorsal horn pathway may
provide significant insight into pain processing as well as into the mechanisms
underlying SCS.

Finally, we based our model connectivity on the Gate Control Theory and similar
simplified architectures of dorsal horn networks that account primarily for spinal
segmental inhibitory mechanisms (Melzack and Wall, 1965, Britton and Skevington,
1996, Aguiar et al., 2010) and short-term monosynaptic responses to SCS. Segmental
mechanisms are sufficient to explain suppression of WDR neuron activity during SCS
(Hillman and Wall, 1969, Foreman et al., 1976a, Smits et al., 2012), but modulation of
neuronal responses from supraspinal centers also contributes to the response to SCS.
For example, the rostroventromedial medulla receives ascending inputs (Lenz et al.,
2010), projects monosynaptically to the dorsal horn (Fields et al., 1995), and directly
affects the activity of dorsal horn projection neurons (Giesler Jr et al., 1981). In addition,
behavioral studies have implicated descending serotoninergic projections as contributors to pain relief during SCS, as intrathecal application of 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ agonists in neuropathic rats can enhance SCS-mediated increases in paw withdrawal thresholds (Song et al., 2011) even when the dorsal columns are lesioned (Barchini et al., 2012). As well, neurons in the locus coeruleus exhibit more activity during SCS in neuropathic rats that show increased paw withdrawal thresholds than in non-responding rats, suggesting that effective SCS requires supraspinal modulation (Song et al., 2013a). However, the extent of supraspinal contributions to SCS is unknown, as modulation of some supraspinal circuits, such as the endogenous μ-opioid pathway, does not affect SCS efficacy (Freeman et al., 1983) and knowledge of the roles of other supraspinal circuits in chronic pain and SCS, such as the serotoninergic pathways, is sparse (Heinricher et al., 2009).
Table 2.1: Synaptic conductances, time constants, reversal potentials of inputs to EX, IN, and WDR neurons.

<table>
<thead>
<tr>
<th>Synapse (Source)</th>
<th>IN Neurons</th>
<th>EX Neuron</th>
<th>WDR Neuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synapse (Source)</td>
<td>Total Conductance $g_{syn}$ (nS)</td>
<td>Rise Time ((\tau_r)) constant (ms)</td>
<td>Decay Time ((\tau_d)) constant (ms)</td>
</tr>
<tr>
<td>AMPA (A5 Local)</td>
<td>14.6</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>AMPA (A5 Surround)</td>
<td>20</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>Synapse (Source)</td>
<td>AMPA (C)</td>
<td>8</td>
<td>0.1</td>
</tr>
<tr>
<td>Synapse (Source)</td>
<td>NMDA (C)</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Synapse (Source)</td>
<td>NKI (C)</td>
<td>0.02</td>
<td>20</td>
</tr>
<tr>
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<td>GABAa (IN, Local)</td>
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</tr>
<tr>
<td>Synapse (Source)</td>
<td>GABAa (IN, Surround)</td>
<td>7.3</td>
<td>0.1</td>
</tr>
<tr>
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<td>AMPA (A6)</td>
<td>24</td>
<td>0.1</td>
</tr>
<tr>
<td>Synapse (Source)</td>
<td>AMPA (A6)</td>
<td>24</td>
<td>0.1</td>
</tr>
<tr>
<td>Synapse (Source)</td>
<td>NMDA (A6)</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>Synapse (Source)</td>
<td>NMDA (A6)</td>
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<td>2</td>
</tr>
<tr>
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<td>200</td>
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</tr>
<tr>
<td>Synapse (Source)</td>
<td>Glycine (IN, Local)</td>
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<td>0.1</td>
</tr>
<tr>
<td>Synapse (Source)</td>
<td>GABAa (IN, Surround)</td>
<td>14.6</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Figure 2.1: Computational network model of the spinal cord pain processing circuit.

Biophysical network model of nociceptive processing in the dorsal horn. (a) Network architecture and depictions of the connections between primary afferents (Aβ, Aδ, C) and inhibitory interneurons (IN), an excitatory interneuron (EX) and a wide dynamic range (WDR) projection neuron in the dorsal horn. Aβ-fibers originating from the surrounding receptive field were distinct from those originating from the local receptive field, and the surrounding receptive field (thin lines/circles) projected to a distinct IN neuron. SCS was represented as a constant frequency spike train delivered through the "Dorsal Columns/SCS" input, and the average firing rate of the WDR neuron was taken as the model output. (b) Synaptic connections onto each type of neuron in the model. Both the "Local" and "Surround" IN neurons receive AMPA synapse inputs from their respective Aβ-fiber inputs and send inhibitory connections to the EX and WDR neurons.
Figure 2.2: Firing characteristics of isolated model neurons compared to experimental data.

(a) Frequency-current (f-I) relationships between the amplitude of a 1s duration current pulse and the number of action potentials fired by the corresponding neuron. The gray shaded area denotes the range of firing rates in dorsal horn neurons as reported in (Melnick et al., 2004b) and (Ruscheweyh and Sandkühler, 2002). (b) IN and (c) EX neurons exhibit tonic firing that increases in rate with increasing amplitudes of applied current. The IN neuron also exhibits amplitude adaptation at stimulation intensities ≥130 pA. (d) Gating variable and firing behavior of delayed firing neuron compared to data from delayed firing neurons in (Ruscheweyh and Sandkühler, 2002). (e) Transient
firing excitatory interneuron behavior compared to representative neuron and experimental range from (Melnick et al., 2004a) (f) Tonic firing in the WDR neuron when held at a -65 mV resting potential and subjected to constant current injection. Consistent with Morisset and Nagy (1999), the model neuron exhibits prolonged after-discharge following the cessation of the current injection when held at a more depolarized resting potential (-62 mV).
Figure 2.3: Wind-up in model WDR neuron in response to 1 Hz C-fiber threshold stimulation.

(a) Raster plots of the output of the model neuron and a raster plot from a dorsal horn neuron (Herrero et al., 2000) during continuous 1 Hz stimulation. Each dot represents a single action potential and each row represents the post-stimulus time interval following an individual stimulation pulse. (b) Cumulative spike count from the model WDR neuron plotted as a function of the stimulus number. The gray region denotes the experimental range from (Schouenborg and Sjolund, 1983) and (Woolf and King, 1987). The dotted line indicates what the spike count would be if wind-up did not occur. (c) Spike counts from A-fiber and C-fiber evoked model responses from each individual stimulus over time and experimental ranges from (Schouenborg and Sjolund, 1983). In (c), both Aβ- and Aδ-fiber evoked spikes were counted as part of the A-fiber evoked response.
Figure 2.4: Influence of the strengths of model synaptic connections of A-fiber and C-fiber evoked responses.

In (a), the strength of the Aβ-fiber inputs onto the local IN neuron was doubled and halved. In (b), the strength of the synapse between the local IN and WDR neurons was doubled and halved. Experimental ranges are the same as those shown in Figure 2.3C.
Figure 2.5: A-fiber mediated inhibition in the model compared with experimental data (Woolf and Wall, 1982a).

(a) A 0.5 Hz stimulus at C-fiber threshold followed at 20-150 ms by Aδ-fiber threshold pulses was applied to the model. (b) and (c) are raster plots of the output of the model during the simulation shown in (a) in the default model state (b: "strong" inhibition) and when the local IN-WDR neuron synapse strength is reduced by 50% (c: "weak" inhibition).
Figure 2.6: Model neuron responses to 1 Hz SCS when only local inhibition was included.

Schematic (a) and network representation (b) of the simulation. Local primary afferent inputs receive spike trains with statistical properties reported from spike trains recorded from primary afferents during "pinch" stimulation (Campbell and Slugg 2000). Dorsal column stimulation is applied to collaterals from "local" Aβ-fiber inputs. (c) Time course of SCS-induced inhibition in model (black) and experimental (gray) neurons from Foreman et. al. (1976) normalized to the baseline activity of the neuron prior to the SCS pulse, and peri-stimulus time histogram (PSTH) generated from 25 trials of simulated single pulse dorsal column stimulation during an on-going peripheral input depict inhibition following SCS. To be consistent with the experiment, the histogram block at 100 ms includes 25 counts representing detection of the stimulus artifact as a spike. (e) and (f) Effects of altering the strength of the A-fiber inputs to the IN neuron (e) and the strength of the synapse between the IN and WDR neurons (f) juxtaposed with 4 representative responses from Foreman et. al. (1976) as well as PSTHs from halving and doubling each connection.
Figure 2.7: Model neuron responses to 1 Hz SCS including both local and surround inhibition.

Schematic (a) and network representation (b) of the simulation. Local primary afferent inputs receive spike trains with statistical properties reported from spike trains recorded from primary afferents during "pinch" stimulation (Slugg et al., 2000). Dorsal column stimulation is applied to collaterals from both "local" and "surround" Aβ-fiber inputs. (c) Time course of SCS-induced depression in model (black) and experimental (gray) neurons from Foreman et al. (1976) normalized to the baseline activity of the neuron prior to the SCS pulse, and PSTH generated from 25 trials of simulated single pulse dorsal column stimulation during an on-going peripheral input depict inhibition following SCS. To be consistent with the experiment, the histogram block at 100 ms includes 25 counts representing detection of the stimulus artifact as a spike.
Figure 2.8: Effects of modifying model network synaptic connection strengths on the time course of inhibition following single pulses of SCS juxtaposed with 4 representative experimental responses from Foreman et al. (1976).

In (a) and (d), the model range shows the effect of halving and doubling the strength of the Aβ to IN connection. In (b) and (e) the model range shows the effect of halving and doubling the strength of the IN to WDR connection. In (c) and (f), the model range shows the effect of halving and doubling the strength of the IN to EX connection. (a-c) depict the effects of only changing the "local" connections, while (d-f) depict the effects of changing both the "local" and "surround" connections.
Figure 2.9: Modeling the effects of SCS on WDR activity during model primary afferent input mimicking neuropathic pain

(a) Relationship between model WDR neuron firing rate and the frequency of SCS. The dotted line denotes the average firing rate of the WDR neuron during the on-going peripheral input during the five seconds before the onset of SCS. (b-d) Transmembrane potential of the model WDR neuron and the local (Loc. IN) and surrounding (Sur. IN) receptive field interneurons during a neuropathic input when low frequency SCS (10 Hz, b), moderate-frequency SCS (70 Hz, c), and high-frequency SCS (150 Hz, d) are applied. Triangles in (b-d) denote when SCS was delivered.
Figure 2.10: Effects of reducing the influence of both the local and surround GABAergic interneurons on the response of the model WDR neuron to different frequencies of SCS.

(a) Network representation of the changes to the local (magenta) and surround (red) inhibitory receptive field representations. Raw (b) and normalized to baseline (c) response of the WDR neuron to SCS following a 50% reduction of the input to the IN neurons, a 50% reduction in the inhibition from the IN neurons to the EX neuron or a 50% reduction of inhibition from the IN neurons to the WDR neuron. The gray region encompasses the range of responses when all 3 changes were implemented simultaneously. Increases in the intrinsic spontaneous activity of the WDR neuron when SCS was off resulted in the apparent reduction of normalized responses in some cases, as in (c).
Figure 2.11: Effects of a depolarizing shift of anionic reversal potentials as would occur during the loss of KCC2 function.

(a) Anionic reversal potential was shifted for all inhibitory synapses onto the EX and WDR neurons. (b) Average firing rate of the WDR neuron as a function of the anionic reversal potential when only the WDR neuron is affected and when both the EX and WDR neurons are affected. Changing the reversal potential of only the EX interneuron produced negligible changes in the activity of the WDR neuron. (c) Normalized to baseline response of the WDR neuron at different anionic reversal potentials when only the WDR neuron is affected (left) and when both the WDR and EX neurons are affected (right). Increases in the intrinsic spontaneous activity of the neuron when SCS was off resulted in the apparent reduction of the normalized responses at some frequencies of SCS.
3. Spinal sensory projection neuron responses to spinal cord stimulation are mediated by circuits beyond Gate Control

3.1. Introduction

Spinal cord stimulation (SCS) is an effective clinical therapy for neuropathic pain. The putative mechanism underlying neuronal responses to SCS, based on the Gate Control Theory (Melzack and Wall, 1965, Shealy et al., 1972), predicts that SCS-mediated inhibition of spinal sensory projection neurons, and therefore the analgesic response to SCS, depends on stimulation frequency (Zhang et al., 2014b). Supporting this prediction are clinical reports that 50-80 Hz SCS is the "optimal" range for SCS (Oakley and Prager, 2002) and that 30-80 Hz SCS maximally suppresses wide dynamic range (WDR) dorsal horn neuron activity in a computational model of SCS (Zhang et al., 2014b). However, prior studies of neuronal responses to SCS have neglected stimulation frequency dependence, and although the analgesic outcomes of SCS may be correlated with the activity of spinal projection neurons representing the output of spinal nociceptive circuits (Hillman and Wall, 1969, Todd, 2010, Simone et al., 1991, Foreman et al., 1976a), recent studies did not confirm that recorded neurons were projection neurons (Yakhnitsa et al., 1999, Guan et al., 2010). An incomplete understanding of the frequency dependent effects of SCS on spinal projection neurons may contribute to the limited improvement in SCS efficacy since its inception (Zhang et al., 2014a, Taylor et al., 2013).
Moreover, the contribution of SCS-mediated inhibition to the frequency-dependent effects of SCS is poorly understood. GABAergic mechanisms may modulate the analgesic effects of SCS, as intrathecal application of bicuculline, a GABA<sub>A</sub> receptor antagonist, resulted in SCS-mediated excitation (Duggan and Foong, 1985), and intrathecal application of CGP 35348, a GABA<sub>B</sub> receptor antagonist, reversed SCS-mediated pain relief in an animal model of neuropathic pain (Stiller et al., 1996). As well, neuropathic pain involves the specific degeneration of GABAergic mechanisms (Braz et al., 2014, Moore et al., 2002a, Zeilhofer et al., 2012), which may contribute to the diminishing efficacy of SCS over time (Kumar et al., 2007). However, antagonism of GABAergic inhibition from both segmental and supraspinal sources may additionally modulate the activity of interneurons (Takazawa and Macdermott, 2010) and increase spontaneous and evoked activity in spinal projection neurons (Lin et al., 1996), behaviors that are beyond what is predicted by the Gate Control circuit. As a result, the relationship between GABAergic inhibition and neuronal responses to SCS remains unclear.

We hypothesized that projection neuron responses to SCS would be dependent on stimulation frequency in a manner consistent with the Gate Control theory and that blockade of GABAergic receptors would reduce inhibitory responses to SCS. We found that spinal sensory projection neuron responses to SCS did depend on stimulation
frequency in both healthy animals and neuropathic animals following chronic constriction injury (CCI) and were often disinhibited by antagonism of GABA receptors. However, responses were significantly more heterogeneous than anticipated, and the Gate Control circuit was insufficient to explain all observed responses. We propose that spinal microcircuits (Prescott et al., 2014) may underlie the observed heterogeneity, and computational models of the spinal microcircuits were able to reproduce the spectrum of experimental responses to SCS. Our results suggest that a population response underlies analgesic effects of SCS and that GABAergic mechanisms play a key role in modulating responses to SCS.

3.2. Methods

3.2.1. Animal preparation

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of Duke University and were in accordance with The Guide for the Care and Use of Laboratory Animals (8th edition). Male Sprague-Dawley rats (300-500g) were initially anesthetized with 3.0% Isoflurane (inhaled) and urethane (1.2 g/kg subcutaneous). Following the subcutaneous injection of urethane, Isoflurane was stopped, and supplemental doses of urethane (0.4 g/kg/hr i.p.) were administered if a withdrawal reflex occurred in response to pinching of the hindpaw. A tracheotomy was performed, and rats were connected to a pressure-controlled ventilator (Kent Scientific
MouseVent G500) via the tracheal tube for artificial respiration. Oxygen saturation and heart rate were monitored using a pulse oximeter (Kent Scientific MouseVent G500 module) on the hindpaw and maintained within physiological levels (SpO₂ > 90%, HR < 450). Body temperature was monitored using a rectal thermometer and maintained at 35-37°C using a heating blanket (Gaymar T/Pump). The head was mounted in a stereotaxic frame (Kopf Instruments), and the vertebral column was suspended from vertebral clamps (Kopf Instruments) attached to T9 and L2 for mechanical stability. The sciatic nerve was exposed, and a 1.5 mm diameter bipolar cuff electrode with an electrode spacing of 1mm was wrapped around the nerve just proximal to the popliteal fossa. Laminectomies were performed to expose the cervical (C1) and thoracic-lumbar (T10-L2) spinal cord. The dura over these regions was resected, and warm (37°C) saline or mineral oil was applied to the exposed cord. At the completion of the surgery, but prior to neuron search and recording, rats were paralyzed using gallamine triethiodide (Sigma-Aldrich, 20 mg/h) delivered through an intraperitoneal catheter, and a 1.5 mm wide bipolar paddle electrode with 1.0 mm long platinum electrodes spaced 2.0 mm apart was inserted into the epidural space at T10 to deliver SCS (Figure 1A).

3.2.2. Cord Dorsum Potentials

The amplitude of SCS in each experiment was determined by recording the cord dorsum potential (CDP) evoked by single cathodic-first biphasic SCS pulses (pulse...
width = 100 μs) approximately 1 cm caudal from the caudal SCS contact following paralysis but prior to neuron search. The CDP was validated by ensuring that the response did not switch polarities during polarity-inverted SCS (Figure 2A) and observing that the magnitude and timing of the peaks of the CDP varied with proximity to the distal contact of the SCS electrode, i.e., responses propagated (Figure 2B). The amplitude of SCS was set such that a prominent CDP (>50 μV<sub>pp</sub>) was recorded from the surface of the dorsal columns both before and after paralysis; CDPs greater than 50 μV<sub>pp</sub> could typically be evoked post-paralysis at stimulation amplitudes corresponding to 80% -90% of pre-paralysis ipsilateral motor threshold, consistent with amplitudes reported in prior studies of SCS (Guan et al., 2010). However, if the CDP amplitude was ≤ 50 μV<sub>pp</sub> at 90% motor threshold post-paralysis, the SCS amplitude was increased until the CDP threshold criterion was met. In some experiments SCS at amplitudes greater than that required to evoke a 50 μV<sub>pp</sub> CDP and/or up to and past visible ipsilateral motor threshold were verified not to alter substantially neuronal responses to SCS (Figure 2C).

3.2.3. Neuron recording and identification

A stimulation microelectrode (platinum-iridium, 3-4 μm tip diameter, 0.1 MΩ impedance) was lowered into the cervical spinal cord contralateral to the sciatic incision (Figure 3.1a). A recording microelectrode (stainless steel, 1-2 μm tip diameter, 1.5 MΩ
impedance, Microprobes Inc.) was lowered into lumbar spinal cord ipsilateral to the sciatic incision (Figure 1A). Following electrode placement and paralysis, a search stimulus (maximum 500μA, 0.2 ms) was applied to the C1 stimulation electrode at no greater than 5 Hz while the recording electrode was advanced and retracted through the dorsal horn until a neuron was observed to follow the search stimulus, i.e., a candidate projection neuron was found. Neuronal signals were amplified and filtered using an X-Cell amplifier (FHC, Inc.) (gain = 10,000, passband = 500 Hz-2000 Hz), recorded using a Powerlab data acquisition system (AdInstruments, Inc.), and sorted post-hoc using manual principal component-based clustering in Offline Sorter (Plexon Inc.).

Once a neuron was isolated, three criteria were used to verify that the neuron was indeed a projection neuron activated by the C1 stimulus: the neuron followed single pulse C1 stimulation with a constant latency that varied less than the pulse width of the search stimulus (< 200 μs, Figure 3A), the neuron followed triplets of C1 stimulation delivered at ≥200 Hz (Figure 3B), and collisions between orthodromic and antidromic spikes occurred during spontaneous activity or activity evoked by mechanical stimulation of the neuron's receptive field (Figure 3C) (Lipski, 1981).
3.2.4. Characterization of peripheral response

Responses of each neuron to mechanical stimulation of the hindlimb were recorded during successive brushing of the receptive field using a camel hair paintbrush (brush), mild pressure using a loose arterial clip (press), moderate pressure using a tight arterial clamp (pinch), and heavy manual pressure using forceps (crush) with 10 s pauses between stimuli. Neurons were classified as "low threshold" (LT) if they responded to innocuous stimuli and/or were inhibited by more noxious stimuli, "wide dynamic range" (WDR) if they responded to all stimuli in a graded manner, and "nociceptive specific" (NS) if they only responded to "pinch" and/or "crush" stimulation (Figure 3D). At least 30s after the brush, press, pinch, crush (BPPC) test, sciatic stimulation at C-fiber threshold (50x-100x motor threshold with a limit of 8 mA) was applied at 1 Hz for 60s to verify that sciatic nerve stimulation could alter activity of the recorded neuron.

3.2.5. SCS protocol

After antidromic identification and sensory characterization of an isolated neuron, we applied SCS at 1 Hz, 10 Hz, 30 Hz, 50 Hz, 100 Hz, and 150 Hz, both with and without accompanying 1 Hz sciatic stimulation during randomized experimental blocks (Figure 1B). The order of SCS frequencies delivered was randomized between experiments as well, and, among responders to SCS, neither raw nor normalized
neuronal responses to SCS were significantly associated with the trial number, i.e., the time course of the experiment (Raw firing rate: \( p = 0.396 \), Repeated Measures ANOVA; Normalized response: \( p = 0.580 \), Repeated Measures ANOVA). To account for possible potentiation or adaption of a neuron during SCS and sciatic stimulation, each individual application of SCS was preceded and followed by 20 s periods with no stimulation or 1 Hz sciatic stimulation at C-fiber threshold (Figure 3.1b) (Herrero et al., 2000, You et al., 2003, Guan et al., 2010), and individual trials were separated by 60 s. The stability of the isolated neuron was verified through antidromic C1 stimulation at the beginning and end of the experimental block.

### 3.2.6. GABAergic modulation

In two subsets of rats, we administered intrathecally the GABA\(_A\) receptor antagonist bicuculline methiodide (BIC) (Tocris Biosciences) or the GABA\(_B\) receptor antagonist CGP 35348 (Tocris Biosciences) locally to the recording site immediately following control recordings. Either 10 μL of 0.3 μg/μL (0.6 mM) BIC in 0.9 % saline solution (\( n = 12 \)) or 10 μg/μL of CGP 35348 (44.3 mM) in 0.9 % saline solution (\( n = 8 \)) were applied using a 250 μL microsyringe (Hamilton, Model 1725) positioned within 1mm of the recording site. Dosages were subconvulsive but sufficient to produce behaviors associated with hyperalgesia and allodynia in awake rats (Sivilotti and Woolf, 1994, Hao et al., 1994). Ten minutes elapsed before BPPC characterization and sciatic
stimulation tests were repeated to assess changes in the response profile of the neuron due to GABAergic antagonism, and the SCS experiment was then repeated. In a few cases, both blockers were administered together, after recording the effects of one blocker in isolation (BIC + CGP, n = 1; CGP + BIC, n = 2).

3.2.7. Chronic constriction injury

Neuropathic pain was produced in 8 animals using the model of chronic constriction injury of the sciatic nerve (CCI) (Bennett and Xie, 1988), and the effects of CCI were validated with quantitative behavioral measurements. Briefly, adult SD rats (male, 250 ± 20 g) were anesthetized with isoflurane, and the site of the incision was disinfected with povidone-iodine and 70% ethanol. The left sciatic nerve was exposed, three ligatures (4-0 Prolene) were placed around the nerve proximal to the popliteal fossa with a distance of 1 mm between each ligature, and the ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed. For 7-10 days following the surgical procedure, rats were doubly housed under a 12-hour light/dark cycle and provided with food and water ad libitum; no supplemental antibiotics were necessary or provided. At the end of this period, animals were subjected to single unit recordings under urethane anesthesia during SCS in a terminal experiment as described above.
3.2.8. Behavioral analysis

Animals were habituated to the testing environment daily for at least 2 d before baseline testing. Prior to testing for mechanical sensitivity, animals were put in boxes on an elevated metal mesh floor and allowed to habituate for 30 min. A series of von Frey hairs with logarithmically increasing stiffness (0.4–26 g; Stoelting) was then presented to the plantar surface of the ipsilateral hindpaws of each animal several times for 3–5 s per hair. Once paw withdrawal behavior was observed, 6 von Frey hair tests centered around the minimum hair stiffness which resulted in 50% paw-withdrawal incidence were recorded per animal, and the final paw-withdrawal threshold was determined from the composite response using Dixon’s up-down method (Chaplan et al., 1994, Dixon, 1980).

3.2.9. Single unit activity analysis

To determine if neurons were responsive to SCS, we constructed post-stimulus time histograms (PSTHs) of each neuron’s activity and accounted for stimulus artifacts by applying a 0.5-2ms width blanking mask corresponding to the duration of the averaged stimulus artifact; PSTH bin widths were set according to the number of stimulation trials per SCS On period (i.e., by SCS frequency) (Shimazaki and Shinomoto, 2007). For comparison of activity between SCS On and SCS Off periods, stimulus times and artifact blanking periods from the On periods were superimposed onto the Off
periods, and PSTHs of activity in the Off periods were generated using this virtual stimulus train. We then normalized each neuron’s PSTH by expressing the number of spikes/bin in the SCS On condition as Z-scores based on the mean and standard deviation of the neuron’s virtual PSTH during a time-matched pre-SCS interval (Montgomery, 2006). A neuron’s response to SCS at a specific frequency was defined as significant if the Z-scores from at least 3 contiguous PSTH bins met or exceeded a magnitude of 1.96, with Z = 1.96 corresponding to an individual response magnitude whose likelihood of occurrence was <5% by random chance. Some neurons, in particular nociceptive-specific neurons, exhibited relatively low firing rates (Craig et al., 2001, Kawamata et al., 2005) and these neurons’ PSTHs during both the SCS Off and SCS On conditions were occasionally insufficient to calculate z-scores (<4 spikes/bin) (Dayan, 2001). In those cases, if the total number of spikes exceeded 30 in both the SCS On and SCS Off intervals, the Kolmogorov-Smirnoff (K-S) test (cutoff p<0.05) was used to determine if SCS altered the distribution of post-stimulus responses between the SCS Off and SCS On cases (Xu et al., 2008). Neurons were classified as being non-responsive if they did not meet both the Z-score and the K-S test criteria or if they exhibited insufficient activity (<30 spikes, <4 spikes/bin, <1.5 spikes/s (Dayan, 2001)) in pre-SCS and SCS On conditions to conduct statistical analyses. Unless otherwise noted, only responders were included in our data analysis.
Mean firing rates were calculated by dividing the total number of spikes by 20 s minus the total amount of time included in artifact blanking periods. Because baseline firing rates fluctuated between neurons, and to a lesser extent within neurons, we quantified the effects of SCS on neuron activity using the change in firing rates between 20 s on periods and the 20 s off period immediately prior to SCS. For clustering analysis, relationships between firing rate and SCS frequency were normalized such that the magnitude of the largest firing rate change between off and on conditions in a neuron corresponded to +1 (excitatory) or -1 (inhibitory); this scaling prevented large responses from dominating the field in clustering analysis (Lemay and Grill, 2004). A principal component analysis was performed on the entire field of normalized responses, and the resulting principal component loadings were used to classify neurons with k-means clustering and hierarchial clustering algorithms in MATLAB (MathWorks, Natick, MA).

3.2.10. **Histology**

At the end of each experiment, an electrolytic lesion (15 μA, 20 s) was made at the recording site in the lumbar spinal cord. Rats were euthanized with an intraperitoneal injection of 0.5 mL Euthasol® (Virbac Corp.) and perfused with 200 mL 0.9% NaCl followed by 100 mL of 10% formalin + 1% ferrocyanide solution to produce a Prussian Blue reaction at the site of the electrolytic lesion. The lumbar spinal cord was removed, fixed in 10% formalin + 1% ferrocyanide for several days, and then blocked
and sectioned into 50 μm-thick sections using a microtome. The tissue was counterstained with nuclear red dye (NovaUltra, IHCWorld, LLC) to facilitate visualization of the electrolytic lesion.

### 3.2.11. Computational modeling of spinal microcircuits

We implemented computational network models of dorsal horn circuits representing each of four observed SCS response classes by connecting biophysically-based compartmental models of dorsal horn neurons via representations of excitatory and inhibitory synapses. Membrane dynamics of the individual neurons, synaptic properties, and the strengths and types of synaptic connections were based largely on a prior model of the dorsal horn network (Zhang et al., 2014b) and were tuned to match experimental observations.

Networks architectures were based on spinal microcircuits hypothesized to underlie inhibitory ("opponency"), excitatory ("coloring"), and mixed ("mixing") responses (Prescott and Ratté, 2012) to increasing frequencies of SCS. The connections of 15 Aδ and C-fiber afferents onto the projection neuron and the C-fiber connections onto an intervening interneuron were unaltered from the original model (Zhang et al., 2014b). However, to account for the full range of interactions between SCS and peripherally evoked activity, a template motif was developed that included monosynaptic excitatory
connections between 15 Aβ afferents and the projection neuron as well as a disynaptic inhibitory connection between an inhibitory interneuron that received inputs from 15 Aβ afferents and the projection neuron. In addition, we included GABAergic and glycinergic inputs onto the inhibitory interneuron ($g_{\text{GABA}} = 19.9 \text{nS}$, $g_{\text{Glycine}} = 5.32 \text{nS}$) and projection neuron ($g_{\text{GABA}} = 15.0 \text{nS}$, $g_{\text{Glycine}} = 4.0 \text{nS}$) from spontaneously active tonic neurons whose activity was decoupled from SCS, as tonic inhibition from segmental or supraspinal sources has been hypothesized as a regulatory influence on excitatory or inhibitory inputs to projection neurons (Peng et al., 1996, Takazawa and Macdermott, 2010). We applied Ornstein-Uhlenbeck noisy currents (Lánský and Sacerdote, 2001) with a mean amplitude of 15 pA, a variance of 5 pA, and a relaxation time of 100 ms to these neurons, to induce firing rates in the inhibitory neurons (40-50 spikes/s) that represented the combined effect of inhibition from segmental (Takazawa and Macdermott, 2010) and supraspinal inhibitory inputs (Li et al., 1998, Carlson et al., 2007).

Conversion of the template circuit to the "mixing", "opponency", and "coloring" microcircuits was accomplished weakening the excitatory or inhibitory paths of the template circuit and/or modulation of the degree of tonic inhibition of the inhibitory interneuron and projection neuron. The "mixing" microcircuit was generated by setting total excitatory and inhibitory synaptic weights from SCS to 75% of their values in the original model (Zhang et al., 2014b); tonic inhibition of the projection neuron and the
inhibitory interneuron were excluded to be consistent with the original model and because excitatory and inhibitory influences on the projection neuron were balanced in this circuit. To generate "coloring" (excitatory) and "opponency" (inhibitory) circuits, the synaptic weight onto the projection neuron of the opposing connection was weakened to 20% of its strength in the "mixing" circuit, while the synaptic weight of the dominant connection was kept at its default strength. To modulate the dominant pathway, external tonic inhibition onto the projection neuron ("coloring") or the inhibitory interneuron ("opponency") via GABAergic and glycinergic synapses was preserved from the template circuit while tonic inhibition onto the weaker pathway was removed.

To model the adaptation to SCS observed in some projection neurons, the model projection neuron was replaced with a previously published model of an adapting neuron (Melnick et al., 2004a). In addition to already existing adaptation mechanisms in the model neuron, a slow potassium current (I_{km}) was added to the soma and dendrites to account for transient adaptation. Activating and inactivating gating variables for I_{km} (n and h) and their respective rate constants (\alpha_n, \beta_n) described as functions of temperature (T) in degrees Celsius, membrane potential V_m, and the potassium reversal potential E_k of -84 mV, are shown in equations 3.1-3.3.

\[ I_{km} = g_{km} n (V_m - E_k) \]  \hspace{1cm} (3.1)
Additionally, to account for slow adaptation (Powers et al., 1999, Benda and Herz, 2003), a slow inactivating gate, \( s \), was introduced to the Hodgkin-Huxley sodium conductance in the adapting firing neuron with rate constants (\( \alpha_s, \beta_s \)) defined in equations 3.4-3.6; other variables governing the sodium current (\( m, h \)) were modeled as previously described (Melnick et al., 2004a).

\[
\alpha_s = 3^{(T-t_{\text{ref}})/10} \cdot \frac{2.2 \times 10^{-8}}{m^2 \cdot \exp\left(\frac{(15-(V_m+65)}{5}\right)} - 1
\]  

(3.2)

\[
\beta_s = 3^{(T-t_{\text{ref}})/10} \cdot 0.5 \cdot \exp\left(\frac{(10-(V_m+65))}{40}\right)
\]  

(3.3)

Tonic inhibition was also implemented onto the adapting projection neuron, but the synaptic conductance of tonic inhibition onto the adapting projection neuron was reduced to 50% of the strength of the connection between the tonic inhibition and projection neuron in the "coloring" microcircuit.
3.2.12. Simulations and Analysis

All simulations of microcircuit responses to SCS were conducted with background activation of the model to generate baseline activity required to visualize inhibitory responses. Sciatic stimulation, as delivered in the experiments, was simulated by applying a 1 Hz train to the local Aβ, Aδ, and C-fiber afferent inputs with arrival latencies determined according to published conduction velocities (Aβ: 14-30 m/s; Aδ: 2.2-8 m/s; C: 0.6-1.5 m/s) and an assumed conduction distance of 100 mm (Harper and Lawson, 1985). Additionally, spike trains with individual interspike intervals drawn from a homogeneous Poisson process (mean = 0.75 spikes/s) were delivered through C-fibers to simulate stochastic activity after surgical sciatic nerve exposure (Michaelis et al., 1995). SCS was simulated by applying a constant frequency train through separate inputs representing dorsal column collaterals of both the local and surround Aβ fibers. Simulations were conducted in the NEURON v. 7.2 simulation environment (Hines and Carnevale, 1997a) using a time step of 0.0125 ms and 2^{nd} order implicit Crank-Nicholson integration.

The output of the computational model prior to and during 20 s of simulated SCS was quantified and normalized in the same manner as the experimental data. PSTHs were generated from model and responder neuron spike trains for 10 Hz, 50 Hz, and 150 Hz SCS using a 10 Hz virtual SCS train; generation of smoothed PSTHs using a "virtual"
10 Hz SCS train enabled visualization of the interactions between transient and longer-duration features at higher frequencies of SCS. Model and experimental PSTHs were smoothed by convolving raw peri-stimulus times with a bin-width optimized Gaussian kernel ($\sigma = 0.870$ ms) in Chronux (Bokil et al., 2010) and then normalized by setting the maximum increase or decrease versus the corresponding virtual PSTH during the SCS off-periods across SCS frequencies for a given neuron to 1 and scaling all responses accordingly. This scaling prevented large responses from dominating analysis of groups of simulations and allowed analysis of small but significant responses.

### 3.3. Results

#### 3.3.1. Population of recorded neurons

We recorded the responses of 50 antidromically identified sensory neurons to at least one repetition of all frequencies of epidural SCS. 11 of these neurons were classified as LT neurons, 18 neurons were classified as WDR neurons, and 19 neurons were classified as NS neurons (Figure 3.3d). The response types of 2 neurons could not be determined, but they exhibited strong A-fiber and C-fiber evoked responses during sciatic stimulation. The widespread locations of 22 neurons, confirmed by electrolytic lesions, were consistent with previous reports of locations of sensory projection neurons (Burstein et al., 1990) (Figure 3.3e).
Analysis of PSTHs using Z-scores and Kolmogorov-Smirnoff testing identified 33 neurons that were responsive to SCS and 17 neurons as non-responsive to SCS at SCS amplitudes sufficient to evoke a >50 μV CDP. (Figure 3.4). Responders and non-responders were located throughout the dorsal horn; there was no significant difference in the dorsal-ventral depths of the responders versus non-responders for which location data were recovered: responder n = 24, median = 565 μm, non-responder n = 13, median = 750 μm, p = 0.12, Kruskal-Wallis, and the distribution of responder and non-responder depths was also not different (p = 0.31, Kolmogorov-Smirnoff test). The amplitude of SCS applied to the population of responders (125 ± 9 μA, mean ± SEM, n = 33) vs. non-responders (121 ± 9 μA, mean ± SEM, n = 17) was also not different (p = 0.75, Welch’s t-test). Although the proportion of responders that were LT neurons (10/33) was higher than the proportion of non-responders that were LT neurons (1/17), and the proportion of responders that were NS neurons (10/33) was lower than the proportion of non-responders that were NS neurons (9/17), the overall distribution of LT, WDR, and NS neurons was not different between responders and non-responders (p = 0.12, Pearson’s χ²). Taken together, these data suggest that responsive and non-responsive sensory spinal projection neurons are intermingled throughout the superficial and deep dorsal horn and that these neurons do not receive a homogeneous set of inputs from Aβ/dorsal column fibers.
3.3.2. Responses to SCS are dependent on SCS frequency and heterogeneous

Neither the mean firing rate during SCS nor the mean change in firing rates between SCS On and SCS Off were significantly different across the frequencies of SCS tested \( (p = 0.43, \text{Repeated Measures ANOVA}) \) (Figure 3.5a). Mean firing rates and mean firing rate differences were also not significantly different across SCS frequencies when 1 Hz sciatic stimulation was applied concurrently with SCS \( (p = 0.92, \text{Repeated Measures ANOVA}) \) (Figure 3.5c). Responses to SCS were heterogeneous both across neurons and within individual neurons: neurons could be inhibited or excited at any of the applied frequencies of SCS, and some frequency-response relationships were non-monotonic with increasing SCS frequency. Inhibitory and excitatory responses were present during both SCS only and SCS + sciatic conditions, and the averaging of excitation and inhibition across neurons resulted in the lack of significant change in mean firing rates across SCS frequencies (Figures 3.5b, 3.5d). However, individual neurons exhibited stereotyped responses to SCS: some neurons showed progressive inhibition with increasing SCS frequency, some neurons showed progressive excitation with increasing SCS frequency, and some neurons exhibited a non-monotonic relationship between changes in firing rate and SCS frequency consistent with prior computational modeling predictions (Figure 3.6a) (Zhang et al., 2014b). The heterogeneous but stereotyped
responses suggested that quantitative classification would provide insight into the origins of the responses to SCS.

We quantitatively classified neurons by clustering the results of a principal component analysis (PCA) on the normalized SCS frequency response curves, with the normalized response to SCS as the dependent variable and SCS frequency as the independent variable (Figure 3.6b). We pooled normalized SCS only and SCS + sciatic response curves together because changes in baseline activity due to sciatic stimulation occasionally unmasked responses that were not evident in the SCS only case. K-means clustering of the resulting first 2 principal component scores revealed four different groupings of frequency-dependent responses to SCS (Figure 3.6b). These groupings represented the minimal total squared euclidean centroid distance out of 100 repetitions of k-means clustering with 4 centroids and accounted for 81% of the total variance of the distribution. Increasing the number of centroids in the k-means analysis to 5 accounted for only 6% more of the total variance, and increasing the number of centroids beyond 5 did not increase the total variance accounted for by more than 2.9% per additional centroid, suggesting that 4 clusters were sufficient to classify the responses.

Hierarchial clustering using squared Euclidean distance between cluster centroids as the linkage distance measure (Distance Cluster Combine) between clusters and visualized through a dendrogram confirmed the results of the k-means clustering
(Figure 3.6d). Although five clusters are evident in the dendrogram, the fifth cluster was small, comprising responses that were classified by the k-means analysis with other inhibitory responses (i.e., Cluster 1), and the small cluster merged with the branch corresponding to the other inhibitory responses on the dendrogram. In addition, only 4 out of 59 responses, on the boundaries of their respective clusters, were inconsistent between k-means and hierarchial analysis clustering (Figures 3.6b, 3.6d), indicating that the observed clusters are independent of the clustering algorithm.

Averaging the normalized frequency response curves corresponding to each cluster revealed distinct SCS frequency-dependent neuronal responses (Figure 3.6c). Cluster 2 responses ("gate") were non-monotonic and exhibited significant inhibition to 30 Hz and 50 Hz SCS (n = 10, RMANOVA p = 0.001) consistent with predictions of neuronal responses to SCS by a computational model of the Gate Control circuit (Zhang et al., 2014b). However, clusters with behaviors that could not be described by the Gate Control circuit model alone were also present: Cluster 1 ("inhibitory") responses were inhibited with increasing SCS frequency (n = 17, RMANOVA p<0.0001), cluster 3 ("excite") responses exhibited increased excitation with increasing SCS frequency (n = 18, RMANOVA p<0.0001), and cluster 4 ("plateau") responses exhibited excitation up to 50 Hz SCS followed by accommodation of their responses at higher SCS frequencies (n = 14,
RMANOVA p=0.0006. All clusters, including the "gate" cluster, contained LT, WDR, and NS neurons.

Fluctuations in baseline activity resulted in the frequency response curves of 20/33 responders being classified differently between SCS only and SCS + sciatic conditions. Changes in response classifications when baseline activity changed were expected, as a response evoked by sciatic stimulation was sometimes necessary to reveal the inhibition characteristic of "inhibitory" (n = 8) or "gate" (n = 3) responses, and high levels of activity evoked by sciatic stimulation sometimes caused neurons to adapt to additional excitation, converting them from "excite" to "plateau" (n = 3) or obscuring excitation entirely (n = 2). The 3 remaining neurons exhibited distinct response class transitions between SCS only and SCS + sciatic conditions. In 10/20 neurons, the features of the SCS-related PSTH, in particular sharp transient excitation at 2-3 ms, diffuse excitation, and/or inhibition immediately following excitation if present, were preserved across SCS only and SCS + sciatic conditions. In 4/20 neurons, lack of baseline activity (<1.5 spikes/s) in either the SCS only (n = 3) or the SCS + sciatic (n = 1) conditions both before and during SCS obscured the inhibitory effect during SCS observed in the other condition. Only 6/20 neurons exhibited significant differences in PSTH features between SCS only and SCS + sciatic conditions. Taken together, these observations indicate that changes in classification between conditions were generally due to changes in baseline
activity resulting from coincident sciatic stimulation rather than changes in the effects of SCS.

### 3.3.3. Stereotyped responses are also evident in chronic constriction injury rats

In a subset of 8 rats, we induced a chronic constriction injury (Bennett and Xie, 1991) and conducted terminal electrophysiology 7-10 days following the injury. CCI animals (n = 8) exhibited lower paw withdrawal thresholds 7 days post-injury (3.97 ±0.31 g, mean ± sem) than at baseline (9.96 ± 0.65 g, p = 9.5 X 10⁻⁷, Student’s t-test; Figure 3.7a); all experiments on CCI animals were conducted 7-10 days post injury, as the injury response peaks and remains steady within that time interval (Bennett and Xie, 1988, Kim et al., 1997). 7 antidromically identified projection neurons were recorded, and all recorded neurons exhibited either WDR or NS behavior during BPPC testing of the ipsilateral hindpaw, confirming that the transmission of sensory information through the sciatic nerve was preserved following CCI (Figure 3.7b). 6 of the 7 neurons were classified as responders based on z-score comparisons of SCS on vs. SCS off PSTHs. In addition, these 6 neurons were classified into the "excite" (n = 2), "inhibit" (n = 1), and "adapt" (n = 3) groups with least squares regression using a generalized linear model with the principal components used to classify the responses shown in Figure 3.6 as
predictors (Figure 3.7c), suggesting that spinal microcircuits underlying response heterogeneity are also present in an animal model of chronic pain.

3.3.4. Dorsal horn microcircuits are sufficient to explain heterogeneous responses to SCS

We implemented computational models of "coloring", "mixing", and "opponency" spinal microcircuits (Prescott and Ratté, 2012, Prescott et al., 2014) to determine whether different spinal microcircuits could account for the different classes of responses to SCS by weakening or removing specific elements in an overall template circuit (Figure 3.8a). Single parameterizations of the computational microcircuits reproduced 3 of the 4 normalized SCS-frequency response relationships observed experimentally (Figures 3.8b-3.8d), and each microcircuit model reproduced features of PSTHs from experimental neurons classified into the corresponding cluster, supporting the connectivity delineated by the specific microcircuit as the driver for the corresponding frequency-response relationship. The "coloring" microcircuit corresponding to cluster 3, with weak SCS-mediated inhibition and strong excitation modulated by SCS-independent tonic inhibition of the projection neuron, reproduced progressive excitation at SCS frequencies exceeding 50 Hz as well as recurring excitatory peaks in neuron activity that occurred 100 ms (10 Hz SCS), 20 ms (50 Hz SCS), and 6.7 ms (150 Hz) apart with latencies similar to those observed experimentally (Figure 3.8b).
The "mixing" microcircuit corresponding to cluster 2, featuring strong excitatory and inhibitory connections from SCS-affected inputs but no tonic inhibition, reproduced the non-monotonic frequency response relationship, the pattern of strong transient excitation followed by prolonged inhibition following 10 Hz and 50 Hz SCS, and recurring excitatory events observed during 150 Hz SCS (Figure 3.8c). Model-predicted suppression of transient excitation by prolonged inhibition at 50 Hz SCS was also observed in experimental neurons classified to cluster 2. Finally, the "opponency" microcircuit corresponding to cluster 1, which included weak SCS-mediated excitation and strong inhibition modulated by SCS-independent tonic inhibition of the interneuron, reproduced experimentally observed progressively inhibited responses with increasing SCS frequency and total suppression by 150 Hz SCS, as well as PSTH peaks corresponding to weak SCS-mediated excitation during 10 Hz and 50 Hz SCS (Figure 3.8d).

### 3.3.5. Spike frequency adaptation mechanisms reproduce the "Plateau" response

The non-monotonic relationship between neuron firing rate and SCS frequency observed in cluster 4 could not be reproduced by any parameterization of the microcircuits described above. Specifically, the decline in firing rate from 50 Hz SCS to 100 Hz SCS, coupled with the absence of an increase in the recurring excitatory
component in the PSTHs from 50 Hz to 150 Hz SCS, suggested that adaptation
mechanisms might underlie the responses observed in Cluster 4. Therefore, we replaced
the tonically firing projection neuron in the "coloring" microcircuit with a model of an
adapting dorsal horn neuron. As few data exist regarding the specific mechanisms of
spike frequency adaptation in dorsal horn neurons, we conducted a sensitivity analysis
wherein we varied the parameters underlying 3 possible drivers of adaptation—Na
conductance (g_{Na}), fast K⁺ conductance (g_k), and slow K⁺ conductance (g_{Km})—within
ranges centered around previously published values (Melnick et al., 2004a, Prescott and
De Koninck, 2005) (Figure 3.9a).

Inclusion of these adaptation mechanisms in the projection neuron was sufficient
to reproduce individual features of but not the overall normalized SCS response curve
representing cluster 4 (Figure 3.9a). Increasing fast or slow K⁺ conductances and
reducing Na⁺ conductances generated the convex increase in responses to SCS up to 50
Hz and the reduction from 50 Hz to 100 Hz SCS but could not reproduce the plateau at
150 Hz. Reducing K⁺ conductances and increasing Na⁺ conductances reproduced the
downturn at 100 Hz and the plateau at 150 Hz but could not reproduce the convex
increase in response from 1-50 Hz SCS. However, consistent with the experimental
data, averaging the model responses across parameterizations reproduced the overall
cluster 4 response (Figure 3.9b), and the range of individual model PSTH responses
spanned the range of responses observed experimentally, including the excitatory peaks at similar latencies and the absence of progressive growth of the recurring excitatory component (Figure 3.9c). Increasing the strength of tonic inhibition onto the projection neuron to its original value from the "coloring" microcircuit did not prevent adaptation but resulted in a peri-stimulus response delay in A-fiber mediated excitation in the model that exceeded the delays observed experimentally, suggesting that adapting neurons may be subject to less tonic inhibition than non-adapting neurons. Taken together, these results suggest that biophysical rather than network mechanisms account for the differences between the responses observed between experimental clusters 3 and 4 and that heterogeneity in the biophysics driving projection neuron adaptation could underlie the diversity within cluster 4.

3.3.6. Disruption of GABA<sub>A</sub> mechanisms alters responses to SCS

In a subset of 20 neurons, we applied intrathecally bicuculline methiodide (BIC), a GABA<sub>A</sub> receptor antagonist, and/or CGP 35348 (CGP), a GABA<sub>B</sub> receptor antagonist, and repeated measurements of the response of the neuron to different frequencies of SCS. BIC was applied in 14 instances, and CGP was applied in 9 instances; these instances include 2 neurons to which BIC was applied after an experimental block with CGP and 1 neuron to which CGP was applied after an experimental block with BIC. BIC administration increased baseline firing in 6/14 neurons and unmasked responses
during BPPC testing in 9/14 neurons (Figure 3.10a). In contrast, CGP administration increased baseline firing in 1/9 neurons and only enhanced the response to crushing of the peripheral receptive field in 1/9 neurons, suggesting that tonic GABA\textsubscript{A} mechanisms had a greater modulatory effect than GABA\textsubscript{B} mechanisms on the behavior of the recorded neurons.

Application of BIC but not CGP had substantial effects on SCS-evoked responses in sensory projection neurons. Based on z-score comparisons of PSTH responses during SCS between control and BIC/CGP conditions, BIC significantly disinhibited neuronal responses to SCS in either the SCS only or SCS + sciatic conditions in 7 of the 14 neurons (Example: Figure 3.10b), while CGP only altered significantly post-stimulus responses to SCS in 1 of 9 neurons. 5/7 neurons in which BIC had a disinhibitory effect also exhibited increased baseline activity and enhanced responses to BPPC stimulation. In the 7 neurons for which BIC had an effect on the SCS response, the primary effects of BIC application were either the accentuation of transient excitation, suggesting disinhibition of excitatory inputs, or a reduction in the period of transient inhibition following the SCS pulse, suggesting a shift to other inhibitory mechanisms, such as glycinergic mechanisms, that are unaffected by BIC (Figure 3.10c) (Braz et al., 2014). Disinhibition was evident even in neurons classified into excitatory clusters: 5/7 neurons were classified into either the "excite" or "plateau" clusters in SCS only or SCS + Sciatic
conditions, and 1/7 neurons was classified into the "gate" cluster during SCS only and the "excite" cluster during SCS + sciatic due to a reduction in baseline activity during sciatic stimulation. Included in these counts is one neuron that was classified into the "inhibit" cluster during both SCS only and SCS + sciatic conditions and for which BIC was applied after CGP; BIC administration disinhibited this neuron's response to BPPC and SCS (Figure 3.10d), particularly at SCS frequencies < 50 Hz, whereas CGP administration had only a modest effect on the neuron's normalized response to SCS (Figures 3.10e, 3.10f). In addition, 2 neurons that did not respond to SCS during the control block responded to SCS following BIC administration, whereas CGP did not unmask responses in any neuron that was non-responsive in the control case.

Finally, 3 neurons exhibited adaptation during SCS + sciatic stimulation following BIC, and one of these 3 neurons exhibited an enhanced response during SCS when sciatic stimulation was off but exhibited adaptation to SCS when sciatic stimulation was on. For all 3 neurons that adapted following BIC, BIC administration resulted in substantially increased baseline firing rates when SCS was off, suggesting that removal of GABA\(_A\)-mediated inhibition resulted in excessive excitatory drive onto these neurons sufficient to cause adaptation or an increase in SCS-independent modulation, such as from supraspinal nuclei, as has been previously reported (Sorkin et al., 1998). CGP did
not induce adaptation during the SCS response in any of the tested neurons, including the neuron for which CGP application raised the baseline activity level.

3.4. Discussion

We hypothesized that SCS-mediated inhibition of spinal sensory projection neurons would be dependent on stimulation frequency and follow a non-monotonic trend predicted by a computational model of the Gate Control circuit (Zhang et al., 2014b). Although the responses of antidromically identified spinal sensory projection neurons were dependent on SCS frequency in both healthy and CCI animals, responses were unexpectedly heterogeneous, and the Gate Control circuit was insufficient to describe the 4 clusters of SCS frequency dependent responses. However, computational models of excitatory, mixed, and inhibitory spinal microcircuits (Prescott and Ratté, 2012), could reproduce the range of observed responses, supporting the proposed microcircuits as a plausible explanation for the experimentally observed heterogeneous responses to SCS (Prescott et al., 2014). The administration of BIC but not CGP altered baseline neural activity and unmasked or disinhibited responses to SCS in some neurons, suggesting that GABA\textsubscript{A} pathways modulate SCS frequency dependence and that attenuation of these pathways may contribute to reductions in SCS efficacy over time (Kumar et al., 2007).
3.4.1. Projection neuron responses and spinal microcircuits

This is the first study to assess the effects of different frequencies of SCS on antidromically verified projection neurons. Quantitative analysis of projection neuron activity during SCS directly tests the Gate Control-based hypothesis that SCS acts to inhibit the output neurons of the dorsal horn circuit (Shealy et al., 1972, Guan, 2012). As the output of projection neurons correlates with the magnitude and temporal characteristics of perceived pain (Simone et al., 1991, Craig, 2003), these results establish a stronger link between neuronal inhibition and SCS-mediated pain relief or neuronal excitation and possible sensory side effects (e.g., paresthesia) than did prior studies that did not identify neurons as projection neurons (Yakhnitsa et al., 1999, Guan et al., 2010).

Despite the existence of neurons whose behavior was consistent with predictions by the Gate Control theory, projection neuron responses to SCS were unexpectedly heterogeneous in both healthy and CCI animals, suggesting that other networks may mediate different aspects of the total SCS response. Spinal microcircuits that describe inhibitory and excitatory interactions between peripheral inputs and SCS beyond the modulation proposed by the Gate Control Theory may explain the heterogeneous interactions between SCS and peripheral stimulation (Prescott and Ratté, 2012, Prescott et al., 2014). Our experiments included co-activation of nociceptive and non-nociceptive afferents necessary to test the proposed interactions, and both frequency-response
curves and PSTHs were reproduced by computational models of the proposed microcircuits, supporting proposed microcircuits as drivers of the SCS frequency dependent responses. Furthermore, the dominant response of the "coloring" and "opponency" microcircuits could be reproduced with attenuation rather than deletion of the opposing pathway, and PSTHs during SCS were not affected by 1 Hz sciatic stimulation at C-fiber threshold in 14 of 20 neurons for which the change in baseline activity resulted in a dual classification. These observations suggest that microcircuits may share common features or represent points along a continuum of a population of dorsal horn circuits responsible for processing nociception and mediating the analgesic effects of SCS. In addition, heterogeneous membrane properties of the model adapting neuron were required to reproduce the overall adaptation response, suggesting that heterogeneity among individual neurons may also contribute to the population code underlying nociception. For example, diverse biophysics affect neuronal synchrony in response to simulated synchronized synaptic inputs, and adaptation mechanisms may act as a safeguard against noise from primary afferent inputs (Ratte et al. 2013). These findings motivate the future consideration of pain as the result of a population response rather than the result of direct information transfer from a specific group of neurons (e.g., a "labeled line") (Prescott et al., 2014).
3.4.2. Effects of SCS are modulated by GABA$_A$ receptor pathways

Our finding that SCS frequency dependence is modulated by activation of GABA$_A$ receptors builds on a prior study showing that SCS-mediated inhibition in healthy animals may be removed by bicuculline (Duggan and Foong, 1985) and supports the computational prediction that as GABAergic inhibition is lost, SCS becomes less effective at inhibiting neuronal activity and may even excite projection neurons, resulting in altered SCS-frequency-dependence following the loss of GABAergic inhibition (Kumar et al., 2007). We did not observe disinhibition following antagonism of GABA$_B$ receptors as in prior studies in animals with sciatic nerve mononeuropathy (Meyerson et al., 1995, Cui et al., 1998). This difference may reflect a conversion of inhibitory mechanisms to GABA$_B$ receptor mediated pathways following the loss of GABA$_A$ receptor mediated pathways during the transition to neuropathic pain. In addition, inhibitory responses to SCS were preserved following CCI in 1 neuron, suggesting that other inhibitory processes may contribute to SCS-mediated pain relief.

Tonically active neurons from segmental (Torsney and Macdermott, 2006, Takazawa and Macdermott, 2010) and supraspinal (Lin et al., 1996, Carlson et al., 2007) sources may also mediate GABAergic inhibition of dorsal horn inhibitory interneurons and projection neurons that modulate neuronal responses to pain and SCS. Altering the magnitude of tonic GABAergic inhibition in the models enabled tuning of the
frequencies at which spinal microcircuits exhibited SCS frequency dependent inhibition and excitation. Furthermore, that BIC had a disinhibitory effect even on 6 neurons that exhibited "excite" or "plateau" excitatory responses during SCS, and that excitatory responses to SCS were unmasked in 2 non-responders by BIC supports tonic inhibition as a general modulator of sensory neuron activity regardless of peripheral afferent inputs. However, we could not determine the extent to which BIC administration affected direct SCS-mediated inhibition versus indirect inhibition from SCS-independent tonic inhibitory interneurons or to what extent the inhibition from these neurons may have been due to other inhibitory neurotransmitters, such as glycine (Takazawa and Macdermott, 2010, Braz et al., 2014).

3.4.3. General limitations

We conducted acute experiments on healthy animals using 1-150 Hz SCS, whereas clinically SCS is used to treat chronic neuropathic pain. Changes involved in the transition from the healthy state to neuropathic pain include but are not limited to altered network connections (Todd, 2010), glial cell activation (Scholz and Woolf, 2007, Ji et al., 2013), modifications in synaptic dynamics due to KCC2 malfunction (Coull et al., 2005), and loss of GABAergic inhibition in the dorsal horn (Braz et al., 2014), not all of which could be assessed in our experiments. Importantly, we addressed some of the possible differences in responses between healthy and neuropathic pain states by
demonstrating that block of GABA\_A mechanisms disinhibited responses to SCS in some neurons and that the same microcircuit responses to SCS were observed in CCI animals.

The presence of non-responders despite a robust SCS-evoked CDP over their resident spinal segment and a lack of correlation between microcircuit classification and peripheral sensory type (LT, WDR, NS) among responders suggests that primary afferents may modulate dorsal horn neuron responses to SCS through more complex, polysynaptic circuits beyond the spinal microcircuits (Zheng et al., 2010, Braz et al., 2014). The existence of non-responders to SCS has not been previously explicitly reported, suggests that not all projection neurons receive inputs affected by SCS, and may explain why SCS does not inhibit acute pain or sensation from the affected dermatome (Lindblom and Meyerson, 1975). That the microcircuit classification of a neuron was independent of its peripheral sensory type supports the hypothesis that SCS-evoked effects may override baseline activity resulting from low frequency (<2.3 Hz) A\_\delta/C-fiber afferent drive (Zhang et al., 2014b). However, in 6/33 responder neurons, the SCS response was significantly different between SCS only and SCS + sciatic conditions, suggesting the existence of more complex interactions between non-nociceptive and nociceptive inputs requiring further study.
Finally, we did not account for the effects of SCS on supraspinal circuits (Heinricher et al., 2009) or on descending pathways, such as the dorsolateral funiculus, that could have been activated by SCS. Although segmental mechanisms have been shown to be sufficient in describing inhibitory and excitatory responses to SCS (Foreman et al., 1976a, Smits et al., 2012), supraspinal loops may also modulate analgesia by SCS (Barchini et al., 2012). For example, neurons in supraspinal nuclei exhibited more activity during SCS in neuropathic rats that showed increased paw withdrawal thresholds than in non-responding rats, suggesting that effective SCS for chronic pain requires supraspinal modulation (Song et al., 2013a), but knowledge regarding the extent of supraspinal contributions to SCS is sparse.
Figure 3.1: Recording the responses of single lumbar dorsal horn projection neurons to epidural SCS in urethane-anesthetized rats.

(a): Rats were implanted with sciatic nerve cuffs (right, bottom) for peripheral stimulation and bipolar paddles (right, top) for SCS. Peripheral stimuli ("Brush, Press, Pinch, Crush;" BPPC) were applied to the hindpaw ipsilateral to the recording electrode for neuron characterization. A Pt-Ir microelectrode was lowered into the ventral aspect of the contralateral cervical spinal cord for antidromic identification of ascending projections from lumbar dorsal horn neurons. (b): Timeline of experimental recordings: Antidromic identification and neuron characterization preceded randomized blocks of SCS.
Figure 3.2: Cord dorsum potentials were used to determine SCS amplitudes.

(a): Inverting the polarity of biphasic SCS (100 μs pulse width) inverted the artifact (red) but not the CDP signal (blue) recorded 1 cm caudal to the site of SCS and over the approximate neuron search region. (b): Moving the electrode 3 mm and 6 mm caudal to the original recording site (A) resulted in a dispersed CDP with later peak latencies consistent with compound action potential propagation at ~10 m/s. (c): Representative examples of peri-stimulus responses to SCS at 50 Hz applied at different amplitudes corresponding to different evoked CDP amplitudes. Increasing the amplitude of SCS beyond the threshold required to evoke a ~50 μV CDP (e.g., to motor threshold) did not significantly affect peri-stimulus responses to SCS.
Figure 3.3: Characterization of antidromically identified neurons.

A-C: All included neurons met 3 criteria required for antidromic identification (Lipski, 1981): neurons followed single pulse stimulation of the contralateral ventral cervical spinal cord (A), neurons followed trains of 3 pulses of stimulation at 200-333 Hz (B), and neurons exhibited orthodromic-antidromic collisions during activation of the neuron's peripheral receptive field (C). Axes in B and C are the same as in A. D: Neurons fell into established low-threshold (LT), wide dynamic range (WDR), and nociceptive-specific (NS) categories as determined based on firing rates during brush-press-pinch-crush (BPPC) stimulation of peripheral receptive fields. E: Neuron locations (right) based on 22 recovered Prussian Blue lesions (example left).
Figure 3.4: Analysis of PSTHs was used to differentiate responders to SCS from non-responders.

(a): Representative peri-stimulus responses during SCS off (left) and SCS on (middle) during 10 Hz, 30 Hz, 50 Hz, 100 Hz, and 150 Hz SCS. Normalization of the SCS on response to the SCS off response using Z-scores (right) was used to determine if a response to SCS was significant. (b): Responder/non-responder, sorted by incidences of significant peri-stimulus responses. A neuron was classified as a responder if the Z-score result from two or more contiguous frequencies in either the SCS only or SCS + sciatic condition was significant. Neurons for which Z-score analysis could not be conducted due to low firing rates but for which K-S tests yielded significant responses were included. (c): Map of neurons that were included in firing rate (Figure 5), principal component, and clustering analyses (Figure 6). Only individual frequency-response relationships that met the contiguous frequency criterion were included in PCA and clustering.
Figure 3.5: Effect of SCS Frequency on firing rate of projection neurons.

(a), (c): Median and 25th -75th percentiles (boxes) of responder neuron firing rates between SCS off and SCS on conditions when only SCS was applied (a) or when SCS was applied with sciatic stimulation (c). Outliers beyond 1.5 interquartile ranges (whiskers) are denoted with the "+" symbol. (b), (d): Changes in firing rates of individual responder neurons between SCS on and SCS off conditions of individual neurons when only SCS was applied (b) or when SCS was applied with sciatic stimulation (d). Each row represents one neuron, and each column represents the continuum of responses across neurons for a given SCS frequency. All columns in the color maps are sorted according to response during SCS.
Figure 3.6: Classification of individual neuron responses to different frequencies of SCS.

(a): Representative examples of increasing (red), non-monotonic (violet), and decreasing (blue) responses relative to "Off" baselines (dotted line) with increasing SCS frequency. Although the magnitudes of deviations versus "Off" baselines were heterogeneous, all examples exhibited significant on vs. off PSTH responses to SCS, and the shapes of the responses were stereotyped, motivating classification of normalized responses. (b): Principal component based clustering of normalized responses. (c): Aggregate normalized responses (mean ± s.e.m of normalized responses shown) formed by averaging individual responses from each cluster in (b). (d): Hierarchial analysis using squared Euclidean distance between points corresponding to the first two PCA loadings as the proximity measure. Discrepancies between K-means and hierarchial clustering are highlighted in (b), with each inconsistent point in (b) highlighted using the case number and color corresponding to its hierarchial clustering in (d).
Figure 3.7: Heterogeneous responses of dorsal horn projection neurons to SCS were also present in animals following CCI.

(a): CCI produced a significant reduction in paw withdrawal thresholds (n = 8, p < 9.5 X 10^{-7}, Student's t-test). (b): Representative brush-sensitive wide dynamic range neuron, brush-insensitive wide dynamic range neuron, and nociceptive-specific neuron indicating that neurons are responsive to stimulation of the peripheral receptive field following CCI. (c): Normalized responses from 6 responsive projection neurons out of 7 neurons found from CCI animals. Insets in (c) depict results of least squares regression classification of the frequency-response curves plotted against the original classification scheme (Figure 3.6). Data from one non-responsive neuron are not shown.
Figure 3.8: Computational models of spinal microcircuits reproduced frequency-response curves and features of PSTHs.
(a): Template circuit that describes all features that contributed to the individual microcircuits. (b-d): Individual spinal microcircuits, normalized frequency response relationships, and normalized smoothed PSTHs constructed using a "virtual" 10 Hz SCS train time-aligned onto real 10 Hz, 50 Hz, and 150 Hz SCS comparing model and experimental responses. In PSTH comparisons, the model response (bold) was overlaid onto all experimental PSTH responses (faint) from neurons whose frequency-response relationships were classified into the cluster corresponding to the microcircuit. The colors of the graph lines indicate the cluster to which each microcircuit corresponds (Figure 3.6).
Figure 3.9: Spike frequency adaptation mechanisms allowed reproduction of the aggregate response representing neurons in cluster 4 (Figure 3.6)

(a): Normalized model projection neuron responses to different frequencies of SCS across different values for $g_{Na}$, $g_K$, and $g_{Km}$. In all cases, the Na$^+$ conductance slowly inactivates according to equations 4-6. $g_{Na} = 100\%$ refers to a hillock Na$^+$ conductance of 2.19 S/cm$^2$. $g_K = 100\%$ refers to a hillock K$^+$ conductance of 0.076 S/cm$^2$, a soma K$^+$ conductance of 0.0043 S/cm$^2$, and a dendritic K$^+$ conductance of 0.034 S/cm$^2$. $g_{Km} = 100\%$ refers to a soma and dendritic K$^m$ conductance of 0.0005 S/cm$^2$. (b): Normalized individual and average experimental responses (left) juxtaposed with the average of the normalized model responses shown in (A) (right). (c): Experimental (left) and all model (right) smoothed normalized PSTHs generated using a virtual 10 Hz train for real 10 Hz, 50 Hz, and 150 Hz SCS responses.
Figure 3.10: Administration of bicuculline (BIC) unmasked or disinhibited responses to peripheral stimulation and SCS.
(a): Brush, press, pinch, crush (BPPC) response profiles of two example neurons during control conditions (blue) and after application of BIC (red). BIC unmasked responses to BPPC in the top neuron and increased baseline firing/disinhibited evoked responses in the bottom neuron.  
(b): Raw frequency-response relationship of neuronal activity vs. SCS frequency before and after the application of BIC in a representative example.  
(c): Peri-stimulus responses to SCS before (blue) and after (red) intrathecal application of BIC for the representative example shown in (b).  
(d): BPPC response of neuron to which CGP 35348 (CGP), then CGP + BIC was applied.  
(e): Normalized relationship between SCS frequency and neuron response to SCS before and after the application of CGP, then CGP + BIC in 1 neuron.  
(f): PSTHs during SCS after application of CGP (orange) and after application of CGP + BIC (red) in a representative example. Raw neuron firing rate vs. SCS frequency curves are not shown because the application of CGP and CGP + BIC resulted in increased baseline activity (d) that would have distorted the plot axes.
4. Dual frequency patterns improve the efficacy and efficiency of spinal cord stimulation

4.1. Introduction

Spinal cord stimulation (SCS) is a therapy for managing chronic pain when conventional treatments including physical therapy, pharmaceuticals, and surgery are not effective. SCS is delivered to the dorsal columns at a constant frequency, most often 50-80 Hz, through an epidural electrode, and stimulation parameters including amplitude, pulse width, electrode configuration, and frequency are tuned to provide pain relief (Cameron, 2004, Taylor et al., 2013). However, despite substantial advances in stimulation technology, only 58% of patients experience 50 % or greater reported pain relief at the end of a postoperative follow-up period, and there has been no significant increase in efficacy between 1972 and 2013 (Zhang et al., 2014a).

The putative mechanism of SCS is based on the Gate Control Theory (Melzack and Wall, 1965) and posits that activation of the dorsal column collaterals of Aβ-fiber primary afferents originating from the site of pain engages inhibitory mechanisms in the dorsal horn that, in turn, suppress the activity of "Gate" neurons responsible for the transmission of pain to spinal nociceptive-specific pathways (Prescott et al., 2014, Luz et al., 2014) or supraspinal centers (Foreman et al., 1976a). However, SCS may also excite Gate neurons (Foreman et al., 1976a, Dubuisson, 1989), and inhibition due to activation
of dorsal column fibers from a "surround" receptive field is required for the net inhibitory effects of SCS (Zhang et al., 2014b, Hillman and Wall, 1969). In addition, SCS must occur at a relatively high frequency for surround inhibition to overcome SCS-mediated excitation, and the efficacy of conventional SCS declines apparently as a result of the loss of GABAergic inhibition over the progression of chronic pain (Zeilhofer et al., 2012, Braz et al., 2014, Moore et al., 2002a).

Prior attempts to increase the efficacy of SCS focused largely on the design of electrode configurations to activate dorsal column fibers at lower amplitudes and with greater selectivity (Holsheimer and Wesselink, 1997, Wesselink et al., 1998a). However, this strategy considers neither the temporal properties of the SCS pulse train nor the effects of SCS-evoked neural activity on spinal circuits that relay pain information to the brain. The lack of attention to the neuronal circuit effects of stimulation may contribute to the lack of improvement in SCS efficacy over time (Kumar et al., 2007, Zhang et al., 2014a). Recently developed novel methods of SCS, such as kilohertz frequency SCS (Al-Kaisy et al., 2014) and burst SCS (De Ridder et al., 2013) are thought to work by altering SCS-evoked activity through conduction block or asynchronous activation of dorsal column fibers (Shechter et al., 2013). As such, both of these novel therapies modulate the temporal rather than the spatial effects of the applied SCS, and both high frequency SCS and burst SCS are clinically effective in producing pain relief. However, the
mechanisms underlying the analgesic effects of high frequency and burst SCS are unknown, and as such, neither high frequency SCS nor burst SCS are necessarily optimized for pain relief.

We hypothesized that SCS could be improved by delivering two different frequencies of SCS concurrently to different groups of dorsal column fibers. Dual frequency (dfSCS) is intended to exploit the underlying biophysical mechanisms of SCS by dispersing in time SCS-mediated excitation while preserving SCS-mediated inhibition. We simulated the effects of dfSCS on the output neuron of a validated computational model of the Gate Control circuit and identified dfSCS pairs inhibited model neuron activity more than single frequency SCS (sfSCS) at the average of the constituent frequencies or individual frequency SCS delivered to half of the dorsal columns (ifSCS) at the higher or lower of the two constituent frequencies. We conducted in vivo experiments in anesthetized rats and confirmed that dfSCS using appropriately selected constituent frequencies produced greater inhibition of spinal sensory neurons than sfSCS, ifSCS, and dfSCS using a suboptimal frequency pair. Our computational and experimental findings indicate that dfSCS may be a novel approach to improve the efficacy and efficiency of SCS and is the first approach that accounts explicitly for sensory neuron responses to SCS.
4.2. Methods

4.2.1. Simulations of dual frequency SCS

We simulated the responses of spinal sensory neurons to dual frequency SCS (dfSCS) using a validated computational network model based on the Gate Control circuit (Zhang et al., 2014b). Briefly, the network consists of biophysically-based compartmental models of excitatory and inhibitory dorsal horn interneurons connected to each other and to a "Gate" neuron receiving polymodal inputs via Alpha function representations of excitatory and inhibitory synapses with rise time constants, decay time constants, and peak conductances based on prior literature (Figure 4.1). The network receives as inputs 15 Aβ-fibers, 15 Aδ-fibers, and 30 C-fibers from a "local" receptive field that make monosynaptic and disynaptic excitatory and inhibitory connections onto the output Gate neuron and 15 Aβ-fibers from a "surround" receptive field that make disynaptic inhibitory connections onto the output Gate neuron (Hillman and Wall, 1969). The activity of the Gate neuron was used as the outcome measure, as the firing rate of WDR neurons correlate with reports of pain following capsaicin injection (Simone et al., 1991), and changes in the firing rate of WDR neurons during SCS parallel behavioral effects of SCS on pain (Wallin et al., 2003, Guan et al., 2010).

dfSCS was implemented by delivering each of two frequencies of SCS ranging from 1 Hz to 70 Hz through 15 of the Aβ fibers split between the local and surround
receptive field inputs. In the default case, 7 local and 8 surround Aβ fibers received one frequency, and 8 local and 7 surround Aβ fibers received the other frequency; the total number of Aβ inputs was kept at 30 to remain consistent with the original computational model (Figure 4.1). Included in these simulations were trials of single frequency SCS (sfSCS), implemented by delivering one frequency of SCS to all dorsal column fibers simultaneously. Sciatic stimulation, to evoke baseline activity required to visualize inhibitory responses and as delivered in the experimental assessments of dfSCS, was simulated by applying an irregular spike train to the Aβ, Aδ, and C-fiber afferent inputs with statistical properties based on recordings from primary afferents following a peripheral nerve injury (Wall and Gutnick, 1974, Liu et al., 2000) (Poisson process, inter-spike interval mean and standard deviation = 667 ms, no ISIs < 300ms). The arrival latencies of peripheral inputs were determined according to published conduction velocities (Aβ: 14-30 m/s; Aδ: 2.2-8 m/s; C: 0.6-1.5 m/s) and an assumed conduction distance of 100 mm (Harper and Lawson, 1985, Woolf and Wall, 1982a). To be consistent with experiments, the propagation distance between the site of SCS and the neurons receiving the Aβ/dorsal column inputs affected by SCS was assumed to be 1 cm. Throughout all simulations, we accounted for the possibility of collisions between orthodromic and SCS-triggered antidromic action potentials along each Aβ-fiber and transmitted the net spike train to the network model prior to simulation (Iggo, 1958).
Simulations of neuronal responses to SCS were conducted in the NEURON v. 7.2 simulation environment (Hines and Carnevale, 1997a) using a time step of 0.0125 ms and 2nd order implicit Crank-Nicholson integration.

The effects on the responses of the Gate neuron to all dfSCS pairs ranging from 1 Hz/1Hz to 70 Hz/70 Hz were simulated both under default conditions and conditions representing non-ideal electrode selectivity or non-ideal electrode placement. Non-ideal selectivity ("overlap") was simulated by setting 66% of all Aβ fiber inputs to receive the merged trains from both dfSCS frequencies and omitting pulses delivered at the same time. Non-ideal placement ("local/surround bias") was simulated by changing the proportion of Aβ-fibers from the local and surround receptive fields that received each constituent frequency. The local bias group consisted of 12 local Aβ-fiber inputs and 3 surround Aβ-fiber inputs, while the surround bias group consisted of 12 surround Aβ-fiber inputs and 3 local Aβ-fiber inputs.

A primary contributor to the transition to chronic neuropathic pain is the loss of GABAergic inhibition in the spinal cord following injury (Zeilhofer et al., 2012, Braz et al., 2014). Lower levels of GABA production result in smaller amplitude post-synaptic inhibitory events and a shift from GABAergic inhibition to glycinergic inhibition in animal models of neuropathic pain (Moore et al., 2002a). In the model, the transition to
chronic neuropathic pain was simulated by reducing the strength of GABAergic inputs onto the excitatory interneuron and the Gate neuron by 50%. To simulate the effects of progressive GABAergic loss (Campbell and Meyer, 2006) on the efficacy of dfSCS, the GABAergic inputs from only the local inhibitory interneuron were weakened to simulate the "early" disease state, and GABAergic inputs from both the local and surround interneurons were weakened to simulate the "late" disease state.

4.2.2. Quantitative assessments of dfSCS

To quantify the effects of SCS, we calculated the difference between Gate neuron firing rate during SCS and Gate neuron firing rate before SCS and converted that difference to a percentage of the Gate neuron firing before SCS, hence forth " % absolute effect" (Equation 4.1). A negative % indicates that SCS has an inhibitory effect, a positive % indicates that SCS has an excitatory effect, and a 0 % indicates that the firing rate of the Gate neuron was the same both before and during SCS.

\[
\%_{\text{absolute effect}} = \frac{\text{SCS On - SCS Off}}{\text{SCS Off}} \times 100\%
\] (4.1)

To compare the relative efficacy of dfSCS to sfSCS, the firing rate of the Gate neuron during dfSCS was subtracted from the firing rate of the Gate neuron during sfSCS, and this difference in firing rates was converted to a percentage of the firing rate
prior to SCS, henceforth the "% relative effect" (Equation 4.2). Within an experimental condition, the activity of the Gate neuron prior to SCS was identical across dfSCS and sfSCS simulations. A negative % indicates that dfSCS produced more inhibition than sfSCS, a positive % indicates that dfSCS produced less inhibition than sfSCS, and 0% indicates that Gate neuron activity was identical during dfSCS and sfSCS.

$$\%\text{relative effect} = \frac{\text{dfSCS} - \text{sfSCS}}{\text{SCS off}} \times 100\%$$ (4.2)

### 4.2.3. Experimental preparation

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of Duke University and were in accordance with *The Guide for the Care and Use of Laboratory Animals* (8th edition). Male Sprague-Dawley rats (300-500g) were sedated with 3.0 % Isoflurane (inhaled), urethane was administered (1.2 g/kg SQ), Isoflurane was stopped, and supplemental doses of urethane (0.4 g/kg/hr i.p.) were administered if a withdrawal reflex occurred in response to pinching of the hindpaw. A tracheotomy was performed, and rats were connected to a pressure-controlled ventilator (Kent Scientific MouseVent G500) via the tracheal tube for artificial respiration. Oxygen saturation and heart rate were monitored using a pulse oximeter (Kent Scientific MouseVent G500 module) on the hindpaw and maintained within physiological levels ($\text{SpO}_2 > 90\%$, HR < 450). Body temperature was monitored using a
rectal thermometer and maintained at 35-37°C using a heating blanket (Gaymar T/Pump). The head was mounted in a stereotaxic frame (Kopf Instruments), and the vertebral column was suspended from vertebral clamps (Kopf Instruments) attached to T9 and L2 for mechanical stability. The sciatic nerve was exposed, and a 1.5 mm diameter bipolar cuff electrode with an interelectrode spacing of 1 mm was wrapped around the nerve just proximal to the popliteal fossa. A laminectomy was performed across the T10-L2 vertebrate to expose the spinal cord, the dura over the lumbar cord was resected, and warm (37°C) saline or mineral oil was applied to the exposed cord. At the completion of the surgery, but prior to neuron search and recording, rats were paralyzed using gallamine triethiodide (Sigma-Aldrich, 20 mg/h i.p.), and a 1.5 mm wide bipolar paddle electrode, with two pairs of 0.5 mm x 0.5 mm platinum electrodes separated by 0.16 mm and spaced 2.0 mm apart, was inserted into the epidural space at T10 to deliver SCS (Figure 4.2a).

4.2.4. Cord dorsum potentials

The amplitudes of SCS applied through the medial and lateral electrode pairs during dfSCS and sfSCS were determined using the N1-P2 amplitude of the cord dorsum potential (CDP) evoked by single cathodic-first biphasic SCS pulses (pulse width = 100 μs) measured approximately 1 cm caudal to the caudal SCS contact, as N1-P2 CDP amplitude is a proxy for dorsal column activation (Honmou et al., 1996). The
amplitude of sfSCS, delivered through both electrodes simultaneously, was set to
generate a CDP with an amplitude > 50 μV_{pp} from the surface of the dorsal columns. To
replicate experimentally the dfSCS simulations, in which approximately half of the
dorsal column inputs to the network affected by SCS were activated by each of the
constituent frequencies, the amplitudes of SCS from each electrode pair were set
individually to evoke a 25 μV CDP (Figure

4.2.5. Extracellular recording and characterization

A recording microelectrode (stainless steel, 1-2 μm tip diameter, 1.5 MΩ
impedance, Microprobes Inc.) was inserted into the spinal cord at the T12-L1 vertebrae,
corresponding to the L4-L6 dermatomes, ipsilateral to the sciatic nerve cuff and
maneuvered until a neuron that exhibited a long-latency excitatory response (> 100 ms)
following 1 Hz sciatic stimulation at C-fiber threshold (> 50 x motor threshold with an
upper limit of 8 mA) was isolated (Figure 4.2). Once a neuron was isolated, responses to
mechanical stimulation (BPPC) of the hindlimb were recorded during successive
brushing of the receptive field using a camel hair paintbrush ("brush"), mild pressure
using a loose arterial clip ("press"), moderate pressure using a tight arterial clamp
("pinch"), and heavy manual pressure using forceps ("crush") with 10 s pauses between
stimuli. Neurons were classified as "low threshold" (LT) if they responded to innocuous
stimuli and / or were inhibited by more noxious stimuli, "wide dynamic range" (WDR) if
they responded to all stimuli in a graded manner, and "nociceptive specific" (NS) if they responded only to "pinch" and / or "crush" stimulation (Figure 4.3b). Although 3 LT neurons were detected along with the targeted WDR or NS neurons being characterized, only WDR and NS neurons were actively sought and recorded during SCS. In addition, only neurons that were excited by sciatic nerve stimulation at C-fiber threshold were recorded. Data from continuous extracellular recordings during all trials were amplified and filtered using an X-Cell amplifier (FHC, Inc.) (gain = 10,000, passband = 500 Hz-2000 Hz), collected using a Powerlab data acquisition system (ADInstruments, Inc.), and sorted post hoc using manual principal component-based clustering in Offline Sorter (Plexon Inc.).

4.2.6. SCS protocol

After sensory characterization of an isolated neuron, we applied both mediolateral configurations of 25 Hz / 40 Hz dfSCS, a combination predicted by the model to inhibit the Gate neuron robustly across conditions, along with several other modes of SCS for comparisons of efficacy with dfSCS (Figure 4.2c). First, to assess the efficacy of dfSCS versus sfSCS, we applied sfSCS at the average of the two constituent frequencies of dfSCS—32 Hz—and sfSCS at the "clinical standard" frequency of 50 Hz (Guan et al., 2010, Song et al., 2014). To determine whether the effects of dfSCS were due primarily to SCS at one of the two constituent frequencies, we delivered all medial
and lateral configurations of individual frequency SCS (iSCS) at the constituent frequencies (i.e., 25 Hz / 0 Hz and 0 Hz / 40 Hz). Finally, to determine if the effects of dfSCS were dependent on the specific frequency pair, we delivered both mediolateral configurations of 15 Hz / 20 Hz dfSCS and the appropriate sfSCS control (17 Hz)—both of which were predicted by the computational model inhibit the Gate neuron less than 25 Hz / 40 Hz dfSCS. Trials of different modes of SCS were order-randomized between neurons and were applied during the middle 20 s of a concurrent 60 stimulation train applied to the sciatic nerve that was identical to that applied to the model. Stimuli were generated using a National Instruments DAQ card (NI-6216e) controlled using MATLAB (Figure 4.2b) and delivered via two battery-powered stimulus isolators (A-M Systems 2200).

**4.2.7. GABAergic modulation**

In a subset of rats, immediately following isolation of a neuron, we administered intrathecally to the lumbar spinal cord 10 μL of 0.3 μg/μL (6 nmol total) bicuculline methiodide (BIC) (Tocris Biosciences), a GABA\(_A\) receptor antagonist, in 0.9 % saline solution using a 250 μL microsyringe (Hamilton, Model 1725) protected from light and positioned within 1mm of the recording site. Dosages were subconvulsive but sufficient to produce behaviors associated with hyperalgesia and allodynia in awake rats (Sivilotti and Woolf, 1994). Ten minutes elapsed before BPPC characterization and sciatic
stimulation tests were conducted to assess the response profile of the neuron as previously described, and the effects of SCS were measured immediately following characterization. Supplemental doses of 10 μL of BIC solution were given every 1-2 hrs to maintain a baseline level of GABAergic antagonism.

4.2.8. SCS response classification and analysis

To determine whether neurons responded to SCS, we constructed post-stimulus time histograms (PSTHs) of each neuron's activity during 50 Hz sfSCS and accounted for stimulus artifacts by applying a 0.5-2ms blanking mask corresponding to the duration of the average stimulus artifact. PSTH bin widths were set to 0.25 ms, as this bin width results in the minimum mean integrated squared error between binned and actual firing rate given the number of stimulation trials in the SCS On period during 50 Hz sfSCS (Shimazaki and Shinomoto, 2007). For comparison of activity between SCS On and SCS Off periods, stimulus times and artifact blanking periods from the SCS On periods were superimposed onto the SCS Off periods, and PSTHs of activity in the SCS Off periods were generated using the virtual stimulus train. We then normalized each neuron's PSTH by expressing the number of spikes/bin in the SCS On condition as Z-scores based on the mean and standard deviation of the neuron's PSTH during a time-matched pre-SCS interval (Montgomery, 2006). A neuron's response to SCS at a specific frequency was defined as significant if the Z-scores from at least 3 contiguous PSTH bins met or
exceeded a magnitude of 1.96, with $Z = 1.96$ corresponding to an individual response magnitude whose likelihood of occurrence was $< 5 \%$ by random chance (Figure 4.3c).

Some neurons, in particular nociceptive-specific neurons, exhibited relatively low firing rates (Craig et al., 2001, Kawamata et al., 2005) and the spikes/bin in these neurons’ PSTHs during both the SCS Off and SCS On conditions were occasionally insufficient to calculate z-scores ($< 4$ spikes/bin) (Dayan, 2001). In those cases, if the total number of spikes exceeded 30 in both the SCS On and SCS Off intervals, the Kolmogorov-Smirnoff (K-S) test (cutoff $p < 0.05$) was used to determine if SCS altered the distribution of post-stimulus responses between the SCS Off and SCS On cases (Xu et al., 2008).

Neuron mean firing rates were calculated by dividing the total number of spikes by 20 s minus the total amount of time included in artifact blanking periods. Because baseline firing rates fluctuated between neurons, and to a lesser extent within neurons, we quantified the effects of SCS on neuron activity using the percent change in firing rates between 20 s on periods and the 20 s off period immediately prior to SCS. Data from WDR and NS neurons were pooled together because the activity of both WDR and NS neurons are correlated with pain behaviors (Simone et al., 1991, Blomqvist and Craig, 2000), and the inhibitory connections onto WDR and NS neurons that are affected by Aβ-fiber inputs appear to be similar (Braz et al., 2014, Luz et al., 2014). However, unless otherwise noted, only WDR and NS neurons that were significantly excited or
inhibited by 50 Hz sfSCS according to the PSTH Z-score test and inhibited by at least one medial/lateral configuration of 25 Hz / 40 Hz dfSCS were included in our statistical analyses, as the computational network model most likely depicts such neurons. To quantify the efficiency of dfSCS relative to 50 Hz sfSCS, we estimated the ratio of energy delivered by dfSCS and sfSCS, henceforth "% energy efficiency," across all individual neurons (Equation 4.3). A negative % indicates that dfSCS uses less energy, a positive % indicates that dfSCS uses more energy, and 0 % indicates that dfSCS uses the same energy as 50 Hz sfSCS. For rectangular biphasic pulses, energy is proportional to the product of the square of the amplitude of the applied current (I1, I2 for dfSCS constituent frequencies, I_dual for 50 Hz), the stimulation frequencies (dfSCS1, dfSCS2, 50), and the pulse width (Wongsarnpigoon et al., 2010); the pulse widths used in dfSCS and sfSCS were the same and were therefore not included in equation 4.3:

\[
\text{% Energy Efficiency} = \frac{\text{dfSCS1} + \text{dfSCS2}}{\text{sfSCS1} + \text{sfSCS2}}
\]  
(4.3)

Statistical comparisons of neuron responses between experimental conditions and energy consumption versus 50 Hz sfSCS were made using the non-parametric paired Wilcoxon Signed-Rank test.
4.3. Results

We used a validated computational model based on the Gate Control Theory to determine combinations of dual frequency SCS (dfSCS) that inhibited the model spinal sensory neuron more than single frequency SCS (sfSCS) at the average of the constituent frequencies. We then evaluated computationally the efficacy of dfSCS across a range of stimulation conditions, and we experimentally measured the responses of spinal sensory neurons to dfSCS in anesthetized rats.

4.3.1. Computational analysis of idealized dual frequency SCS

We quantified the activity of the model Gate neuron during dfSCS consisting of independent but concurrent stimulation of 2 groups of 15 Aβ fibers with constituent frequencies ranging from 1 - 70 Hz. dfSCS produced greater inhibition of the model Gate neuron (Equation 4.1) for all combinations tested, but the degree of inhibition versus sfSCS at the average of the frequency pair (Equation 4.2) depended strongly on the constituent frequencies (Figures 4.4a-4.4b). dfSCS using constituent frequencies whose averages ranged from 20 - 40 Hz and using non-harmonic constituent frequencies (i.e., not factor-multiple pairs) that differed by > 5 Hz produced the most inhibition. For example, 25 Hz / 40 Hz dfSCS produced a -95.8 % absolute effect and a -63.2 % relative effect vs. 32 Hz sfSCS. Non-harmonic dfSCS pairs using constituent frequencies with averages < 20 Hz and > 40 Hz, using constituent frequencies that were < 5 Hz, or using
constituent frequencies that differed by < 5 Hz, produced inhibition of the Gate neuron that was equivocal with the effects of sfSCS. 15 Hz / 20 Hz SCS produced a -53.8% absolute effect but a +6.5% relative effect versus 17 Hz sfSCS. Single frequency SCS above 38 Hz produced -80 % or greater absolute effects, and as a result, dfSCS pairs using constituent frequencies with averages > 38 Hz were relatively not as effective. Finally, dfSCS using harmonic frequency pairs (i.e., factor-multiple pairs) produced less inhibition than sfSCS. The effects of dfSCS using harmonic frequency pairs were similar to the effects of SCS using the higher constituent frequency applied only to half of the dorsal column inputs and resulted in less activation of inhibitory interneurons than sfSCS at the average of the constituent frequencies. For example, 20 Hz / 60 Hz SCS produced a -64.9% absolute effect but a +16.4 % relative effect versus 40 Hz sfSCS.

4.3.2. Effects of imperfect selectivity

We quantified the effects of non-ideal selectivity among the fibers receiving dfSCS by delivering both constituent frequencies concurrently (i.e., as if both electrode pairs activated the fibers) to 20/30 Aβ-fiber inputs to the network; an equal number of local (10) and surround (10) Aβ-fiber inputs received both frequencies (Figure 4.5a). In contrast to the default case, dfSCS using harmonic frequency pairs performed better when selectivity was imperfect: 20 Hz / 60 Hz dfSCS produced a -96.0% absolute and a -5.3 % relative effect versus 40 Hz sfSCS. dfSCS using constituent frequencies whose
averages ranged from 15 - 30 Hz and dfSCS using constituent frequencies whose averages were > 40 Hz performed worse when selectivity was imperfect: for example, 15 Hz / 20 Hz dfSCS provided a -35.2% absolute effect but a +26.2 % relative effect versus 17 Hz sfSCS. Importantly, the performance of some dfSCS frequency pairs, such as dfSCS using constituent frequencies whose averages ranged from 30 - 40 Hz and dfSCS frequency pairs with common factors, were robust to non-ideal selectivity: 25 / 40 Hz dfSCS still produced a -85.9 % absolute effect and a -53.2 % relative effect versus 32 Hz sfSCS. These results indicate that imperfect selectivity can have substantial effects on the efficacy and relative benefits of dfSCS but that some frequency pairs are robust to this condition.

4.3.3. Effects of local-surround bias

We quantified the effects of bias in the proportion of local inputs vs. surround inputs activated between frequencies in each dfSCS pair by skewing the proportion of local Aβ inputs to surround Aβ inputs activated by each constituent frequency from 8:7 to 12:3 for one frequency and from 7:8 to 3:12 for the other frequency (Figure 4.5c). The performance of dfSCS remained the same or improved versus sfSCS when more surround inputs received the higher frequency of the pair. The performance of dfSCS declined versus sfSCS applied at the constituent frequency average when the higher frequency, usually > 30 Hz, was delivered to 12 of the local inputs and when the lower
frequency, usually < 25 Hz, was delivered to 12 of the surround inputs. For example, 20 Hz / 60 Hz dfSCS produced a -7.8% relative effect versus 40 Hz sfSCS when 60 Hz was preferentially delivered to the surround inputs, but 20 Hz / 60 Hz dfSCS produced a +58.4% relative effect vs. 40 Hz sfSCS when 60 Hz was delivered preferentially to the local inputs. However, the performance of dfSCS when the frequency delivered to the surround inputs was > 25 Hz was generally similar to that in the default condition and therefore robust to local-surround bias, as inhibitory effects from activation of the surround inputs were still sufficient to inhibit the activity of the Gate neuron despite increased excitation from local inputs. For example, 25 Hz / 40 Hz SCS produced a -57.0% relative effect versus 32 Hz sfSCS when 12 surround inputs received 40 Hz and a -38.2% relative effect versus 32 Hz sfSCS when 12 local inputs received 40 Hz SCS (Figure 4.5d), supporting the robustness of 25 Hz / 40 Hz dfSCS to changes in local-surround bias.

4.3.4. Effects of disease progression

We quantified the effects of the loss of GABAergic inhibition in the dorsal horn that accompanies the progression to chronic neuropathic pain (Zeilhofer et al., 2012, Braz et al., 2014) on the performance of dfSCS by weakening the GABAergic synapses in the model (Figure 4.6A, 6C). To simulate an early disease state, we first reduced by 50% the strengths of the GABAergic synapses from the local inhibitory interneuron onto the excitatory interneuron and the Gate neuron (Figure 4.6a). Reducing local inhibition
substantially diminished the reduction in Gate neuron firing rate by sfSCS: the maximum % absolute effect on Gate neuron activity provided by sfSCS over 1 Hz – 70 Hz decreased from -95.2% in the default state to -68.2 %. However, Gate neuron inhibition by dfSCS was quite robust and followed similar trends as in the default case, as inhibition due to activation of surround input activation was sufficient to overcome the loss of local inhibition (Figure 4.6b). For example, 25 Hz / 40 Hz dfSCS still produced a -86.7 % absolute effect, 15 Hz / 20 Hz dfSCS still produced a -56.0 % absolute effect, and 20 Hz / 60 Hz dfSCS still produced a -60.6% absolute effect.

The reduction by 50% of both local and surround inhibition onto the Gate neuron, representing an advanced neuropathic pain state, resulted in substantially less suppression of the Gate neuron by all modes of SCS (Figure 4.6d). Following the reduction of GABAergic inhibition, the maximum drop in Gate neuron firing rate produced by sfSCS decreased to -59.4 % from -95.2 % in the default model, and the frequency at which maximal inhibition by sfSCS occurred shifted to 17 Hz from 66 Hz, the latter now generating an +128.2 % absolute effect on the activity of the Gate neuron. Additionally, the efficacy of dfSCS was much more sensitive to even a difference of one fiber between the local and surround inputs activated by each frequency, and both dfSCS using constituent frequencies with averages exceeding 57 Hz and sfSCS at
frequencies > 57 Hz now excited the Gate neuron. Despite these changes, some dfSCS frequency pairs, in particular dfSCS using constituent frequencies that differed by < 25 Hz and dfSCS using constituent frequencies whose averages ranged from 35 - 50 Hz, continued to inhibit the Gate neuron; 25 Hz / 40 Hz SCS still produced a -32.9 % absolute effect. Other dfSCS frequency pairs continued to inhibit the Gate neuron more effectively than sfSCS using the constituent frequency average; for example, 30 Hz / 45 Hz dfSCS produced a -65.1 % absolute effect on the activity of the Gate neuron and a -38.3 % relative effect versus 37 Hz sfSCS. That some pairs of dfSCS still inhibited the Gate neuron and produced more inhibition of the Gate neuron than sfSCS suggests that dfSCS may be a strategy to overcome the loss of SCS efficacy that occurs during the progression of neuropathic pain (Kumar et al., 2008).

4.3.5. Experimental measurement of the effects of dfSCS

We assessed the utility of dfSCS by recording and quantifying the activity of wide dynamic range (WDR) and nociceptive-specific (NS) spinal sensory neurons in response to dfSCS and appropriate controls. We used 25 Hz / 40 Hz dfSCS, as this combination inhibited the activity of the model Gate neuron, produced greater inhibition than 32 Hz sfSCS, and was relatively robust to non-ideal selectivity and local-surround bias.
We isolated and recorded the responses of 21 spinal WDR and NS neurons to both mediolateral configurations of 25 Hz / 40 Hz dfSCS during concurrent C-fiber threshold stimulation of the sciatic nerve and quantified the absolute effects (Equation 1) and the relative effects on sensory neuron activity compared to sfSCS applied at the average of the constituent frequencies (32 Hz) and sfSCS applied at a standard clinical frequency (50 Hz) (Figure 4.7a). 15/21 neurons were inhibited by at least one mediolateral configuration of 25 Hz / 40 Hz dfSCS, 5 neurons were excited by both mediolateral configurations of dfSCS, and 1 neuron was unresponsive to SCS. Although 5 neurons, including 3 WDR neurons and 2 NS neurons, were excited by dfSCS, there were no cases when either 32 Hz or 50 Hz sfSCS had an inhibitory effect while dfSCS had an excitatory effect. Although 50 Hz sfSCS produced a greater inhibitory effect than dfSCS in 6 neurons, dfSCS had an inhibitory effect in 3 WDR neurons for which either 32 Hz or 50 Hz sfSCS had excitatory effects.

Dual frequency SCS inhibited sensory neuron activity (-57.8 % ± 6.9 % absolute effects, mean ± s.e.m) significantly more than 32 Hz sfSCS (-34.0 % ± 10.7 % absolute effects, p = 0.015, Wilcoxon Signed-Rank test, Figure 4.7b). dfSCS also produced substantially more inhibition than 50 Hz sfSCS (-35.3 ± 16.9 % absolute effect, p = 0.12, Figure 4.7b). Furthermore, dfSCS inhibited the Gate neuron using significantly less energy than 50 Hz sfSCS (Equation 3, -45.0 % ± 4.22 % energy consumption, p = 6.1X10^-
Finally, there was a striking parallel between model predictions of the efficacy of 25 Hz / 40 Hz dfSCS versus 32 Hz sfSCS and 50 Hz sfSCS and the effects measured experimentally (Figure 4.7c).

4.3.6. Comparison of dfSCS to ifSCS at the constituent frequencies

To determine whether the effects of dfSCS were predominately due to the effects of one of the two constituent frequencies, we compared the effects of 25 Hz / 40 Hz dfSCS with the effects of SCS through individual electrode pairs using each of the constituent frequencies (ifSCS) in 13/15 neurons inhibited by dfSCS. The pairs of electrodes used to deliver 25 Hz or 40 Hz ifSCS had the same mediolateral configuration as the configuration of 25 Hz / 40 Hz dfSCS that provided maximal inhibition. For example, if 25 Hz medial/40 Hz lateral dfSCS produced more inhibition, then dfSCS was compared to 25 Hz medial ifSCS and 40 Hz lateral ifSCS (Figures 4.2c, 4.8a). Maximal inhibition by dfSCS varied across but did not depend on the mediolateral configuration of dfSCS: in 6/13 neurons, the 25 Hz medial/40 Hz lateral configuration of dfSCS produced more inhibition, and in 7/13 neurons, the 40 Hz medial/25 Hz lateral configuration of dfSCS produced more inhibition. 25 Hz / 40 Hz dfSCS inhibited the sensory neuron (-56.3 % ± 7.8 % absolute effects) more than 25 Hz ifSCS (-2.98 ± 15.8 % absolute effects, p = 4.9X10^-4, Figure 4.8b) in all but 1/13 neurons and more than 40 Hz ifSCS (-28.4 ± 9.6 %, p = 0.034, Figure 4.8b) in 9/13 neurons. The mean reduction in
activity, measured using % absolute effect, during dfSCS was greater than the arithmetic sum of the inhibition produced individually by 25 Hz and 40 Hz ifSCS in 9/13 neurons. Again, there was a striking parallel between the measured effects of dfSCS and ifSCS on spinal sensory neurons and the predictions of the computational model (Figure 4.8c).

4.3.7. Comparison to suboptimal dfSCS

To test the predictions from the computational model that the inhibition generated by dfSCS depends on the constituent frequencies, we selected a dual frequency pair (15 Hz / 20 Hz) predicted to be less effective than 25 Hz / 40 Hz dfSCS at inhibiting Gate neuron activity. We compared inhibition of spinal sensory neuron activity by 15 Hz / 20 Hz dfSCS versus inhibition produced by the appropriate sfSCS control (17 Hz) and 25 Hz / 40 Hz dfSCS in 12/15 neurons (Figure 4. 9A). Consistent with model predictions (Figure 4. 9C), 15 Hz / 20 Hz dfSCS produced more inhibition than 17 Hz sfSCS (p = 0.065) but produced less inhibition than 25 Hz / 40 Hz dfSCS (p = 0.042) (Figures 4.9b, 4.9c). In addition, 25 Hz / 40 Hz dfSCS inhibited sensory neuron activity more than 17 Hz sfSCS despite the average of the constituent frequencies being higher (p = 0.018), while 32 Hz sfSCS did not produce significantly more inhibition than 17 Hz sfSCS (p = 0.47). This latter observation indicates that the greater inhibition produced by 25 Hz / 40 Hz dfSCS is not simply due to an increase in the average of the constituent frequencies but rather required concurrent dual frequency stimulation.
4.3.8. Effects of GABAergic modulation

We quantified the effects of reduced GABAergic inhibition on the performance of dfSCS by recording the responses of 4 WDR and 3 NS neurons to dfSCS following intrathecal administration of BIC. We recorded responses to 25 Hz / 40 Hz dfSCS, 30 Hz/45 Hz dfSCS, selected because the model predicted that the efficacy of the pair would surpass that of 25 Hz / 40 Hz dfSCS following loss of GABA, and sfSCS controls at the averages of the respective constituent frequencies (32 Hz, 37 Hz) and at 50 Hz (Figure 4.6d). 4/7 neurons recorded after BIC application were inhibited by dfSCS, and all neurons that were excited by dfSCS were also excited by all frequencies of sfSCS. Both 25 Hz / 40 Hz dfSCS (Figure 4.10a) and 30 Hz / 45 Hz dfSCS (Figure 4.10b) produced more inhibition than each of their respective sfSCS controls (32 Hz, 37 Hz) and 50 Hz sfSCS in all neurons (Figure 4.10c). Although, 30 Hz/45 Hz dfSCS produced greater inhibition than 25 Hz / 40 Hz dfSCS in 3/4 neurons, as predicted by the model, additional neurons must be recorded to verify this trend.

4.4. Discussion

The objective of this study was to assess the utility of dual frequency SCS, in which two distinct frequencies are delivered concurrently to the dorsal columns, to increase the efficacy of SCS. Simulations of dfSCS using a validated computational model of the Gate Control circuit identified frequency pairs that were more effective at
suppressing sensory model neuron activity than sfSCS at the average of the constituent frequencies or at the clinical standard of 50 Hz. Further, sensitivity analyses revealed that appropriately selected frequency pairs maintained efficacy across non-ideal stimulation conditions and after loss of GABAergic inhibition associated with the progression of chronic pain. Experimental assessments of dfSCS in an anesthetized rat confirmed model predictions of the superior efficacy of dfSCS versus sfSCS at the average of the constituent frequencies and at 50 Hz, and in the case of the latter, greater efficacy by dfSCS was achieved using less energy. As well, the experimental results confirmed model predictions that dfSCS produced greater inhibition than SCS at either of the constituent frequencies through individual electrode pairs. These computational and in vivo data support the efficacy of dfSCS, a novel method for implementing SCS, that, unlike prior approaches to improve SCS, relies on the temporal properties of stimulation and the response to SCS of dorsal horn neural circuits underlying nociceptive processing.

4.4.1. Mechanisms and utility of dual frequency SCS

Dual frequency SCS produced greater inhibition of spinal sensory neurons, both in silico and in vivo, than sfSCS at the average of the two constituent frequencies. The interaction between excitatory and inhibitory inputs from Aβ-fiber afferents onto WDR and possibly NS neurons (Foreman et al., 1976a, Luz et al., 2014) underlies stimulation
frequency-dependent responses to SCS (Zhang et al., 2014b). In dfSCS, the two constituent frequencies each activate inhibitory mechanisms that suppress the activity of the Gate neuron. However, unlike in sfSCS, the total excitation onto the Gate neuron from activation of local Aβ-fiber afferents is split between the two constituent frequencies and therefore not as strong. As a result, SCS-evoked excitation is not as strong as in sfSCS, and inhibition of the Gate neuron by dfSCS is greater. Support for this mechanism comes from the experimental observation that in 6/9 neurons for which 25 Hz / 40 Hz dfSCS produced greater inhibition than 50 Hz sfSCS, sfSCS produced an excitatory peri-stimulus response (e.g., Figure 4.3c). This includes 3 neurons for which peri-stimulus excitation by 50 Hz sfSCS was sufficient to produce a net increase in the firing rate of the sensory neuron, suggesting that dfSCS may also reduce sensory side effects associated with 50 Hz sfSCS, such as unpleasant paresthesias (Shealy et al., 1972), whose mechanisms may be related to spinal sensory neuron activity (Simone et al., 1991). Taken together, these computational and experimental data support dfSCS as a strategy to improve the efficacy of SCS by exploiting the interaction of excitatory and inhibitory effects of SCS through manipulation of the temporal pattern of the stimulation train.

The finding that dfSCS produced equal or greater inhibition of sensory neuron activity as clinical standard 50 Hz SCS, but at lower average frequencies, suggests that
dfSCS may also improve the energy efficiency of SCS. In sfSCS, greater inhibition is required to overcome excitation from activation of local Aβ-fiber inputs, but the only way to generate more inhibition using sfSCS is to increase the stimulation frequency. Higher frequencies of stimulation require more energy, and higher energy consumption results in shorter battery life, more frequency battery replacement procedures for non-rechargeable implantable pulse generators (IPGs), and shorter intervals between recharging sessions for rechargeable IPGs. In contrast, dfSCS produces more inhibition at lower average stimulation frequencies because less additional activation of inhibitory surround inputs is required to overcome SCS-mediated excitation than in sfSCS (Zhang et al., 2014b). Experimentally, 25 Hz / 40 Hz dfSCS consumed on average 46 % less energy than clinical standard 50 Hz sfSCS, even after accounting for individual electrode pair amplitudes (Figure 4.3a), suggesting that dfSCS may also be a more energy efficient approach to SCS.

The total inhibitory effect of dfSCS could not be reproduced via stimulation using either of the constituent frequencies delivered individually to half of the dorsal column fibers (ifSCS). Although ifSCS inhibits the Gate neuron while only affecting half of the excitatory inputs activated by sfSCS, all inhibitory events are still time locked to a preceding excitatory event in ifSCS, making it more challenging to overcome inhibition. In contrast, during dfSCS, inhibition due to stimulation of half of the Aβ-fibers at one
frequency suppresses the excitation due to stimulation of Aβ-fibers at the other frequency, preventing excitatory events that are time locked to SCS from overcoming overall inhibition. This mechanism also explains why frequency pairs that are not factors or multiples of each other suppress the Gate neuron more effectively than factor/multiple frequency pairs in most simulations of dfSCS. Further, these results indicate that asynchronous activation of populations of dorsal column collaterals from Aβ-fibers may represent a general strategy to increase the efficacy of SCS, and the asynchronous excitation of the dorsal columns may also reduce paresthesias.

4.4.2. Robustness of dfSCS across conditions

The effects of dfSCS with appropriately selected frequency pairs were preserved under conditions of imperfect selectivity between the populations receiving each of the two constituent frequencies or bias in the proportion of local to surround receptive field inputs receiving each constituent frequency. In simulations of imperfect selectivity, the network received the merged pulse trains from each constituent frequency in the dfSCS pair through 66% of the inputs. When the two frequencies were not factors or multiples of each other, excitatory events from closely spaced pulses in the composite pulse train were occasionally close enough to each other to excite the Gate neuron, producing less overall inhibition. However, when frequencies shared common factors or were multiples of each other, the composite pulse train was more regular, there were fewer
short inter-pulse intervals, and dfSCS inhibited Gate neuron activity without evoking more excitation. In simulations of the effects of local-surround bias, dfSCS was sometimes no more effective than ifSCS using the higher frequency, as activation of inputs from inhibitory surround receptive fields does not excite the local Gate neuron. However, activation of surround inputs is likely to activate neighboring Gate neurons via excitatory synapses (Hillman and Wall, 1969), whereas the inhibition from each constituent frequency in a dfSCS pair may suppress excitation due to the other frequency via inhibitory surround inputs, resulting in less excitation across a population of Gate neurons. Nonetheless, some frequency pairs were robust to these imperfect conditions, and 25 Hz / 40 Hz SCS performed consistently with model predictions in most individual neurons across experiments with different stimulation amplitudes and electrode placements, further demonstrating the potential utility of dfSCS.

4.4.3. dfSCS following loss of GABAergic inhibition

GABAergic inhibition plays an important role in spinal nociceptive processing, as blocking GABAergic inhibition in the dorsal horn unmaskspain-related behaviors in otherwise healthy rats (Yaksh, 1989, Sivilotti and Woolf, 1994), and the development of pathological hyperaglesia and allodynia in animal models of neuropathic pain has been linked to the reduction of GABAergic inhibition in the spinal cord (Moore et al., 2002a, Zeilhofer et al., 2012, Braz et al., 2014). Weakening the GABAergic synapses lessened the
inhibition of the Gate neuron by SCS and unmasked excitation, resulting in increased firing during clinical standard 50 Hz sfSCS, thus providing a possible mechanism for the clinically observed decline in SCS efficacy over time (Kumar et al., 2007). However, some frequency combinations, including 25 Hz / 40 Hz dfSCS chosen for experimental assessments, continued to inhibit the Gate neuron after attenuation of GABAergic inhibition. Other frequency combinations, such as 30 Hz / 45 Hz dfSCS, performed more effectively than both sfSCS applied at the average of the constituent frequencies (37 Hz) and at 50 Hz.

Remaining GABAergic inhibition and glycinergic inhibition not affected by the progression of neuropathic pain (Braz et al., 2014) underlie the persistent inhibition of the Gate neuron by dfSCS even after both local and surround inhibition were weakened. The important contribution of preserved glycinergic inhibition in SCS-mediated inhibition was supported by the observation after application of BIC of transient post-stimulus inhibition during SCS, with a time course consistent to that of glycinergic synapses in the spinal cord (Figure 4.10d) (Moore et al., 2002b). In addition to confirming model predictions of the possible role of glycinergic inhibition in dfSCS, this observation suggests that dfSCS may be a means to exploit glycinergic mechanisms in the spinal cord that are not affected by the progression of chronic pain and, thus, dfSCS may be more effective in treating advanced neuropathic pain than sfSCS.
4.4.4. General limitations

The experimental assessments of dfSCS were conducted in an acute preparation using healthy rats, while clinical SCS is used to treat chronic neuropathic pain. Although the loss of GABA during the progression of chronic pain was incorporated into computational and experimental studies of dfSCS through modulation of GABAergic inhibition, other changes involved in the transition from the healthy state to neuropathic pain, such as anatomical changes within the spinal sensory network (Von hehn et al., 2012), glial cell activation (Ji et al., 2013), and modifications in synaptic dynamics due to loss of KCC2 transporter function (Coull et al., 2003) were not fully represented in the computational model or experiments. Behavioral studies in an animal model of neuropathic pain, such as the spared nerve injury model (Decosterd and Woolf, 2000), are needed to determine the effectiveness of dfSCS in the presence of all the plastic changes that occur during the transition to neuropathic pain, and clinical assessments of dfSCS are needed to translate this innovative approach to practice.

In addition, we had limited control of electrode position and no ability to select contacts within an array, and thus the selectivity by each electrode pair and local/surround bias during experimental dfSCS were unclear. Stimulation intensity was determined systematically and consistently using CDPs, whose amplitudes represent the proportion of dorsal column fibers activated by SCS (Honmou et al., 1996), but CDPs
do not provide sufficient spatial information to discern the extents to which inputs from local and surround receptive fields of a sensory neuron were activated by SCS. The degree of inhibition of sensory neuron activity by SCS is sensitive to the balance of local excitatory and surround inhibitory inputs received by the neuron. Therefore, understanding the correspondence between dorsal column fiber populations and receptive field organization in the dorsal horn and using spatially selective electrodes capable of targeting specific dorsal column fibers (Sankarasubramanian et al., 2011) may enable further improvement of dfSCS.

We did not antidromically identify the recorded neurons explicitly as projection neurons that represent the overall output of the sensory spinal cord network, and 95% or more of all spinal sensory neurons are interneurons (Todd, 2010). Although responses were heterogeneous, the neurons we analyzed were all either WDR or NS neurons, and all were excited by C-fiber threshold sciatic stimulation. The neurons that were excited by SCS may also have been inhibitory interneurons receiving Aβ-fiber inputs (Dubuisson, 1989, Baba et al., 2003), and interneurons receiving predominately non-nociceptive afferent inputs may inhibit pronociceptive excitatory interneurons or projection neurons according to the original Gate Control Theory (Melzack and Wall, 1965) and more recent work (Luz et al., 2014, Braz et al., 2014, Prescott et al., 2014). That 3 additional neurons excited by dfSCS were LT neurons that responded predominately
to "brushing" of the neuron's hindpaw receptive field, i.e., activation of non-nociceptive afferents, supports this possibility. In addition, "Gate" interneurons may play an important role in modulating the activity of nociceptive-specific "labeled line" circuits (Luz et al., 2014) and in segregating nociceptive and non-nociceptive inputs in the dorsal horn (Braz et al., 2014, Prescott et al., 2014). The progression of neuropathic pain involves the disinhibition of these intervening interneurons (Braz et al., 2014), and SCS may act through modulation of the activity of both interneurons and spinal projection neurons. Understanding the specific aspects of nociception encoded by the activity of sensory interneurons and reconciling the activity of these neurons with sensory projection neuron activity, in particular Lamina I NS projection neurons and Lamina IV/V WDR neurons (Todd, 2010, Braz et al., 2014), may provide further insight into the mechanisms underlying dfSCS.

Finally, we did not account for the effects of SCS on supraspinal circuits (Heinricher et al., 2009) or on descending pathways, such as the dorsolateral funiculus, that may be involved in the effects of SCS. Although segmental mechanisms are sufficient in describing inhibitory and excitatory responses to SCS (Foreman et al., 1976a, Smits et al., 2012), supraspinal loops may also modulate analgesia by SCS (Barchini et al., 2012). For example, neurons in supraspinal nuclei exhibited more activity during SCS in neuropathic rats that showed increased paw withdrawal thresholds than in non-
responding rats, suggesting that effective SCS for chronic pain requires supraspinal modulation (Song et al., 2013a), but knowledge regarding the extent of supraspinal contributions to the effects of SCS is sparse.
Figure 4.1 Computational modeling of the effects of dual frequency SCS (dfSCS) on the activity of the spinal Gate neuron.

dfSCS is applied concurrently with random peripheral afferent activity with statistical properties consistent with primary afferent activity following nerve injury. The changes in Gate neuron activity produced with dfSCS by all integer combinations of frequency 1 and frequency 2, each ranging from 1 Hz to 70 Hz, were quantified.
Figure 4.2 Recording the responses of single lumbar dorsal horn neurons to epidural SCS in urethane-anesthetized rats.

(a): Rats were implanted with a nerve cuff on the sciatic nerve for peripheral stimulation and a two-channel bipolar paddle electrode in the epidural space for SCS. Mechanical stimuli ("Brush, Press, Pinch, Crush;" BPPC) were applied to the hindpaw ipsilateral to
the recording electrode to characterize neuron receptive field and response properties. The four contact SCS electrode comprising two rostrocaudally oriented bipolar electrode pairs is comparable in dimensions to the electrodes used in Chapter 3 and was used to deliver dfSCS (Inset). (b): Timeline of experimental recordings: Neuron characterization preceded randomized blocks of SCS. (c): Experimental comparisons to assess the utility of dfSCS. 25 Hz / 40 Hz dfSCS was tested against single frequency SCS delivered simultaneously through both electrode pairs (sfSCS) at the constituent frequency average of 32 Hz, sfSCS at the clinical standard 50 Hz, and individual frequency SCS delivered through individual electrode pairs using one or the other constituent frequency (ifSCS). A suboptimal dfSCS pair (15 Hz / 20 Hz) was tested against sfSCS at its constituent frequency average (17 Hz) and optimal 25 Hz / 40 Hz dfSCS.
Figure 4.3 Systematic selection of SCS amplitude using cord dorsum potentials, and characterization of neuronal responses to peripheral stimulation and SCS.

(a): Recordings of cord dorsum potentials (CDP) were used to set the amplitudes of SCS. The amplitude of sfSCS, with both bipolar pairs set to the same amplitude, was set to the minimum required to evoke a 50 μV or greater CDP. To match the half-activation of the dorsal columns by each constituent frequency, the amplitudes of each bipolar pair for use with dfSCS and ifSCS were set to be the minimum required to evoke a 25 μV or greater CDP. (b): The effects of SCS were measured in wide dynamic range (WDR) neurons (top) that responded in a graded manner to brush, press, pinch, crush and nociceptive-specific (NS) neurons (bottom) that were preferentially responsive to pinch and crush. (c): Peri-stimulus analysis using Z-scores was used to determine if neurons responded to 50 Hz sfSCS. Neurons that exhibited significant (|z|>1.96) responses in three or more contiguous bins were characterized as responsive to SCS. The Z-score normalized PSTH shown is truncated to ±5 to allow visualization of the inhibitory response.
Figure 4.4 Computational modeling of the effects of dfSC on the activity of the output Gate neuron.

(b): Representative examples of the effects of dfSCS quantified by calculating the absolute effect in the firing rate of the Gate neuron during 20 s of dfSCS as compared to the firing rate during 20 s prior to SCS (Equation 1) and the relative effect versus the change in firing rate produced by sfSCS using the average of the two constituent frequencies (Equation 2).  
(c): % absolute effect on firing rate of the Gate neuron by dfSCS at all integer combinations of frequency 1 and frequency 2, from 1 - 70 Hz.  
(d): % relative effect in firing rate of the Gate neuron versus sfSCS at the average of the two constituent frequencies of each dfSCS pair.
Figure 4.5 Model-based analysis of the effects of non-ideal selectivity and local/surround bias.

(b): % absolute effects (left) and % relative effects (right) versus sfSCS on Gate neuron activity during dfSCS with imperfect selectivity. (d): % absolute effects (left) and % relative effects (right) versus sfSCS on Gate neuron activity during dfSCS with local-surround bias.
Figure 4.6 Model-based analysis of the effects of a 50 % reduction in GABAergic inhibition on the efficacy of dfSCS.

(a): Effects of a 50 % reduction in local GABAergic inhibition representing early disease state. (b): % absolute effects (left) and % relative effect (right) versus sfSCS on Gate neuron activity when local GABAergic inhibition was reduced by 50 %. (c): Effects of a 50 % reduction in both local and surround GABAergic inhibition representing advanced disease state. (d): % absolute effects (left) and % relative effect (right) versus sfSCS on Gate neuron activity when local and surround GABAergic inhibition was reduced by 50 %.
Figure 4.7 Experimental assessments of the utility of dfSCS versus sfSCS during concurrent sciatic stimulation.

(a): Comparisons of 25 Hz / 40 Hz dfSCS with 32 Hz sfSCS and 50 Hz sfSCS. (b): Paired comparisons of individual neuron responses to dfSCS versus 32 Hz sfSCS and 50 Hz sfSCS (*p<0.05, Wilcoxon Signed-Rank Test). Dotted line denotes no effect in sensory neuron firing rate by SCS. (c): Model prediction of effects of dfSCS and sfSCS Gate on neuron firing rate (left) compared with experimental responses (mean ± s.e.m.) to dfSCS and sfSCS (right).
Figure 4.8 Experimental assessments of the utility of dfSCS versus ifSCS during concurrent sciatic stimulation.

(a): Comparisons of 25 Hz / 40 Hz dfSCS with 25 Hz ifSCS and 40 Hz ifSCS. The mediolateral configurations of ifSCS were the same as the configuration of dfSCS that produced the most inhibition. (b): Paired comparisons of individual neuron responses to dfSCS versus ifSCS using each of the constituent frequencies (*p<0.05, **p<0.01, Wilcoxon Signed-Rank Test). (c): Model prediction of effects of dfSCS and ifSCS on Gate neuron firing rate (left) compared with experimental responses (mean ± s.e.m.) to dfSCS and ifSCS (right).
Figure 4.9 Experimental assessments of the utility of optimized versus suboptimal dfSCS during concurrent sciatic stimulation.

(a): Comparisons of suboptimal 15 Hz / 20 Hz dfSCS with 17 Hz sfSCS and 25 Hz / 40 Hz dfSCS. (b): Paired comparisons of individual neuron responses to suboptimal dfSCS versus sfSCS and optimized dfSCS (*p<0.05, Wilcoxon Signed-Rank Test). (c): Model prediction of effects of suboptimal dfSCS, sfSCS, and optimal dfSCS on Gate neuron firing rate (left) compared with experimental responses (mean ± s.e.m.) to suboptimal dfSCS, sfSCS, and optimized dfSCS (right).
Figure 4.10 Experimental assessment of the effect of intrathecal administration of bicuculline on the utility of dfSCS.

(a): % absolute effects on activity of 4 spinal sensory neurons of 25 Hz / 40 Hz dfSCS, 32 Hz sfSCS, and 50 Hz sfSCS following application of BIC. (b): % absolute effects of 30 Hz/45 Hz dfSCS, 37 Hz sfSCS, and 50 Hz sfSCS following application of BIC. (c): % absolute effects of 25 Hz / 40 Hz dfSCS, 30 Hz/45 Hz dfSCS, and 50 Hz sfSCS. (d): Representative peri-stimulus response of a WDR neuron during 50 sfSCS, which excited the neuron (i.e., a positive % absolute effect). This neuron was inhibited by both frequency pairs of dfSCS.
5. Conclusions

5.1. Summary of Results

Spinal cord stimulation (SCS) is an increasingly prevalent therapy for the treatment of intractable chronic pain: between 14,000 and 50,000 individuals receive implants annually in North America alone, and SCS comprises a $1.8 billion market worldwide (Neurotech Reports, 2014). However, the mechanisms underlying the neuronal effects of SCS are poorly understood, and efforts to improve therapy focus on the hardware (e.g., implantable pulse generator, stimulation leads, programmer) used to deliver SCS rather than determining and exploiting the underlying physiological mechanism(s) for therapy. The lack of progress in understanding of the neurophysiological mechanisms underlying pain and the effects of SCS contribute to the stagnant efficacy of SCS since its inception over 40 years ago (Chapter 1) (Taylor et al., 2013, Zhang et al., 2014a).

The objectives of this dissertation were to provide a greater understanding of the mechanisms underlying SCS through the development of computational and experimental models of SCS and to devise novel strategies for the optimization of SCS through a model-based design approach. These objectives were met through 3 specific aims: 1) the development of a biophysically accurate computational model of the spinal cord pain processing circuit that can be used to model the effects of SCS on sensory...
neurons, 2) the \textit{in vivo} validation of computational modeling predictions of the effects of SCS on spinal sensory projection neurons, and 3) the computationally driven design and experimental validation of novel temporal patterns of SCS.

A computational model consisting of individual spinal neurons with realistic biophysics connected using an architecture based on the Gate Control Theory (Melzack and Wall, 1965) reproduced neuronal responses to peripheral stimulation important to pain processing, notably wind-up and A-fiber mediated inhibition (Chapter 2) (Zhang et al., 2014b). Computational analysis predicted that responses to SCS are dependent on stimulation frequency, inhibition due to activation of dorsal column fibers from surrounding receptive fields, and the level of GABAergic inhibition in the spinal cord (Chapter 2). Computational model predictions were partially verified through single unit recordings of antidromically identified sensory spinal projection neurons during different frequencies of SCS, and some neurons responded in a manner consistent with the Gate Control Theory (Chapter 2, Chapter 3). Although the Gate Control Theory could not account for other heterogeneous responses, computational models of spinal microcircuits representing distinct sensory computations could reproduce the full gamut of experimentally measured frequency response relationships (Chapter 3). Finally, dual frequency SCS, formed by applying two pulse trains at distinct frequencies simultaneously to two distinct populations of dorsal column fibers, was designed using
the computational model to be more effective than constant frequency SCS (Chapter 4). Experimental assessments of dual frequency SCS in an acute anesthetized rat preparation confirmed model predictions of the improved efficacy of select frequency pairs of dual frequency SCS, supporting this as a novel strategy for improving therapy (Chapter 4). The outcomes of this dissertation are a greater understanding of the neurophysiological mechanisms underlying pain relief by SCS and a novel biophysically based strategy that may improve the clinical efficacy of SCS and thereby the quality of life of individuals with chronic pain.

5.1.1. Aim 1: Development of a computational model of the dorsal horn pain processing circuit and use of this model to simulate the effects of spinal cord stimulation

The first aim was to develop a biophysically-based network model of the dorsal horn circuit consisting of interconnected dorsal horn interneurons and a wide dynamic range (WDR) projection neuron to model the effects of SCS on spinal sensory neuron activity. The computational model reproduced cellular and network responses relevant to pain processing including wind-up, A-fiber mediated inhibition, and surround receptive field inhibition. The activity of the WDR projection neuron in response to different frequencies of SCS was simulated, and the results indicated that neuronal responses to SCS are dependent on stimulation frequency. In addition, a sensitivity analysis on the effects of reducing GABAergic inhibition on neuronal responses to SCS
revealed that the efficacy of SCS applied at 1 - 150 Hz decreases with less GABAergic inhibition, providing an explanation for the diminishing efficacy of SCS over treatment time (Kumar et al., 2008). The outcomes of this aim included the first biophysical model of spinal cord stimulation, predictions regarding the relationship between SCS frequency, GABAergic inhibition, and WDR neuron activity—a validated proxy for pain (Simone et al., 1991, Wallin et al., 2003)—and an increased understanding of the cellular mechanisms underlying SCS.

The computational model reproduced both neuronal activity in response to peripheral stimuli (Woolf and Wall, 1982a, Herrero et al., 2000) and dorsal column stimulation (Foreman et al., 1976a) and revealed network parameters to which the responses to stimulation were most sensitive. Increasing the strengths of A-fiber inputs to the model increased the magnitude of the short latency response of the WDR neuron to 1 Hz peripheral stimulation but did not affect the occurrence of wind-up, defined as progressive potentiation of neuronal responses to sustained peripheral stimulation (Herrero et al., 2000). Increasing or decreasing the strength of GABAergic inhibition from the inhibitory interneuron to the WDR neuron proportionally affected the A-fiber evoked response and the magnitude of A-fiber mediated inhibition in the WDR neuron. The inhibitory interneuron also sent an inhibitory connection to the excitatory neuron that exclusively received excitatory inputs from C-fibers, but weakening this synapse
did not affect substantially the C-fiber response. Conversely, altering the firing properties of the excitatory interneuron from tonic firing to delayed or transient firing eliminated the C-fiber evoked response and wind-up in the WDR neuron but did not affect the A-fiber evoked response. Responses to SCS were dictated by A-fiber mediated excitation and inhibition, and altering the ratio of A-fiber mediated excitation to inhibition significantly affected both the evoked responses by SCS and SCS-frequency dependent effects on the WDR neuron. However, altering the properties of the excitatory interneuron or the strength of inhibition from the inhibitory interneurons to the excitatory interneuron had negligible effects. Taken together, these findings suggest that although both A-fiber and C-fiber mediated responses are important to nociceptive processing, SCS exerts its effects primarily through A-fiber mediated circuits.

Although the Gate Control architecture could reproduce responses to peripheral stimulation, inclusion of only a local excitatory receptive field was insufficient to reproduce the magnitude and time course of inhibition of the WDR neuron due to SCS. The failure of the Gate Control architecture alone to reproduce the SCS response additionally highlights a paradox in WDR neuron behavior: WDR neurons are inhibited by SCS but excited by stimulation of Aβ-fiber afferents originating from the neuron's receptive field (Foreman et al., 1976a). These observations and this paradox suggest that other mechanisms underlie the inhibitory effects of SCS, and inhibition due to activation
of inputs from surround receptive fields represents one such mechanism (Hillman and Wall, 1969). Further, surround inhibition was demonstrated through direct mechanical stimulation of the surround receptive field (Menetrey et al., 1977) and electrical stimulation of primary afferents arising from a surround receptive field (Foreman et al., 1976a, Yang et al., 2011). Although clinical observations indicate that the best pain relief provided by SCS occurs when SCS-evoked paresthesias overlap with the region of pain (Aló and Holsheimer, 2002, Barolat et al., 1993), SCS likely also activates surround receptive field inputs, as pulses of SCS may activate extensive regions of the dorsal columns corresponding to the affected dermatomes (Sankarasubramanian et al., 2014, Kent et al., 2014). The finding that inclusion of surround inhibition was both necessary and sufficient to reproduce SCS-evoked responses without affecting peripheral response profiles supports this mechanism as a contributor to the therapeutic effects of SCS.

The computational model predicted that the responses of sensory neurons to SCS depend on stimulation frequency and GABAergic inhibition. SCS frequencies of 30 Hz - 100 Hz maximally inhibited the model WDR neuron, while frequencies under 30 Hz and over 100 Hz excited the model WDR neuron. The optimally inhibitory range of SCS frequencies represents those frequencies for which SCS-mediated inhibition overcomes both peripherally-evoked and SCS-evoked excitation and for which SCS-mediated excitation does not exceed SCS-mediated inhibition, and this finding was consistent with
the most often cited clinical frequencies (50 Hz – 80 Hz) (Oakley and Prager, 2002). In addition, reducing GABAergic inhibition onto the projection neuron—the primary driver of SCS-mediated inhibition—either by weakening synaptic connections from GABAergic interneurons or by increasing the reversal potential of GABAergic and glycineergic channels to mimic the loss of KCC2 function, reduced the range of SCS frequencies that had an inhibitory effect and lowered the optimally inhibitory SCS frequency. The loss of GABAergic inhibition plays a significant role in the progression of neuropathic pain (Von hehn et al., 2012, Zeilhofer et al., 2012, Braz et al., 2014), and the model-predicted reduction in the efficacy of SCS with loss of GABA may provide a mechanism for the reduction of clinical SCS efficacy over treatment time (Kumar et al., 2007). Validating predictions of the dependence of neuronal responses to SCS on stimulation frequency and GABAergic inhibition was the goal of experimental assessments of SCS (Chapter 3), and exploitation of the interaction between excitatory and inhibitory effects due to SCS inspired dual frequency SCS (Chapter 4).

5.1.2. Aim 2: Development of an experimental protocol to test predictions generated from the computational model regarding the effects of SCS on dorsal horn projection neurons.

The second aim was to test the model-generated predictions of the effects of SCS frequency and GABAergic inhibition on the activity of sensory projection neurons in the spinal cord. The responses of antidromically identified sensory projection neurons in
the lumbar spinal cord were recorded during different frequencies of SCS in acute experiments in urethane anesthetized healthy and neuropathic rats with chronic constriction injury (CCI). In some experiments, either bicuculline methiodide (BIC), a GABA\textsubscript{A} receptor antagonist, or CGP 35348, a GABA\textsubscript{B} receptor antagonist, was applied intrathecally to assess the effects of modulating GABAergic inhibition on neuronal responses to SCS. Finally, computational models of spinal microcircuits (Prescott and Ratte, 2012; Prescott 2014) were developed to account for the full range of observed SCS-frequency dependence relationships. The outcomes of this aim validate the observations of SCS-frequency and GABAergic dependence of neuronal responses to SCS predicted by the model but also challenge the long-held assertion that the output of the Gate Control circuit solely represents neuronal responses to SCS (Guan, 2012).

The experimental measurements of the effects of SCS described in Chapter 3 were the first to quantify the relationship between SCS frequency and neuronal response in antidromically identified sensory projection neurons, and the outcomes may explain several features of the sensory effects of SCS. First, the presence of non-responders, in particular that a higher proportion of nociceptive-specific neurons were non-responders than responders, provides a possible explanation for why SCS does not prevent the perception of acute, nociceptive pain. Second, SCS-mediated excitation at stimulation frequencies from 100 Hz to 150Hz may underlie SCS-evoked uncomfortable paresthesias
that preclude the clinical use of frequencies > 100 Hz. Finally, the finding that a subset of neuronal responses to SCS followed predictions made by the computational model in that their responses were maximally inhibited by SCS at 30 Hz-50 Hz validated the model and provided justification for the commonly cited clinical use of 30 Hz – 80 Hz SCS (Oakley and Prager, 2002).

One unexpected finding from the experimental measurements was that "spinal microcircuits" (Prescott and Ratté, 2012) beyond the Gate Control theory were required to reproduce the full gamut of frequency dependent responses to SCS (Chapter 3). Spinal microcircuits may underlie both labeled lines for the transmission of specific types of sensory information to supraspinal centers and more complex networks responsible for filtering and processing peripheral input (Prescott and Ratté, 2012, Prescott et al., 2014). For example, the connectivity onto Lamina I nociceptive specific neurons whose activity is strongly associated with nociceptive behavior in both healthy and neuropathic animal models of pain (Todd, 2010) may contain of modules consisting of microcircuits. In addition, interactions between non-nociceptive and nociceptive "labeled lines" that are governed by complex interneuronal circuits (Torsney and Macdermott, 2006, Daniele and Macdermott, 2009, Takazawa and Macdermott, 2010) may be modeled by networks with modular internal operations that are represented by microcircuits (Prescott et al., 2014). Further study of the spinal microcircuits may
provide insights regarding the nature of other sensory circuits (e.g. thermal pain, C-fiber mediated inhibition) (Braz et al., 2014) and explain the lack of an observed correlation between peripheral response class (e.g. low threshold, WDR, nociceptive-specific) and SCS response.

Finally, experimental measurements demonstrated that blockade of GABA_A receptor mediated inhibition using BIC but not GABA_B receptor mediated inhibition using CGP 35348 disinhibited responses to SCS as predicted by the computational model. However, BIC also unmasked excitatory responses to SCS, occasionally in prior non-responders, and enhanced spontaneous activity across neurons from all microcircuits. The unmasking of responses following antagonism of GABAergic inhibition supports the Gate-Control Theory-based hypothesis that the loss of inhibition from "gating" interneurons, for example via reduction of GABA production, may underlie abnormal sensory coding, such as hyperalgesia and allodynia (Sandkühler, 2009). However, GABAergic antagonism also unmasked SCS responses in non-responders and increased baseline firing, suggesting that tonic GABAergic inhibition from segmental interneurons (Takazawa and Macdermott, 2010) or from supraspinal sources (Heinricher et al., 2009) play a modulatory role beyond that predicted by the Gate Control Theory. Supporting this possibility is the finding that adjusting the strength of tonic inhibition onto inhibitory interneurons or the projection neuron in
microcircuit models could modulate the threshold frequency at which the principal excitatory or inhibitory responses were observed. This result additionally raises the possibility that tonic, SCS-independent inhibition acts as a thresholding and tuning mechanism for the sensitivity of sensory neurons to peripheral afferent inputs (Chapter 3).

5.1.3. Aim 3: Design of more optimal strategies for SCS through temporal patterning of the stimulation train.

The final aim was to use the validated computational model of the spinal pain processing circuit (Chapter 2) to design improved strategies for SCS through the use of non-regular temporal patterns and to test the efficacy and relative benefit of novel stimulation trains in an experimental preparation (Chapter 3). Two initial approaches were taken to improve the efficacy of SCS: First, non-regular temporal patterns of stimulation were optimized using a genetic algorithm (Goldberg, 1989) to suppress the output of the model output neuron more effectively than constant frequency SCS (cfSCS) (Appendix I). Second, properly selected pairs of dual frequency SCS (dfSCS) were predicted by the computational model to be more effective at inhibiting sensory neuron activity than cfSCS applied simultaneously to all dorsal column fibers at the average of the two constituent frequencies (Chapter 4). In addition to being more effective versus cfSCS than the GA-designed patterns, the effects of select frequency pairs of dfSCS on
the output of the computational model were robust to stimulation conditions and the level of GABAergic inhibition in the computational model. The larger effect size and robustness of dfSCS motivated experimental assessments of dfSCS rather than GA-designed patterns. Experimental measurements of neuronal responses to dfSCS confirmed model predictions of the improved efficacy of appropriately selected pairs as compared to cfSCS. Preliminary measurements further indicated that dfSCS is more effective than clinical standard 50 Hz cfSCS following antagonism of GABAergic inhibition using (BIC), suggesting that dfSCS may be more effective at treating chronic pain than conventional SCS.

Concurrent activation of inhibitory mechanisms in the Gate Control circuit by the two constituent frequencies of a dfSCS pair with dispersion of excitation between the two frequencies underlies the effects of dfSCS. During cfSCS, evoked inhibitory events are temporally locked to excitatory events, as evidenced by transient post-stimulus excitation of neurons observed in vivo during 50 Hz cfSCS. In some cases, transient excitation during cfSCS was sufficient to overcome inhibitory effects, resulting in net excitation of sensory neurons by cfSCS. Transient excitation may be suppressed by more inhibitory input, but more inhibition can only be generated in cfSCS by increasing the stimulation frequency until inhibitory events overlap. However, increasing stimulation frequency also has deleterious effects, such as increasing current consumption and
thereby reducing the battery life of an implantable pulse generator, and increasing
evoked activity in affected dorsal column fibers, which in turn may activate more dorsal
column nuclei neurons (Qin et al., 2009, Song et al., 2014) and result in uncomfortable
paresthesias (Shealy et al., 1972). However, during dfSCS, inhibition from one
constituent frequency mitigates SCS-evoked excitation from the other constituent
frequency, and excitatory events are themselves weaker, as only half the dorsal column
inputs receive each frequency. As a result, lower average stimulation frequencies are
required to generate inhibition sufficient to suppress sensory neuron activity. This
observation suggests that dfSCS may be used as a strategy to improve the efficiency of
SCS and to reduce unpleasant side effects associated with SCS, such as paresthesias.

The effects of dfSCS with appropriately selected frequency pairs were preserved
even under conditions of imperfect selectivity between the populations receiving the
two frequencies or bias in the proportion of local to surround receptive field inputs
receiving each frequency. In simulations of imperfect selectivity, the network received
through 66% of its inputs a composite non-regular pattern representing the composite
pulse trains from each constituent frequency in the dfSCS pair. When the two
frequencies were not factor-multiple pairs, excitatory events closely spaced in time from
closely spaced pulses in the composite pulse train were occasionally close enough to
each other to excite the sensory neuron, yielding less inhibition. However, when
frequencies shared common factors or were multiples of each other, the composite pulse train was more regular, the inter-pulse intervals were not as close, and dfSCS suppressed model Gate neuron activity. In simulations of local-surround bias, dfSCS was sometimes no more effective than SCS using the higher frequency applied only to the surround inputs, as activation of inputs to inhibitory surround receptive fields did not excite the local sensory neuron. However, activation of surround inputs is likely to activate other sensory neurons to which these inputs connect via excitatory synapses (Hillman and Wall, 1969), whereas the mutual inhibition provided by close but not time locked dfSCS pairs mitigated this occurrence. Nonetheless, some frequency pairs shared common factors and were close to each other, making them robust to both imperfect selectivity and local-surround.

Experimental measurements in changes in spinal sensory neuron firing rates supported the utility of dfSCS, as 25 Hz/40 Hz dfSCS suppressed neuron activity more than cfSCS and consumed less energy to generate suppression than clinical standard 50 Hz cfSCS. In addition, dfSCS produced more inhibition of sensory neurons than SCS using the individual constituent frequencies delivered through individual electrode pairs, indicating that concurrent delivery of both frequencies is necessary for the full inhibitory effect of dfSCS. Furthermore, the efficacy of dfSCS depended on the specific frequency pair. Although a suboptimal 15 Hz/20 Hz dfSCS pair still produced greater
inhibition than 17 Hz cfSCS, 25 Hz/40 Hz dfSCS inhibited sensory neurons to a greater degree than 15 Hz/20 Hz dfSCS. In addition, the stronger inhibition produced by 25 Hz/40 Hz dfSCS was not due to an increase in the average SCS frequency alone, as no significant difference was observed in the degree of inhibition generated by cfSCS applied at the respective pair-average frequencies. Finally, dfSCS continued to produce stronger inhibition than either average frequency or 50 Hz cfSCS following loss of GABAergic inhibition, and all neurons that exhibited peri-stimulus excitation during 50 Hz SCS following antagonism of GABAergic inhibition were inhibited by dfSCS. More data are necessary to confirm the trends observed following loss of GABA, and a combination of behavioral assessments during multifrequency SCS using animal models of neuropathic injury and clinical trials are necessary to determine the potential clinical utility of this novel innovative to SCS.

5.2. Future Work

5.2.1. Modeling multiple nodes and spatial effects of SCS

Although sufficient to account for inhibition due to stimulation of afferents from surround receptive fields and SCS (Foreman et al., 1976a, Menetrey et al., 1977), the computational model in Chapter 2 represents a simplification of the dorsal horn circuit. Classical single unit recordings during mechanical and electrical stimulation support the existence of surround inhibitory receptive fields (Applebaum et al., 1975, Gerhart et al.,
In addition, recent structural and functional mapping provide evidence of surround inhibition at a cellular level by demonstrating that some sensory neurons possess extensive dendritic arborizations that receive inhibitory inputs from across several dermatomes (Szucs et al., 2013, Kato et al., 2013). However, the model incompletely depicted the influence of the activation of primary afferents from surrounding receptive fields, such as the activity of Gate neurons representing the surround nodes (Hillman and Wall, 1969) and the effects of C-fiber mediated excitation from surrounding nodes on projection neuron activity (Luz et al., 2010). In addition, dorsal horn circuits may be organized as interconnected modules (Lu and Perl, 2003, Lu and Perl, 2005), and both sensory projection neurons and interneurons receive excitatory or inhibitory inputs from others (Szucs et al., 2013, Takazawa and Macdermott, 2010). These findings indicate that a single node is insufficient to describe the nociceptive response (Chapter 2) and that the population output of a multinodal network with a center-surround architecture is more appropriate for assessing sensory responses to SCS (Figure 5.1).

Multinodal extensions of the computational model may provide insight regarding the neuronal effects of SCS, but to reconcile fully the role of spatial selectivity in the efficacy of SCS with the neuronal effects of SCS, the network model must be coupled to a spatial model of spinal cord activation (Holsheimer and Wesselink, 1997,
Howell et al., 2014a). A major assumption regarding the inputs to the present model is that all dorsal column fibers relevant to the receptive fields from which pain originates were activated. This assumption is in line with clinical observations that paresthesias induced by conventional SCS must at least partially overlap with the region of pain for effective relief (Oakley and Prager, 2002, Barolat et al., 1993, Aló and Holsheimer, 2002). The population of dorsal column fibers activated by SCS is dependent on physical parameters such as the thickness of the cerebrospinal fluid layer beneath the dura (Wesselink et al., 1998a) and the electrode-tissue interface (Merrill et al., 2005, Howell et al., 2014b), as well as on stimulation parameters such as electrode configuration and stimulation amplitude (Sankarasubramanian et al., 2011, Sankarasubramanian et al., 2014) that cannot be represented without a spatial model. Spatial models are also required to account for the geometries of the dorsal column fibers themselves, such as the presence of axon collaterals, which affect activation thresholds in ways that depend on the spatial distribution of extracellular potentials (Struijk et al., 1992).

Realistic finite element models (FEMs) of the spinal cord can be used to assess how physical properties, anatomical features, and stimulation parameters can affect the distributions of extracellular potentials and current densities from SCS. Coupling FEM-generated field solutions to biophysical models of dorsal column fibers that, in turn, propagate to a network model may be used to assess directly the effects of varying the
spatial or stimulation parameters of SCS on the activity of spinal sensory neurons (Howell et al., 2014a, Struijk et al., 1993b). Accounting for physical properties and parameters affecting stimulation in this manner become particularly important in modeling novel high frequency (Al-Kaisy et al., 2014) and burst (De Ridder et al., 2013) SCS, for which, unlike in conventional SCS, neural activation is not locked to and may be blocked by the stimulation waveform (Bhadra and Kilgore, 2005) and for which the stimulation waveform may be filtered by the electrode-tissue interface (Howell et al., 2014a, Medina and Grill, 2014). Modeling the spatial extent of activation by SCS also becomes important in assessing the efficacy and relative benefit of dual frequency SCS versus conventional single frequency SCS (Chapter 4), as interactions between anatomical features and electrode geometries may distort the proportion of dorsal column fibers activated by electrode pairs corresponding to distinct frequencies. In addition, dorsal column fibers are roughly somatotopically organized, with fibers originating from rostral dermatomes positioned more laterally and dorsally than fibers originating from caudal dermatomes (Werner and Whitsel, 1967). This assumption has been used with FEMs to design electrode configurations capable of targeting specific dermatomes but without consideration on the effects of targeted SCS on the spinal nociceptive circuit (Holsheimer and Wesselink, 1997, Howell et al., 2014a). Coupling axon models of dorsal column fibers to a spatial FEM and linking the dorsal column
fibers to a multinodal computational model with center and surround representations (Figure 5.1) will enable assessments of the effects of the spatial distribution of currents and potentials generated by SCS (resulting, for example, from different electrode geometries) on the center-surround network. Implementation of such a spatial model may then enable the design of electrodes, waveforms, and pattern combinations that preferentially engage surround inhibition onto dermatomes affected by pain, thereby improving therapy.

5.2.2. Experimental characterization of spinal microcircuits

The computational models of spinal microcircuits beyond Gate Control, described in Chapter 3, were sufficient to reproduce responses to SCS, and this supports the existence of these microcircuits. The observation of a distribution of SCS-evoked responses across microcircuits and the preservation of microcircuit-classifiable responses in chronic constriction injury animals suggest that responses to pain and SCS may be mediated by a population code rather than a labeled line. In particular, the specific distribution of microcircuits among projection neurons in the dorsal horn may represent the population code, and shifts in the distribution to more excitation may contribute to the progression of neuropathic pain and the reduction in the effects of SCS over treatment time (Kumar et al., 2008). Supporting this hypothesis are two observations. First, sensory thresholds of specific populations of sensory projection neurons decrease.
as a neuropathic pain state develops (Lavertu et al., 2013). Second, from Chapter 3, a higher proportion of projection neurons recorded from CCI animals (5/6) were classified into predominately excitatory microcircuits versus those from healthy animals (32/59). Linking microcircuits and microcircuit transitions to behavioral changes over the development of neuropathic pain could lead to optimization of SCS through targeted suppression of pronociceptive microcircuits and targeted excitation of antinociceptive microcircuits. However, more recordings and behavioral studies using other neuropathic pain models, such as the spared nerve injury model (Decosterd and Woolf, 2000) are needed to determine the feasibility of this approach.

Relating the phenotypes of spinal neurons with their function and specific microcircuitry may provide further insight into the specific operations in sensory processing encoded by the microcircuits and allow the explicit identification of specific neuron pathways affected by SCS. Immunohistochemistry conducted in concert with recording studies has revealed that excitatory and inhibitory interneurons may exhibit distinct cell marker expression profiles. In particular, a group of PKCγ-positive interneurons in Lamina IIi/Lamina III of the dorsal horn receive convergent direct excitatory and indirect inhibitory inputs from non-nociceptive afferents and send excitatory projections to more superficial neurons (Miraucourt et al., 2007, Neumann et al., 2008) that may ultimately terminate onto NK1 expressing Lamina I projection...
neurons (Todd, 2010). In addition, knockdown of spinal PKCγ in mice prevented the
development of allodynia following nerve injury without affecting acute pain behaviors
(Malmberg et al., 1997). This observation suggests that these PKCγ positive cells
represent an interneuron "gate" between nociceptive and non-nociceptive transmission
upon which SCS may exert inhibitory effects (Braz et al., 2014, Prescott et al., 2014).
Determining the expression profiles of neurons affected by SCS, for example by labeling
neurons with antibodies targeted at PKCγ or other cell-specific markers, and staining for
c-Fos, an activity-dependent immediate early gene indicating synaptic activity, in these
neurons following SCS will provide information about the neurons and pathways
affected directly by SCS. Assessments of activity levels, gene expression patterns, and
locations of neurons in the dorsal horn can then be compared to existing motifs of dorsal
horn connectivity (Zheng et al., 2010) to track signal flow from dorsal columns to
projection neurons and thereby map the neural circuits affected by SCS.

5.2.3. SCS Optimization: Targeting glycinergic pathways

Dual frequency SCS (dfSCS) exploits the balance of excitatory and inhibitory
inputs onto sensory neurons in the spinal cord by preserving GABAergic inhibition from
local and surround receptive fields while dispersing excitation. However, GABAergic
inhibition is lost during the progression of chronic pain due to mechanisms including
glial cell death and resultant down-regulation of GABA production (Scholz and Woolf,
2007, Ji et al., 2013). Network changes and phenotypic switches among neurons in spinal sensory networks following a prolonged inflammatory response further contribute to loss of inhibitory mechanisms in the spinal cord (Von hehn et al., 2012). This loss of GABAergic inhibition unmasks or accentuates the effects of excitatory inputs onto nociceptive neurons (Chapter 3) and, as such, is detrimental to the efficacy of both conventional SCS and dfSCS (Chapter 4). Therefore, targeting other inhibitory pathways that are relatively unaffected by the transition to the neuropathic pain state may yield improvements in therapeutic outcomes.

Segmental glycinergic mechanisms play an important role in regulating interneuronal activity in the dorsal horn and present a possible future target for optimizations. The administration of the glycinergic antagonist strychnine in subconvulsive doses to healthy animals is sufficient to evoke hyperalgesia and allodynia, suggesting that glycinergic mechanisms play a significant role in modulating nociceptive responses (Sivilotti and Woolf, 1994, Yaksh, 1989). Furthermore, co-administration of bicuculline and strychnine is necessary to unmask fully excitatory responses from non-nociceptive afferents in excitatory superficial dorsal horn neurons (Torsney and Macdermott, 2006). Importantly, a subpopulation of glycine-dominant interneurons in Lamina IIi receive non-nociceptive afferent inputs, exert tonic inhibition onto other interneurons, and may synapse directly onto PKCγ-positive "Gate"
interneurons that segregate nociceptive information flow from non-nociceptive information flow (Takazawa and Macdermott, 2010, Miraucourt et al., 2007). No studies to date have identified subpopulations of Aβ/dorsal column fibers that terminate specifically onto these glycine-dominant interneurons, but glycine and GABA often colocalize in the dorsal horn (Todd et al., 1996). In addition, glycinergic activity is relatively unaffected by the changes that occur during the progression of neuropathic pain (Moore et al., 2002a, Braz et al., 2014), suggesting that targeting previously GABAergic pathways may still engage glycinergic mechanisms.

That 50 Hz cfSCS evoked peri-stimulus inhibition in spinal sensory neurons for up to 6-10 ms following each stimulus pulse after administration of BIC supports a role for glycine in mediating inhibition dorsal horn neurons by SCS. However, the ratio of remaining GABAergic to glycinergic inhibition following the transition to neuropathic pain is not constant (Takazawa and Macdermott, 2010), and this ratio may affect the utility of dfSCS. For computational assessments, the ratios of GABAergic to glycinergic inhibition originating from the local and surround receptive fields may alter the impact of GABAergic loss on therapeutic efficacy in neuropathic pain. Both the inclusion of glycinergic inhibition to inputs from surround receptive fields and an analysis on differing ratios of GABA to glycine on the optimal frequency pairs in dfSCS may address this issue. For experimental assessments, applying strychnine, a glycinergic
antagonist, intrathecally either alone or following the application of BIC, and assessing the effects on the responses to and utility of dfSCS may offer insight into the relative contributions of GABAergic and glycinergic inhibition to the effects of dfSCS. Finally, behavioral assessments of dfSCS in a chronic animal model of peripheral nerve injury (Decosterd and Woolf, 2000) or back injury (Lee et al., 2004), with and without administration of subconvulsive doses of strychnine (<30 μg) (Yaksh, 1989), may link glycinergic inhibition with symptom relief by SCS.

5.2.4. SCS Optimization: Winning the battle for inhibition

Computational modeling (Chapter 2) and experimental measurements (Chapter 3) of the neuronal effects of SCS indicate that the balance of excitatory and inhibitory inputs onto projection neurons mediates neuronal responses to SCS. Changing this balance by weakening the GABAergic inputs onto projection neurons reduces the ability of SCS to inhibit neuronal activity and may reflect diminished therapeutic efficacy with advanced chronic pain (Kumar et al., 2008). The strategies taken in this dissertation to improve SCS involve restoring the excitatory-inhibitory balance onto Gate neurons using SCS, either by generating temporal summation of inhibitory events using non-regular patterns of stimulation (Appendix I) or by dispersing excitatory inputs through dual frequency SCS (Chapter 4). Although effective at producing greater inhibition than conventional SCS at the equivalent frequency, these strategies only begin to explore the
possible ways in which SCS may be used to control the excitatory-inhibitory balance in the spinal nociceptive circuit.

Random SCS, in which all dorsal column fibers receive different randomized patterns of stimulation, may disperse excitation while preserving inhibition, thereby producing greater suppression of spinal sensory neurons without SCS-mediated excitation. In addition, if internal segments of the random pattern generate sufficient temporal summation, subsequent periods of low frequency stimulation may still produce net inhibition of the Gate neuron and thereby make random stimulation more efficient (i.e., use fewer pulses/s) than cfsCS. In support of this hypothesis, preliminary simulations of random SCS (Figure 5.2a) with each dorsal column input receiving distinct pulse trains at 10 pulses/s, 22 pulses/s, 34 pulses/s, and 50 pulses/s indicate that random SCS produces nearly total suppression of the Gate neuron, even when individual dorsal column fibers only receive 10 pulses/s. However, implementing truly random SCS is challenging, as microelectrode arrays with each contact coupled to an independent current source are likely needed to activate individual dorsal column fibers in the manner described. The use of novel stimulation patterns, such as sustained kilohertz frequency stimulation, may asynchronously and stochastically activate dorsal column fibers to generate these effects (Rubinstein et al., 1999), but these waveforms are not energy efficient and may result in an impractically short battery life of an implanted
A more feasible approach, similar to that taken in dual frequency SCS, involves the concurrent application of different random patterns to distinct groups of dorsal column fibers. Concurrently applying and optimizing multiple patterns to inhibit the Gate neuron (e.g., using genetic algorithms) may thereby yield practically applicable pattern combinations that are more effective than equivalent cfSCS and more efficient than clinical standard 50 Hz cfSCS. Understanding how features of pattern combinations interact with the excitatory and inhibitory input onto spinal sensory neurons will provide further insights regarding how to design temporal patterns to improve SCS.

As well, the results of testing dual frequency SCS suggest that asynchronous activation of distinct populations of dorsal column collaterals of Aβ fibers, at the same or different stimulation frequencies, represents a general strategy to enhance the inhibitory effects of SCS. One realization of this strategy is to stagger the timing of the pulse trains delivered at the same frequency through two distinct electrodes. "Staggered" SCS thereby disperses the SCS-mediated excitation endemic in conventional constant frequency SCS (Zhang et al., 2014b, Dubuisson, 1989) while preserving SCS-mediated inhibition, resulting in substantially more inhibition of the Gate neuron at SCS frequencies > 20 spikes/s (Figure 5.3). Clinically, staggered SCS could be implemented by delivering pulse trains offset by a constant time interval through two or more
independent electrode sets or by delivering stimulation at different locations on the spinal cord. Implementing true multi-frequency SCS through the application of 3 or more concurrent frequencies to dorsal column fibers represents an additional extension of this strategy. Multi-frequency SCS may improve the efficacy of some suboptimal dfSCS pairs, such as factor multiple pairs, by adding frequencies that produce inhibition at times not locked to simultaneous stimulation events in dfSCS. The added inhibition may increase the effectiveness of dfSCS combinations with lower average frequencies and, in turn, reduce the overall average stimulation frequency, thereby improving the efficiency as well as the efficacy of SCS. Computational assessments of multi-frequency SCS are necessary to determine both the optimal number of frequencies and the specific frequency combinations that produce the best efficacy at the lowest average frequency.

Finally, activation of dorsal column nuclei neurons may underlie paresthesias, and asynchronously activating the dorsal columns may also be a way to reduce uncomfortable paresthesias associated with SCS (Shealy et al., 1972) by exploiting the center-surround arrangement of the dorsal column nuclei (Sánchez et al., 2004). Notably, asynchronous activation of dorsal column fibers is theorized to underlie pain relief without paresthesia produced by novel burst (De Ridder et al., 2013) and kilohertz frequency SCS (Shechter et al., 2013), and unlike conventional SCS, kilohertz frequency SCS does not activate dorsal column nuclei neurons (Song et al., 2014). However, the
true effects of burst and kilohertz frequency SCS on dorsal column fibers are as of yet unknown. In addition, patterning of the stimulation train may represent a more energy efficient method of asynchronous activation than the continuous application of burst or kHz frequency waveforms. Developing a network model of the dorsal column nuclei will allow assessment of the activity of dorsal column nuclei projection neurons during different methods of asynchronous stimulation. Coupling of the dorsal column nuclei network model to the spinal nociceptive circuit will then allow the development of asynchronous SCS patterns that inhibit pain while minimizing paresthesias (Figure 5.4).

**5.2.5. Supraspinal Mechanisms**

Beyond the segmental mechanisms explored in this dissertation, changes in supraspinal nuclei and descending projections play an important role in the development of neuropathic pain. Supraspinal mechanisms contribute to the disruption of the balance of descending facilitation and inhibition that, in turn, may alter the excitability of spinal sensory neurons and induce pathological sensory changes (Figure 5.5) (Millan, 2002, Suzuki et al., 2004, Heinricher et al., 2009). Behavioral and electrophysiological studies also demonstrated that descending mechanisms play a role in SCS. Descending contributions may be independent of segmental mechanisms (Tabet et al., 1986), and their temporal characteristics correlate with the period of pain relief that occurs after cessation of SCS (Barchini et al., 2012). In particular, serotoninergic (5-
HT) pathways have been implicated in pain relief by SCS: SCS efficacy and levels of 5-HT in the spinal cord of neuropathic rats are correlated (Song et al., 2009), and descending serotonergic connections affected by SCS modulate both GABA\textsubscript{A} and GABA\textsubscript{B} synapses (Song et al., 2011) independently of segmental mechanisms (Barchini et al., 2012). The relationship between 5-HT and GABA, particularly as it relates specifically to 5-HT\textsubscript{2}, 5-HT\textsubscript{3}, and 5-HT\textsubscript{4} receptors, is also a topic of recent interest, as activation of distinct 5-HT pathways has heterogeneous effects on behavioral responses to SCS (Guan, 2012). Understanding these mechanisms may lead to the development of novel treatment regimens that exploit serotonergic pathways.

Despite progress in understanding supraspinal mechanisms underlying SCS, knowledge regarding the specific neuronal connections and circuits that mediate descending modulation remains sparse. The rostroventromedial medulla (RVM), considered to be a major source of descending facilitation and inhibition, receives ascending inputs from dorsal horn neurons (Millan, 2002, Heinricher et al., 2009), sends direct axonal projections to the dorsal horn (Fields et al., 1995), and affects the activity of both interneurons and projection neurons in the dorsal horn (Giesler Jr et al., 1981, Heinricher et al., 2009). Recent work has also revealed direct effects by SCS on supraspinal neurons. Both "off" cells in the RVM and serotonergic neurons in the locus coeruleus exhibit more activity during SCS in neuropathic rats that show increased
paw withdrawal thresholds than in non-responding rats, and the combined effects of activation of these nuclei by SCS may account for up to 50% of the inhibition due to SCS (Song et al., 2013a, Song et al., 2013b, Barchini et al., 2012). These observations strongly support the hypothesis that SCS acts by additionally modulating the activity of a spinal-supraspinal loop and possibly a supraspinal circuit. The addition of a supraspinal network based on knowledge of the locus coeruleus and RVM circuits and linking this model via excitatory and inhibitory projections to the existing computational model may result in more realistic representations of pain phenomena, such as diffuse noxious inhibitory controls (Bars et al., 1979), that cannot be accounted for by purely segmental mechanisms (Heinricher et al., 2009). The effects of supraspinal modulation are also likely to affect the outcomes of model-based optimization of SCS, and novel temporal patterns generated with a combined spinal-supraspinal model may result in further improvements in therapy.

5.3. Summary

Spinal cord stimulation (SCS) has emerged as a promising therapy for the prevalent, distressing, and expensive syndrome of intractable chronic pain. The combination of scientific knowledge of the mechanisms and engineering design principles is required to optimize SCS, but most attempts to improve therapy have focused only on the latter, resulting in a plateau in clinical efficacy. The work in this
dissertation addressed the unmet need of understanding how the neurophysiological mechanisms underlying SCS affect therapeutic efficacy through three aims: 1) Computational modeling of the cellular effects of SCS, 2) Experimental assessments of computational model predictions of the neuronal effects of SCS, and 3) Model-based design and experimental validation of dual frequency SCS based on biophysical mechanisms. The outcomes of this dissertation are an improved understanding of the mechanisms underlying SCS, computational and experimental tools with which to continue the development and improvement of SCS, and a novel strategy for potentially improving the efficacy of SCS. The insights and knowledge gained from the work described in this dissertation may result in translational applications that significantly improve the therapeutic outcomes of SCS and the quality of life of individuals affected by chronic pain.
Figure 5.1 Extension of the computational model to a multinodal architecture.

Modular "Gate Control" or microcircuit nodes will be arrayed such that central neurons (e.g., WDR # 3) receive excitatory and inhibitory inputs from their local (A3/C3) and adjacent (A2/C2, A4/C4) nodes and inhibitory inputs from farther surround nodes (A1, A5) (Hillman and Wall, 1969). The model will comprise a sufficiently large number of nodes such that edge effects will not affect the activity of a group of central nodes. To
assess the effects of spatial selectivity in SCS activation on neuronal responses, SCS may be applied to all, some, or only one set of A-fiber/dorsal column inputs.
Figure 5.2 Preliminary assessment of random SCS.

(a) Schematic depicting simulation of random SCS. Random pulse trains are all set to 10 pulses/s, 22 pulses/s, 34 pulses/s, or 50 pulses/s and are all set to the same frequency within a simulation. Trains are applied to individual dorsal column collaterals of Aβ fibers during an on-going peripheral input. No dorsal column input receives the same pattern as another. (b) Comparison of SCS frequency vs. Gate neuron output relationships at 10 pulses/s, 22 pulses/s, 34 pulses/s, and 50 pulses/s produced by conventional SCS (black) and random SCS (blue).
Figure 5.3 Preliminary assessment of staggered SCS.

(a) Schematic depicting simulation of staggered SCS. Two pulse trains of equal frequency staggered by 0.5x the inter-pulse interval (IPI) corresponding to the stimulation frequency are applied to two distinct populations of dorsal column collaterals of Aβ fibers during on-going peripheral input. Propagation distance was set to 1 cm. (b) Comparison of SCS frequency vs. Gate neuron output relationships produced by conventional (black) and staggered (red) SCS.
Figure 5.4 Schematic of the combined spinal/dorsal column nuclei (DCN) model.

This model will use WDR neuron output for optimization for pain reduction as well as dorsal column nuclei (DCN) neuron outputs for optimization of paresthesia reduction.
Figure 5.5: Changes that occur to the circuitry and neurochemistry of supraspinal structures that exert descending modulation on the dorsal horn pain processing network.

A schematic showing changes that occur to the circuitry and neurochemistry of supraspinal structures that exert descending modulation on the dorsal horn pain processing network during the induction and maintenance of neuropathic pain and during SCS. Ascending pathways from the superficial dorsal horn and descending pathways from the brain send collaterals to the locus coeruleus, the raphe nuclei, and the rostroventromedial medulla (RVM). These nuclei, in turn, send descending facilitatory (F) and inhibitory (I) projections to the spinal dorsal horn network. The transition from acute to neuropathic pain involves the accentuation of facilitatory
pathways (e.g., from "On" cells in the RVM, Inset) and the attenuation of inhibitory pathways (e.g. from locus coeurleus, raphe nuclei, "Off" cells in RVM, Inset) which have the net effect of disinhibiting nociceptive transmission from the spinal cord. Spinal cord stimulation may enhance the activity of descending serotonergic and inhibitory pathways, thereby restoring some of the balance between descending facilitation and inhibition. (Zhang et al., 2014a). Image credit: Stan Coffman, MedMedia Solutions.
Appendix A. Design of optimized non-regular temporal patterns of spinal cord stimulation using a genetic algorithm

A.1. Introduction

Spinal cord stimulation (SCS) is a therapy for managing chronic pain when conventional treatments (including physical therapy, pharmaceuticals, and surgery) are not effective. In conventional SCS, stimulation is delivered to the dorsal columns at a constant frequency, most often 50-80 Hz, through an epidural electrode, and stimulation parameters such as amplitude, pulse width, electrode configuration, and frequency are tuned to provide individualized pain relief (Cameron, 2004, Taylor et al., 2013). However, the proportion of patients who experience 50% or greater reported pain relief remains at 58% with no significant upward trend between 1972 and 2013 (Zhang et al., 2014a). Prior attempts to increase the efficacy of SCS focused largely on the design of electrode configurations that may activate dorsal column fibers at lower amplitudes and with greater selectivity (Holsheimer and Wesselink, 1997, Wesselink et al., 1998a). However, this strategy considers neither the temporal properties of the SCS pulse train nor the effects of SCS-evoked neural activity on spinal circuits that relay pain information to the brain. The lack of attention to the dynamic neural circuit effects of stimulation may contribute to the lack of improvement in SCS efficacy over time (Kumar et al., 2007, Zhang et al., 2014a).
Non-regular temporal patterns of stimulation may be a strategy to improve the efficacy of SCS by exploiting the balance of excitatory and inhibitory effects of SCS on sensory neurons, and this strategy has not been previously explored. However, devising the optimal temporal pattern of stimulation cannot be accomplished analytically or through brute force due to the complexity of the dorsal horn network and the infinite number of possible temporal patterns. A genetic algorithm (GA) is an iterative global optimization algorithm based on a mathematical representation of biological evolution that can be used to design temporal patterns according to a quantitative "cost function" based on the desired outcomes (Goldberg, 1989). In this approach, a population of "organisms" containing a collection of "genes" defining the stimulation pattern is applied to a model, assessed for "fitness" using a cost function, and then "mated" to form new candidate patterns for the next iteration. This process continues until the best solutions from subsequent generations no longer show improvement compared to those from prior generations, indicating convergence to an optimal solution. This process has been used previously to optimize neuromodulation parameters for efficacy and efficiency in other domains, such as the parameters of periodic DBS (Feng et al., 2007), the optimal energy-efficient waveform for neural stimulation (Wongsarnpigoon and Grill, 2010), and non-regular temporal patterns of DBS (Brocker et al., 2013), but this technique has never been applied to SCS.
We used genetic algorithms to design non-regular temporal patterns of spinal cord stimulation (GA-SCS) intended to be more effective than constant frequency SCS (cfSCS) in suppressing the activity of the output neuron of a validated computational model of the spinal nociceptive circuit (Zhang et al., 2014b). We used the firing rate of the model output (Gate) neurons as a proxy for the level of pain, as there is a strong correlation between wide dynamic range (WDR) neuron firing rate and pain ratings (Simone et al., 1991), and changes in WDR neuron firing rate during SCS parallel behavioral effects on pain by SCS (Wallin et al., 2003, Guan et al., 2010). We changed the parameters of the cost function to assess how the balance of stimulation efficacy, quantified as neuron activity during SCS, and stimulation efficiency, quantified as the average stimulation frequency of the pulse train, affected the resulting GA-SCS patterns. We also altered the propagation distance between SCS and the output neuron of the computational model and the strength GABAergic inhibition in the model to assess how altering the balance of excitation and inhibition onto sensory neurons affected the GA-SCS patterns.

The results of this computational study suggest that GAs can be used to design temporal patterns of SCS that are more effective and efficient than constant frequency SCS (cfSCS) under certain conditions. We identified non-uniform temporal patterns of stimulation that suppressed Gate neuron activity more effectively than equivalent
frequency regular SCS and at lower stimulation frequencies than clinical SCS (50-80 Hz). Multiple trials of the GA converged to similar optimized patterns despite randomized initial populations, matings, and mutations between trials, suggesting that the final patterns truly represented an optimized solution. However, improved efficacy versus equivalent frequency cfSCS drove the GAs more strongly than efficiency, and final GA-SCS patterns with average frequencies between 10 pulses/s and 34 pulses/s all exhibited similar efficacy. In addition, the characteristics of optimized patterns were very sensitive to the nature of the interaction between SCS-mediated excitation and inhibition on the output Gate neuron. As a result, GA-SCS was not as effective after the loss of GABAergic inhibition at inhibiting the Gate neuron, suggesting that GA-SCS would not be as effective in treating advanced chronic pain. The latter observations suggest that a different implementation of GA-SCS is needed to produce stimulation patterns that more effectively balance efficacy and efficiency and stimulation patterns that exhibit greater efficacy during advanced chronic pain.

A.2. Methods

A.2.1. Computational model

We simulated the responses of spinal sensory neurons to SCS using a published computational network model based on the Gate Control circuit (Zhang et al., 2014b). The network model consists of biophysically-based compartmental models of excitatory
and inhibitory dorsal horn interneurons connected to each other and to a "Gate" neuron receiving polymodal inputs via Alpha function representations of excitatory and inhibitory synapses with rise time constants, decay time constants, and peak conductances based on prior literature (Figure A.1). The network receives as inputs 15 Aβ-fibers, 15 Aδ-fibers, and 30 C-fibers from a "local" receptive field that send monosynaptic and disynaptic excitatory and inhibitory connections onto the output Gate neuron and 15 Aβ-fibers from a "surround" receptive field that send disynaptic inhibitory connections onto the output Gate neuron (Hillman and Wall, 1969). The activity of the Gate neuron was used as the outcome measure, as the firing rate of these neurons has been shown previously to correlate with reports of pain following capsaicin injection (Simone et al., 1991).

### A.2.2. Genetic algorithms

A genetic algorithm (GA) is an iterative global optimization and search heuristic akin to computational evolution and the method by which optimal non-regular patterns were designed. All non-regular patterns were encoded in the GA using series of 1000 bits, with each "0" (no pulse) or "1" (pulse) bit representing a "gene" corresponding to the 1 millisecond bin during a 1 s interval. Composite GA-SCS pulse trains were built using successive repeats of a 1 s pattern through the total stimulation time (20 s) and then applied to the model through the dorsal column inputs (Figure A.2). The efficacy of a
GA-SCS pattern versus constant inter-pulse interval SCS at the same average frequency (cfSCS) was quantified using the difference between the activity in spikes/s of the output Gate neuron during GA-SCS and cfSCS (G, Equation A.1). The "fitness" of each pattern was evaluated using a cost function C, calculated based on the weighted sum of G and the efficiency of GA-SCS, quantified as the average frequency of GA-SCS in pulses/s (F). The relative weighting of efficacy and efficiency on C was determined by constants A and B, as shown in Equation A.2:

$$G = \text{Gate} \text{(GA-SCS)} - \text{Gate} \text{(cfSCS)} \quad (A.1)$$

$$C = A \times G + B \times F \quad (A.2)$$

An optimal stimulation pattern generates a minimal response from the Gate neuron using as few pulses/s as possible, so patterns of stimulation yielding lower costs (i.e., negative C) were deemed to be more fit. The initial GA generation comprised 25 patterns each constructed using 20 randomly generated bit sequences containing no fewer than 10 "1"s (i.e., 10 pulses/s) and no more than 100 "1"s (i.e., 100 pulses/s) and 5 cfSCS pulse trains with frequencies between 10 - 100 Hz. Each generation after the first was constructed using the 2 fittest (lowest cost function) "survivors" from the previous generation, 5 randomly generated "immigrants" including one 10 – 100 Hz cfSCS pulse train to preserve constant inter-pulse interval segments in the total gene pool, and 15
"children" created from mating two patterns in the previous generation (Figure A.2). Crossings between distinct parent organisms were conducted using a uniform cross, in which the probability of any bit in the child originating from either parent is independent of that of any other bit, i.e., multiple crossover points were used to recombine parental patterns (Figure A.2). Although all patterns in the previous generation could be represented in these offspring, the probability of a pattern being represented in these crossings was determined using a random decaying exponential distribution built such that patterns that were more fit (i.e., produced lower C) had a higher probability of being selected for uniform crosses than less fit patterns. Point mutations, in which individual genes were inverted (e.g., from "0" to "1") with a probability of 0.02% per bit, were then applied randomly to individual genes in all offspring. Unless otherwise noted, this iterative process continued until the cost function changed by fewer than 10 points over at least 50 generations, typically after 200-400 generations (Figure A.2). The seeds used to generate initial organism populations and immigrants, the pairs of parents used to generate offspring patterns, uniform cross sites, and mutation sites were randomized between generations and across simulations.
A.2.3. Simulations

In simulations of SCS, pulse trains were delivered concurrently with and 20 s after the beginning of background primary afferent activity. Primary afferent activity, representative of recordings from primary afferents and dorsal root ganglion neurons following a peripheral nerve injury (Wall and Gutnick, 1974, Liu et al., 2000) evoked baseline activity that was required to visualize inhibitory responses. Afferent activity was simulated by applying an irregular spike train to the Aβ, Aδ, and C-fiber afferent inputs with statistical properties based on a Poisson process with inter-spike interval mean and standard deviation = 667 ms, and no ISIs < 300ms. The arrival latencies of peripheral inputs were determined according to published conduction velocities (Aβ: 14-30 m/s; Aδ: 2.2-8 m/s; C: 0.6-1.5 m/s) and an assumed conduction distance of 100 mm (Harper and Lawson, 1985, Woolf and Wall, 1982a). To account for the sensitivity of GA-SCS to the propagation distance from the SCS electrode, the assumed dorsal column propagation distance was set to either 1 cm or 10 cm. To account for the sensitivity of GA-SCS to GABAergic inhibition in the spinal cord, which is known to lessen with the progression of neuropathic pain (Braz et al., 2014), the strengths of GABAergic synapses from the local and surround inhibitory interneurons were reduced by 50 %. We accounted for the possibility of collisions between orthodromic and SCS-triggered antidromic action potentials along each Aβ-fiber and transmitted the net spike train to
the network model prior to simulation (Iggo, 1958). All simulations were performed using NEURON v. 7.2 simulation environment (Hines and Carnevale, 1997a) using a time step of 0.0125 ms and 2\textsuperscript{nd} order implicit Crank-Nicholson integration.

**A.3. Results**

We used a validated computational model based on the Gate Control Theory (Zhang et al., 2014b) coupled with a genetic algorithms (Goldberg, 1989) to design non-regular temporal patterns of SCS (GA-SCS) to be more effective and more efficient than constant frequency SCS (cfSCS). Implementing GAs using a cost function to define the relative importance of efficacy, quantified using the activity of the model "Gate" neuron during SCS, and efficiency, quantified as the average frequency of GA-SCS, produced GA-SCS patterns that were more effective than cfSCS at the average frequency and with fewer pulses/s than 50 Hz cfSCS. The characteristics and average frequencies of GA-SCS patterns derived using the default model were robust across multiple iterations of the GA using different randomized initial populations, immigrants, uniform cross pairings, and mutations. However, optimizations were driven primarily by improvements in efficacy against equivalent rate cfSCS, resulting in less efficient GA-SCS patterns. In addition, the characteristics of the optimized patterns were sensitive to the interaction between SCS-mediated excitation and inhibition of the Gate neuron. Changing the propagation distance between the site of SCS and the Gate neuron altered
the properties of optimized GA-SCS patterns, and reducing the level of GABAergic inhibition in the network model substantially reduced the efficacy of GA-SCS patterns. The results of this study suggest that under some conditions, GA-based optimization may be used to generate temporal patterns of SCS that are more effective than equivalent frequency cfSCS.

A.3.1. Computational analysis of GA-SCS patterns

We identified optimal GA-SCS patterns that were more effective than equivalent frequency cfSCS using a cost function (Equation A.2) that emphasized the relative efficacy of GA-SCS versus cfSCS (G) 10 x more than the efficiency (F). Over the course of the GA, generations "evolved" from a population of 20 random patterns and 5 constant frequency pulse trains (Figure A.3a) to a population that exhibited evidence of convergence to an optimal solution. The performance of the least fit (i.e., least effective, highest frequency SCS) GA-SCS patterns was never better than C = +290 and was primarily attributed to random immigrant patterns, and the performance of the GA-SCS pattern with median fitness fluctuated across generations, indicative of the influence of matings. However, the fitness of the two fittest patterns improved monotonically with successive generations, and improvements in the fitness of the survivors were accompanied by reductions in the firing rate of the Gate neuron versus equivalent frequency cfSCS (Figures A.3b, A.3c). Furthermore, the offspring of the crosses from the
previous generation were very similar to each other and to the survivors representing the two best solutions from the previous generation (Figure A.3c). Finally, although the firing rate of the Gate neuron was 7.3 spikes/s higher during GA-SCS than during 50 Hz cfSCS, the fittest GA-SCS produced 21.4 spikes/s less Gate neuron firing than cfSCS at the equivalent frequency of the GA-SCS pattern at a substantially lower equivalent frequency (34 pulses/s).

Multiple iterations of the GA using differently randomized initial populations, randomized uniform cross pairs, and randomized point mutation sites produced patterns with similar characteristics. Repeat GAs converged to GA-SCS patterns possessing the same average frequency (34 Hz), during which the Gate neuron exhibited the same firing rate (12.85 spikes/s), as the GA-SCS patterns from the original run (Figures A.3c, A.3e, A.6a). Although the patterns were not identical, the original and repeat optimized GA-SCS patterns both exhibited similar characteristics including alternating high frequency periods and low frequency periods over similar time intervals when aligned (Figure A.3d). This observation suggests that the characteristics of the GA-SCS patterns may be indicative of an underlying mechanism that results in the improved efficacy and efficiency of GA-SCS over cfSCS.
A.3.2. Effects of efficacy-efficiency balance

We altered the weighting on the efficiency term (B) to 5 x and 10 x its default setting (Equation A.2, Figure A.4) to assess how changing the desired balance of efficacy and efficiency affected the outcomes of the GA. Populations converged within 250-400 generations and the survivors of each successive generation were always as fit or more fit than those from the previous generation (Figure A.4a). Unexpectedly, weighting the efficiency term by 5x its default setting had few effects on the characteristics of the final patterns: the GA-SCS patterns exhibited similar frequency fluctuations, converged to the same average frequency, and produced the same firing rate in the Gate neuron as did the default GA-SCS patterns (Figures A.4b, A.4c). Increasing the efficiency term to 10 x its original setting forced resulting GA-SCS patterns to converge to 1 pulse/s SCS after 90 generations (Figures A.4d, A.4e, A.4f), suggesting that GAs using the cost function as defined in equation 2 have limited utility for optimizing patterns for efficiency.

One possible explanation for the inability of the GA to improve efficiency while preserving efficacy is that no patterns of GA-SCS at lower average frequencies exist that show greater efficacy than that achieved at 34 pulses/s (Figure A.3). Unless such solutions existed, GA-SCS patterns at lower frequencies would still be less fit than GA-SCS at 34 pulses/s due to the adverse effect on the cost function of increasing firing rate in the Gate neuron with increasing cfSCS frequency from 10 – 34 Hz (Figure A.3f). To
assess this possibility, we conducted fixed frequency GAs, in which all organisms across all generations were set to have the same average frequencies, at 10 pulses/s, 22 pulses/s, or 34 pulses/s. Because average frequency was fixed, only improvements in efficacy versus equivalent frequency cfSCS drove the evolution of patterns. In all fixed frequency GAs, optimized GA-SCS patterns after 197 generations (10 pulses/s), 225 generations (22 pulses/s), or 200 generations (34 pulses/s) converged in fitness and characteristics, and all patterns were equally or more effective than cfSCS (Figure A.5a, 5b). However, as the frequency of the GA-SCS patterns decreased, the patterns became more similar in appearance to 10 Hz cfSCS (Figure A.5b), and no fixed frequency GA-SCS pattern resulted in lower firing rates in the Gate neuron than 34 pulses/s GA-SCS (Figure A.5c). This finding indicates that improvements in efficacy vs. the 34 pulses/s GA-SCS pattern could not be achieved using lower frequencies.

A.3.3. Effects of excitatory-inhibitory balance

Dispersion in dorsal column input arrival times arising from differences in conduction distance between the SCS electrode and the Gate neuron may affect the interaction between SCS-mediated excitation and SCS-mediated inhibition and thereby change the characteristics of optimal GA-SCS patterns. To test this possibility, we conducted a GA optimization using the default model but with dorsal column input arrival times set assuming a conduction distance of 10 cm (Figure A.6a). Due to the
increased dispersion in excitatory inputs to the Gate network, the relationship between SCS frequency and Gate neuron activity during cfSCS changed at cfSCS frequencies > 35 Hz, with higher cfSCS frequencies producing more suppression of the Gate neuron. The GA converged to common solutions by 400 generations, and the average frequency (33 pulses/s) and firing rate of the Gate neuron during GA-SCS (10.2 spikes/s) were similar to those produced by the GA-SCS patterns optimized at a conduction distance of 1 cm (Figures A.6b-A.6d). Although high frequency/low frequency fluctuations were still present in the optimized GA-SCS patterns, the durations of the fluctuations were different than those in the original GA-SCS patterns, and the optimal GA-SCS patterns from both simulations appear to be out of phase. These results suggest that the characteristics of optimized GA-SCS patterns are somewhat sensitive to electrode placement and that GAs may need to be customized to specific placements and propagation distances to be maximally effective.

The loss of GABAergic inhibition has been linked to the development of pathological symptoms during the progression of chronic pain (Zeilhofer et al., 2012, Braz et al., 2014) and significantly alters the interaction between SCS-mediated excitation and inhibition of sensory neurons (Zhang et al., 2014b) and. To assess how changes in GABAergic inhibition affect the characteristics of GA-SCS patterns, we first conducted a GA after reducing the strength of GABAergic synapses from the local
inhibitory interneuron to the Gate neuron by 50 % to mimic an early disease state (Figure A.7a). Reducing local inhibition substantially diminished the reduction in Gate neuron firing rate by cfSCS, and cfSCS failed to inhibit the activity of the Gate neuron to below 16.4 spikes/s, in contrast to a rate of 2.1 spikes/s in the default model. Although the GA still converged by 250 generations (Figure A.7b), the characteristics of the fittest GA-SCS patterns were substantially different from those of the original GA-SCS patterns (Figure A.7c). In addition, GA-SCS was not as effective at reducing Gate neuron activity relative to equivalent frequency cfSCS: unlike in the original GAs, GA-SCS did not reduce the firing rate of the Gate neuron to below that produced by any frequency of cfSCS < 38 spikes/s. Furthermore, the optimized GA-SCS pattern exhibited a higher frequency (38 spikes/s) than the original GA-SCS patterns following loss of GABAergic inhibition (Figure A.7d).

We then assessed the effect of an advanced disease state on GA-SCS by reducing the strengths of the GABAergic synapses from both the local and surround inputs by 50 %. As with the early disease state, the cost function used to optimize GA-SCS patterns emphasized efficacy 10 x more than efficiency. Reducing local and surround inhibition further reduced the efficacy of cfSCS such that cfSCS did not reduce the firing rate of the Gate neuron to below 20.6 spikes/s. Although optimized GA-SCS converged within 100 generations (Figure A.8b), GA-SCS patterns inhibited the firing rate of the Gate neuron
only marginally more (< 2 spikes/s) than cfSCS. Thus, the GA emphasized efficiency over efficacy and converged to a low frequency (6 spikes/s) pattern. However, the optimal pattern suppressed the Gate neuron by only 1.9 spikes/s more than 6 Hz cfSCS and was less effective than maximally effective cfSCS (16 Hz). These results indicate that the utility of GA-SCS in its current implementation is limited following the loss of GABAergic inhibition in the spinal nociceptive circuit during the progression of chronic pain.

A.4. Discussion

The objective of this study was to design non-regular temporal patterns of SCS (GA-SCS) that were more effective than constant frequency SCS (cfSCS) at inhibiting the activity of model sensory neurons. A secondary goal was to devise patterns that could inhibit model sensory neurons at lower average stimulation frequencies, thereby improving the efficiency of SCS. Genetic algorithms (GAs) with a quantitative cost function that defined fitness were used to evolve stimulation patterns to be more effective and efficient. Initial and repeat GAs using the default computational model produced similar patterns that were more effective at inhibiting Gate neuron activity than cfSCS at the same frequency and that were robust to the random seeds used to initialize the GA. However, GA-SCS patterns were sensitive to the propagation distance between the site of SCS and the Gate neuron (i.e., electrode placement), and GAs were
not as effective in optimizing for efficiency or after loss of GABAergic inhibition associated with chronic pain. These computational results suggest that GA-driven optimization of SCS may produce more effective patterns than cfSCS at a given stimulation frequency under certain conditions, but an alternative approach for implementing GA-SCS is necessary for the design and use of non-regular temporal patterns to treat advanced chronic pain.

A.4.1. Mechanisms underlying GA-SCS

Across all GAs, the fittest pattern always performed equally with or better than both cfSCS at the same frequency and the fittest pattern from the prior generation, indicating that GA-SCS patterns did improve according to the cost function. A non-regular temporal pattern may contain features that produce more inhibition and minimize excitation, but the possible solution space for features or irregular patterns favorable for inhibition of the Gate neuron is infinite and therefore requires an optimization algorithm to resolve. GA-based optimization involves ranking patterns by their performance against a quantitative cost function (“fitness”) and selecting patterns for recombination with probabilities proportional to their fitness levels. Such "computational evolution" promotes the preservation of favorable features and the omission of unfavorable features for a given balance of efficacy and efficiency, spurring convergence towards optimal solutions. That repeats of the GA using converged to
similar solutions suggests that the underlying mechanisms of common motifs between patterns may drive the improved efficacy of GA-SCS patterns over equivalent frequency cfSCS.

The interaction between excitation and inhibition of sensory neurons by SCS (Foreman et al., 1976b) drives stimulation-frequency dependent efficacy of SCS (Zhang et al., 2014b), and non-regular patterns may more effectively exploit this interaction and at lower frequencies than cfSCS. Notably, the GA-SCS patterns assuming 1 cm and 10 cm propagation distance using the default model featured alternating high frequency and low frequency periods. During high SCS frequency segments, inhibitory events occur sufficiently close to each other to overcome SCS-mediated excitation. In addition, residual inhibition due to temporal summation from high frequency segments may further suppress Gate neuron activity after the end of the segment, allowing for a subsequent lower frequency segment. The lower frequency segment in turn lowers the average frequency of the overall pattern without resulting in an increased firing rate from the Gate neuron. In contrast, during cfSCS, each SCS-mediated inhibitory event is time locked to an excitatory event, and the only way to produce sufficient inhibition to overcome excitation in cfSCS is to increase the stimulation frequency. However, the inter-pulse interval in a cfSCS pulse train is constant, resulting in temporal summation of inhibitory events beyond what is required to suppress the Gate neuron. Experimental
assessments of GA-SCS patterns are needed to verify the improved efficacy of GA-SCS at lower frequencies than clinical standard 50 Hz SCS.

A.4.2. Limitations of GA-SCS

Despite the ability of GA-SCS patterns to suppress the Gate neuron firing rate equally to or better than cfSCS, the outcome of the GAs using the default model did not represent the most efficient solutions. Although the GA-SCS solution at 34 pulses/s was more effective than any cfSCS < 35 Hz, fixed frequency GAs at 10 pulses/s and 22 pulses/s generated GA-SCS patterns that produced inhibition of the Gate neuron as GA-SCS at 34 pulse/s. The reason for convergence to 34 pulses/s GA-SCS is that the GA cost function (Equation A.2) emphasized efficacy against equivalent frequency cfSCS ("G", Equation A.1) rather than just the firing rate of the Gate neuron during GA-SCS. Notably, 34 Hz cfSCS produced a local maximum in the SCS-frequency response relationship of the Gate neuron. As a result, GA-SCS patterns that suppressed the Gate neuron at 34 pulses/s produced the largest G and were therefore evaluated as being the fittest by the cost function. However, weighting efficiency and efficacy equally abruptly overcame the effect of the local maximum and resulted in GA-SCS patterns converging to 1 pulse/s. Conducting a finer grained analysis of the effects of weighting of the efficiency term on the resulting GA-SCS patterns may reveal a range of efficiency terms.
that lead to the lower frequency alternatives. The use of non-linear cost functions may also enable a better balance between Gate neuron inhibition and stimulation frequency.

The properties of optimized GA-SCS patterns were sensitive to the excitatory-inhibitory interactions driving the effects of SCS. Although similar to the original GA-SCS patterns, GA-SCS patterns optimized using a 10 cm propagation distance exhibited shorter duration fluctuations between high frequency and low frequency periods. This observation suggests that dispersion due to increased distance sufficiently affected the excitation-inhibition interaction driving SCS effects to alter the characteristics of the GA-SCS solution. More importantly, the reduction of GABAergic inhibition associated with the progression of chronic pain (Moore et al., 2002a, Zeilhofer et al., 2012, Braz et al., 2014) substantially reduced the overall efficacy of both cfSCS and GA-SCS. The loss of local inhibition representing an early disease state weakened SCS-mediated inhibition sufficiently to prevent the temporal summation of SCS-evoked inhibitory events from overcoming SCS-mediated excitation, resulting in less efficacy versus cfSCS by the GA-SCS pattern. When both local and surround inhibition were compromised, insufficient inhibition was present to overcome any SCS-mediated excitation; as a result, GA-SCS patterns converged to solutions with low average frequencies, as higher frequency GA-SCS patterns produced more Gate neuron activity. These results indicate that incorporating a method to disperse SCS-mediated excitation, such as by applying
different GA-optimized patterns to distinct groups of dorsal column fibers, similar to dual frequency SCS (Chapter 4), is necessary for GA-SCS to be effective in advanced chronic pain.

Finally, we did not account for the effects of SCS on supraspinal circuits (Heinricher et al., 2009) or on descending pathways, such as the dorsolateral funiculus, that may be involved in the effects of SCS. Although segmental mechanisms are sufficient in describing inhibitory and excitatory responses to SCS (Foreman et al., 1976a, Smits et al., 2012), supraspinal loops may also modulate analgesia by SCS (Barchini et al., 2012). For example, noradrenergic neurons in locus coeruleus (Song et al., 2013a) and serotoninergic neurons in the rostroventromedial medulla (Song et al., 2013b) exhibited more activity during SCS in neuropathic rats that showed increased paw withdrawal thresholds than in non-responding rats. These data suggest that effective SCS for chronic pain requires activation of supraspinal centers, and that up to 50 % of SCS-mediated inhibition may originate from supraspinal activation (Barchini et al., 2012) motivates the development of a combined spinal-supraspinal model. Optimizing temporal patterns of SCS using a model that includes supraspinal mechanisms may produce more effective and efficient temporal patterns of SCS than clinically used 50-80 Hz cfSCS.
Figure A.1 Computational modeling of the effects of genetic algorithm optimized patterns of SCS (GA-SCS) on the activity in the spinal Gate neuron.

GA-SCS was applied concurrently with random peripheral afferent activity with statistical properties consistent with primary afferent activity following nerve injury. The output of the Gate neuron was used as a proxy for efficacy.
Figure A.2 Schematic of the model-based design process of GA-SCS.

An initial generation of random patterns was generated and applied to the model, after which GA-based optimization proceeded iteratively until the generation or convergence limit was reached.
Figure A.3 Optimization of temporal patterns of SCS using a genetic algorithm strongly weighted for efficacy.

(a, c): Initial and final populations of stimulation patterns. Each row is a stimulation pattern, and each line represents the time point at which a stimulation pulse would be delivered by the stimulator. In (c), blue patterns denote the two most fit "optimized" GA-SCS patterns (i.e. the patterns with the lowest cost functions) from the previous generation, red patterns denote randomly generated immigrants, and green patterns denote the children from the crossing of patterns from the previous generation. (b): Cost
function scores from the best pattern, median pattern, and worst patterns during the original (top) and repeat (bottom) GAs. Dotted line denotes equivalence with cfSCS. The Y-axis was broken at C = 300 to allow visualization of the progression of the best and worst patterns. (d): Comparison of two successive 1 s repeats of final generation survivors from the original and a repeat GA conducted using different random seeds. Red lines denote high stimulation rate periods and blue lines denote low stimulation rate periods. (e): Plot of Gate neuron firing rate during optimized GA-SCS compared to Gate neuron firing rate during cfSCS between 1 – 60 Hz.
Figure A.4 Optimization of temporal patterns of SCS using a genetic algorithm with strong weightings of efficiency.

(a, d): Cost function scores from the best pattern and median pattern over the course of the GA using a 5 x weight on efficiency (a) or a 10 x weight on efficiency (d). The worst pattern, which never produced C < 200 in either case, is not shown. (b, e): Final populations of survivors (blue), immigrants (red), and children (green) after GA using a 5 x weight on efficiency (b) or a 10 x weight on efficiency (e). (c, f): Plot of Gate neuron firing rate during optimized GA-SCS using a 5 x weight on efficiency (c) or a 10 x weight
on efficiency (f) compared to Gate neuron firing rate during CF-SCS between 1 – 60 Hz. In (f), the default and 5x efficiency weight performance of GA-SCS are shown in gray.
Figure A.5 Optimization of temporal patterns of SCS using a genetic algorithm with patterns fixed to have average frequencies of 10 pulses/s, 22 pulses/s, or 34 pulses/s.

(a, d): Cost function scores from the best pattern, median pattern, and worst pattern over the course of the GA using 10 pulses/s (top), 22 pulses/s (middle), and 34 pulses/s (bottom).  (b): Final populations of survivors (blue), immigrants (red), and children (green) after fixed frequency GA using 10 pulses/s (i), 22 pulses/s (ii), and 34 pulses/s (iii).  (c): Plot of Gate neuron firing rate during optimized GA-SCS using 10 pulses/s (i), 22 pulses/s (ii), and 34 pulses/s (iii).
Figure A.6 Optimization of temporal patterns of SCS using a genetic algorithm with a 10 cm conduction distance.

(a): Depiction of simulation.  (b): Cost function scores from the best pattern and median pattern over the course of the GA.  The worst pattern, which never produced C < 200, is not shown.  (c): Final populations of survivors (blue), immigrants (red), and children (green).  (e): Plot of Gate neuron firing rate during optimized GA-SCS compared to Gate neuron activity during CF-SCS between 1 – 60 Hz.
Figure A.7: Optimization of temporal patterns of SCS using a genetic algorithm following 50% reduction of local GABAergic inhibition

(a): Depiction of simulation with computational network. Red dotted lines denote inhibitory synapses weakened by 50%. (b): Cost function scores from the best pattern and median pattern over the course of the GA. The worst pattern, which never produced C < 200, is not shown. (c): Final populations of survivors (blue), immigrants (red), and children (green). (e): Plot of Gate neuron activity during optimized GA-SCS compared to Gate neuron firing rate during cfSCS between 1 – 60 Hz.
Figure A.8 Optimization of temporal patterns of SCS using a genetic algorithm following 50% reduction of local and surround GABAergic inhibition.

(a): Depiction of simulation with computational network. Red dotted lines denote inhibitory synapses weakened by 50%. (b): Cost function scores from the best pattern and median pattern over the course of the GA. The worst pattern, which never produced $C < 200$, is not shown. (c): Final populations of survivors (blue), immigrants (red), and children (green). (e): Plot of Gate neuron activity during optimized GA-SCS compared to Gate neuron firing rate during cfSCS between 1 – 60 Hz.
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**Zhang TC, Janik JJ and Grill WM** 2014a Mechanisms and models of spinal cord stimulation for the treatment of neuropathic pain *Brain Research*
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