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Los Angeles

**Electrical Stimulation and Recording of the  
Spinal Cord for Autonomic Neuromodulation**

A thesis submitted in partial satisfaction  
of the requirements for the degree  
Master of Science in Bioengineering

by

**Paymon Garakani Rezaii**

2016

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ABSTRACT OF THE THESIS

# **Electrical Stimulation and Recording of the Spinal Cord for Autonomic Neuromodulation**

by

**Paymon Garakani Rezaii**

Master of Science in Bioengineering

University of California, Los Angeles, 2016

Professor Wentai Liu, Chair

Electrical stimulation of the spinal cord has been demonstrated to facilitate recovery of motor functions. Here, the effects of spinal cord stimulation on autonomic functions is demonstrated via two series of experiments. The first series involved epidural stimulation of the dorsal cervical spinal cord in anesthetized patients for respiratory modulation. Application of epidural stimulation resulted in acute changes in respiratory rate and tidal volume, dependent on the location and frequency of stimulation. The second series involved transcutaneous electrical stimulation (tSCS) of the thoracolumbar spinal cord of spinal cord injury subjects to enable bladder function. Results demonstrate improvements in bladder function via repeated application of tSCS. Lastly, epidural thoracic potentials were analyzed to determine if the waveforms contained information regarding the onset of bladder sensations. Distinct differences in spectral characteristics of the waveforms were demonstrated; however, a larger sample size is needed to confirm whether the waveforms can decode urge onset.

The thesis of Paymon Garakani Rezaii is approved.

Victor R. Edgerton

Warren Grundfest

Wentai Liu, Committee Chair

University of California, Los Angeles

2016

*To those who have experienced  
neurological damage. Together, we will find  
a solution.*

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# CHAPTER 1

## Introduction

### 1.1 Consequences of Spinal Cord Injury

For healthy individuals, normal activities such as breathing, using the restroom and walking may be overlooked or taken for granted. After a spinal cord injury (SCI), these ‘normal’ activities can become extremely challenging or even impossible. For instance, an injury to the spine can cause weakness and result in functional deficits as severe as not being able to move the arms and/or legs. Depending on the location and severity of the damage along the spinal cord, SCI may also result in lost or diminished respiratory, sexual, bladder and bowel functions, among others. SCI can severely impact a person’s life and affect one’s ability to perform daily activities.

SCI currently affects about 273,000 people in the U.S. alone [26]. SCI of the cervical spine normally results in tetraplegia while injury to the thoracic and/or lumbar spine can lead to paraplegia. Less than 1% of all individuals with SCI experienced full neurological recovery at the initial hospital discharge [26]. Life expectancy of those affected by SCI is significantly less than those without SCI [26]. Spinal cord injuries negatively impact thousands of people and diminish quality of life.

The overarching and ultimate goal of the research reported here was to help restore independence of those affected by SCI. Multiple series of experiments were conducted in which electrical stimulation was applied to the spinal cord in or-

der to facilitate the recovery of respiratory and lower urinary tract functions. In addition, a separate experiment was conducted in order to determine if neurological potentials recorded from the spinal cord can aid in determination of optimal stimulation-based therapy regimes to restore functions lost or hindered due to SCI.

Before delving into the details of these experiments, a brief introduction on the neural basis of respiration and micturition is mentioned, followed by commentary on current state-of-the-art technology to help recover functions lost due to SCI, ensued by a primer on neurological potentials and how they have been used to understand various aspects of the spinal cord.

## **1.2 Neural Regulation of Respiration**

Respiration is essential for life; without our respiratory system, we would not be able to adjust to our body's demands. Regulation of respiration requires: (1) generation and preservation of a respiratory rhythm, (2) rhythm modulation via sensory feedback loops and reflexes, and (3) activation of respiratory muscles that contract in a coordinated manner.

### **(1) Generation and preservation of respiratory rhythm**

Central control of breathing is accomplished via control centers in the brainstem that transmit descending efferent signals to the motor neuron pools in the spinal cord to activate associated respiratory muscles [68, 135, 158, 167]. The rhythmicity of breathing is generated within the medulla; specifically, the rhythm-generating neurons are located within the pre-Bötzinger complex (pre-BötC) and the para-facial respiratory group (pFRC) found in the ventral lateral medulla [69, 172].

These neurons synapse onto propriobulbar neurons and premotor neurons lo-

cated in the ventral respiratory group which is found in the medulla oblongata [157]. The medulla contains two dense bilateral groups of neurons: the ventral respiratory group (VRG) and the dorsal respiratory group (DRG) [157]. These groups send information to the diaphragm and intercostal muscles in order to regulate breathing. The DRG, which is located in the ventrolateral portion of the nucleus of the tractus solitarius, is always functioning during breathing and regulates respiratory rate and rhythm [157]. The DRG responds to changes in blood pH, carbon dioxide and oxygen levels. The DRG primarily consists of cells that are active during inspiration [19]. DRG neurons transmit signals to phrenic motoneurons located in the cervical spinal cord to control respiratory muscles (i.e. diaphragm and external intercostals) [214]. The VRG, which is associated with the nucleus ambiguus and nucleus retroambiguus, consists of inspiratory and expiratory cells [97]. The VRG expiratory cells activate expiratory muscles (i.e. abdominal and internal intercostals) during active expiration and also sends inhibitory signals to suppress activity of inspiratory neurons [97]. The VRG becomes involved when breathing demands are increased, such as during forceful breathing; it works harmoniously with the DRG and has connections to the accessory muscles of respiration.

The Böttinger complex (BötC), located in and near the nucleus retrofacialis in the medulla, is primarily composed of expiratory neurons as well as vagal and glossopharyngeal motoneurons [153, 210]. It exhibits an inhibitory effect on inspiratory cells of the DRG and VRG as well as spinal motoneurons [16, 127, 143, 211].

There are also respiratory centers located in the pons: the Pontine Respiratory Group (PRG) and the Apneustic center. These work with centers in the medulla to adjust respiration via reciprocal connections [28, 202]. The PRG, consisting of expiratory and inspiratory cells, receives input from the cerebral cortex, limbic system and hypothalamus, then sends information to the medullary respiratory

centers to adjust the depth and length of each breath [3, 28, 29, 70]. The Apneustic center is active during inhalation; it works with the inspiratory neurons in the medulla to adjust inhalation [15].

## **(2) Rhythm modulation via sensory feedback loops and reflexes**

The respiratory control system can be altered via various inputs. For instance, inputs from higher brain centers modulate breathing. The cerebrum, limbic system and hypothalamus send information to the brainstem centers in order to regulate breathing [97, 151]. Non-specific and specific proprioceptors of muscles, joints and tendon organs provide feedback to the medullary respiratory center [15]. Mechanoreceptors, central and peripheral chemoreceptors found in the brainstem, lungs, and circulatory system feedback to the medullary respiratory neurons to influence respiration [15]. Temperature and pain information is also fed-back onto the respiratory centers [151]. Also, level of arousal and emotional state will influence breathing [151]. In addition, breathing can be modulated via voluntary control from the cerebral cortex; these voluntary pathways bypass the medullary respiratory centers to directly affect spinal respiratory motoneurons [151].

## **(3) Activation of respiratory muscles**

The respiratory centers in the brainstem communicate with the spinal cord in order to activate respiratory muscles to accomplish pulmonary ventilation. Ventilation requires alteration of intrapulmonary pressure achieved by contraction of inspiratory muscles followed by passive recoil of the lungs [99]. The primary inspiratory muscles consist of the diaphragm (innervated by the phrenic nerve) and the external intercostal muscles (innervated by the external intercostal nerves) [151]. The phrenic nerve is composed of axons originating from motoneurons in the ventral horn of cervical spinal cord segments C3-C5 [185], whereas the external intercostal nerves consist of axons that originate from motoneurons in the tho-

racic spinal cord segments T1-T12. Accessory inspiratory muscles consist of the scalene muscles and sternocleidomastoid muscles [151]. The primary expiratory muscles of respiration include the rectus abdominus (innervated by motoneurons in the L1-L5 spinal segments), the internal intercostal muscles (innervated by motoneurons in the T1-T12 spinal segments), and the internal and external oblique muscles (innervated by lower intercostal, iliohypogastric, and ilioinguinal nerves) [151]. For quiet (shallow) breathing, the diaphragm performs the majority of work required for breathing.

During inspiration, inspiratory neurons in the DRG and VRG send signals to cervical and thoracic motoneurons, activating the diaphragm and external intercostal muscles [99]. The diaphragm subsequently protrudes downward into the abdominal cavity while moving the abdomen outward to create a negative pressure inside the lungs [99]. The external intercostal muscles pull the ribs upward and outward, which mildly increases the negative pressure in the chest required for ventilation. Paralysis of the external intercostal muscles does not have a significant effect on breathing because the diaphragm plays the largest role of all respiratory muscles during respiration [129]. During quiet breathing, expiration is a passive process which is accomplished through termination of inspiration mainly via inhibitory signals from neurons in the medulla and spinal cord [99]. During passive expiration, the inspiratory muscles are allowed to relax and the compliance of the chest wall returns the ribs and abdominal contents back to their relaxed position, causing air to be pushed out from the lungs. Forced expiration requires expiratory neurons in the brainstem to send signals to the internal intercostal and abdominal muscles for contraction, which compresses the chest with greater force.

The respiratory system constantly adjusts breathing in order to keep oxygenated blood flowing to our cells. Failure of the respiratory system (such as in some cases of spinal cord injury) will cause severe impairment in quality of life, and can even be life-threatening. Motor-complete SCI of higher cervical levels (such as C1-C3) results in respiratory failure, due to the interruption of neural signals from the respiratory centers in the brain stem to the respiratory motoneurons in the spinal cord [50]. Fortunately, the respiratory muscles and their respective neural innervations below the spinal lesion remain intact and are therefore amenable to stimulation techniques for restoration of function [50].

### 1.3 Neural Regulation of Micturition

Urine is produced in the kidneys and travels down to the bladder via the ureters [99]. Once the bladder is sufficiently full, control centers in the brain and spinal cord cause the release of urine from the bladder down the urethra and out of the body. The bladder consists of a layer of smooth muscle fibers called the detrusor muscle [99]. There exists a smooth muscle called the internal urethral sphincter (present in males, absent in females) at the junction of the urinary bladder and the urethra. Caudal to the location of the internal urethral sphincter in males exists another smooth muscle called the external urethral sphincter [99]. The external urethral sphincter is present in both males and females.

The following discussion will focus on the male urinary tract, since all subjects involved in the micturition experiments (Chapter 3) were male.

**Control of micturition is accomplished via the nervous system.** Various parts of the central nervous system play roles in micturition, including the brain, brainstem, as well as the thoracic, lumbar and sacral spinal cord. The pons,



located in the brainstem, contains the pontine micturition center (PMC) [150]. The PMC acts as a control center for regulating micturition. For instance, an activated PMC communicates with the lower urinary tract by sending signals that relax the external sphincter and allow for voiding [207]. Communication between the central nervous system and the urinary tract is accomplished via efferent and afferent nerves.

Three primary efferent nerves control the urinary tract: the efferent pelvic nerve, the pudendal nerve, and the hypogastric nerve (Figure 1.1). The parasympathetic efferent pelvic nerve exits from the sacral spinal cord and synapses onto the  $M_3$  muscarinic receptors on the bladder wall [73]. Stimulation of the pelvic nerve from the spinal cord releases acetylcholine into the synaptic cleft at the  $M_3$  receptors on the bladder, which in turn causes detrusor muscle contraction [73]. The somatic pudendal nerve also exits from the sacral spinal cord, and acts on the nicotinic receptor on the external urethral sphincter [73]. Stimulation of the pudendal nerve causes release of acetylcholine, which when bound to the nicotinic receptors causes contraction of the external urethral sphincter [73]. While the activation of the pudendal nerve is under voluntary control, the activation of the efferent pelvic nerve does not function via voluntary control.

The pre-sympathetic nerve fibers exit from the thoracic-lumbar area of the spinal cord and synapse at a ganglion, secreting neurotransmitters that bind to a post-sympathetic nerve fiber called the hypogastric nerve [73]. The hypogastric nerve synapses onto the  $\beta_3$  adrenergic receptors of the detrusor muscle as well as the  $\alpha_1$  adrenergic receptors of the internal urethral sphincter [73]. Stimulation of the hypogastric nerve causes release of norepinephrine at these receptors. When norepinephrine binds to the  $\beta_3$  receptors, it causes relaxation of the detrusor muscle [73]. When norepinephrine binds to the  $\alpha_1$  receptors, it causes contraction of the internal urethral sphincter [73]. Therefore, the sympathetic nervous system is primarily responsible for urine retention, whereas the parasympathetic nervous

system is primarily responsible for urine expulsion.

The primary afferent nerve involved in control of micturition is called the sensory pelvic nerve, which sends signals from the detrusor muscle to the spinal cord [43, 73]. This sensory pelvic nerve is stimulated in response to stretching of the bladder (i.e. when the bladder is full of urine) [43, 73].

**Neural activity during empty bladder.** A bladder which contains little to no urine is one that is not experiencing much stretching of its walls due to the urine. The sensory pelvic nerve senses the lack of significant bladder wall stretching, and sends low frequency impulses to neurons in the sacral spinal cord [208]. These sacral neurons have ascending projections to the thoracic level of the spinal cord [73]. When the low frequency impulses from the sensory pelvic nerve are received by the sacral neurons, it stimulates the thoracic pre-sympathetic nerve that synapses onto the hypogastric nerve, causing increased hypogastric nerve activity [73]. Stimulation of the hypogastric nerve causes relaxation of the detrusor muscle and contraction of the internal urethral sphincter, which helps keep the urine in the bladder [73].

When the bladder is empty, the brain and micturition center of the pons send signals to the thoracic level of the spinal cord to cause stimulation of the hypogastric nerve [42, 73, 208]. In addition, the neurons in the brain and pons send signals to the sacral spinal level, which inhibit the afferent pelvic nerve (causing inhibition of detrusor contraction) and stimulate the pudendal nerve (causing external urethral sphincter contraction) [42, 73, 208]. These combined effects allow one to store urine in the bladder voluntarily.

**Neural activity during full bladder.** A bladder which contains a large amount of urine experiences a significant amount of stretch of its walls. Stretching of the bladder walls causes increased stimulation of sensory nerves connected

to the bladder walls, resulting in an increased frequency of firing of the sensory pelvic nerve which enter the sacral spinal cord [208]. The neurons in the sacral spinal cord that receive the high frequency signal from the sensory pelvic nerve send ascending signals that bypass the thoracic spinal level and synapse onto neurons in the pontine micturition center in the pons [73, 208]. Stimulation of the pontine micturition center causes inhibition of the hypogastric nerve (which inhibits detrusor muscle relaxation and internal urethral sphincter contraction) [73]. In addition, excited neurons of the pontine micturition center stimulate the efferent pelvic nerves (causing detrusor muscle contraction) and inhibit the activity of the pudendal nerve (causing inhibition of external urethral sphincter contraction which effectively relaxes the external sphincter) [73]. These combined effects allow one to expel urine from the bladder voluntarily.

**Voiding Reflex.** At the initiation of voiding (expulsion of urine from the bladder), the voiding reflex occurs. During this reflex, the afferent pelvic nerve fibers continue to send impulses to neurons in the sacral spinal cord [73]. These neurons synapse onto interneurons in the sacral level, which in turn stimulate the parasympathetic efferent pelvic nerve fibers [73]. Stimulation of the efferent pelvic nerve fibers causes detrusor muscle contraction. This allows for the continuation of voiding once it has been initiated.

## 1.4 Spinal Cord Stimulation (SCS)

### 1.4.1 Suitability of Spinal Cord for Neuromodulation

Spinal cord injuries vary based on the location along the spine the injury occurred, as well as the severity of the injury. Those who experience ‘incomplete’ spinal cord injuries have some spared functional connections between the neurons above the lesion and neurons below the lesion. Patients with incomplete

spinal cord injuries can experience various forms of recovery of lost motor and sensory functions over time, especially with rehabilitation. For instance, it has been demonstrated that locomotor training improves recovery of walking in individuals with incomplete SCI [98, 103, 104, 115, 125, 131, 148, 213, 216, 217, 218, 219, 220, 221]. However, those who have experienced complete spinal cord injuries, unfortunately do not generally recover as well as those with incomplete injuries, presumably due to the lack of intact connections between the brain and spinal cord below the lesion [223]. Nevertheless, the cellular structures and neural circuits below the spinal lesion remain intact even though they are not functionally connected to supraspinal centers [50]. When these neural networks below the lesion receive suitable sensory input, and are sufficiently excited, they are able to function in manners similar to those when the spinal cord is not injured. One such potential way to sufficiently excite the neural networks below the lesion is via the use of electrical stimulation; this intervention has been referred to as electro-enabling motor control (eEmc) [62]. eEmc has been shown to restore weight bearing [6, 189] and voluntary motor function [5, 102] in individuals with motor-complete spinal cord injury.

Recent research has shown that it is possible to recover voluntary motor and autonomic functions, such as locomotion and micturition, by the use of electrical stimulation of the spinal cord. Although the complete mechanism of action of spinal electrical stimulation is still unclear, the functional gains of subjects who have undergone this form of intervention are encouraging and command further research. The following is a brief review of the results obtained from the use of electrical stimulation for spinal neuromodulation in both humans and animals.

### 1.4.2 Epidural SCS for Facilitation of Postural and Locomotor Recovery

Of the various functions lost or hindered due to spinal cord injury, postural and locomotor function may be the most extensively researched to date. Recently, a number of rehabilitative interventions, including assisted motor training, epidural spinal cord electrical stimulation, and/or pharmacological facilitation have yielded astonishing recovery of motor function in humans and animals [187].

In 2011, Harkema *et al.* reported a case study in which an individual with motor-complete and sensory-incomplete spinal cord injury regained voluntary movement after seven months of epidural electrical stimulation in combination with stand and step training. This study was motivated by results that demonstrate the generation of rhythmic and tonic motor patterns of the legs of patients with motor-complete SCI by the use of epidural electrical stimulation [58, 78, 154, 156, 123]. These findings suggest that locomotor circuits exist in the human spinal cord; however, locomotion cannot normally be accomplished in humans with SCI due to the absence of a particular level of excitability from supraspinal centers. Therefore, Harkema *et al.* hypothesized that epidural spinal cord stimulation can sufficiently modulate the human spinal networks into a physiological state that permits sensory input from standing/stepping movements to act as a source of neural control to promote locomotor activities [102]. To test the hypothesis, they conducted a series of experiments in which epidural stimulation was administered to a man who had a clinically complete SCI. The results were astonishing. The subject exhibited robust and consistent rhythmic stepping-like activity during manually facilitated stepping only when tonic epidural stimulation and stepping-associated proprioception was present. Epidural stimulation was shown to enable sensory input that might serve as a controller of spinal circuitry during standing and manually facilitated stepping, without clinically detectable supraspinal input [102]. It was presumed that the epidural stimulation enabled motor function by

stimulating afferent fibers in the dorsal root to involve populations of interneurons responsible for integrating lower limb proprioceptive input in order to coordinate activity of the motor pools. Harkema *et al.* explains that this phenomena may be due to the reactivation of residual supraspinal connections that cannot be clinically detected, or due to the formation of new supraspinal connections in the spinal cord. Specifically, the epidural stimulation may have provided ample excitation of the lumbosacral interneurons and motoneurons [122, 148], which when combined with the otherwise insufficient excitatory activity of residual descending axons, created a sufficient level of excitation to activate motoneurons to accomplish postural and locomotive functions. Another possible mechanism is the idea that axonal regeneration or sprouting may have been induced due to the experimental regime over seven months. Regardless of potential mechanism, the subjects was able to exhibit conscious movement control presumably from increased interneuronal excitability via subthreshold epidural electrical stimulation, which allowed for control via descending pathways.

Results from Harkema *et al.* prompted Angeli *et al.* to conduct another set of experiments on four individuals who had experienced SCI, two of which were diagnosed with both motor and sensory complete SCI [5]. These individuals were shown to elicit intentional movements only in the presence of epidural stimulation, which indicates that the influence of remaining functional supraspinal connections alone were not sufficient to activate spinal motor pools. Angeli *et al.* therefore proposed that epidural stimulation may drive the functional state of spinal networks closer to their appropriate activation threshold, effectively enabling voluntary movement [14, 180, 222]. Epidural stimulation may have facilitated intentional motor drive via transmission of rostro-caudal signal propagation through propriospinal interneuronal projections [35, 36, 72, 224, 225]. Spinal plasticity may also play a role after repetitive epidural stimulation and postural/locomotive training, potentially forcing disrupted spinal pathways into a more functional

state. Presumably, new neuronal pathways and synapses were established, yielding novel functional connectivity [182]. Although Harkema *et al.* previously stated that a potential mechanism of epidural stimulation to induce voluntary movement may result from axonal regeneration or sprouting, Angeli *et al.* report that this mechanism is unlikely for execution of volitional movement in three of the four individuals present in their study. Overall, they showed that neuromodulation of the sub-threshold motor state of excitability of the lumbosacral spinal networks played a key role to recover voluntary movement in all of their studied individuals who exhibited complete paralysis, even years after injury.

A handful of other reports have demonstrated the effects of lumbosacral epidural spinal cord stimulation on motor movements. Results from Sayenko *et al.* [189] show that epidural electrical stimulation of specific locations of the lumbar spinal cord results in selective recruitment of proximal and distal leg muscles, while Sherwood *et al.* [194] showed that epidural stimulation increased motor performance in patients experiencing motor neuron disorders. Barolat *et al.* demonstrated that epidural stimulation enhanced voluntary motor function [11]. Herman *et al.* showed that patients with severe incomplete SCI who received epidural stimulation were able to walk faster and expend less energy over a 15-meter distance [105]. Other studies have demonstrated that the use of lumbar stimulation reduces the motor threshold for activation of locomotor spinal circuitry [6, 132, 162]. Results from Gilge *et al.* illustrate that sustained extension of the lower limbs can be accomplished using lumbar spinal epidural stimulation in subjects with motor complete SCI [123]. Carhart *et al.* demonstrated that epidural spinal cord stimulation facilitates recovery of functional walking in a subject experiencing chronic incomplete spinal cord injury [23]. Dimitrijevic *et al.* [58], Pinter *et al.* [179] and Gerasimenko *et al.* [85] have demonstrated that epidural stimulation of the spinal cord can induce rhythmic limb movements in subjects with motor-complete SCI.

Similar results have been demonstrated in animal models. For instance, epidu-

ral stimulation has been shown to augment hindlimb stepping functions in normal rats and rats with complete spinal cord transections [80, 83, 117]. Gerasimenko *et al.* [81] and Ichiyama *et al.* [119] showed that administration of quipazine in combination with epidural stimulation facilitates walking in the rat model. Electrical stimulation applied dorsally over the lumbar spinal cord of decerebrate cats was demonstrated to induce active and fictive hindlimb locomotion [120, 140, 164, 165]. Gerasimenko *et al.* demonstrated similar results in both spinalized and decerebrate cats [80, 82]. Musienko and coworkers state that locomotor activity in spinal as well decerebrate cats, facilitated by epidural stimulation, is controlled by peripheral sensory feedback from the limbs [164, 165]. Activation of spinal circuits involved in inducing motor outputs has also been demonstrated in spinal rats [33, 75, 117, 118, 137, 138, 139].

#### **1.4.3 Postulated Mechanisms of Action of Epidural Stimulation for Locomotor Recovery**

The mechanisms of action by which epidural electrical stimulation improves functions lost or impaired due to spinal cord injury are currently not fully understood, despite the extensive amount of research on the topic.

Hofstoetter and coworkers hypothesized that the basic excitability of the lumbar spinal locomotor circuitry can be enhanced by the use of spinal cord stimulation, and presumed that spinal cord stimulation modified the state of the spinal networks in a way such that they become more responsive to inputs from supraspinal structures via the remaining intact descending pathways [108, 110]. Young *et al.* hypothesized that widespread activation of both ascending and descending activity due to spinal cord stimulation may strengthen synaptic activity in motor centers of the spinal cord through Hebbian mechanisms that play a role in walking, micturition and defecation [223]. Gerasimenko and coworkers stated that the combination of descending supraspinal input, peripheral sensory input



and interneuronal networks that have been modulated by spinal cord stimulation activates motor pools that generates muscle activity [84]. Spinal networks above, within and below a lesion can be rehabilitated into a functional state in the presence of a sufficient amount of spinal activation [84].

Three important factors aid in recovery of function via spinal cord electrical stimulation [187]: automaticity of the spinal cord, sensory input to the spinal cord and learning capacity of the spinal cord circuitry (plasticity).

Automaticity of the spinal cord refers to the cord's ability to self-regulate and function without volitional control [187], specifically its ability to interpret complex sensory information and subsequently process this information to produce, for example, postural and locomotor functions [63, 66]. Central pattern generating networks (CPG), which produce rhythmically patterned outputs, are key to the automaticity of the spinal cord, and can elicit activity in response to sensory input [187].

Sensory input to the spinal cord plays an important role in spinal cord automaticity. Upon receiving a set of sensory information from various sources, the spinal cord makes decisions for the most appropriate response at that point in time. In the case of complete spinal animals, spinal interneurons can predict the next logical sequence of neurons to activate based on the specific groups of neurons that were activated immediately prior to that time point [187]. It has been shown that in the absence of supraspinal input, CPG neuronal cells interpret nonspecific afferent signals, providing the necessary information required to produce effective locomotor function [63, 66]. However, without sensory input to the spinal cord, CPG activity is limited [187].

The spinal cord is able to learn; it exhibits plasticity with repetitive training. It has been shown that adult cats with spinal lesions could be trained to step [9, 10, 44, 61, 64, 65, 144]. Based on these and similar experiments, it has become clear that the spinal neural networks can learn a task with repeated practice

[45, 46], and can also forget these tasks if not practiced [27, 47].

Although the specific mechanisms are not fully understood, results from the literature indicate that spinal cord stimulation can serve as an enabler of neural control and improve normal physiology such as voluntary motor control [62, 109].

#### **1.4.4 Neuronal Elements Activated by Epidural SCS**

Epidural spinal cord stimulation has become a widely used treatment for patients with intractable pain [17, 114, 116, 133, 136, 160, 166, 170, 197, 199]. As stated in previous sections, epidural stimulation has also been shown to improve voluntary and involuntary functions in those who have been affected by spinal cord injury.

The mechanisms of action by which epidural electrical stimulation improves functions lost or impaired due to spinal cord injury are currently not well understood. In order to obtain a better understanding of how the electrical stimulation may be affecting the neural circuitry in the spinal cord to induce various functions, it is helpful to review the neural structures that are presumably activated by epidural electrical stimulation.

Intraspinal structures can be viewed as non-homogenous conductors. The following structures are most relevant for spinal electrical stimulation, in order of most conductive to least conductive: cerebral spinal fluid (CSF), longitudinal fibers, grey/white matter, transverse fibers, epidural space, vertebral bone, and dura mater [12]. The CSF is by far the largest contributor towards the spread of electrical current (approximately 80-90 percent of current flow contained in CSF [12]), since its conductivity is approximately twice the conductivity of the next most conductive spinal tissue [21]. Therefore, the volume of the CSF space between the electrode and neural structures is an important factor that determines the current spread due to epidural spinal cord stimulation. Each spinal segment

has a different thickness of dorsal CSF layer; the thoracic levels (especially T5-T6) contain the largest thickness of dorsal CSF layer [12].

It was initially thought that the intensity of epidural spinal cord stimulation used to reduce intractable pain generates electric fields and current distributions that cause both dorsal root and dorsal column recruitment [4]. Dorsal root fibers typically enter the spinal cord and diverge into ascending and descending branches [24, 40]. These fibers then diverge forming rootlets that enter the spinal cord at various angles. The angles at which these rootlets enter the spinal cord plays a significant role in how much electrical stimulation is required to activate these rootlets [12]. The area where dorsal root fibers enter the dorsal horn is the region that requires the least amount of electrical stimulation for activation [123]. The dorsal columns are aggregates of fibers with large diameters which transmit sensory information (i.e. tactile sense, limb proprioception, bladder sensations, etc.) [99, 207]. The fibers that exist in the dorsal columns also project collateral branches that enter the dorsal horn and end in the laminae of the gray matter [152].

There are three primary anatomical factors that affect the excitation threshold of neural fibers: 1. fiber diameter (larger fibers are recruited first; therefore dorsal root fibers are recruited before dorsal column fibers), 2. fiber curvature and orientation (curvature away from the cathode decreases excitation threshold; fibers that cross the electric field longitudinally have a higher threshold than those that run transversely), and 3. collateral branching (fibers with collaterals exhibit lower excitation thresholds than those without collaterals) [12]. The excitation threshold for the following fibers increases from least to greatest: dorsal root fibers, lateral dorsal column fibers, and medial dorsal column fibers [12].

The position of the stimulation electrodes as well as the inter-electrode distance also affects the excitation threshold of spinal structures [12]. Increasing the distance between stimulation electrodes, when applying bipolar stimulation for

pain control, will result in a decrease in body surface paresthesia [13]. In addition, the intensity of electrical stimulation for the perception threshold decreases, on average, when electrodes are moved laterally from the midline [12].

Epidural spinal cord stimulation produces intricate field potentials originating in the epidural space and emanating into various neural elements inside and outside of the spinal cord (Figure 1.2). Barolat *et al.* states that at stimulation intensities at or below those used clinically for pain control, stimulation of large myelinated afferent fibers can be achieved at the dorsal root, the area where dorsal root enters the spinal cord, the dorsal horn, and the dorsal columns [12]. Increasing the intensity of stimulation to large enough intensities will lead to motor contractions. This can be due to the activation of the segmentary motor system (consisting of the ventral roots and motoneurons) and/or the activation of descending corticospinal pathways [12]. Hunter and Ashby accredit the nonspecific motor contractions to antidromic activation of the afferent muscle fibers found in the dorsal column [116].

It has been reported that bipolar epidural electrical stimulation at intensities within the clinical range produces activation of dorsal column fibers; however, these findings have been directly challenged by more recent research. Rattay *et al.* demonstrated that dorsal roots are recruited during epidural electrical stimulation at stimulus voltages within clinical range, followed by recruitment of ventral roots at higher stimulus intensities [183]. According to Rattay *et al.*, the dorsal column fibers are not activated due to bipolar epidural electrical stimulation [183]. Computer modeling studies of the spinal cord conclude that dorsal root fibers most likely have lower stimulation thresholds than dorsal column fibers; however, these findings are dependent on various factors, including the thickness of the CSF layer, local geometry of the spinal cord and dorsal/ventral roots, electrode configuration, and stimulation parameters used [4, 205, 113, 112]. Moreover, Minassian *et al.* reported that muscle activity in response to bipolar epidural stimulation of the

dorsal lumbosacral spinal cord is principally due to activation of the large afferent fibers found in the dorsal roots [154].

Jilge *et al.* state that epidural stimulation does not play a major role, if any role, in directly activating dorsal column fibers or interneurons [123]. Instead, Jilge *et al.* hypothesize that the large afferent fibers within the dorsal roots are excited, in turn synaptically transmitting signals to the inherent spinal neuronal circuitry responsible for generation of lower limb movement patterns as well as generating monosynaptic responses that elicit motor activity [123].

In summary, epidural electrical stimulation of the spinal cord chiefly stimulates large sensory axons in the dorsal roots which in turn can indirectly generate muscle responses [184]. At a fixed stimulation intensity and spinal cord level, altering the frequency of electrical pulse trains applied epidurally has been shown to produce either sustained tonic motor responses together with lower limb muscle contractions, or rhythmic movements of the lower limbs mimicking locomotion [184]. Tonic responses are presumably due to modulation of spinal reflex pathways, while rhythmic activities are putatively due to modulation of central pattern generators in the spinal cord [184]. Epidural stimulation has been demonstrated to be an effective method to activate spinal neuronal networks (such as those involved with locomotion and micturition) [5, 76, 102].

#### **1.4.5 Transcutaneous Spinal Cord Stimulation**

The promising results from epidural electrical stimulation of the spinal cord led to the idea that non-invasive methods of stimulation, such as transcutaneous electrical stimulation (tSCS), can be used to modulate spinal circuitry to facilitate motor functions in similar ways. Researchers demonstrated that locomotor-like movements can be generated in healthy, uninjured humans using tSCS [79, 93]. Transcutaneous spinal cord stimulation has also been shown to generate multi-

segmental reflexes in lower limb muscles of uninjured individuals as well as individuals who have experienced spinal cord injury [34, 60, 155, 186]. Experiments investigating the efficacy of transcutaneous spinal cord stimulation were subsequently conducted on individuals with incomplete as well as complete spinal cord injury [84, 110, 130]. Gerasimenko *et al.* demonstrated that application of transcutaneous spinal cord stimulation to the lower thoracic, lumbosacral and coccygeal vertebrae, in combination with a monoaminergic drug, increases the ability of individuals with spinal cord injury to generate voluntary stepping-like movements of their lower limbs [84]. In addition, computer modeling studies propose that similar afferent neurons that project to the locomotor spinal circuitry are activated in response to either transcutaneous electrical stimulation or epidural electrical stimulation [134]. The results of these studies imply that transcutaneous electrical stimulation of the spinal cord can modulate spinal cord neural circuitry after SCI.

Gerasimenko *et al.* state that the combination of somatosensory inputs, descending motor inputs, and interneuronal and motorneuron activity modulated by electrical and pharmacological facilitation results in the recurrence of locomotor function and voluntary control in individuals who have experienced spinal cord injuries [84]. Repetitive training may lead to generation of new synaptic connections among interneurons that project to motorneurons which can improve motor output [84]. Thus, training the spinal networks that are largely inactive following a spinal cord injury in the presence of electrical and/or pharmacological treatments can induce motor activity and can lead to permanent long-term improvements in functional abilities.

## 1.5 Neural Potentials

Neural activity of the spinal cord recorded from the epidural space contains information that can potentially be used to guide epidural electrical stimulation

protocols. A brief review of neural potentials, recording and analysis is discussed next.

### 1.5.1 Sources of extracellular fields

Active cellular processes generate electric currents that can be measured and quantified [106]. Synaptic activity, fast action ( $\text{Na}^+$ ) potentials, calcium spikes, intrinsic currents and resonances, spike afterpolarization and down states, gap junctions and neuron-glia interactions, as well as ephaptic currents all contribute to the electric currents in a volume of neural tissue [20]. The electric currents of excitable membranes arise primarily from synaptic activity and to a lesser extent from calcium spikes/various voltage-dependent events and from action potentials and spike afterpotentials [20].

The sum of all electric currents within a volume of neural tissue results in a measurable potential with respect to a reference potential [20]. The difference in potentials between two points yields an electric field (defined as the negative spatial gradient of the potential difference) which can be detected by electrodes placed outside of cells. A recorded electric field can be used to interpret various features of neural communication and activity, such as how exactly transmembrane currents give rise to a recorded electric potential [20]. Although all neural structures generate extracellular fields, the brain may be the most widely studied neural structure to date. However, much of what has been determined from studying the electric potentials that arise from the brain (especially cortex) can be applied to other neural structures, such as the spinal cord.

In general, all types of excitable membranes (i.e. soma, axon, dendrite, spine, etc.) as well as all types of transmembrane currents influence the extracellular field potential [20]. The net summation of all cellular ionic processes at a particular location yields a potential that reflects the superimposition of all local

currents [106]. When using electrodes to record from a particular location near a neural source, voltage deflections are acquired at sub-millisecond resolution, yielding waveforms that can be quantitatively analyzed. The characteristics of the waveform, such as its frequency components and amplitudes, are dependent on the contribution of various neural sources as well as the different properties of neural tissue. The spatial distribution of distinct current sources, as well as their magnitude and sign, influence the extracellular field [20]. In addition, the temporal coordination (or synchrony) of these current sources affects the amplitude and frequency of the recorded waveform [20].

Widely-used recording methods of extracellular events include electroencephalography (EEG), electrocorticography (ECoG), electrospinography (ESG), local field potentials (LFP), and voltage-sensitive dye imaging. LFP recordings are performed by inserting electrodes into neural structures' extracellular space and collecting information regarding action potentials as well as other membrane potential-derived fluctuations in a minute volume of neural cells [20]. EEG, ECoG and ESG primarily sample electrical activity at superficial levels of the neural tissue, and thus have been speculated to contain less information regarding specific cellular events. The EEG recording is a spatiotemporally smoothed representation of the LFP integrated over a relatively large area [20]. Bypassing the signal-distorting structures such as bone and intermediate tissue as well as decreasing the size and inter-electrode distance of the recording electrode improves spatial resolution of recordings, yielding more information regarding the underlying cellular events.

### **1.5.2 Determinants of Extracellular Recordings**

Under the assumption that the recorded medium is homogeneously composed, the two largest factors that determine the LFP extracellular field strength are the spatial arrangement of neurons and their temporal synchrony [20]. Synchrony of



neural cells, often due to network oscillations, significantly contributes to the magnitude of the extracellular current [20]. Synchronous fluctuations of the membrane potentials of large groups of neurons increases the magnitude of the extracellular recording. The position and orientation of adjacent neural cells also influences the extracellular field. For example, adjacent pyramidal cells in the cortex lie parallel to one another, which yields a geometry that is ideal for superposition of synchronously active dipoles [20]. Therefore, LFPs recorded from the cortex are usually larger in magnitude than LFP recordings from other neural structures [20].

Although all neuronal cells play a role in generating an extracellular field, the shape of a neuronal cell determines the extent of its contribution to the overall extracellular field. Cells that can generate strong dipoles along their somatodendritic axis (such as pyramidal cells) cause substantial ionic flow into the extracellular space, thereby contributing a significant amount to the extracellular field [20]. On the other hand, neurons that are spherically symmetric contribute less significantly to the extracellular field than those that have long apical dendrites, and theoretically do not contribute much, if at all, when several of its dendrites are simultaneously activated [20].

The distance between the recording electrode and the population of neurons of interest also influences the characteristics of the waveform. There are two primary reasons why increasing the distance between the neural source and the recording site decreases the amount of information regarding the events causing the local potential that can be obtained: (1) the amplitude of the recorded potential is inversely proportional to the distance between the neural source and the recording site, and (2) the effects of signals that contaminate the true neural signal increases as the distance between neural source and recording site increases, which leads to spatial averaging [20].

Volume conduction also influences the extracellular field. Electric fields can

be transmitted through various volumes (such as spinal tissue), termed volume conduction [20]. The extent to which volume conduction occurs is dependent on the current dipole as well as the conductive medium [126, 141]. Therefore, some LFP patterns can be recorded at a distance from the source, while others must be recorded relatively locally. The effects of volume conduction have been clearly demonstrated in hemispherectomized individuals [31]. Unfortunately, volume conduction hinders the ability to interpret the functional meaning of the relationship between signals recorded from distinct neural locations [20].

### **1.5.3 Neural Potentials Recorded from the Spinal Cord**

In 1947, Sawa conducted a set of experiments in which he recorded spontaneous electrical activity of the spinal cord of 15 individuals [188]. A concentric needle electrode was inserted into a lumbar segment of the human spinal cord at five different locations: top half of dorsal horn, bottom half of dorsal horn, top half of ventral horn, bottom half of ventral horn (more lateral than top half of ventral horn), ventral portion of white matter, and lateral portion of white matter. Recordings from each of these sites yielded waveforms with distinct amplitudes and frequency components [188]. These differences can be attributed to the distinct cellular processes occurring at each of those regions.

Magladery and coworkers also recorded the electrical activity of the human spinal cord intrathecally using long bared-tip insulated steel needle electrodes [149]. Methods for obtaining intrathecal recordings have higher risk of complication than methods for obtaining epidural spinal cord recordings [196]. Therefore, considerable amount of research has been conducted on the acquisition and recordings of spinal cord electrical activity via epidural means.

Intraoperative neuromonitoring of the spinal cord during neurosurgical procedures has been demonstrated to be an effective tool in detecting potential neuro-

logical events and possibly avoiding revision surgery [209]. Real-time recordings of the spinal cord from epidurally placed electrodes aid in examination of sensory and motor function, and are used for diagnostic purposes [30, 124, 146, 198, 204]. Stimulation of the cortex or peripheral sensory nerves activates primarily motor or sensory pathways, respectively, and the responses to these stimuli can be recorded by epidural electrodes placed over the spinal cord [204].

Parker *et al.* demonstrated the feasibility of recording spinal cord evoked compound action potentials (ECAPs) in human patients treated with spinal cord stimulator systems [175]. Evoked potentials in response to electrical stimulation of the spinal cord were recorded from the epidural space. The electrical response from the spinal electrodes contained components generated by the stimulus current as well as inherent neurological sources [175]. These ECAPs represent the sum of all responses from a group of single fibers, and its characteristics describe properties of the responding fibers, such as conduction velocity, stimulation threshold, response amplitude, and even fiber diameter [175]. This measurement technique has the potential to determine the combinations of active electrodes that induce the most sensitive responses to spinal cord electrical stimulation.

#### 1.5.4 Signal Analysis of Neural Potentials

Electrical activity is typically recorded from an ensemble of neurons using systems with the following components: micro- or macroelectrodes positioned near/in the neural tissue, amplifiers to increase the recorded signal's strength, filters to minimize certain frequency components, and an analog-to-digital converter.

There are various ways of analyzing neuronal field data [95, 176]. Data recorded from neuronal fields often exhibit rhythms, and these rhythms can be analyzed using spectral analysis techniques to determine important features of the recorded signal. Fourier transform is a well-established and widely used technique that can

be used to generate the power spectrum of a recorded neural signal. The Fourier transform decomposes a signal into its component frequencies. The power spectrum is the magnitude squared of the Fourier transform of a signal, and is useful for obtaining the amplitude and rhythmic activity in a dataset as a function of frequency. Power spectra can be computed for an entire signal at once (called a periodogram) or it can be computed as periodograms of segments of the signal averaged together, forming what is called the power spectral density. Since neural signals are non-stationary, performing a periodogram on an entire signal at once is not entirely appropriate. When a signal is non-stationary, it implies that the signal's statistical characteristics are time-variant. The neural signals' non-stationary characteristics are in part due to the various distinct neural structures that contribute to the overall waveform, as well as the distinct time scales involved in their dynamical processes [128].

One of the many methods to modestly address the non-stationary concern is the use of Short-Time Fourier Transform (STFT). STFT is based on the Fast Fourier Transform, which is derived from the Discrete Fourier Transform [173]. The STFT technique segments the signal into successive time windows and subsequently calculates the frequency components present in each time window. The window length used should correspond to the segment of the signal that is validly assumed to be stationary. The requisite of using windows creates a drawback for the use of STFT: there is an inverse relationship between time and frequency resolutions. In other words, increasing the temporal resolution will correspondingly cause a decrease in frequency resolution, and vice-versa. This is an extension of the Heisenberg-Gabor uncertainty principle, which states that it is not possible to know what specific spectral component exists at any given time instant [173]. Although the spectral and temporal resolution trade-off cannot be circumvented, it can be optimized by the selection of the optimal parameters used for STFT. The various derivations of the Fourier Transform, including STFT, are presented

in detail by Sethares, Smith, and Christian [173, 191, 203].

Plotting the squared magnitude of the discrete STFT of a time series results in what is called a spectrogram. A spectrogram is a 2D plot of the relative power content of each frequency bin at different instances of time. The spectrogram has been widely used to perform time-frequency analysis of neural signals (i.e. ECoG, EEG studies).

## 1.6 Summary

In this introduction, we briefly reviewed the impact of spinal cord injury on individuals, basic neural control of the respiration and micturition, examples of neuromodulation techniques that can improve functional abilities, basic theories on mechanism of action of spinal cord stimulation based on experimental and modeling studies, and a brief introduction to neural potentials and recordings. The following discussion can be broken down into two overarching goals: (1) demonstrate that spinal cord electrical stimulation can be used to induce acute and chronic changes in respiratory and bladder function, and (2) record neural potentials from the epidural space of the thoracic spinal cord and determine if the recorded waveforms contain information that can be used to help determine optimal electrical stimulation parameters. The first goal was accomplished via two series of experiments. The first experimental set involved the use of epidural electrical stimulation applied to the cervical spinal cord to induce changes in respiratory function, confirmed by monitoring of tidal volume and respiratory rate. The second experimental set involved the use of transcutaneous electrical stimulation applied to the thoracolumbar spinal cord to aid in micturition, confirmed by recorded voided volume and flow rate. The second goal of this thesis was accomplished by recording epidural potentials from the thoracic cord while the subject received a bout of spinal cord stimulation for clinical pain relief.

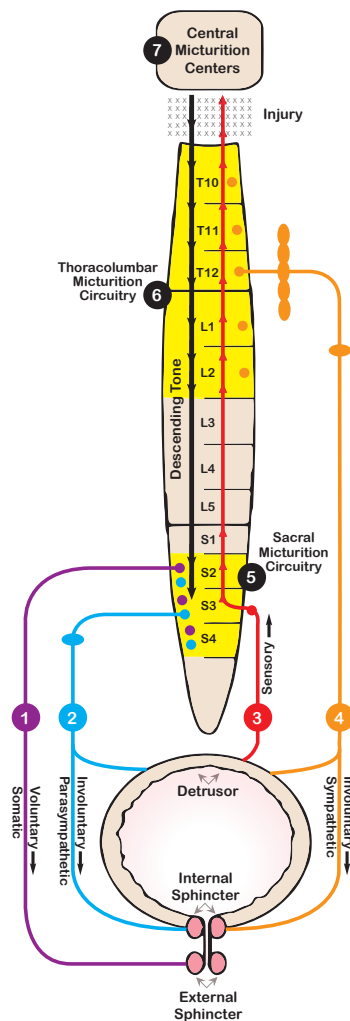


Figure 1.1: **Elements of Micturition:** 1. The pudendal nerve innervates the external urethral sphincter, providing voluntary control. 2. Parasympathetic pelvic nerves innervate the internal urethral sphincter as well as the bladder walls, providing involuntary control of micturition. 3. Involuntary control involves hypogastric nerve sympathetic activity as well. 4. Sensory nerves from these centers send afferent signals towards the spinal cord and supraspinal centers. 5,6. There exists neural circuitry in the sacral and thoracolumbar levels of the spinal cord that aid the micturition process. 7. Descending signals interact with circuitry at various levels to effect micturition. Spinal cord injury can minimize the effects of descending activity and diminish micturition ability.

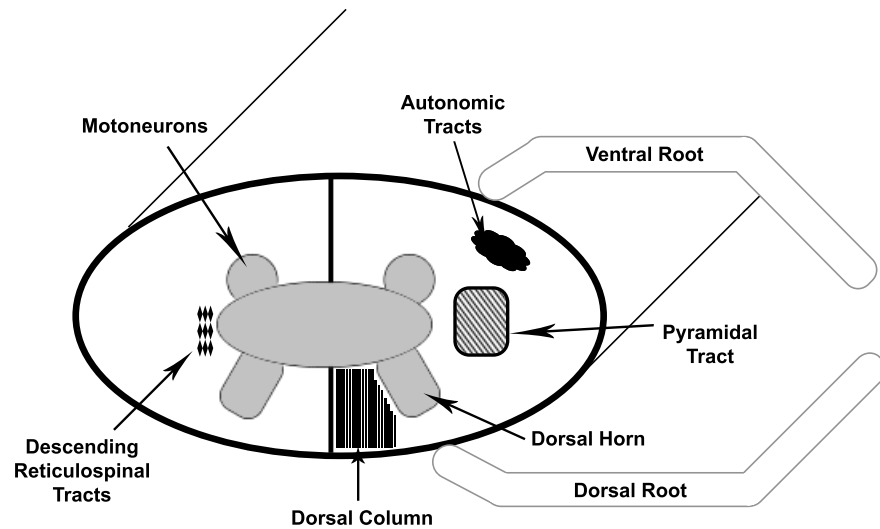


Figure 1.2: **Representative illustration of intraspinal structures that directly and indirectly respond to dorsally-applied epidural stimulation.** Epidural electrical stimulation of the dorsal spinal cord presumably activates the dorsal roots, eliciting a response from deeper neural structures. Stimulation of dorsal columns and horns results in paresthesia of body segments ipsilateral to the stimulating electrode. Activation of the descending reticulospinal tracts yields reduction in tone or involuntary movements [12]. Activation of the pyramidal tract and motoneurons causes motor contractions. Stimulation of autonomic tracts results in feeling of warmth [12]. Although not illustrated here for the sake of simplicity, the labeled spinal structures exist bilaterally. Structures are not drawn to scale.

## CHAPTER 2

# Intraoperative Assessment of Respiratory Function in Response to Cervical Epidural Electrical Stimulation

### 2.1 Introduction

Depending on the level of injury, spinal cord injury (SCI) may result in disrupted communication between the respiratory centers in the brainstem that control breathing and the muscles responsible for the physical act of breathing. The more rostral the injury, as in the case of an upper cervical SCI, the more likely the respiratory system will be severely diminished post-injury. Respiratory system collapse may occur in spinalized individuals due to disruption of the bulbospinal pathways innervating the phrenic and intercostal motoneuron pools and/or damage to the motoneurons themselves [54, 74, 94, 159]. Since the phrenic motoneurons innervate the diaphragm (which is the main inspiratory muscle in humans), interruption of the bulbospinal pathways due to cervical SCI leads to paralysis of the diaphragm, causing reduction in forced vital capacity as well as potentially complete apnea [77, 142].

Effective treatment options for respiratory dysfunction are limited for those with SCI [111]. Most individuals experiencing respiratory deficiencies following SCI require the use of ventilator-assist devices as well as long-term care focused towards minimizing complications due to respiratory causes [7, 8].



However, since the neural circuitry below the level of injury is often intact, researchers have been able to leverage this knowledge to create methods of activating peripheral nerves that innervate inspiratory muscles to ultimately restore respiratory function.

Electrical stimulation of these peripheral nerves has been demonstrated to induce inspiratory muscle function [100, 174]. Bilateral phrenic nerve stimulation (or phrenic nerve pacing) has become a clinically accepted modality to manage respiratory function in tetraplegics reliant on pulmonary ventilators [2, 18, 25, 37, 48, 49, 50, 56, 57, 59, 67, 86, 87, 88, 89, 90, 91, 107, 163, 171, 177]. However, most patients who are dependent on mechanical ventilation due to a cervical SCI are not candidates for phrenic nerve stimulation [49]. In addition, the associated risks with phrenic nerve stimulation include the risk of phrenic nerve damage, muscle fatigue, and the surgical risks inherent to a thoracotomy [49]. Other techniques such as combined intercostal and unilateral phrenic nerve pacing as well as intramuscular diaphragm pacing offer advantages over conventional phrenic nerve stimulation [49].

Recently, DiMarco and colleagues demonstrated that epidural electrical stimulation of the ventral upper thoracic spinal cord activates both the diaphragm and intercostal muscles in animals [51, 52, 53, 55]. This technique resulted in activation of inspiratory motoneurons and increased physiological activation of inspiratory muscles (i.e. diaphragm and intercostals) [55]. DiMarco *et al.* state that in theory, epidural electrical stimulation of the ventral spinal cord will result in larger inspired volumes and increased fatigue resistance when compared to conventional diaphragm pacing [55].

Unfortunately, some techniques mentioned above do not entirely mimic the complex muscle recruitment patterns during ‘normal’ breathing and do not take into consideration sensory drive to affect respiratory pattern (i.e. changes in blood gases, lung volume, etc.).

Control of the respiratory motoneurons recruitment pattern occurs at the level of the spinal cord and therefore does not require input from the bulbospinal premotorneurons [50]. In addition, within the neural network of respiratory control there exists spinal mechanisms that affect plasticity in response to intermittent hypoxia which lead to increase in phrenic motor output [39]. Based upon the control system of the spinal cord and its intrinsic ability to exhibit plasticity [66], electrical stimulation may provide a means to modulate the spinal circuitry responsible for controlling respiratory function.

We suggest that epidural electrical stimulation applied to the dorsal cervical spinal cord stimulates respiratory circuits (i.e. central pattern generators) in such a way that respiratory function can be modulated. By monitoring the respiratory rate and tidal volume before and during the application of epidural stimulation, we determined the effects of cervical spine stimulation on respiration. In addition, we demonstrated that cervical spine stimulation rescued respiratory function in an individual who was administered opiates that completely depressed the respiratory network.

## **2.2 Methods**

The use of epidural electrical stimulation of the spinal cord is part of a clinical research protocol on the evaluation of intraoperative spinal cord stimulation and monitoring (Protocol 11-000043) that was approved by the institutional review board of the University of California, Los Angeles. Informed consent was obtained from each patient.

### **Subjects**

9 subjects receiving clinical surgical treatment (i.e. laminectomy) that exposed the epidural space were included in this study\*. Upon receiving informed consent, the neurosurgeon performed the clinical procedure. Once the clinical procedure

was complete, the neurosurgeon gave the cue to begin the experimental protocol.

### **Epidural Stimulation**

A ball-tipped electrode (Ball-Tip Probe, Cadwell, Kennewick, WA) was placed dorsally over the epidural fat at various exposed cervical levels while the subject was under anesthesia (remifentanil 0.05-0.07 mcg/kg/min as tolerated). Not all subjects had every cervical spinal level exposed. The ground electrode was placed approximately 5 inches from the base of the surgical incision. Monopolar epidural stimulation was applied using an intraoperative electrical stimulator (ES-IX Stimulator, Cadwell, Kennewick, WA). Stimulation pulse width was set to 500 microseconds. The following stimulation parameters were adjusted based on the neurosurgeon's discretion: amplitude (mA) and frequency (either 5, 30, 60, or 90 Hz).

Data were recorded for at least 30 seconds before the application of epidural stimulation (a.k.a. baseline segment) as well as at least 30 seconds during the application of epidural stimulation (a.k.a. stimulation segment). Throughout the entire recording, the ventilator-assist device was turned off to allow for spontaneous breathing.

### **Opiate-Induced Respiratory Depression**

In one subject, we tested whether epidural electrical stimulation applied to the cervical spinal cord can rescue respiratory function after administration of opiates at a concentration that completely depresses respiration. The ventilator was initially turned off and the patient began spontaneous respiration. A bolus of remifentanil was administered. The ventilator was then turned on briefly by the anesthesiologist in order to check that the end-tidal CO<sub>2</sub> was within an acceptable range. The ventilator was turned off again and this time the patient was unable to respire due to the anesthetic bolus. Epidural stimulation was applied at the C3-C4 spinal level approximately 30 seconds after the ventilator was turned off last. The entire recording lasted approximately 5 minutes, and blood oxygen

levels remained above 90% throughout the entire recording, as monitored by the anesthesiologist.

As a control, the same procedure was administered on the same subject, except without the use of epidural stimulation. The ventilator was turned off and the patient was able to spontaneously breathe. The opiates were administered at the same dose, and the patient subsequently stopped breathing. The entire recording lasted approximately 5 minutes, and blood oxygen levels remained above 90% throughout the entire recording, as monitored by the anesthesiologist.

### **Data Capture and Analysis**

Tidal volume was collected using an Apollo anesthesia ventilator system (Dräger, Telford, PA). Due to limited capabilities of the Apollo system for outputting data, a camcorder was used to record the screen of the Apollo monitor. The recorded video was subsequently analyzed frame-by-frame by an optical character recognition program created in-house using LABVIEW. Respiratory rate was calculated by collecting inspiratory and expiratory pressure data real-time. A data acquisition device (Low Cost USB DAQ, EMANT, Singapore) with peripheral pressure adapter (Pressure Application Adapter, EMANT, Singapore) was used to record pressure data. A tube was connected to one end of the differential pressure sensor; the opposite end of the tube was connected the patients' tracheal tube. A custom LABVIEW program was written to interface with the pressure device and record the data. The use of event markers in this custom program allowed for synchronization of various recorded data types. An algorithm was written in MATLAB that determined respiratory rate from the pressure traces.

\*This is an ongoing study; subjects continue to be actively enrolled.

## 2.3 Results

Epidural electrical stimulation of the dorsal cervical spinal cord was demonstrated to modulate respiratory rate as well as tidal volume. Results indicate that stimulation parameter combinations that yielded the largest change in respiratory rate averaged across all patients include: C6 at 60Hz (70.6% change), C2 at 90Hz (59.1% change from baseline), C6 at 90Hz (52.4% change), and C3 at 30Hz (50.8% change) (Figure 2.1). If only taking into consideration the effect due to location of epidural stimulation on respiratory rate and tidal volume, the results show that stimulation at C3 produced largest average change in respiratory rate and tidal volume from baseline (with largest variability) (Figure 2.2). Stimulation at C6 resulted in the next highest averaged change in tidal volume from baseline, while stimulation at C5 resulted in the third highest averaged change in respiratory rate from baseline.

It appears that, overall, the greatest positive change in respiratory rate from baseline occurred when higher frequencies were applied to the cervical spinal cord (Figure 2.1). On the other hand, the results show based on two stimulation bouts that epidural stimulation applied at C5 at a lower frequency (5Hz) resulted in a decreased respiratory rate of more than 59% change from baseline.

Cervical epidural electrical stimulation was not only shown to modulate an ongoing respiratory rhythm. This stimulation technique was also demonstrated to enable respiration in a subject that was not able to breathe before or after the application of electrical stimulation (Figure 2.3, bottom trace). In almost all cases, the patients were breathing rhythmically before the administration of epidural stimulation.

In a separate experiment, it was demonstrated that epidural stimulation of the cervical spinal cord has the capacity to rescue respiratory function in an individual experiencing opiate-induced respiratory cessation (Figure 2.4). For this

experiment, the ventilator was initially turned off and a bolus of remifentanyl was subsequently administered. The ventilator was turned on to check the end-tidal  $\text{CO}_2$  for clinical purposes. Once the ventilator was turned off again, the patient was unable to breathe due to the remifentanyl bolus. Epidural stimulation was applied between the C3-C4 spinal levels once the patient was apneic for 30 seconds. The patient was able to conjure one breath after 50 seconds of stimulation. The patient established a regular respiratory rhythm in the presence of epidural stimulation, which continued even after the cessation of stimulation (Figure 2.4).

In order to confirm that the commencement of respiration was not due to end-tidal  $\text{CO}_2$  caused respiratory resuscitation, the opiate-induced respiratory depression experiment was once again carried out on the same patient, but this time without the use of epidural stimulation. The ventilator was turned off and the patient exhibited a spontaneous breathing rhythm. The bolus of remifentanyl was administered, causing the cessation of respiration. The patient was monitored for approximately 300 seconds, yet the patient was unable to spontaneously respire (Figure 2.4).

The results demonstrate that under opiate-induced respiratory depression, the patient was able to generate a single breath after 50 seconds and spontaneous respiratory rhythm after 110 seconds of epidural stimulation. Under opiate-induced respiratory depression, the patient was not able to respire when the epidural stimulation was not applied for 290 seconds. This suggests that epidural stimulation of the cervical spinal cord may have played an important role in decreasing the time it takes to resuscitate respiration when the patient is deeply under anesthetics.

## 2.4 Discussion

Administration of epidural stimulation of the intact cervical spinal cord in anesthetized patients resulted in acute changes in respiratory function, depend-

ing on the location and frequency of stimulation. We have demonstrated that epidural stimulation is also capable of rescuing respiratory function in an individual who experienced opiate-induced respiratory depression. These results have widespread implications for addressing various potentially life-threatening conditions/events (such as sudden infant death syndrome, neurodegenerative disorders, strokes, trauma and/or pharmacological overdose) in which the brainstem may be inactivated resulting in respiratory depression.

Recovery of postural and locomotor function via the use of epidural spinal cord electrical stimulation has been demonstrated in animal and humans after complete paralysis, and we hypothesized that a similar approach can be used to recover respiratory function after neurological debilitations. Before testing this hypothesis on individuals experiencing neurological damage that affects respiration, we tested the hypothesis that epidural spinal cord stimulation can modulate the respiratory networks in individuals that exhibit normal respiratory function. The results of the current set of experiments suggest that respiratory neural networks in the adult human spinal cord can be modulated in order to alter respiratory rate and tidal volume. Although further research is required to determine the exact mechanism of action, dorsal roots at and near the spinal level of applied stimulation are presumably activated and in turn modulate the interneuronal and potentially central pattern generating respiratory networks in the spinal cord which control the respiratory musculature output.

For individuals who have experienced high cervical spinal cord injury, the projections from the medulla (specifically, rostral ventral respiratory group) that normally provide excitatory inspiratory drive to the phrenic nuclei are interrupted [192]. However, the spinal structures and circuitry below the spinal lesion remain intact [102]. Therefore, epidural stimulation of the cervical spinal cord can modulate the intact circuitry below the spinal lesion and potentially improve respiratory function with repeated respiratory training. Presumably, the epidural

stimulation may cause a change in transient excitability levels (i.e. lower the threshold of activation) of spinal respiratory neurons to a sufficient level that allows for the modulation of respiratory activity when the neurons receive impulses from remaining intact descending projections from the respiratory centers above the lesion. The acute effects on respiratory rate and tidal volume in individuals with intact spinal cords due to epidural stimulation may occur in a similar fashion in those who have experienced spinal cord injury; yet, further research is required for confirmation.



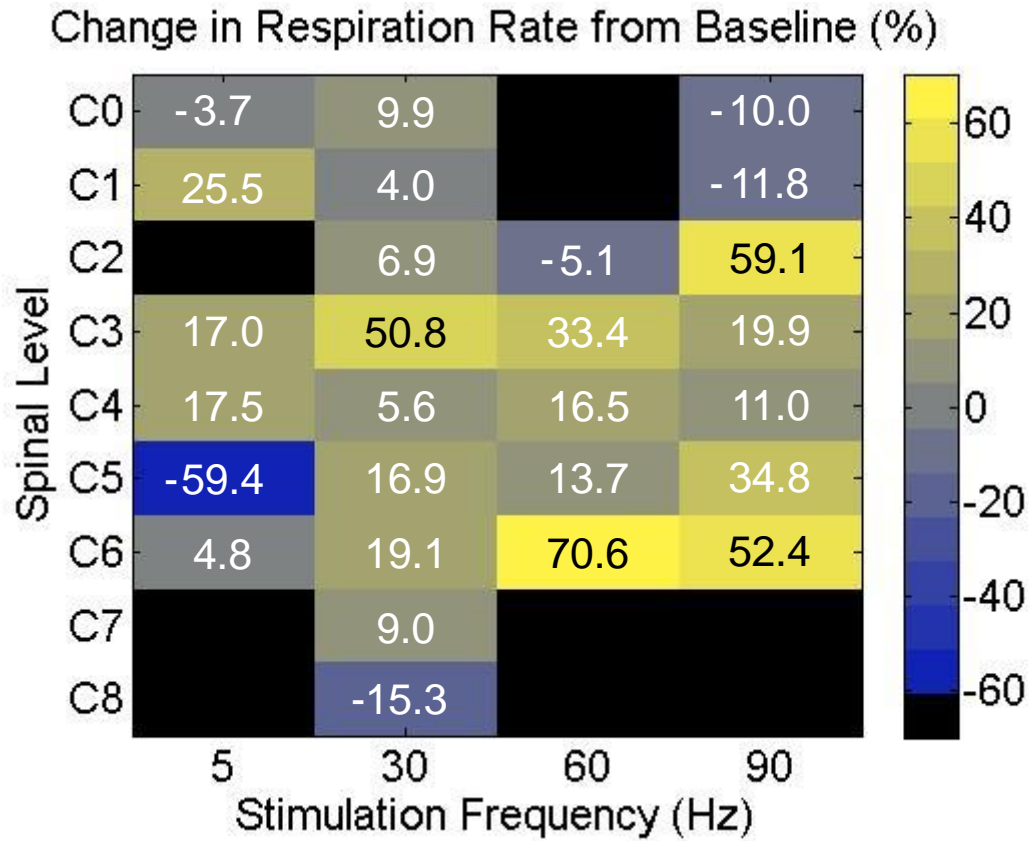


Figure 2.1: **Changes in respiratory rate in response to various stimulation frequencies and locations.** In subjects who exhibited an ongoing respiratory rhythm, the changes in respiratory rate between the baseline states (no stimulation) and stimulation states (during epidural stimulation) were calculated. The colors represent different values of percent change from baseline; blue indicates a negative change (i.e. the subject's respiratory rhythm slowed down during stimulation) while yellow indicates a positive change (i.e. subject began breathing faster during stimulation). Black represents data that has yet to be collected.

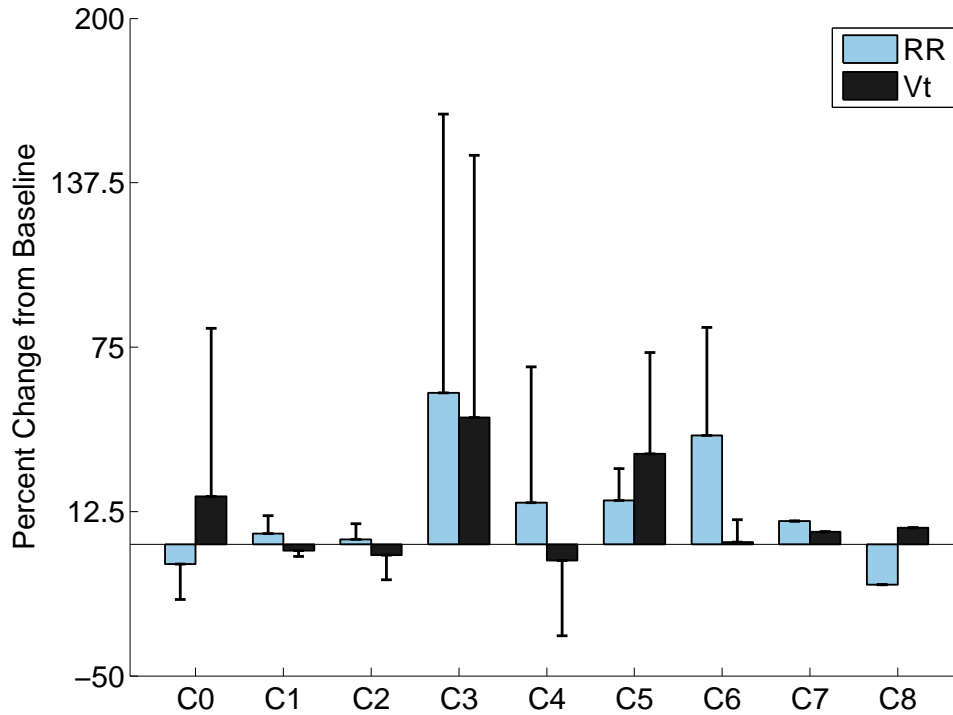


Figure 2.2: **Effects of epidural stimulation on respiratory rate (RR) and tidal volume (Vt) during spontaneous respiration.** Electrical stimulation applied to the cervical spinal cord resulted in changes in respiratory rate (blue) as well as tidal volume (black); the magnitude of the change is dependent on location of stimulation. Respiration rate and tidal volume were calculated before the application of stimulation (baseline) as well as during stimulation. The percent change from baseline was subsequently calculated and plotted for each cervical level.

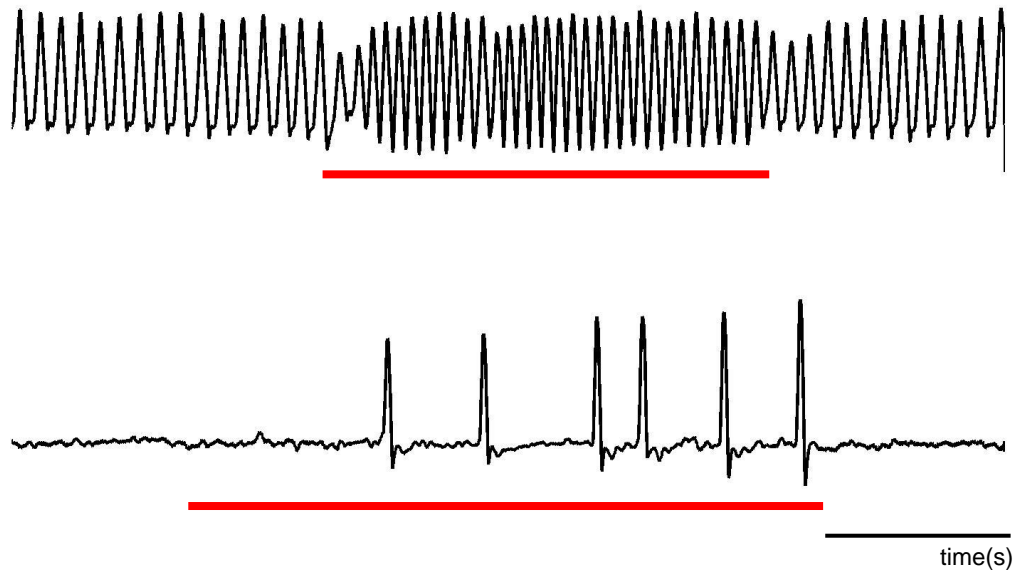


Figure 2.3: **Representative changes in respiratory pressure that demonstrate the effects of epidural stimulation on respiratory rate.** Respiratory pressure was monitored before stimulation, during stimulation (red bar) and after stimulation. The pressure recordings displayed are from two subjects. The time bar represents 55 seconds for the top trace and 60 seconds for the bottom trace. The stimulation frequency was set to 90 Hz (top) and 60 Hz (bottom) and the stimulation was applied between the C3 and C4 spinal levels.



Figure 2.4: **Changes in respiratory rate due to the presence (top) and absence (bottom) of cervical epidural stimulation after opiate-induced respiratory cessation.** Respiratory pressure was monitored for a single subject during opiate-induced respiratory cessation. Onset and duration of stimulation is represented by the red bar. The time bar represents 82 seconds for the top trace and 62 seconds for the bottom trace. The stimulation frequency used during onset of respiration was 30 Hz and applied between the C3 and C4 spinal levels. Downward deflections in the pressure recordings represent breaths administered by the ventilator, while the upwards deflections represent breaths taken without aid from the ventilator. The ventilator was used (top trace) in order to determine if the patient's end-tidal CO<sub>2</sub> was within an acceptable physiologic range, as determined by the anesthesiologist.

## CHAPTER 3

# Transcutaneous Electrical Stimulation of the Thoracolumbar Spinal Cord for Micturition

### 3.1 Introduction

Depending on the level and severity of injury to the spinal cord, bladder function can be significantly impaired. The lower urinary tract must be able to retain and empty urine from the bladder in a coordinated manner [41, 193]. Impairments to the lower urinary tract can result in conditions known as overactive bladder and detrusor-sphincter dyssynergia (DSD) [41, 193]. In humans with normal bladder function, the bladder and urethral sphincters contract and relax, respectively, in a coordinated and specific manner. When normal bladder function is impaired, the external urethral sphincter (EUS) and detrusor muscles of the bladder contract asynchronously (simultaneously), yielding uncoordinated voiding reflexes, also known as DSD [1]. Instead of complete relaxation of the EUS during voiding, the EUS contracts, which increases bladder pressure and interrupts urine flow [22]. DSD results in urinary retention, which over time can lead to urinary tract infections as well as renal damage and failure [215]. Thus, solutions to ameliorate bladder dysfunctions due to SCI are necessary to improve the quality of life of those affected with SCI.

Many treatments have been or are currently being developed to address the issues associated with bladder dysfunction. For example, electrical stimulation of the conus medullaris, pelvic nerves, sacral nerve roots, and the bladder have

been attempted, yet produce unreliable results [181]. Some of these methods require a posterior rhizotomy in order to discontinue the reflex arc, which leads to irreversible loss of sexual functions [38, 147]. Intraspinal electrical stimulation has also been under investigation, however, these methods are far too invasive for human application [96, 178]. Unfortunately, the treatments mentioned above just moderately reiterate the coordinated sequence of muscle contractions and relaxations that occur during normal micturition [190]. To date, there does not exist a clinically-approved neural interface that allows for full bladder control without undesirable side effects [32].

An attractive new approach for solving the issues associated with the neurogenic bladder is the use of epidural electrical stimulation of the spinal cord. Recovery of postural and locomotor function via the use of spinal cord electrical stimulation has been demonstrated in animals and humans after complete paralysis, and we hypothesized that an analogous approach can be used to recover micturition ability after neurological debilitations. This method does not require surgically cutting nerves that result in loss of motor and/or sensory function, which is usually the case for the strategies such as direct bladder wall stimulation, pelvic nerve stimulation, and sacral nerve stimulation [76]. Because the spinal cord contains the necessary neural networks to control micturition when delivered the requisite afferent information from the bladder and EUS, it has been hypothesized that repeated epidural stimulation training sessions influences spinal networks that control micturition and potentially aid in the recovery of bladder function after a complete SCI.

It has been previously demonstrated that motor training in combination with epidural electrical stimulation of the spinal cord facilitated the unexpected recovery of voluntary voiding in an individual with motor complete SCI [102]. Harkema *et al.* stated that the region of the spinal cord that received epidural electrical stimulation not only included the neural circuitry to control locomotor and pos-

tural functions, but also autonomic functions such as bladder function. Plastic reorganization of the spinal circuitry as a result of this long-term step training in the presence of epidural stimulation is a proposed explanation for the recovery of bladder function [33].

In a separate series of studies (clinical research protocol 11-001720), tSCS was applied to the spinal cord of complete SCI individuals to determine its effects on motor output. The protocol was approved by the institutional review board of the University of California, Los Angeles, and informed consent was obtained from the patients. During these studies, we observed urination in subjects during and after the administration of tSCS. Based on these observations, we conducted a set of studies on two of the individuals in order to determine the effects of tSCS on bladder function.

## **3.2 Methods**

### **Subjects**

Patients included in this study had previous exposure to locomotor training sessions in the presence of tSCS as well as at least 4 bladder training sessions with tSCS.

#### ***Patient 1***

Patient 1 is a 45-year-old male who injured his thoracic spinal cord from falling off a horse. Initially, the patient was not able to move or sense touch below thoracic level 3 (T3), and was assessed an ASIA A score. A sternal fracture, left pneumothorax and destructed spinal canal at T3 was confirmed via imaging. A posterior fusion was performed with instrumentation at T1-T5 in order to stabilize the spine. The subject was not able to demonstrate motor function of the lower extremities nor voluntary bladder function assessed by urodynamic studies. He relied on clean intermittent catheterization, and suffered from urinary accidents

roughly twice per week as well as urinary tract infections (UTI) nearly once per month. Patient 1 experienced involuntary bladder voiding in the presence of lumbosacral tSCS to improve leg motor function, which suggests that tSCS influenced sensory and/or motor centers in the spinal cord that aid in bladder function.

### ***Patient 2***

Patient 2 is a 58-year-old male who injured his cervical spinal cord due to a bicycle accident. He received T1-T6 pedicle screw fixation as well as arthrodesis for T3-T4 dislocation and subluxation which resulted in a motor-complete spinal cord injury (scored ASIA-B). He also received an occiput to C4 arthrodesis for a C1 burst fracture, odontoid fracture as well as cervical instability. This subject was not able to demonstrate motor functions of the lower extremities nor volitional bladder function. He was also reliant on clean intermittent catheterization, and experienced roughly one urinary accident per week as well as approximately one UTI every two to three months.

### **Transcutaneous Spinal Cord Stimulation (tSCS)**

Transcutaneous electrical stimulation was applied to the lumbosacral spinal cord using a KULON stimulator (St. Petersburg State University of Aerospace Instrumentation, St. Petersburg, Russia) [92]. The cathodes used were 2.5 cm round electrodes (Lead-Lok, Sandpoint, ID), and were positioned on the skin between T11-T12 and L1-L2 spinal processes. Two 5.0 x 10.2 cm<sup>2</sup> anode electrodes were placed symmetrically on the skin over the iliac crests. The stimulation protocol called for the use of trains of 10 kHz constant-current bipolar rectangular stimuli with 0.5ms pulse width (with amplitudes ranging from 30-100mA) at frequencies of 1-40Hz. Voltage applied was approximately 30V when the stimulator was set to 100mA. Patient 1 underwent 5 tSCS bladder training sessions, while Patient 2 underwent 4 tSCS bladder training sessions.

### **tSCS Bladder Training Sessions**

At first, the patient's bladder was emptied via the insertion of a Foley catheter.



The patient's bladder was then filled at a rate less than 30 mL/min until the total volume injected reached 500 mL or until the patient determined that the bladder was full. tSCS was then applied while the patient was asked to voluntarily void. A uroflow system was used to measure total voided volume and flow rate, consisting of ATmega168 microcontroller (Atmel Corporation, San Jose, CA), AD620 amplifier (Analog Devices, Norwood, MA), strain gauge taken from an American Weigh AMW-1000 scale (American Weigh Scale, Norcross, GA), and a 1 L graduated urinal. Data acquisition software was written in Python programming language. The data was subsequently filtered and processed using IGOR Pro 6 (Wavemetrics, Portland, OR). The uroflow device was calibrated for each session, and the final voided volume reading consistently matched the amount of volume in the graduated urinal.

### **Urodynamic Testing**

The Aquarius XT system (Laborie Medical Technologies, Toronto, Ontario) was used to conduct the pressure-void volume studies (please note that the system was not able to record flow measurements due to the patient being in supine position for the duration of the study). Initially, the patient's bladder was completely emptied via the insertion of a Foley catheter. Two cathode electrodes were placed on the skin over the spinal cord vertebral levels T11 and L1, and two ground anode electrodes were placed bilaterally on the skin of the iliac crests. The patient's bladder was then filled at a rate of approximately 30-50 mL/min. Fluoroscopy was used to assess the shape of the bladder as well as the presence of vesicoureteral reflux. The patient was then asked to cough in order to assess leakage as well as increases in abdominal pressure. Subsequently, the patient was asked to voluntarily void for 5 minutes without any interventions. Voided urine was collected in a graduated urinal. The patient was then asked to voluntarily void in the presence of tSCS (described above) applied at 1Hz at L1-L2. Detrusor, abdominal and intravesicular pressures were monitored and recorded. Upon observing an increase

in intravesicular pressure, tSCS was applied at 30Hz at T11-T12 in addition to the tSCS applied at 1Hz at L1-L2. The voided urine volume was then measured, and the residual volume was collected via use of Foley catheter. Urodynamic testing was performed in one subject on two occasions: once before and once after tSCS bladder training sessions.

### 3.3 Results

Prior to the tSCS bladder training sessions, neither patient 1 nor patient 2 were able to volitionally void. However, during tSCS training, both patients were able to voluntarily void (Figure 3.1). Interestingly, the combination of lumbar and thoracic stimulation allowed for increased voided volume when compared to single-site stimulation of either the lumbar or thoracic levels (Figure 3.1).

During tSCS bladder training sessions, patient 1 was able to exhibit voluntary voided flow rates of greater than 10mL per second, while patient 2 exhibited voluntary flow rates of approximately 4 mL per second (Figure 3.2). These values must be taken with a grain of salt, since the flow rates reported are not true flow rates. During the tSCS bladder training sessions, patients were placed in supine position and a condom catheter was subsequently applied. A tube was then connected to the condom catheter that traversed across the bed and fed into the graduated urinal which collected the expelled urine. We expect that the flow rates reported here are less than the true flow rates had their not been a condom catheter and tube that slowed down urine flow as it was collected in the urinal. The total voided volume, on the other hand, is reported accurately and represents true total voided volume.

Patient 1 underwent urodynamic testing prior tSCS bladder training (Figure 3.3, Session 1) as well as during the tSCS training regime (Figure 3.3, Session 2). Initially, patient 1 was asked to voluntarily void without the presence of tSCS,

and was unable to expel any urine. However, once the tSCS was applied, patient 1 volitionally voided approximately 25mL during his first urodynamic study. The volume voided under volitional control corresponded with increased intravesicular pressure as opposed to increased abdominal pressure. Approximately 6 months later, patient 1 underwent a second urodynamic study in which he demonstrated volitional voiding without the presence of stimulation (Figure 3.3, Session 2). An increase in vesicular pressure corresponded to the voided volume. The patient was then asked to volitionally void again without the presence of stimulation. The patient was able to increase vesicular pressure without increasing abdominal pressure, but was not able to expel urine. Once the tSCS was applied, however, the patient was able to void and produced a voided volume of approximately 75 mL. The results suggest that patient 1 recovered ability to voluntarily void after several tSCS locomotor and bladder training sessions, and voiding ability was improved in the presence of tSCS. Fluoroscopic imaging verified the voiding events (Figure 3.3).

Upon the conclusion of the study, both patients reported reductions in daily catheterizations and UTIs. The patients stated that catheterization is not required in the morning and have not experienced a UTI within a 6 month follow period after the end of the study. Both patients also reported increased voluntary voiding, FIM bladder scores, ASIA sensory improvements, and I-QOL scores.

### **3.4 Discussion**

Spinal cord injury results in the impairment of coordinated neuromuscular control of micturition, negatively impacting millions affected by SCI. We have demonstrated that bladder function can be recovered by the repeated application of transcutaneous spinal cord electrical stimulation in individuals with SCI that are dependent on catheterization to expel urine. Urodynamic studies confirmed

that transcutaneous stimulation applied over the lower thoracic and upper lumbar spinal cord resulted in adequate voiding to decrease or abolish the use of catheters. In the two patients reported here, quality of life was improved and urinary tract infection rate was reduced after this intervention.

Previous studies have demonstrated that certain regions of the spinal cord contain neural circuitry required to perform complex motor behaviors [145, 206]. In the presence of electrical stimulation, these complex motor behaviors have been shown to improve in animals and human subjects with functionally-hindering spinal cord injuries and even result in volitional movements [5, 102]. The results of the present study suggest that under conscious control, parasympathetic and sympathetic preganglionic motoneurons were activated by transcutaneous electrical stimulation [92]. Due to the coordinated activity resulting from the stimulation, we hypothesize that central pattern generating circuits in the spinal cord are activated as well as peripheral and efferent pathways to a lesser extent.

Volitional control of bladder function requires intact neural connects between higher centers (i.e. brain, brain stem) and the spinal centers responsible for micturition. After a complete SCI, these connections are destroyed or diminished to the point that supraspinal signals are not able to adequately excite bladder spinal circuitry. However, our findings suggest that these connections can be enabled to function following repeated sessions of non-specific stimulation [35]. Similar to how motor commands have been shown to be transmitted through commissural projections of propriospinal pathways [36] and trained to improve voluntary control of lower extremity muscles [187], we demonstrated that the neural pathways can also be trained to induce and improve bladder function. Our results suggest that dormant spinal pathways due to a spinal cord injury are capable of being modulated and trained to learn a specific task in the presence of repeated electrical spinal cord stimulation, presumably by strengthening neural pathways between the brain centers and spinal centers controlling micturition.

Transcutaneous spinal cord stimulation was demonstrated to enhance bladder function and restore volitional control in two subjects. Further research must be conducted in order to refine stimulation parameters to induce increased voided volumes and larger flow rates. The results presented here are promising, yet call for further attention so that individuals reliant on catheterization can eventually void independently.

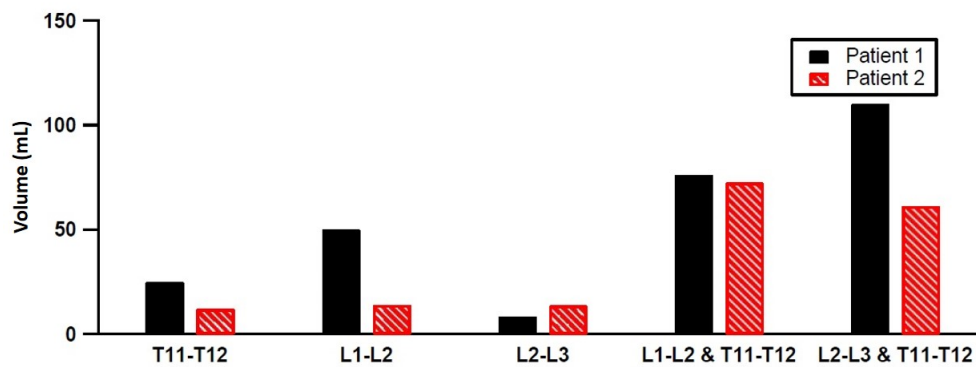


Figure 3.1: **Effects of transcutaneous spinal cord stimulation at various spinal levels on voided volume in catheter-dependent individuals.** In the presence of a particular tSCS protocol (described in methods), voluntary micturition was accomplished by two individuals with SCI who are normally reliant on catheterization. tSCS applied at both thoracic and lumbar levels concurrently resulted in, on average, increased urine output when compared to tSCS applied at either thoracic or lumbar regions.

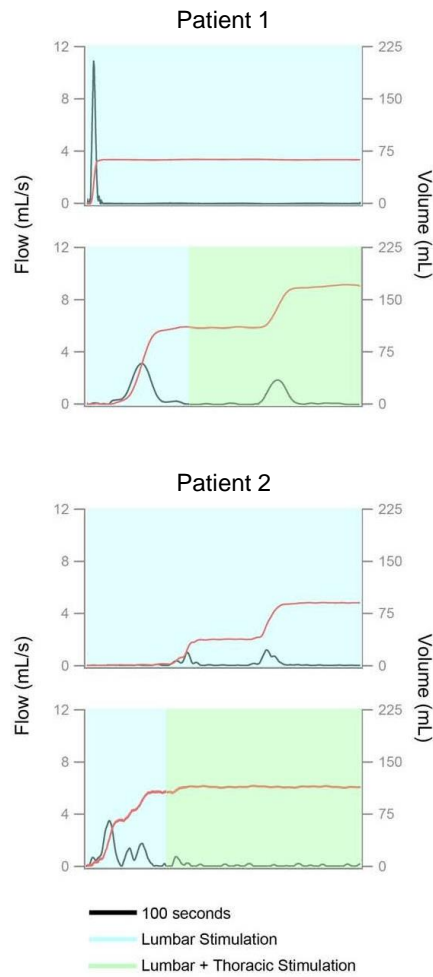


Figure 3.2: **Representative examples of voided volumes and flow rates during tSCS bladder training sessions.** Lumbar stimulation was initially solely applied at 1 Hz (blue shading), yielding modest urine output. When thoracic stimulation was applied at 30 Hz simultaneously with lumbar stimulation at 1 Hz (green shading), subjects were able to increase the total volume of urine voided. Red traces represent voided volume (mL), while the black traces represent flow rate (mL/s).

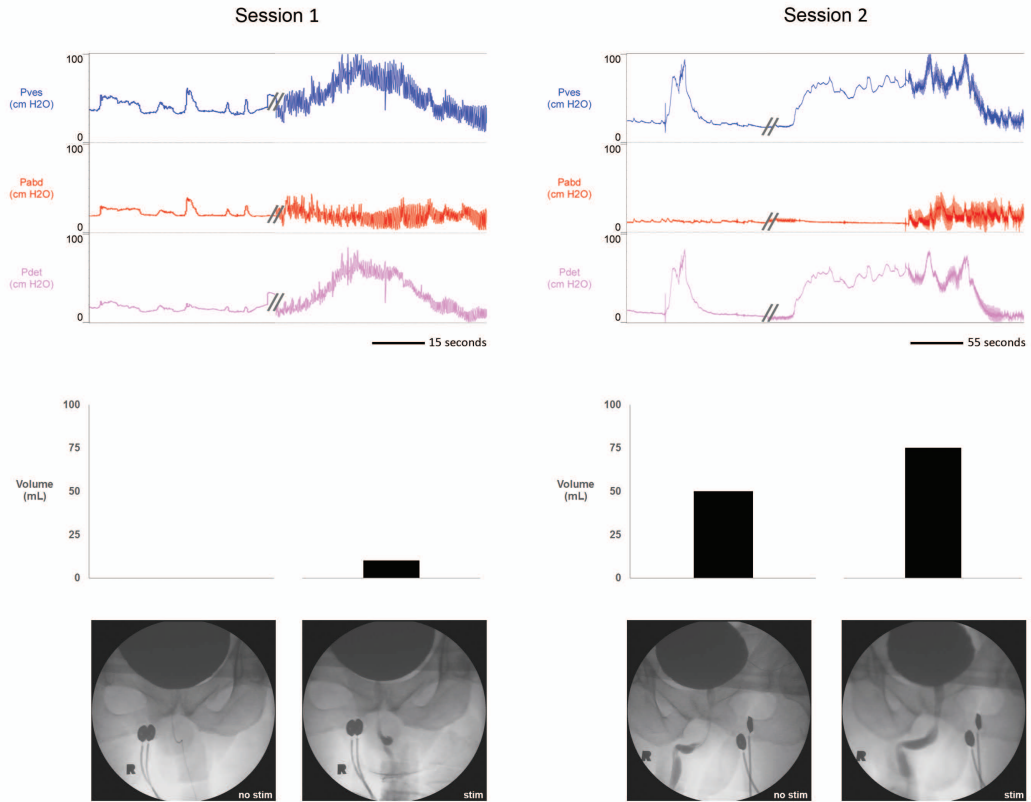


Figure 3.3: **Urodynamic assessment of voluntary bladder function in the presence and absence of tSCS, before and after tSCS bladder training sessions.** Patient 1 was not able to voluntarily void in the absence of tSCS prior to the bladder training sessions (left column of ‘Session 1’). After administration of tSCS, patient 1 voided approximately 25 mL. Six months after the initial urodynamic testing session, patient 1 exhibited voluntary control of his bladder without the use of tSCS (left column of ‘Session 2’). In the presence of tSCS, the patient was able to expel more urine than without the use of tSCS. Session 1 occurred prior to any tSCS bladder training sessions; Session 2 occurred after tSCS therapy was administered. Urodynamic assessment involved monitoring of intravesicular pressure (blue trace), abdominal pressure (red trace), detrusor pressure (pink trace), total voided volume (black bars) and fluoroscopic imaging.

## CHAPTER 4

# Epidural Recordings for Determination of Spinal States Before and After the Urge to Void

### 4.1 Introduction

Although the results from the two series of experiments mentioned in the previous chapters are remarkable, there exists room for improvement. For instance, only a small subset of stimulation parameters were tested in both experiments, and these stimulation parameters may have yielded sub-optimal results. Determining the best method to choose stimulation parameters for experimentation can be a difficult task, especially if there are many combinations of stimulation parameters sets available.

In addition, the modality of electrical stimulation (i.e. epidural, transcutaneous) that elicits optimal acute and chronic responses for various functions, such as respiration and micturition, has yet to be determined. Ideally, non-invasive means of spinal cord electrical stimulation (i.e. tSCS) are preferable to invasive means (epidural SCS). However, in the case of facilitating micturition, the electrical current density generated by transcutaneous electrical stimulation of the spinal cord may be too diffuse to solely penetrate the required neural structures to elicit maximal voided volumes and urinary flow rates. Focal application of electrical stimulation via epidural electrodes may prove to be more effective in activating distinct cell populations involved in neural processes (i.e. locomotion, micturition) than application of widespread electrical stimulation via transcuta-



neous electrodes.

Based on literature findings, I postulated that electrophysiological recordings of the spinal cord from the epidural space contain information that can help guide the selection of optimal stimulation modalities and parameters for subjects undergoing long-term electrical stimulation-based functional therapy. Specifically, the neural responses generated by epidural electrical stimulation pulses were quantified via time- and frequency-domain analysis to determine if information present in the neural waveforms can aid in determination of optimal stimulation parameters.

Various studies have demonstrated that field potentials recorded from the epidural surface (also known as epidural field potentials, or EFPs) contain information that can be used to decode arm movement direction in animal models [71, 195, 200, 201]. For instance, Flint *et al.* demonstrated that EFPs from the primate motor cortex contain significant information regarding movement, and this information was used to decode target of reaching movements and endpoint trajectories [71]. They found that EFPs showed characteristic power changes in distinct frequency bands starting just before movement onset and ending after movement termination [71]. Thus, they concluded that, based on their findings, epidural potentials may provide a useful signal source for brain-machine interfaces.

Other studies have demonstrated that epidurally-recorded electrical activity of the human spinal cord in response to a stimulus (also known as spinal cord evoked potentials, or SCEP) can be used to determine various states of the spinal cord. For instance, real-time intraoperative recordings of the spinal cord from epidurally placed electrodes aid in examination of sensory and motor function, and are used for diagnostic purposes [30, 124, 146, 198, 204]. Stimulation of the cortex or peripheral sensory nerves activates primarily motor or sensory pathways, respectively, and the responses to these stimuli can be recorded by epidural electrodes placed over the spinal cord [204]. SCEPs have also been collected and analyzed in order to determine the contributions of specific spinal pathways to the recorded

waveforms. For instance, Tsyuama *et al.* found that SCEPs epidurally recorded from spinal cords of intact humans contained two prominent negative peaks, the first of which was proposed to be derived from superficial layers of the posterior regions of the lateral columns while the second peak was suggest to be derived from the posterior columns [212]. In slight contrast, Machida *et al.* proposed that the first peak of recorded SCEPs were due to activation of spinocerebellar tracts while the second peak was generated by the posterior columns [146]. Results from animal studies led to the proposition that ventral pathways contribute to the first negative peak of SCEPs [101, 168]. Morioka *et al.* state that various afferent and efferent tracts (i.e. spinothalamic and corticospinal) possibly contribute to the SCEPs [161]. Although the sources of SCEPs are still controversial, SCEPs can reflect the transient state of the spinal cord, which can be useful for determining optimal stimulation parameters.

To confirm whether spinal cord recordings contain information useful for determining spinal states, a set of experiments were designed in which epidural spinal cord stimulation is applied with simultaneous recording of epidural spinal cord potentials in individuals with intact spinal cords (who underwent a spinal cord stimulation implantation procedure for clinical pain management) and those with SCI.

The initial phase of the experimental design involved determination of the stimulation intensity that produced initial sensation, the optimal intensity for paresthesia, and the maximal intensity before discomfort. Recordings were obtained from the spinal cord throughout experimentation. During this initial phase, we observed that at a certain amplitude of stimulation, the subject attained an immediate urge to void. I hypothesized that there may be a detectable difference in the spinal recordings of the subject before and after the subject had an urge to void, as assessed quantitatively via spectrogram power (an application of the Short-Time Fourier Transform). By analyzing the data in the time and frequency

domains, I postulated that spinal recordings can provide useful information about (1) the location of the spinal circuitry in the thoracolumbar region involved in micturition, (2) special characteristics of the spinal recordings that distinguish the spinal states when the bladder is full (experiences large intravesicular pressures) and when it is empty (experiences small intravesicular pressures). Knowing when the bladder is full is useful, especially for those with spinal cord injury. A method to determine the fullness of bladder can be the first step in creation of a closed-loop system that can first detect if the subject has to void, then warn the subject to enter a restroom, and lastly stimulate with the optimal settings in order to aid in voiding.

## **4.2 Methods**

The use of epidural electrical stimulation of the spinal cord is part of a research protocol on the evaluation of spinal cord activity after pain stimulator implantation (Protocol 14-001544) that was approved by the institutional review board of the University of California, Los Angeles. Informed consent was obtained from the subject.

### **Patients**

Only one subject was enrolled in this study at the time this thesis was completed. This subject underwent a routine trial spinal cord stimulator implantation procedure to verify whether or not she is a candidate for permanent implantation. Two standard octopolar leads (Octrode Trial Lead Kit 60cm Length, St. Jude, Plano, TX) were percutaneously inserted into the epidural space under fluoroscopic guidance. Each lead was composed of 8 electrodes, with inter-electrode distance of 4mm and electrode size of 3mm. The leads were placed parallel to one another, and positioned over T8-T10 spinal levels. During the 10-day trial period, these leads exited the subject's back and were allowed to connect to an external

stimulator and/or bio-amplifier system.

### **Epidural Stimulation, Recordings and Analysis**

The subject was placed in supine position during the entire experiment. Electrical stimulation parameters were selected postoperatively with feedback from the patient by routine clinical adjustment. Epidural electrodes were connected via a custom-fabricated connector box to an electrophysiological workstation (RZ5D, Tucker-Davis Technologies, Alachua, FL) and a clinical electrical stimulator (Clinical External Stimulator, St. Jude, Plano, TX) used for routine trial SCS procedures. The external stimulator was connected to the first and second most rostral electrodes of the right set of leads, while all other electrodes were connected to the neural recording system. The ground electrode was connected to the subject's left knee. A 10 Hz, 120 microsecond square-pulse was applied across the two stimulation electrodes. The maximum stimulation level was established based on the maximum level that produced effective pain relief during the routine clinical adjustment of the SCS system. The maximum injected charge was kept in a neurologically-safe range as determined by the preset limits of the external stimulator. The amplitude of the stimulus was increased from zero until the maximum level of tolerance, in steps of 1 mA.

Epidural potentials were recorded continuously at a sampling rate of 24.4 kHz in the presence of a 60 Hz notch filter. Data were processed off line with custom software (MATLAB, Mathworks, Natick, MA). Data were referenced to the most caudal electrode of the right set of leads (electrode 16 in Figure 4.2), and filtered using a first-order butterworth bandpass filter (lowpass: 20Hz, highpass: 1000Hz). Referenced and filtered data were then segmented into two sections: a 9 second segment immediately before the subject experienced an urge to void and a 9 second segment immediately after the subject experienced an urge to void. Each segment was subsequently divided into data frames; each data frame consisted of a digitized waveform that spanned 50 ms (which started 20 ms after the onset

of the stimulus pulse in order to minimize analysis of data contaminated by the stimulation artifact). The oscillations that occur within this time window can be viewed as the late-response of the evoked potentials, or as the induced responses of the stimulation pulse. Some of the data frames were contaminated by pulsations of the CSF due to the heart beat. These data frames were removed via a detection algorithm that was based off thresholding method. The remaining data frames were ensemble averaged across approximately 90 frames (depending on the number of contaminated frames that were removed).

Spectral analysis was performed on the ensemble averaged waveforms via computation of the Short Time Fourier Transform spectrogram using MATLAB (window size: 82, window type: hamming, overlap: 81). The 2-dimensional spectrogram is a matrix that stores the relative power for each associated time bin (x-axis) and frequency bin (y-axis). A spectrogram was generated for the averaged stimulus response immediately prior to obtaining the urge to void (*no urge* state), and also for the averaged pulse response immediately after obtaining the urge to void (*urge* state). The powers of each spectrogram time-frequency bin of the *no urge* state were subtracted from the respective powers of each spectrogram time-frequency bin of the *urge* state to yield a difference map, in order to highlight differences in spectral information between the two states.

### 4.3 Results

During routine clinical adjustment of the stimulation parameters to determine optimal parameters for paresthesia, the stimulation intensity was increased in a step-like fashion from 0 mA to 23 mA. Epidural signals were recorded from the spinal leads during stimulation (Figure 4.1). When the stimulation intensity was increased from 14 mA to 15 mA, the subject reported an immediate urge to void. This urge persisted throughout the remainder of the epidural recording. To

determine if urgency to void can be decoded by epidural spinal cord recordings, a segment of recording immediately prior to the urge to void (purple box, Figure 4.1) was directly compared to the segment of recording immediately after the urge to void (yellow box, Figure 4.1).

The data were first analyzed in the time-domain (Figure 4.2) by averaging the responses due to each stimulus for both the *no urge* state (stimulation intensity: 14.0 mA) and the *urge* state (stimulation intensity: 15.0 mA). In general, the averaged waveforms between the *no urge* and *urge* states exhibit similar morphology, especially near the beginning of the window where the contributions due to the stimulus pulse were most prominent. For recordings from electrodes 6, 7 and 8, the onsets of peaks of the averaged *no urge* and *urge* waveforms occur at approximately the same time points (i.e. 3.2 ms, 9.3 ms, 15 ms, 20.7 ms, 21.7 ms) granted small phase shifts (about 1 ms) and slight differences in amplitudes. For instance, the average amplitude of the averaged signal (electrode 8, Figure 4.2) is larger for the *urge* state than *no urge* state from 0 - 16.2 ms, then becomes smaller between 16.2 ms and 24.5 ms, then larger again between 24.5 and 33.5 ms, then smaller again between 24.5 - 49.0 ms. This illustrates how the lower-frequency components of the averaged waveforms are slightly out of phase between the *no urge* and *urge* states. In order to describe the spectral characteristics of the average waveforms more thoroughly, spectrograms were generated for the averaged stimulus response of the *no urge* and *urge* states.

”Difference map” is the term used to describe a spectral plot of Figure 4.3. The difference maps can be understood in the following way: if we assume that there is absolutely no change in the spectrograms of the *no urge* and *urge* states, then the difference maps for each channel would be uniformly green in color (i.e. the power of each frequency bin at each time bin is the same for the *no urge* and *urge* states). On the other hand, if we assume that there is a change in the spectrograms between the two states such that the *urge* state has larger powers

for all frequency bins and at all time bins compared those of the *no urge* state, then the difference maps would contain solely shades of red.

If the noise associated with the stimulus artifact is identical in the two states, then we would assume that the noise components are cancelled out when the difference maps of the two states are computed. If this holds true, then any disparity between the powers in the spectrograms of the *no urge* and *urge* states can be associated to the signals of interest (neurologically-based). In addition, if these neurological sources behave in the exact same fashion during the *no urge* state and the *urge* state, we would not expect to see a disparity between the respective powers of the spectrogram of the two states, yielding a uniformly green difference map. But, if these neurological sources behave in distinct manners in the *no urge* state versus the *urge* state, and the behavior is adequately captured by the recording electrodes in the form of characteristic oscillations, we would expect to see differences between the respective powers of the spectrograms of the two states, yielding a difference map with regions of blue and red.

As apparent in Figure 4.3, there are distinct differences in the powers associated with each time-frequency bin between the two states, across all electrodes. The differences are most apparent between the frequencies of 400 - 1000 Hz across all time bins. The number of frequency bins that differ in power between the *no urge* and *urge* states increases along the x-axis of the difference maps. In other words, spectral differences are least apparent near the beginning of the difference map (where the contribution of the stimulation artifact is greatest) and most apparent near the end of the difference map (where the stimulation artifact contribution is least).

The differences in the powers associated with each time-frequency bin between the two states are least prominent at lower frequencies (less than 400 Hz), especially within the first 15 - 25 ms of the difference map window (depending on the electrode). This can be primarily associated to the large contributions of the

stimulus artifact, which contaminate or drown the lower-frequency components that may originate from neurological sources, making them undetectable via this analysis technique. Increasing the stimulation amplitude from 14 mA to 15 mA (*urge* state) did not cause a remarkable difference in power of the low-frequency components for the first 15 - 25 ms of the window, as demonstrated by the green shading in the difference maps (Figure 4.3). Thus, the power of spectrogram time-frequency bins that correspond to low frequencies (less than 400 Hz) and times near or during the stimulation artifact do not show remarkable differences between the *no urge* state and the *urge* state. These time-frequency bins are therefore least informative in detecting urge onset. However, low-frequency bins become more informative after approximately 25ms of the difference map window (which corresponds to 45 ms after stimulus onset).

Based on the number of differences visible in the difference maps, recordings from electrodes 6 - 8 and 11 - 15 may contain the most information regarding the underlying processes that are involved in urge onset and persistence. Frequency components greater than 400 Hz appear to contain information throughout the 50 ms analysis window (20 - 70 ms after stimulus onset), while frequency components under 400 Hz seem to contain information primarily after the first 25 ms of the analysis window (depending on the electrode). Difference maps of recording electrodes farthest from the stimulation electrodes exhibited the greatest amount of difference in powers of respective time-frequency bins.

## 4.4 Discussion

Epidural recordings of the thoracic spinal cord may contain information that can be used to determine neurological states of the spinal cord, and this information may be used to determine optimal stimulation parameters for stimulation-based functional therapy. A goal of this experiment was to determine whether



an electrical stimulus applied epidurally over the thoracic spinal cord produces a response (recorded by epidural electrodes) that contains frequency components that remarkably change in power (calculated as the squared magnitude of the STFT) when the subject transitions from having no urge to void to having an urge. Analysis was conducted to determine how differently the spectrograms of the averaged stimulus response before the urge to void differs from those when the subject obtained the urge to void.

The recorded responses of the spinal cord to the electrical stimulation did not contain characteristic peaks that were visibly apparent in the time-domain. This is interesting to note as results from Parker *et al.* demonstrate that at large enough stimulation amplitudes, a series of visibly apparent compound action potentials should be present in the epidural recordings [175]. The absence of these compound action potentials in the averaged waveforms obtained from the *no urge* and *urge* states can be explained by the presence of a large-duration stimulation artifact and/or the sub-threshold intensities administered. For instance, Parker *et al.* reported that the stimulation artifact decays well below the neurological component within 1 ms after the stimulus termination [175]. However, the stimulation artifact observed in our experiment lasted approximately 22 - 30 ms after stimulus termination (depending on the proximity of the recording electrode to the stimulation electrodes), which may have drowned the neurologically-based compound action potentials. Another possibility is that the stimulation intensities used to generate the averaged responses in this experiment (14 mA and 15 mA; 120 microsecond pulse width) were smaller than those used by Parker *et al.* to elicit compound action potentials (19+ mA; 120 microsecond pulse width) [175].

The analysis of the stimulus responses of our experiments were therefore performed on time segments 20 ms after the onset of the stimulus, to reduce the contribution of the stimulus artifact to the analysis. Since the analysis window is relatively far from the onset of the stimulus, time-locked evoked potentials were

not discernibly present. Thus the oscillations present in the analysis window can be considered as signatures that are related to certain events (i.e. the stimulus pulse, urge onset and persistence). However, it may not be possible to undeniably associate certain oscillations to urge onset using this data set, as the sample size is too small to make statistical comparisons. Thus, the features present in the difference maps may be associated with an urge to void, or they may be a result of, for example, increasing the stimulus amplitude. Differentiating the contributions of the stimulus artifact to the contributions due to the changes in neural states requires a more complex experimental design that additionally monitors perineal EMG activity and/or urodynamics testing.

In healthy individuals, the urge to void is correlated with bladder fullness. The sensation of bladder fullness are transmitted to the spinal cord via the  $A\delta$ -fibers of the pelvic and hypogastric nerves [73]. Specifically, the  $A\delta$ -fibers respond to passive distension as well as active contraction [121]. The cell bodies of these fibers are located in the dorsal root ganglia (DRG) of the sacral and thoracolumbar (T11-L2) segments [73]. The axons of these cell bodies synapse with spinal interneurons as well as spinal-tract neurons that project to supraspinal centers involved in bladder control [73]. These spinal-tract neurons that carry ascending information regarding the sensations of bladder fullness are located in the medial portions of the dorsal columns of the mid-thoracic level (Nyberg-Hansen 1966). Thus, I postulated that the electrical activity of the neurons involved in transmitting sensations of bladder fullness (i.e. DRG cell bodies, spinal interneurons, dorsal column projections) may be epidurally recorded and identified.

To identify whether neurons involved in bladder fullness sensation exhibit discernable features in epidural spinal cord recordings, epidural electrical stimulation was applied to the thoracic spinal cord of our experimental subject in increasing intensity steps. Upon reaching a certain stimulation intensity, the subject reported an immediate urge to void. In healthy individuals, the first desire to void is re-

ported when about 60% of the bladder capacity is reached [42]. Since our subject did not initially report an urge to void, we can assume that the subject's bladder was insufficiently full to cause a discernible urge to void; however, the effects of the epidural stimulation modulated the physiological state of the subject in such a way that a compelling urge was reported. Under the assumption that the amount of urine in the subject's bladder did not increase significantly during the experimental protocol, the sense of urgency brought about via epidural stimulation may be distinct from the sense of urge due to fullness of bladder. For instance, each stimulus applied to the spinal cord may be directly or indirectly activating motor pools which in turn cause detrusor and sphincter muscle contractions, yielding an increase in bladder pressure (i.e. detrusor pressure). This increase in pressure may be the source for the sensation of bladder fullness, rather than an increase in urine volume. On the other hand, the epidural stimulation may have activated the sensory pathways involved in sensations of bladder fullness that travel to higher brain centers, 'tricking' the brain into thinking that the bladder is full. Since the subject exhibited slight visible twitches in lower extremity and abdominal muscles at the stimulation intensity that initially caused the onset of urge to void, it is more likely that the sensation of bladder fullness may be due to the induced contractions of lower urinary tract muscles.

It is possible that this method of recording from the epidural space is not suitable for detecting the onset of urge to void. Perhaps the contributions of the neurons at the DRGs that relay bladder fullness sensation to the epidural recordings may be insufficient to exhibit characteristic signatures in the recorded waveforms, as less than 3% of the total number of neurons in a single DRG innervate the lower urinary tract [43]. Perhaps the orientation, physical morphology, and spatial locations of the neurons involved with bladder fullness sensation are in such a way that activity from these neurons do not summate to form noticeable potentials in the recorded waveforms. The distance of the epidural electrodes

from the neural sources that transmit sensations of bladder fullness may be too large for electrodes to detect activity from these neural sources in the recorded waveforms, since the detectable potentials are inversely related to the distance between neural source and the electrodes. The effects of volume conduction may also diminish higher frequency information that may arise from activity of these neural sources via the global attenuation of the distant low-amplitude potentials [169].

However, if the results of further experiments determine that epidural recordings in fact contain information that can be used to detect bladder fullness, the signals can be used to inform those with spinal cord injury and SCS implants when the bladder is full and thus when to excuse themselves to empty the bladder. A method to determine the fullness of the bladder can also be used in closed-loop systems that aid in determination of optimal stimulation settings for those undergoing long-term electrical stimulation-based functional therapy.

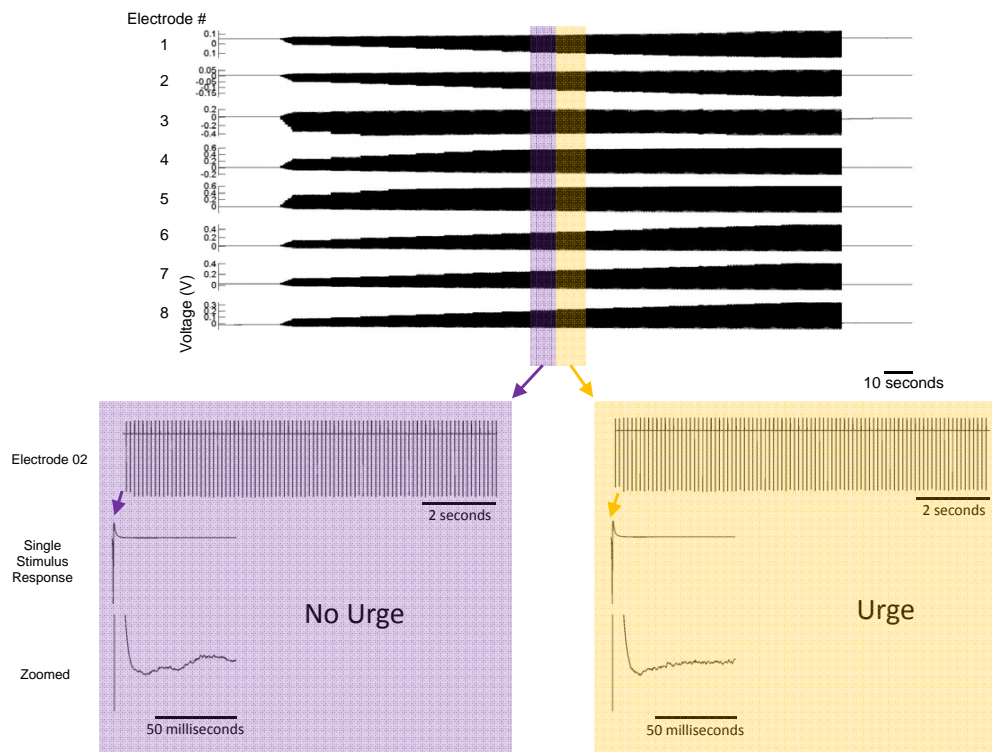


Figure 4.1: **Epidural field potential recordings of the thoracic spinal cord.**

(a) Neural recordings from 8 of the 16 electrodes are shown (top) during the stimulation procedure. The stimulation intensity was increased in a step-like fashion from zero until the maximum tolerable level that reduced pain according to the subject. At the transition between two specific stimulation intensity levels, the subject reported an immediate urge to void. A segment of data before the reported urge (purple) as well as a segment of data after the reported urge (yellow) were analyzed. The top trace in each colored box illustrates the segment of data analyzed; the middle trace illustrates a response to a single stimulus pulse; the bottom trace illustrates a zoomed-in version of the stimulus response.

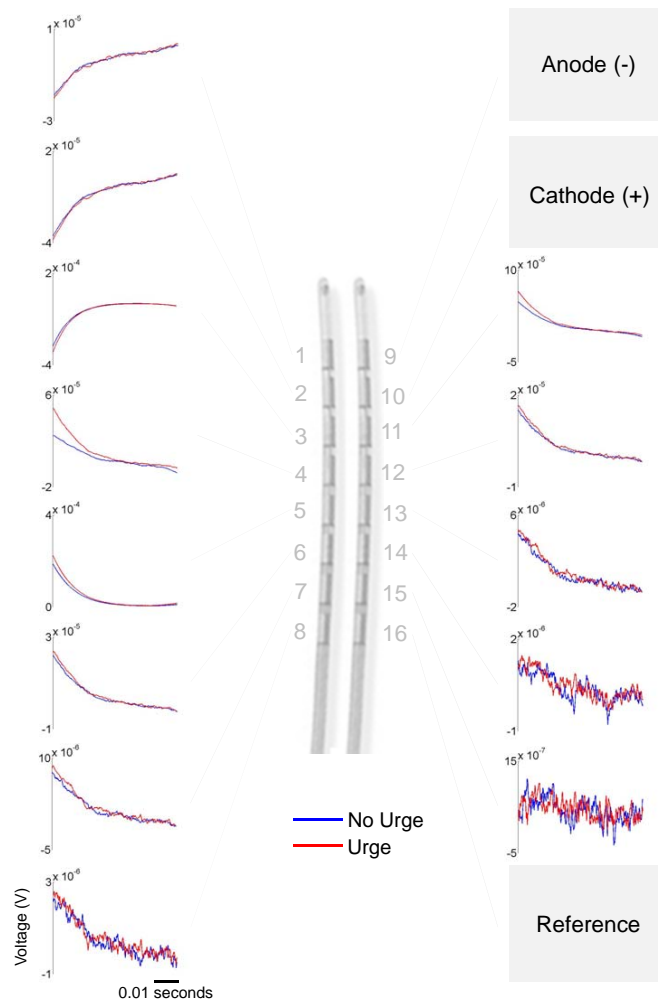


Figure 4.2: **Averaged epidural spinal cord potentials in response to electrical stimuli before (blue) and after the urge to void (red).** Electrode 16 served as the reference electrode, while electrodes 9 and 10 were configured as the anode and cathode, respectively. Each trace spans 50 milliseconds and represents the average evoked/induced potentials 20 milliseconds after the onset of stimulus pulse (to reduce effects of stimulus artifact on analysis). The contribution of the stimulus artifact to the neural recording is apparent and predominately affects recordings of electrodes closest to the stimulation electrodes.

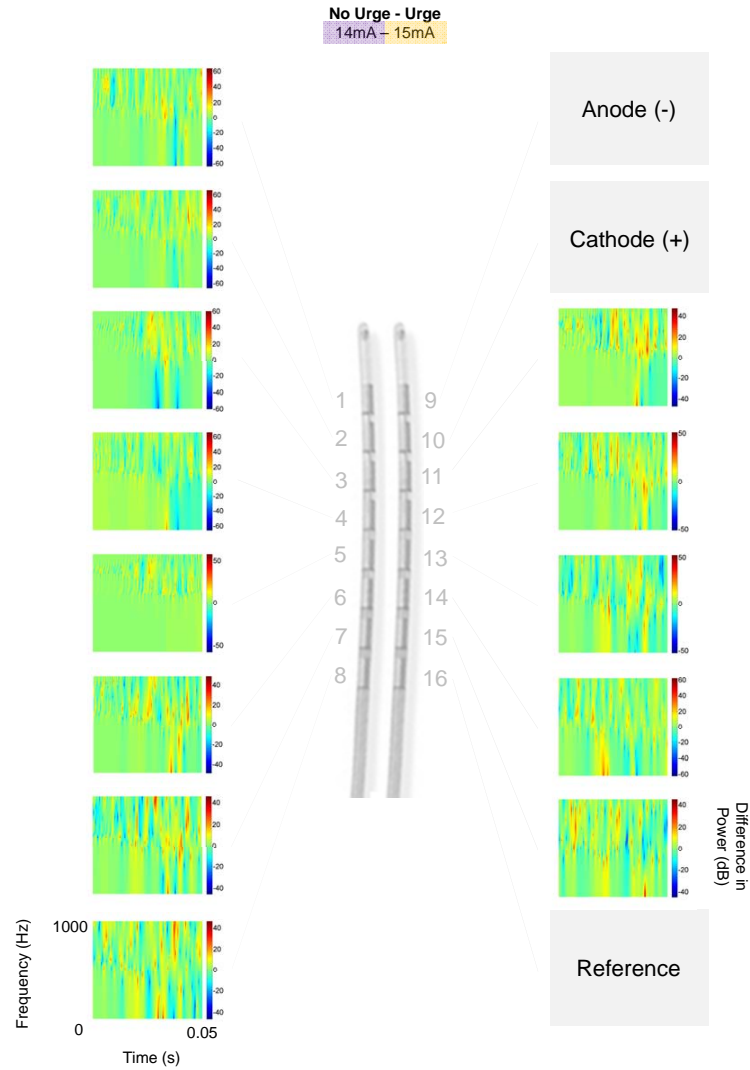


Figure 4.3: **Difference Maps: Difference in spectrogram power (dB) of averaged stimulus responses between *no urge* and *urge* states.** To determine the amount of change of the average stimulus response between the *no urge* and *urge* states, the power of each time-frequency bin in the *no urge* spectrogram was subtracted from the power of the respective time-frequency bin in the *urge* spectrogram. The green color in the colorbar represents little to no difference in power for a particular time-frequency bin between the *no urge* and *urge* states. Red color represents a large increase in power from the *no urge* to *urge* states. Blue color, on the other hand, represents a large decrease in power from the *no urge* to *urge* states.

## CHAPTER 5

### Conclusion

Electrical stimulation of the spinal cord has been demonstrated to facilitate the recovery of motor functions. Here, the ability of spinal cord stimulation to neuromodulate autonomic functions was demonstrated via two series of experiments. The first series involved the use of epidural electrical stimulation applied to the dorsal cervical spinal cord to alter excitability levels of respiratory circuits to modulate respiratory function. Epidural stimulation of the intact cervical spinal cord in anesthetized patients resulted in acute changes in respiratory rate and tidal volume, dependent on the location and frequency of stimulation. Application of epidural stimulation was also demonstrated to rescue respiratory function in an individual under opiate-induced respiratory depression. These results have widespread implications for addressing various potentially life-threatening conditions or events in which the brainstem may become unresponsive resulting in respiratory failure.

The second series of experiments demonstrating the effects of spinal cord stimulation on autonomic functions involved the use of transcutaneous electrical stimulation applied to the thoracolumbar spinal cord of individuals with spinal cord injury, specifically to assess changes in bladder function. We demonstrated that bladder function can be recovered by the repeated application of transcutaneous spinal cord electrical stimulation for individuals with SCI. The results suggest that dormant spinal pathways due to a spinal cord injury are capable of being modulated and trained to learn a specific task in the presence of repeated electrical



spinal cord stimulation, presumably by strengthening neural pathways between the brain centers and spinal centers controlling micturition.

Lastly, in an effort to test whether epidural spinal cord potentials contain information that can determine onset of bladder sensations, recordings from the thoracic spinal cord were obtained and analyzed from one individual before and during the urge to void. Distinct differences in spectral characteristics of the epidural waveforms were demonstrated; however a larger sample size is needed to determine if these characteristics can decode for urge onset.

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