NEUROLOGICAL OUTCOMES AFTER CERVICAL SPINAL CORD INJURY

by

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Abstract

In addition to overcoming biological barriers that limit regeneration and repair in the central nervous system after spinal cord injury, clinicians and researchers are faced with the daunting task of assessing the safety and efficacy of therapeutics proposed to ameliorate neurological deficits in humans. While the selection of an appropriate clinical endpoint will depend on a number of factors, including the phase of study and the underlying biological activity, the estimated effect size based on preclinical studies in animal models of spinal cord injury of most therapeutics are expectedly small. A potential promising strategy to detect subtle but clinically meaningful changes in humans is to focus on individual spinal segments partially damaged adjacent to the level of injury.

The primary aim of this thesis was to evaluate the validity of segmental sensorimotor outcomes for the purpose of devising clinical trial endpoints for spinal cord injury. In Chapters Two through Five, I focus this investigation on evaluating sensory outcomes. Chapter Two is chiefly intended to introduce clinical sensory testing methods (i.e. light touch and pinprick) and provide a better understanding of the relationship between the neuropathology of spinal cord injury and afferent anatomy and physiology. In order to address limitations of clinical sensory testing methods, the subsequent three chapters are focused on the application of segmental neurophysiological approaches. More specifically, this involves a series of studies in individuals with spinal cord injury aimed at objectively measuring conduction deficits in the dorsal column and spinothalamic tract based on outcomes from sensory evoked potentials. The thesis then shifts to the segmental assessment of motor function. In Chapter Six, changes in muscle strength are examined in
the first year after cervical spinal cord injury, with a specific interest in documenting the
relationship between improvements in motor scores in the upper extremities and the
recovery of motor levels. This chapter concludes by linking functional independence and
neurological outcomes. The discussion that follows is intended to provide an outline of how
the knowledge acquired during the course of this doctoral thesis could be translated into
phases of a clinical trial program.
Preface

The works in preparation, submitted or accepted for publication, and published in peer-reviewed journals or book chapters are indicated below. My contributions are highlighted for each Chapter. Chapters Four and Five were completed in collaboration with laboratory of Dr. Armin Curt at the Spinal Cord Injury Center, Balgrist Hospital, University of Zurich. Chapter Six was completed in collaboration with members of the European Multi-Center Study about Spinal Cord Injury and the Spinal Cord Outcomes Partnership Endeavour. The research ethics conducted herein were reviewed and approved by the University of British Columbia Offices of Research Services (H07-02410, H08-03141, and H08-01087). The European Multi-Center Study about Spinal Cord Injury has been reviewed by the Research Ethics boards of each participating center. A data sharing agreement was in place with the University of Zurich.

General Introduction

Sections and sub-sections of the General Introduction have been published or are accepted for publication.


- Wrote the Book Chapter.


- Wrote a section for this Book Chapter. Only the section I authored appears in the General Introduction.
Steeves, JD, Zariffa, J, Kramer, JLK. Are you tilting at windmills or undertaking a valid clinical trial? Yonsei Medical Journal (Invited Review). Accepted.

- Co-authored this manuscript. Only a brief portion of this review has been included in this thesis (re: phases of clinical trials).

**Chapter Two:**

A version of *Chapter Two* will be submitted for publication.

Kramer, JLK, Zariffa, J, Curt, A, Steeves, J. Discontinuous sensory preservation is not limited to sacral segments. (In preparation).

- Designed the methods of analysis, analyzed the data, and wrote the manuscript.

**Chapter Three:**

A version of *Chapter Three* has been published.


- Designed the methods of analysis, analyzed the data, and wrote the manuscript.

**Chapter Four:**

A version of *Chapter Four* has been accepted for publication.


- Designed the study, collected the data, analyzed the data, and wrote the manuscript.

**Chapter Five**

A version of *Chapter Five* has been accepted for publication.


- Designed the study, collected the data, analyzed the data, and wrote the manuscript.
Chapter Six

A version of Chapter Six has been published:

*
*, both authors contributed equally to this work.

- Analyzed the data and wrote the manuscript. A second manuscript is in preparation regarding additional material presented in this thesis not previously published (re: relationship between SCIM and motor levels) (Kramer et al).
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Lists of abbreviations

ACC  anterior cingulate gyrus
ADL  activities of daily living
AIS  American Spinal Injury Association International Standards
CHEP contact heat evoked potential
CMAP compound muscle action potential
CNS  central nervous system
CSF  cerebrospinal fluid
dSSEP dermatomal somatosensory evoked potential
EEG  electroencephalography
EMG  electromyography
EPT  electrical perception threshold
EM-SCI European Multi-Center Study about Spinal Cord Injury
GRASSP Graded Redefined Assessment for Strength, Sensibility, and Prehension
ICC  intraclass correlation coefficient
ISNCSCI International Standards for the Neurological Classification of Spinal Cord Injury
LEP  laser evoked potential
MCID minimally clinically important difference
MEP  motor evoked potential
MRI  magnetic resonance image
MVA  motor vehicle accident
NRS  numerical rating scale
QoL  quality of life
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>QST</td>
<td>quantitative sensory testing</td>
</tr>
<tr>
<td>RCT</td>
<td>randomized controlled trial</td>
</tr>
<tr>
<td>RTM</td>
<td>regression towards the mean</td>
</tr>
<tr>
<td>S1</td>
<td>primary somatosensory cortex</td>
</tr>
<tr>
<td>SCI</td>
<td>spinal cord injury</td>
</tr>
<tr>
<td>SCIM</td>
<td>Spinal Cord Independence Measure</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SSEP</td>
<td>somatosensory evoked potential</td>
</tr>
<tr>
<td>UEMS</td>
<td>upper extremity motor score</td>
</tr>
<tr>
<td>VGH</td>
<td>Vancouver General Hospital</td>
</tr>
<tr>
<td>WISCI</td>
<td>walking index for spinal cord injury</td>
</tr>
<tr>
<td>ZPP</td>
<td>zone of partial preservation</td>
</tr>
</tbody>
</table>
Acknowledgements

*If you can meet with triumph and disaster
And treat those two imposters just the same*

If, Rudyard Kipling (1865-1936)

I would like to thank the members of my supervisory committee, Drs. Tim Inglis, Brian Kwon, and Matt Ramer for their insightful comments and suggestions. I am indebted to Drs. John Steeves and Armin Curt, both of whom have provided wonderful supervision and support, and afforded me countless opportunities over the course of the last four years. A special thank you to John for assuming a supervisory role – I hope it has been worth the trouble. Many of my accomplishments would not have been possible without the financial support of the Canadian Institute of Health Research and the Michael Smith Foundation for Health Research.

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In loving memory of my late Papa, John Kramer  
(June 25, 1922 – July 21, 2010)

Above all else, I have always tried to live up to the name and hoped that I might be a success in your eyes. This is perhaps one step.
1. General introduction

1.1. Anatomical organization of the spinal cord

The mammalian spinal cord, illustrated in Figure 1.1, is organized into two anatomically and functionally distinct regions aptly named for their gray and white appearance (Goshgarian, 2010). The central gray matter, comprised primarily of neuron cell bodies and divided into the dorsal (laminae I-VI) and ventral horn (laminae VII-X), is chiefly responsible for integrating and relaying sensory and motor stimuli. The white matter, orientated around the central gray matter, is comprised of sensory, motor and propriospinal axons positioned in the dorsal, lateral, and ventral funiculi. The major ascending and descending white matter tracts in humans are described in Table 1.1. Active (e.g. saltatory conduction) and passive properties (e.g. diameter) of axons in the white matter tracts convey the action potentials to their intended synaptic target (Table 1.2).

Similar to sub-cortical and cortical structures, the central gray and white matter of the spinal cord is somatotopically organized. In the dorsal columns, the most caudal afferents from lumbosacral and lower thoracic segments are positioned in the gracies fasciculus, medial to afferents from cervical and upper thoracic segments in the cuneate fasciculus. In the spinothalamic tract, afferents from more caudal spinal segments ascend lateral to afferents from more rostral segments. Similarly, the corticospinal tract is organized with sacral efferents lateral to more rostral afferents. The topographical organization of the spinal cord, as well as the relative cross-sectional area of gray matter to white matter, varies according to the rostral-caudal level of the spinal cord.
Spinal nerves functionally divide the spinal cord into 31 distinct segments (8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal), with each spinal segment innervating a group of muscles (i.e. myotome) and a given area of skin (i.e. dermatome) via the ventral and dorsal horn, respectively. The spinal cord is protected by three layers of meninges (dura mater, the arachnoid, and pia mater) and cerebrospinal fluid (CSF), and contained within the spinal column. The spinal column is comprised of cervical, thoracic, and lumbar vertebrae separated by intravertebral discs, the sacrum, and the coccyx. Each cervical, thoracic, and lumbar vertebra has three rotational and three translational axes, for a total of six degrees of freedom.

Figure 1.1 Spinal cord cross-section
The corticospinal tract (lateral and anterior, orange), dorsal columns (red), and spinothalamic tract (blue) are shown. The topographical organization of each pathways is marked.
<table>
<thead>
<tr>
<th>Ascending</th>
<th>Name</th>
<th>Funiculus</th>
<th>Primary functional significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal column – medial lemniscal system</td>
<td>Dorsal</td>
<td>Kinesthesia and discriminative touch</td>
<td></td>
</tr>
<tr>
<td>Posterior Spino cerebellar</td>
<td>Dorsal-lateral</td>
<td>Proprioception</td>
<td></td>
</tr>
<tr>
<td>Anterior-lateral system</td>
<td>Lateral</td>
<td>Pain and temperature</td>
<td></td>
</tr>
<tr>
<td><em>Lateral spinothalamic</em></td>
<td>Lateral</td>
<td>Pain and temperature</td>
<td></td>
</tr>
<tr>
<td><em>Anterior spinothalamic</em></td>
<td>Ventral</td>
<td>Non-discriminative pain</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Descending</th>
<th>Name</th>
<th>Funiculus</th>
<th>Primary functional significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticospinal</td>
<td>Anterior</td>
<td>Precise movements (e.g. hands, feet)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubrospinal</td>
<td>Lateral</td>
<td>Voluntary movement (e.g. large extensors/flexors)</td>
<td></td>
</tr>
<tr>
<td>Lateral Vestibulospinal</td>
<td>Ventral-lateral</td>
<td>Balance</td>
<td></td>
</tr>
<tr>
<td>Medial Vestibulospinal</td>
<td>Ventral</td>
<td>Head position</td>
<td></td>
</tr>
<tr>
<td>Reticulospinal</td>
<td>Ventral</td>
<td>Movement, respiration, inhibitory effects on transmission of sensory stimuli</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Goshgarian, 2010

Table 1.1 Major ascending white matter tracts in humans
<table>
<thead>
<tr>
<th>Fibers</th>
<th>Motor</th>
<th>Sensory</th>
<th>Diameter (µm)</th>
<th>Conduction Velocity (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>I</td>
<td>Aα</td>
<td>12-20</td>
<td>72-120</td>
</tr>
<tr>
<td>Medium</td>
<td>II</td>
<td>Aß</td>
<td>6-12</td>
<td>36-72</td>
</tr>
<tr>
<td>Small</td>
<td>III</td>
<td>Aδ</td>
<td>1-6</td>
<td>4-36</td>
</tr>
<tr>
<td>Unmyelinated</td>
<td>IV</td>
<td>C</td>
<td>0.2-1.5</td>
<td>0.4-2</td>
</tr>
</tbody>
</table>

Adapted from Gardner, et al., 2000

Table 1.2 Axonal conduction properties

1.2. Traumatic spinal cord injury

1.2.1. A brief history of traumatic spinal cord injury

The neurological consequences of spinal cord injury (SCI) were first described in the Edwin Smith Papyrus (17th century BC) (Hughes, 1988; van Middendorp, et al., 2010). Six cases of spinal injuries were reported based on the observation of vertebral fracture/dislocation and the appearance of sensorimotor and autonomic deficits (van Middendorp, et al., 2010). The neurological sequelae of traumatic SCI, including the underlying neuropathology and mechanisms contributing to secondary damage (described below) became more widely known at the turn of the 19th century (Allen, 1911; Wilson, 1911; Allen, 1914). However, SCI remained largely a “medical condition that cannot be healed” (from the Edwin Smith Papyrus) until the early half of the 20th century. Progress in the care of individuals with SCI in 1930’s, particularly for the treatment of those secondary complications (described below) that often were the cause of death (e.g. infections), was led by Dr. Donald Munro in the United States and Sir Ludwig Guttmann in the United
Kingdom. Patients suffering traumatic SCI now were surviving the initial phase of SCI due largely to advances in their care, and undergoing, for the first time, activity based rehabilitation. The importance of accurately diagnosing traumatic SCI for the purposes of predicting long term functional outcomes emerged in the late 1960’s and early 1970’s (Frankel, et al., 1969; Holdsworth, 1970; Stauffer, 1975), and ultimately led to the development of the International Standards for the Neurological Classification of Spinal Cord Injuries (ISNCSI) in 1982 (Maynard, Jr., et al., 1997).

1.2.2. Primary and secondary spinal cord injury

Traumatic SCI is now often divided into primary and secondary injury phases (Tator and Fehlings, 1991). The primary injury results directly from the vertebral column flexion, extension, rotation, distraction, and/or a combination of forces, with or without vertebral fracture that, in turn, mechanically disrupts the spinal cord parenchyma (Figure 1.2). Direct primary mechanical disruption of cell membranes, along with hemorrhagic necrosis, anoxia, and the release of neurotoxic cellular constituents from within disrupted cells generates an “epicenter” of injury that can traverse multiple spinal cord segments (Kakulas, 1984; Hayes and Kakulas, 1997). Acute secondary SCI is broadly defined as “downstream events following primary injury that involve a complex cascade of molecular events, culminating in a progressive degenerative injury to the spinal cord” (Tator and Fehlings, 1991; Park, et al., 2004). Subsequent Wallerian degeneration rostral and caudal to the epicenter of injury, demyelination, the formation of a glial scar and central cavitation in the days and weeks following acute primary traumatic SCI are believed to contribute strongly to long-term neurological impairment and prevent regeneration and remyelination (Houle
and Tessler, 2003). Regardless, neither primary (e.g. flexion-extension of spinal column) nor secondary mechanisms of injury typically result in a complete transection of the spinal cord, but leave an intact rim of white matter (Kakulas, 1984; Hayes and Kakulas, 1997).

![Diagram of spinal cord injury mechanisms](image)

**Figure 1.2 Primary mechanisms of spinal cord injury**
A combination of these forces may also result in a traumatic spinal cord injury (image used with permission, Couris, *et al.*, 2010). These types of closed injuries are difficult to replicate in animal models.

### 1.2.3. Spinal shock

Clinically, the acute phase of traumatic SCI is characterized by a period of spinal shock. During the initial 24 hours post injury, individuals present with absent or pathological reflexes (e.g. delayed plantar response) and muscles caudal to the lesion site are completely paralyzed and flaccid (Ditunno, *et al.*, 2004). Resolution of paralysis, emergence of pathological reflexes and recovery of absent reflexes generally occurs between 1 and 3 days, and is often followed by progressive development of hyperreflexia.
and spasticity over the first year after injury. Several physiological processes have been considered to account for the distinct phases of spinal shock following traumatic SCI. Paralysis and areflexia have been largely attributed to acute neuronal hyperpolarization, lost supraspinal background neural excitation, and alterations in presynaptic inhibition (Ditunno, et al., 2004). The reemergence of absent reflexes and hyperreflexia has been linked to increased neuronal signaling resulting from denervation supersensitivity by receptors to neurotransmitters and the unmasking of new synaptic connections within the central nervous system (CNS) (Ditunno, et al., 2004). Recently, reduced motor axon excitability of peripheral nerves in the upper and lower extremities have been reported during the initial period of SCI, which may also contribute to the emergence of spinal shock symptoms (Boland, et al., 2011).

1.2.4. Spontaneous recovery and chronic spinal cord injury

The severity of neurological deficits associated with acute traumatic SCI varies depending on the degree of disruption to the major ascending and descending white matter tracts. However, even after the most severe spinal injuries, patients suffering an acute traumatic SCI are expected to experience a modest degree of spontaneous neurological recovery over the first year (Frankel, et al., 1969; Maynard, et al., 1979; Bedbrook, 1980; Kiwerski, 1989; Katoh and El Masry, 1994; Katoh and El Masry, 1995; Ditunno, Jr., et al., 2000a; Curt, et al., 2008). Several mechanisms have been proposed as contributing to spontaneous neurological recovery after traumatic SCI, including compensatory strategies, neural plasticity, and neural repair (Table 1.3). Most peer-reviewed research has only noted a limited degree of spontaneous axonal regeneration within the injured spinal cord
(for a review of the descending pathways and regeneration after SCI, see Deumens, et al., 2005). The modest amount of axonal regeneration within the adult cord may be actively inhibited by an environment (CNS myelin and astrogliosis) that is unsupportive of regeneration (Keirstead, et al., 1995; Houle and Tessler, 2003). Neural plasticity caudal to the level of injury has been extensively studied with regards to locomotion. Most notably, the use of weight supported treadmill training has shown to enhance ambulation among individuals with incomplete SCI. Activity dependent plasticity arising from afferent feedback to the lower limb central pattern generators has been proposed as a potential mechanism facilitating such functional recovery (Muir and Steeves, 1997; Harkema, 2008; Dietz, 2009). Rostral to the level of injury, neural plasticity has also been reported in supraspinal structures during spontaneous neurological recovery (Jurkiewicz, et al., 2007) and in response to treadmill training (Winchester, et al., 2005).

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compensation</td>
<td>Changes in function that can be achieved without any change in the neurological deficit (e.g. training adapted or new movement strategies)</td>
</tr>
<tr>
<td>Neural plasticity</td>
<td>Reorganization of neuronal circuits (e.g. sprouting), changes in synaptic strength</td>
</tr>
<tr>
<td>Repair</td>
<td>Reconnection or restoration of conduction in damaged spinal tract fibers (e.g. axonal sprouting, reduced astrogliosis, provision of essential growth factors, remyelination)</td>
</tr>
</tbody>
</table>

Adapted from Curt, et al., 2008

Table 1.3 Mechanisms of spontaneous neurological recovery

The chronic phase of SCI (approximately 1 year post injury) is marked by the resolution of acute pathophysiological processes and the stabilization of neurological
impairment. Late neurological recovery or subsequent neurological deterioration secondary to ongoing traumatic events (e.g. post traumatic syringomyelia) also may be observed, albeit both occur in less than ~5% of all cases (Kirshblum, et al., 2004; Carroll and Brackenridge, 2005). Upon reintroduction to activities of daily living (ADL), individuals with traumatic SCI experience a variety of secondary complications, including; pressure sores, urinary tract infections, upper respiratory tract infections, and neuropathic pain (Cardenas, et al., 2004). The type, incidence, and severity of secondary complications is related to the level of injury and motor function, whereby individuals with higher level injuries and who score lower on measures of independent function are more likely to be hospitalized and be negatively impacted by secondary complications (Cardenas, et al., 2004).

1.2.5. Epidemiology of traumatic spinal cord injury

Based on provincial estimates from recent epidemiological studies, the incidence rate of SCI in Canada is between 40 and 50 people per million, per year (Dryden, et al., 2003; Pickett, et al., 2003; Pickett, et al., 2006; Kattail, et al., 2009; Couris, et al., 2010; Pirouzmand, 2010; van den Berg, et al., 2010; McCammon and Ethans, 2011). Comparable to most countries, these studies indicate that the age at the time of injury is bimodally distributed; the first peak between 15 and 29 years of age and the second in adults older than 65 years. Although motor vehicle accidents (MVAs) generally account for the largest proportion (35 – 56%) of injuries in Canada, the number of individuals sustaining a traumatic SCI due to falls is increasing. In Canada, injuries at cervical segments resulting in tetraplegia are most common (60-75% of all injuries), followed by thoracic segment
injuries resulting in paraplegia (10-22%). While advances in emergency and primary treatment (e.g. management of SCI patients in a specialized spine trauma center, Kattail, et al., 2009) have significantly decreased the mortality associated with acute SCI, the average lifespan of individuals living with SCI remains shorter than that of the general population (DeVivo, et al., 1999; Devivo, 2007).

By all accounts, the estimated lifetime financial impact of traumatic SCI on the universal health care system in Canada is high. This is attributable to the costs associated with acute hospitalization and rehabilitation, but also a function of long term dependence on health care services to treat ongoing secondary health challenges over the chronic stages of living with SCI (Dryden, et al., 2004; Cardenas, et al., 2004). Estimates from a recent analysis of direct health care costs (i.e. hospitalization, physician services, home care, and long-term care) in the first year alone are between $42,000 to $120,000 per individual depending on the spinal level and severity of SCI, with subsequent annual costs of up to $5,000 per individual (Dryden, et al., 2005). The direct costs to health care of traumatic SCI in Canada fail to consider the high indirect costs to society (e.g. lost productivity) and the deleterious impact on the individual’s quality of life (QofL).

1.3. Classification of spinal cord injury

1.3.1. Neurological

1.3.1.1. International Standards for the Neurological Classification of Spinal cord Injury

Since its inception, the ISNCSCI (shown in Figure 1.3) has undergone years of intensive study to examine psychometric properties (Cohen, Ditunno et al. 1998; Mulcahey,
Gaughan et al. 2007; Mulcahey, Gaughan et al. 2007; Savic, Bergstrom et al. 2007; Marino, Jones et al. 2008; Mulcahey, Gaughan et al. 2009), the relationship with functional outcomes (Curt and Dietz 1997; Curt, Rodic et al. 1997; Curt, Keck et al. 1998; Marino and Graves 2004; van Hedel and Curt 2006), and the responsiveness to spontaneous neurological recovery (Katoh and El Masry, 1994; Katoh and El Masry, 1995; Marino, et al., 1999; Ditunno, Jr., et al., 2000a; Fawcett, et al., 2007; Curt, et al., 2008; Spiess, et al., 2009).

Currently in its 7th edition, the ISNCSCI has been adopted by both the clinical and research communities worldwide as a tool for the examination of basic motor and sensory function. The outcomes from the ISNCSCI, including American Spinal Injury Association (ASIA) impairment scale (AIS) grades (shown in Table 1.4), have served as a stratification tool and/or primary outcome measure in most of the randomized controlled trials (RCTs) for SCI to date (Furlan, et al., 2008; Hawryluk, et al., 2008).
Note that there are no key myotomes for thoracic segments (excluding T1).
<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><strong>Sensorimotor complete:</strong> No motor or sensory function is preserved in the sacral segments S4-S5</td>
</tr>
<tr>
<td>B</td>
<td><strong>Motor complete:</strong> Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5</td>
</tr>
<tr>
<td>C</td>
<td><strong>Incomplete:</strong> Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3.</td>
</tr>
<tr>
<td>D</td>
<td><strong>Incomplete:</strong> Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more.</td>
</tr>
<tr>
<td>E</td>
<td><strong>Normal</strong></td>
</tr>
</tbody>
</table>

Table 1.4 American Spinal Injury Association International Standards Impairment Scale grades

The sensory component of the ISNCSCI is based on the theoretical framework that:

1) transduction of light touch and pinprick sensation in peripheral receptors (Meissener’s corpuscle and mechanical nociceptors, respectively) is conducted in different peripheral nerve fibers (Aβ and Aδ, respectively) that enter the spinal cord and ascend in anatomically distinct, somatotopographically organized pathways (i.e. dorsal columns and spinothalamic tract, respectively), and 2) defined peripheral cutaneous boundaries (i.e. dermatomes) innervate individual spinal segments. Large diameter fibers conveying touch sensation primarily enter the spinal cord and ascend ipsilaterally in the dorsal columns to their second order neurons in the medulla. These afferents then decussate to form the medial lemniscus pathway, which ascends to the ventral posterior lateral and medial nuclei (Figure 1.4). The somatosensory cortex (S1) represents the primary target of large diameter projections entering the thalamic nuclei. The majority of small diameter fibers
enter the spinal cord to synapse in the dorsal horn (lamina I and V), decussate within the segment via the anterior white commissure and ascend contralaterally to the ventral posterior inferior nucleus, ventral posterior lateral nucleus, and the ventral posterior medial nucleus (Figure 1.4). A smaller proportion of small diameter central afferents enter the spinal cord and ascend or descend one rostral or caudal segment, respectively, in the tract of Lissauer before decussating across the midline and ascending in the spinothalamic tract (LaMotte, 1977). From the thalamic nuclei, small diameter afferents project to medial and lateral systems. These include the S1 and the secondary somatosensory cortex, and the insular and anterior cingulate cortex (ACC), respectively (Treede, et al., 1999).

Dermatomal boundaries as they are defined in clinical neurological practice today are largely based on a series of observations made by Dr. Orfried Foerster at the turn of the 19th century. In his original Brain publication in 1933, Foerster describes the technique he uses to define dermatomal boundaries:

To Sir Charles Sherrington we owe our knowledge of the complete topography of the dermatomes in the monkey. The method used by him, and called the method of "remaining sensibility" or the "isolation method," consists in dividing a series of contiguous roots above and below a single root which is preserved. The area of the skin, the sensibility of which is preserved after this procedure, represents the sensory dermatome of the intact root (Foerster, 1933)

Although Foerster generally did not discuss his reasoning to perform such a procedure in humans ("I need not discuss here the circumstances under which such a selected procedure may be undertaken." Foerster, 1933), especially why one dorsal root was spared, it was thought to be done for the treatment of pain and spasticity (Lee, et al., 2008). In addition to mapping cervical, thoracic, and lumbosacral dermatomes, Foerster reported that dermatomes overlap with nearby dermatomes, and that this overlap was greater for
epicratic sensation (i.e. large diameter fibers, light touch) than for protopathic sensation (i.e. small diameter fibers, pinprick) (Foerster, 1933; Lee, et al., 2008).

The motor component of the ISNCSCI is less comprehensive than the sensory component, in that the motor assessment only examines muscle strength in key upper and lower extremity myotomes (C5-T1 and L2-S1, respectively), as well as whether there is voluntary anal contraction. There is no direct measurement of motor function from the following spinal cord segments: C1-C4; T2-L1; and S2-S5 due to the difficulty in isolating innervation of a specific muscle from a single spinal segment. Furthermore, most ventral roots innervate more than one muscle and most muscles are innervated by more than one ventral root (Figure 1.5). Muscle strength is graded according to a 5-point scale (Table 1.5), where a grade of three or higher constitutes a ‘functional’ motor score. The primary objective of the motor examination after SCI is to measure the loss of muscle strength, rather than the coordination of motor commands (Wirth, et al., 2008a).
Figure 1.4 Ascending pathways examined after spinal cord injury
Large diameter fibers conveying light touch sensation enter the spinal cord and ascend ipsilaterally, whereas smaller diameter fibers conveying pinprick sensation decussate upon entry or within one or two segments to ascend contralaterally to the stimulation site. This is illustrated at the C5 segment.
<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Total paralysis</td>
</tr>
<tr>
<td>1</td>
<td>Palpable or visible contraction</td>
</tr>
<tr>
<td>2</td>
<td>Active movement, full range of motion, gravity eliminated</td>
</tr>
<tr>
<td>3</td>
<td>Active movement, full range of motion, against gravity</td>
</tr>
<tr>
<td>4</td>
<td>Active movement, full range of motion, against gravity and provides some resistance</td>
</tr>
<tr>
<td>5</td>
<td>Active movement, full range of motion, against gravity and provides normal resistance</td>
</tr>
</tbody>
</table>

, denotes functional motor score

**Table 1.5 Muscle strength grading according to the International Standards**

The reliability of ISNCSCI sensory and motor outcomes are well supported in the literature by a number of large, statistically powerful studies. In general, studies examining the test-retest (i.e. intra-rater) and inter-rater reliability of motor and sensory scores (light touch and pinprick) in the chronic, stable SCI condition demonstrate intraclass correlation coefficients (ICC) that exceed the minimal requirements of clinical standards (0.80 – 1) (Shrout, 1998) and provide strong inter-rater agreement. However, the reliability of sensory and motor scoring may be influenced by a number of factors, including when the examination is performed after SCI (<72 hours), characteristics of the individual being examined (i.e. age), severity of SCI, and formal training of the examiner.
The primary outcomes of the ISNCSCI (i.e. neurological/motor/sensory level, AIS-grades, sensory and motor scores) vary with regards to their responsiveness to spontaneous neurological recovery. Generally speaking, the extent of sensory and motor recovery in the first year after SCI depends on the initial severity of injury, with more complete injuries recovering a fewer number of sensory and motor points, and converting AIS grades less frequently than incomplete injuries. Spontaneous neurological recovery according to the ISNCSCI is best characterized by rapid improvements in the initial months, followed by a period of a decreased rate of change, and a plateau between 6 and 12 months post injury (Ditunno, Jr., et al., 2000a).

1.3.1.2. Quantitative sensory testing

The primary goal for implementing quantitative sensory testing (QST) as an adjunct to the ISNCSCI was to improve the overall sensitivity and clinical meaningfulness of
segmental sensory outcomes after SCI. Specifically, QST methods aim to: 1) detect underlying deficits in dermatomes with “normal” sensation, and 2) distinguish differences in dermatomes with “impaired” sensation. QST methods still require that the individual being tested report the sensation to an examiner, but differ from the ISNCSCI in that the dependent variable is continuous.

A number of different QST modalities have been tested in individuals with SCI (Table 1.6) (Krassioukov, et al., 1999; Hayes, et al., 2002; Finnerup, et al., 2007; Felix and Widerstrom-Noga, 2009). Typically, thresholds are achieved by increasing the intensity of stimulation at a set rate from baseline until the individual verbally reports a change in sensation (i.e. method of limits). This method can be employed in a shorter time-frame, and is considered as accurate as other methods that more precisely determine thresholds (i.e. method of levels, which requires that the stimulus be increased and decreased above threshold). A number of stimulation parameters, including the rate of increase and the size of the area stimulated, as well as properties of the stimulation site (e.g. skin type and thickness) affect QST parameters (Pertovaara, 1999; Iannetti, et al., 2006; Rolke, et al., 2006a; Rolke, et al., 2006b). In general, the construct validity of QST outcomes has been demonstrated with other measures of sensory function, including the ISNCSCI and neurophysiology (see below) (Hayes, et al., 2002). However, most QST methods have not been employed by clinicians due in part to practical limitations (i.e. time consuming and need for expensive equipment). The value of QST over ISNCSCI has also been challenged on the basis of a lack of sufficient data regarding their utility to distinguish differences in dermatomes with clearly impaired sensation (i.e. hypersensitive vs. hyposensitive), as well
as an equally limited capacity to track meaningful changes (Finnerup, et al., 2007; Steeves, et al., 2007).

To address the practical limitations of existing QST measures, electrical perception threshold (EPT) was introduced to SCI in 2006 (Savic, Bergstrom et al. 2006), and has been extensively studied in both healthy control subjects and chronic individuals with SCI. This assessment technique, which requires a subject to report perception to an increasing electrical stimulation from a surface electrode positioned on a key sensory point of each dermatome, requires minimal technical experience, uses relatively inexpensive equipment, and appears sensitive to documenting varying degrees of sensory function (segment by segment) after SCI. At present, the major drawback of EPT as a measure of dorsal column function (Kramer, et al., 2008) is that the stimulation is not physiologically specific, as it broadly activates both small and large diameter afferents in the periphery.
<table>
<thead>
<tr>
<th>Study Author (date)</th>
<th>Study Description</th>
<th>QST modality</th>
<th>Interpretation of reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krassioukov et al. (1999)</td>
<td>Incomplete SCI subjects (n=21), examined dermatomes below the level of SCI at chronic (mean, 6 years) time-points</td>
<td>Warm Cold Cold pain Vibration</td>
<td>Depended on the dermatome and modality tested Day to day variability higher in SCI subjects than uninjured controls Repeated examination on separate days required to establish a baseline</td>
</tr>
<tr>
<td>Felix &amp; Widerstrom-Noga (2009)</td>
<td>Complete and incomplete SCI subjects, examined dermatomes at and below the level of SCI with chronic (mean, 6 years) neuropathic pain (n=10)</td>
<td>*Mechanical Vibration Cool Warm Cold pain Hot pain</td>
<td>Moderate to fair reliability, comparable to uninjured controls Innocuous modalities can be measured in one test session (do not require repeated examinations)</td>
</tr>
<tr>
<td>King et al. (2009)</td>
<td>Incomplete SCI subjects (n=12), examined all ASIA sensory points at chronic (&gt;20 months) and sub-acute (&lt;9 months) time-points</td>
<td>Electrical</td>
<td>Good intra- and inter-rater reliability to measure cutaneous sensory function Depended on the dermatome tested</td>
</tr>
</tbody>
</table>

*, monofilaments

Table 1.6 Quantitative sensory testing reliability findings in individuals with spinal cord injury

1.3.1.3. Neurophysiology

Also largely considered complementary to the ISNCSCI, the use of detailed electrophysiological approaches in clinical practice intends to provide a more objective index of spinal cord function for diagnosis and prognosis of SCI (Rowed, et al., 1978). These
methods are comprised of a number of different techniques, including evoked potentials (sensory and motor) and nerve conduction studies (sensory and motor). Monitoring residual volitional and reflexive motor function has also been proposed to objectively assess motor function (e.g. Brain Motor Control Assessment, Sherwood, et al., 1996). Performed in conjunction with the ISNCSCI, a more detailed understanding of the neurological deficits related to SCI can be determined based on a combined approach (e.g. peripheral nerve impairment). Furthermore, by employing other neurophysiological outcomes (e.g. sympathetic skin response) neurological deficits not assessed by the ISNCSCI (e.g. autonomic function) can be objectively studied (Curt, et al., 1996; Curt and Dietz, 1999).

Conventional somatosensory evoked potentials (SSEP), examined by stimulating mixed nerves in the periphery with electrical pulses and recording electroencephalography (EEG), were first made possible in man by computer averaging techniques (Dawson, 1947). Depending on the peripheral nerve stimulated in the upper extremities, the electrical impulse traverses the brachial plexus in different nerves and enters the spinal cord across multiple vertebral segments at different levels (Figure 1.6). The characteristic waveform of SSEPs, recorded from scalp electrodes positioned over the contralateral somatosensory cortex, following peripheral electrical stimulation of the median (arm) and tibial (leg) nerves (Figure 1.7), reflects an extracellular recording of a population response conducted over many large diameter afferents (Aα and Aβ) ascending in the dorsal column system (York, 1985). Motor evoked potentials (MEPs) are most commonly induced in the motor cortex using transcranial magnetic stimulation, and the accompanying compound muscle action potential (CMAP) is recorded using electromyography (EMG) at the target muscle.
Because stimulation is considered to activate the motor cortex directly, the CMAP is thought to arise from efferent impulses conveyed in the corticospinal tract. However, given the lack of specificity of magnetic stimulation, it also seems likely that other descending pathways are involved (e.g. cortico-bulbar projections which then activate brainstem-spinal pathways such as reticulospinal, rubrospinal or vestibulospinal tracts). For both SSEPs and MEPs, the time it takes from stimulation to the acquisition of the signal (i.e. latency) represents the primary variable interpreted for clinical purposes. Depending on the severity of pathology, the latency of SSEPs and/or MEPs may be delayed (delayed SSEPs illustrated in Figure 1.7) or completely abolished. The latter is almost always the case with complete SCI. Conventional SSEPs and MEPs both represent methods to detect sparing in the spinal cord after injury, and, in general, improve the prediction for long term functional outcomes (Curt and Dietz 1999).
Figure 1.6 The brachial plexus
The median (right) and ulnar (left) nerve innervate multiple muscles in the forearm and hand (highlighted in green). Upon entry into the spinal cord, the median and ulnar nerves traverse multiple segments, making it difficult to localize a level of injury using conventional mixed nerve SSEPs.

In theory, implementing electrophysiology assessments during the first year after SCI would enable a greater ability to track spontaneous recovery and possibly understand the mechanisms underlying functional changes (i.e. sprouting, remyelination and/or regeneration). The responsiveness of conventional SSEPs and MEPs during spontaneous recovery has only recently been studied in a large and statistically powerful cohort of
individuals with SCI (Curt, Van Hedel et al. 2008; Spiess, Schubert et al. 2008). In general, it seems that only minor changes in latency may occur, and only in those recordings that are severely deteriorated but that remain recordable initially. Furthermore, changes in motor activity have been characterized by the Brain Motor Control Assessment (see above for description) (Sherwood, et al., 1996; McKay, et al., 2011), which include a decrease in the time to the activation of a motor command and improved coordination of the movement.

**Figure 1.7 Conventional somatosensory evoked potentials**
Prominent N20 (median) and P40 (tibial) components of the cortical evoked potentials are marked (N – negative, P – positive). The numerical value represents the time (ms) where one would expect to mark the negative (median) or positive (tibial) peak potential based on studies in neurologically healthy subjects. In the above figure, SSEPs from an intact subject are shown in grey and SSEPs from an individual with incomplete cervical SCI are illustrated in black. In comparison to healthy subjects, SSEPs are delayed (median and tibial) and smaller amplitude (tibial) after SCI.
Based on the findings from median and ulnar SSEPs, it is difficult to localize the exact level of SCI. Electrical stimulation of specific individual dermatomes using similar EEG methodological principles and protocols as used for conventional SSEPs provides a more precise segmental neurophysiological assessment of posterior spinal cord innervation (dorsal root entry and ascending dorsal column conduction). Dermatomal SSEPs (dSSEPs) have been used for many years to diagnose lumbar radiculopathy (Dvonch, *et al.*, 1984; Katifi and Sedgwick, 1987). A dermatomal neurophysiological approach shares the advantages of the ISNCSCI and QST to record segment-by-segment sensory outcomes above, at, and below the level of lesion. As with conventional electrophysiological techniques (i.e. SSEPs), but unlike ISNCSCI or QST, an objective measure of sensory function is obtained with little interpretation by the examiner or subject, thus limiting any investigator or patient bias. In the example shown in Figure 1.8, a dSSEP with normal N1/onset latency is recorded in both an individual with SCI (i.e. above the level of the lesion) and a neurologically healthy control subject. In an individual with cervical SCI, as the stimulation site moves to examine a more caudal spinal segment (from C4 to C8), the dSSEP recordings latency is increased relative to healthy control values, revealing impaired spinal conduction at and below the level of injury (C5, incomplete). A comparable motor correlate of dSSEPs (e.g. myotomal MEPs) is not possible because current techniques do not enable the activation of individual muscles (i.e. spinal segments) using surface stimulation of the cranium overlying regions of the motor cortex.

SSEPs and dSSEPs only provide information regarding conduction in large diameter fibers that ascend in the dorsal column. Most studies that attain measures of conduction in the spinothalamic tract have used supramaximal CO₂ laser pulses to irradiate specific areas.
of skin within a dermatome affected by a small-diameter fiber neuropathy (Treede, et al., 2003; Cruccu, et al., 2008). Originally described in 1976 (Carmon, et al., 1976), the painful subjective response (i.e. first and second pain), long latency of the laser evoked potentials (LEPs), and the cortical structures activated, which include key brain regions in the pain matrix (e.g. ACC), corresponds with the known properties of conduction in the spinothalamic tract. To address the practical limitations of acquiring LEPs in a clinical setting (e.g. skin burns, safety precautions), contact heat has emerged as a modality for the neurophysiological assessment of conduction in small diameter fibers with recent technological advances that permit safe, continuous surface contact and rapid warming (70°C/s) and cooling (40°C/s) of the skin's surface up to 55°C (Chen, et al., 2001). Since being re-introduced in 2001 (i.e. the rate of contact heat stimulation using earlier devices was insufficient to elicit reliable cortical potentials(Harkins, et al., 2000), a number of studies have investigated CHEPs in neurologically healthy subjects, primarily focused on the vertex N2P2 (illustrated in Figure 1.9) (Chen, et al., 2001; Chen, et al., 2002; Le, et al., 2002; Valeriani, et al., 2002; Granovsky, et al., 2005; Chen, et al., 2006; Iannetti, et al., 2006; Atherton, et al., 2007; Chao, et al., 2007; Truini, et al., 2007; Granovsky, et al., 2008; Wydenkeller, et al., 2008; Roberts, et al., 2008a; Roberts, et al., 2008b; Warbrick, et al., 2009). The cortical structures involved in processing CHEPs are similar to those for LEPs, and include the secondary somatosensory cortex, dorsal and anterior insular cortex and anterior insular cingulate cortex (Figure 1.9, upper panel). An advantage of contact heat is the physiological relevancy of stimulation, from which large amplitude, long latency evoked potentials can be built based on a small number of stimulation repetitions (i.e. does not require the same averaging methods of SSEPs). Although LEPs have been used to assess a
variety of diseases in the spinal cord, including syringomyelia (Kakigi, et al., 1991), to date, few studies have systematically characterized the effect of traumatic SCI on CHEPs.

Figure 1.8 Dermatomal somatosensory evoked potentials
The N1 (first negative peak) increases from C4 to C6 and remains constant from C6 to C8 because of the changes in peripheral conduction distance. As in the previous figure, neurologically healthy dsSEPs from a representative subject are shown in grey and dsSEPs from an individual with incomplete SCI are shown in black. Note that in comparison to a neurologically healthy subject, the dsSEPs from an individual with cervical SCI are smaller amplitude and delayed.
Figure 1.9 Functional magnetic resonance image following contact heat stimulation and cortical evoked potential

Functional magnetic resonance image (fMRI) reveals activation of secondary somatosensory cortex, dorsal and anterior insular cortex and ACC following contact heat stimulation on the arm (C4 dermatome). The N2P2 CHEPs are similar to LEPs (vertex), but slightly delayed, owing to the longer stimulation duration, and typically smaller in amplitude. The fMRI findings are from an ongoing collaboration with members of the Spinal Cord Center at Balgrist Hospital at the University of Zurich (Dr. Armin Curt and Jenny Haefeli) and were performed in neurologically healthy subjects. The shaded grey line represents the pre-trigger.
1.3.1.4. Neuroimaging

Based on a systematic review of preclinical and clinical studies, MRI has been described as the “imaging modality of choice” for the evaluation of the injured spinal cord (Lammertse, et al., 2007a). Presumably this reflects the ability of MRI to provide high resolution images of the damage in the spinal cord compared to plain computer tomography. During the acute or sub-acute stage of SCI, objective MRI measures of parenchymal hemorrhage/contusion, edema, and spinal cord disruption (e.g. intramedullary high-signal intensity change) contribute to our understanding of severity of injury and prognosis for neurological improvement. In chronic SCI, MRI is most important to track progressive deterioration that may arise (e.g. syringomyelia). During spontaneous recovery, apparent changes in the area of damage (i.e. increased intramedullary high-signal intensity change, reduced size of the damaged area) may or may not correlate with functional improvements in conduction (Figure 1.10).

There is considerable interest in applying advanced neuroimaging techniques including diffusion tensor tractography (Vargas, et al., 2008) and fMRI of the spinal cord (Stroman, et al., 2004) to further evaluate the injured spinal cord. At present, most of these techniques remain investigational. The primary limitations of most clinical and advanced MRI techniques are the low cross-sectional resolution to detect damage in specific white matter tracts in the spinal cord, lack of information regarding specific correlations between functional deficits and anatomical damage within the spinal cord, and difficulty imaging the spinal cord near surgical instrumentation. Examining spinal cord atrophy rostral to the
lesion site may be a useful technique to address the latter problem (Lundell, et al., 2011a; Lundell, et al., 2011b).

![Image: MRI and SSEPs comparison](image)

**Figure 1.10 Magnetic resonance imaging and conventional somatosensory evoked potentials**

Changes in clinical MRI (indicated with arrows) from acute to chronic (+1 and 2 years post injury) are unaccompanied by changes in conduction in the spinal cord according to conventional (tibial) SSEPs (courtesy of Dr. Armin Curt). Red and blue have been used to trace the evoked potential recordings. Neither evoked potential (acute or chronic) are clinically delayed based on normal P40 latencies.
1.3.2. Functional

All of the outcomes described so far are focused on measuring the neurological deficits sustained after SCI. An emerging area of clinical research is to link specific neurological impairments with objective measures of functional capacity. Functional outcome measures more directly track the independent performance of ADL. Compared to neurological outcomes, changes in functional outcomes after SCI may depend on a variety of other factors, including the degree to which an individual can perform tasks by employing compensatory strategies (e.g. use of assistive devices, see Table 1.3). In addition, an improvement in a valid functional outcome measure is an emerging requirement for demonstrating a minimal clinically important difference (MCID) in a phase III clinical trial (described below), which is necessary to demonstrate efficacy of an experimental therapeutic and receive approval for marketing by a regulatory health agency.

The relevance of functional outcomes to individuals with SCI depends on the injury characteristics or specific disabilities of those being evaluated. For example, the Walking Index for Spinal Cord Injury (WISCI), developed by a panel of SCI experts including Dr. John Ditunno (Ditunno, Jr., et al., 2000b), which takes into account the distance walked as well as the amount and type of assistance required for walking, is only useful to classify individuals with some ability to ambulate. The Graded Redefined Assessment of Strength, Sensibility and Prehension (GRASSP), a recently proposed measure of upper limb function that considers both how a movement is preformed (i.e. is the correct movement strategy employed) in addition to if the task could be completed (Kalsi-Ryan, et al., 2011), would only be useful to classify individuals with some upper limb impairment.
The Spinal Cord Independence Measure (SCIM), now in its 3\textsuperscript{rd} revision, was developed as a SCI specific functional independence measure – a universally adopted measure of independence (Catz, et al., 2007). The SCIM is an assessment scale that monitors a broad range of “global” capacities for both individuals with tetraplegia and paraplegia. The activities measured by SCIM are divided into the self-care, respiration/sphincter management, mobility in the room, and mobility in/outdoors subscales. The SCIM is an aggregate measure of function based on the direct observations of patients’ performance completing activities of daily living (e.g. dressing and grooming), or self-report where direct observation is not practical (e.g. bowel habits) (Catz, et al., 2007; Bluvshtein, et al., 2011). In general, the SCIM has been generally found to have high intra- and inter-rater reliability.

The total SCIM score has been validated as a measure of functional capacities in several studies by independent researchers. In general, lower SCIM scores are associated with more severe (e.g. motor complete) and higher level injuries (e.g. tetraplegia) (van Hedel and Curt, 2006). The rostro-caudal extent of SCI is more important for individuals with tetraplegia as loss of arm and hand function limits independence and integration within the community. Conversely, individuals with paraplegia have preserved arm and hand function and thus have retained more opportunity for an independent lifestyle; improvements in SCIM total score or of specific sub-scores are less likely to have an effect on overall independent activities of daily living. With regards to cervical level injury, the SCIM self-care sub-score is closely related to neurological and other functional outcomes (i.e. GRASSP) (Kalsi-Ryan, et al., 2011). During spontaneous recovery in the first 6 months after injury, increased SCIM scores generally accompany neurological improvements. The
development of compensatory movements (e.g. learning to adapt to disability, new movement strategies) may also result in greater independence and confound the relationship between SCIM and neurological outcomes (Curt, et al., 2008).

1.4. Clinical trials: Translating preclinical efficacy to human spinal cord injury

1.4.1. Phases of clinical trials

There are a number of different types of clinical trials to consider, each of which has a distinct goal:

1. Diagnostic trials are conducted to find better tests for diagnosing a particular disease or disorder and are usually conducted with people showing symptoms of that condition. For example, if researchers wanted to compare the diagnostic performance of fMRI for a particular disease, they would conduct a diagnostic trial.

2. Prevention trials look for ways to prevent illness or injury in people who have never had the disease and the approaches may include: vaccines, vitamins, exercise, or lifestyle changes.

3. QoL trials explore ways to improve access, opportunity and integration within the community for individuals with a chronic illness or disability.

4. Screening trials test the best way to detect certain diseases or health conditions. A screening trial may complement or overlap with a diagnostic trial.

5. Treatment trials test experimental interventions designed to improve outcomes for patients whether the therapeutic is a drug, cell transplant, surgical procedure, assistive device, or rehabilitation strategy.
Each phase of a clinical trial program also has distinct goals and thus different parameters, protocols, outcome measures, and endpoints that are ultimately important to consider. The phases of clinical trials include:

- **Phase I** trials are centered on the initial exploration of safety, and in the case of a drug or cell transplant often include an evaluation of the responses to different therapeutic doses. Of course, safety is continuously monitored throughout all subsequent trial phases. Phase 1 trials will sometimes attempt to collect pilot data on functional outcomes, primarily to justify continuation, particularly funding, for the trial program.

- **Phase II** trials are still exploratory with focus on the preliminary demonstration of functional biological activity and/or functional benefit of the intervention. They will usually measure a number of different biological, clinical or functional outcomes to determine which endpoint most reliably measure a clinically meaningful outcome in a sensitive and accurate manner.

- **Phase III** trials are the pivotal confirmatory studies where an intervention must demonstrate benefit in a clinically meaningful manner, which is then weighed against the associated risks before it can be approved by the relevant regulatory body.

After regulatory approval and adoption of the intervention as a standard of clinical practice, most interventions enter a surveillance period where the greatly increased exposure of a more heterogeneous array of patients allows potential detection of less frequent adverse events and may provide additional information on efficacy. At this stage,
it is also possible to perform **Phase IV** or post-market use studies that continue to examine additional questions of efficacy, interactions with other treatments, risks, optimal treatment approaches, and safety in a more controlled way. Early phase studies may also be combined, largely for the purpose of identifying the potential therapeutic efficacy/biological activity while demonstrating safety (i.e. phase I/IIa). Despite convincing evidence of therapeutic effects in animal models, few treatments have shown preliminary efficacy in early phase studies (I/IIa), and none aimed at ameliorating the neurological and/or functional deficits associated with traumatic SCI in humans have succeeded in a pivotal phase III RCT (Hawryluk, *et al.*, 2008).

**1.4.2. Spinal cord injury clinical trials: Difficulties translating preclinical findings**

Although it is difficult to determine the exact cause of failure, there are a number of possibilities and combinations of possibilities why preclinical findings have yet to be translated in humans. Firstly, the lack of effect in humans may be attributable to the absence of biological activity of the therapeutic. This may arise from problems with determining the correct concentration/dose and/or differences between animal models and human SCI. Incorrect dosing was cited as a cause for failure in the first methylprednisone study and led to the second North American Spinal Cord Injury Study (Braughler and Hall, 1984; Bracken and Holford, 2002). There are a number of differences between animal models (e.g. rodents) and human SCI to consider, perhaps none more obvious than those differences related to quadruped versus bipedal locomotion (Dietz and Curt, 2006). As an example of a more subtle but important difference, the impactor devices
that have allowed researchers to re-create the injured spinal cord in rodents in a time frame comparable to most traumatic human spinal injuries (<10 ms) cannot recreate the mechanical damage that may simultaneously compress, dislocate and distract the spinal cord along the translational axes. The importance of primary injury mechanism has been demonstrated even within animal models, with the type and location of trauma (i.e. dorsomedial versus dorsolateral) ultimately determining the efficacy of a preclinical treatment (Popovich, et al., 2010).

In contrast to animal models, human SCI is also much more heterogeneous. Depending on the initial level (para- versus tetraplegia) and severity (complete versus incomplete) of injury, the trajectory of spontaneous recovery may vary considerably according to neurological and functional outcomes described above. Unfortunately this presents a major problem, one that is difficult to overcome for SCI researchers and clinicians; on one hand, traumatic SCI is rather infrequent in the general population (see epidemiology above), which necessitates broad inclusion criteria to complete a study in a reasonable time-frame and cost-effective fashion, but on the other, the inclusion of some subjects may make it more difficult to detect a statistical and clinical meaningful benefit. This “conundrum” was recently discussed with regards to the phase II autologous macrophage study, for which over 1800 subjects were prescreened and 50 subjects (or 3%, “funnel effect”) were randomized to the control or treatment group (Jones, et al., 2010). The alternative problem, the inclusion of “all-comers”, is perhaps more limiting to the success of a clinical trial, and there is an anatomical rationale for why some therapies might only work in a particular subset of individuals. For example, a therapeutic that proposes to remyelinate spared demyelinated axons (e.g. 4-Aminopyridine) (Wolfe, et al., 2001) would
presumably require that some axons were spared by the injury. Therefore, the inclusion of individuals with transected spinal cords or individuals with no evidence of tissue sparing (i.e. clinically complete) would likely be unwise.

Although their results are frequently interpreted for the purposes of informing whether or not a pivotal phase III RCT should be undertaken, early phases of clinical study often rely on historical controls (i.e. natural progression of SCI), and are therefore not appropriately designed (e.g. uncontrolled, nonrandomized, and unblinded) to objectively measure efficacy. The hazards of small, uncontrolled early phase studies can be illustrated by the phase I autologous macrophage study, which reported a 30% conversion rate from sensorimotor complete (AIS-A) to sensory and motor incomplete (3/10 subjects converted to AIS-C, which based on the natural history is 2 to 3-fold higher than would be expected spontaneously, Knoller, et al., 2005; Fawcett, et al., 2007) and that recently failed to find efficacy in a larger RCT (personal communication, Dr. Daniel Lammertse). Furthermore, early phases of study (I, II, or I/IIa) are rarely powered (i.e. of sufficient sample size) to measure statistical significance. This may have been the cause of failure for Sygen (i.e. GM-1 ganglioside, Geisler, et al., 2001b), which was originally undertaken based on evidence of a significant treatment effect in a randomized and placebo-controlled phase II study that enrolled only 37 patients (Geisler, et al., 1991). Despite such a limited capacity to detect a treatment effect, owing in part to study design and underpowered statistics, it is clear that future phase I and II studies will continue to attempt to assess preliminary efficacy (e.g. phase I/IIa recombinant Rho protein antagonist or Cethrin, Fehlings, et al., 2011). Therefore, at this point in the clinical trial program understanding variables that affect
spontaneous neurological recovery are of the utmost importance to avoid making erroneous conclusions.

The problems translating preclinical findings to humans discussed so far primarily concern the limitations of animal models, the heterogeneity of human SCI, and poor study design. Potential solutions to these problems are to develop better models of SCI in larger animals or primates in order to increase the relevance of preclinical findings (Kwon, et al., 2010a), improve the classification of SCI (e.g. biomarkers in the CSF, Kwon, et al., 2010b), and invest more time addressing preliminary efficacy and biological activity in well designed, early phase studies. However, there is another possible explanation as to why preclinical findings have been so difficult to translate from the bench to the bedside: clinical outcomes employed to determine the efficacy of a SCI treatment are NOT sensitive enough to detect subtle therapeutic and functionally meaningful benefits. A challenge for all clinical studies of SCI is the ability to accurately, sensitively and reliably measure a change over time in the subject’s neurological status, their functional capability and/or their performance in an ADL (Steeves, et al., 2007).

Regardless of benefits reported in preclinical studies, the estimated effect size of early treatments translated to humans is expected to be small (Houle and Tessler, 2003). For example, a neuroprotective treatment that limits the spread of secondary damage (e.g. methylprednisone) may indeed spare tissue, but it will not ameliorate all sensorimotor deficits, which remain due to the primary mechanism of injury. Undoubtedly, in order to “cure” SCI (i.e. complete recovery with no neurological deficits), a combination of treatments and effective rehabilitation strategies will need to be employed (Ruff, et al.,...
Therefore, in order to avoid falsely concluding that a single treatment is not effective, outcomes must be employed in clinical trials that can measure subtle changes in sensorimotor function in an accurate, sensitive and reliable manner.

To date, most phases (I, II, and III) of acute SCI clinical trials have adopted changes in AIS grade or changes in total motor score as their primary endpoints (Furlan, et al., 2008; Hawryluk, et al., 2008), including very recent neuroprotection trials (Fehlings, et al., 2011). The ISNCSCI is a common diagnostic tool used throughout SCI clinical research, and, as a consequence, it represents a tool that most SCI clinicians are both familiar and comfortable using. Both AIS grade and total motor score constitute “global” measures of neurological function, and, in light of failed phase II and III clinical trials, have both been recently criticized for their lack of sensitivity (Steeves, et al., 2007; Furlan, et al., 2008). In the case of AIS-A sensorimotor complete individuals, whom are likely candidates to be recruited to an early safety and preliminary efficacy trials (phase I/IIA) because they stand to potentially benefit the most from a therapeutic treatment (i.e. spontaneously recover the least and are left with the greatest degree of disability and neurological impairment), conversion requires that a change occur across the lesion site. Given the obstacles of regeneration and repair through the injury epicenter (e.g. myelin inhibitors, glial scar), measuring differences in the number of individuals converting from sensorimotor complete (AIS-A) to incomplete injuries (AIS-C or D as was proposed by the Sygen study) (Geisler, et al., 2001b) in a treatment and control group as a primary endpoint is now considered overly ambitious. Moreover, other functionally meaningful and significant changes, particularly those in the spinal segments adjacent to the level of injury, could ultimately be missed by such a measure. Using conventional neurophysiological
approaches (SSEPs and MEPs), proposed as an outcome in a phase II study to determine biological activity of a therapeutic (e.g. remyelination), one faces a similar problem in that these techniques would only reflect changes in spinal conduction through the injury epicenter (dorsal columns and corticospinal tract, respectively).

1.5. Thesis objective

Therefore, the primary objective of this doctoral dissertation was to investigate segmental sensory and motor outcomes to predict and track neurological recovery after cervical traumatic SCI. Five original research studies (Chapters Two to Six) were undertaken between September 2007 and May 2011. The objective of the first study reported in Chapter Two was two-fold: 1) describe patterns of sensory function after cervical SCI in individual spinal segments (lower cervical, thoracic, and lumbosacral), and 2) determine whether emerging patterns of sensory preservation predicted spontaneous neurological recovery. A case report and data from the European Multi-Center Study about SCI (EM-SCI) was reviewed. A study follows to introduce a segmental neurophysiological approach to investigate the injured spinal cord, where dSSEPs were examined in individuals with SCI during spontaneous neurological recovery (Chapter Three). While dSSEPs represent an established method to examine conduction in the dorsal columns, study was warranted to investigate the validity of CHEPs to assess conduction in the spinothalamic tract (Chapter Four). The test-retest reliability of cervical CHEPs was examined in neurologically healthy subjects and individuals with cervical SCI. Based on the experience gained in this study, novel techniques were developed to improve the acquisition of CHEPs in individuals with SCI (Chapter Five). At this point, the segmental
evaluation of cervical spinal cord segments transitions to the assessment of muscle strength in Chapter Six for the purpose of examining motor function. Motor recovery in the upper extremities was examined during spontaneous recovery, with an emphasis on motor level changes and describing the relationship with functional outcomes (i.e. SCIM).

1.6. Thesis outline

At the beginning of each research chapter, the primary motivation for performing the study is stated. Where necessary and appropriate, the rationale linking the chapters is provided. The methods used in each chapter are described, but, where possible, previous chapters are referred to in order to avoid repetition. The five original research chapters are followed by a general discussion.
2. Discontinuous segmental afferent sparing after spinal cord injury is not limited to sacral segments

The motivation for Chapter Two came from what were originally considered “anomalous” sensory testing observations borne out of an ISNCSCI measurement error. Specifically, a number of cases (one case report described below) were observed where sensation was absent in lower cervical dermatomes (e.g. C8) but preserved in upper thoracic dermatomes (e.g. T1-T4) (personal communication, Dr. Armin Curt). This was termed “discontinuous sensory preservation” and had not been previously described to our knowledge outside of being reported in sacral segments (e.g. S4S5). Therefore, the primary aim of this chapter was to examine other patterns of sensory preservation in individual dermatomes caudal to the level of cervical SCI and provide an anatomical and physiological rationale for these patterns. The principal finding was that impaired/normal sensation in upper thoracic dermatomes in the absence of sensation in lower cervical dermatomes does reflect anatomical sparing and is meaningful for predicting spontaneous sensorimotor recovery. The physiological and anatomical rationale for this type of afferent sparing in the upper thoracic segments is discussed.

2.1. Introduction

Detecting residual tissue sparing after SCI is important for accurately predicting long-term functional outcomes (Holdsworth, 1970; Stauffer, 1975; Folman and el, 1989; Waters, et al., 1991; Katoh and El Masry, 1995; Weiss, et al., 1996; Curt and Dietz, 1997; Curt, et al., 1997; Curt, et al., 1998; Curt and Dietz, 1999; Oleson, et al., 2005; van
Middendorp, et al., 2011). Owing to the topographical organization of the spinal cord (Smith and Deacon, 1984; Zhang, et al., 2000) and the neuropathology of traumatic SCI (Stauffer, 1975; Kakulas, 1984; Hayes and Kakulas, 1997), sacral afferents in the dorsal columns and spinothalamic tract are ideally positioned in subpial white matter rim for afferent sparing (illustrated in Figure 2.1). For this reason, the determination of clinically “complete SCI” has been made on the basis of absent sensation in the most sacral segments (S4S5) (i.e. evidence that the area affected by SCI involves the entire spinal cord, even the most peripherally located white matter tracts, and is therefore, complete). Also due to the topographical organization of the major ascending pathways and the neuropathology of SCI, sacral sparing (i.e. normal or impaired) may emerge in the absence of sensation in more rostral cervical, thoracic, and lumbar afferents (Stauffer, 1975; Katoh and El Masry, 1995). Little is known regarding “discontinuous patterns of sensory preservation” (i.e. preserved sensation in a spinal segment caudal to the complete loss of sensation in a more rostral spinal segment, illustrated in Figure 2.2) emerging after SCI in other spinal segments, and whether these too may be attributable to the neuroanatomy and pathology of SCI.
Figure 2.1 Neuropathology of spinal cord injury
Depending on the severity of SCI, the lesion area expands out from the center of the spinal cord, ultimately disrupting sacral segments when the injury is most severe (Kakulas, 1984). Lower cervical and upper thoracic afferents are considered “highly intermingled” in the dorsal columns (Smith and Deacon, 1984).
Figure 2.2 Discontinuous sensory preservation in sacral segments

Sensation in the segments immediately adjacent to the level of injury may also be preserved in the zone of partial preservation (ZPP). Impaired or normal sensation in the sacral segments (light green shading) caudal to complete loss of sensation (grey shading) may also emerge after traumatic SCI on account of the topographical organization of the ascending pathways and the neuropathology of traumatic SCI.

Based on a clinical observation (described below), the present study aimed to address the hypothesis that discontinuous patterns of sensory preservation may not be restricted to sacral segments, but rather can also be observed at the transition between lower cervical and upper thoracic segments. The functional significance of discontinuous sensory preservation in upper thoracic dermatomes was examined by determining the extent of spontaneous sensorimotor recovery over the first year after SCI in a representative sample of individuals with cervical SCI.
2.2. Case report

A 65-year-old male subject sustained a C4, clinically incomplete SCI after a fall two years prior to our examination. The outcomes from the clinical pinprick testing according to the ISNCSCI (methods described below) and neurophysiological investigation (contact heat evoked potentials, see Chapter Four and Five for a detailed description of methodology) are illustrated in Figure 2.3. Pinprick sensation was absent in C8 but preserved in the more caudal T2 and T10 dermatomes. The clinical MRI of this subject demonstrated a high intramedullary signal intensity change between C4 and C5 vertebral segments, corresponding with the clinical level of traumatic SCI. The subject perceived contact heat stimulation and CHEPs could be recorded from C4 (at the sensory level of SCI) and T2 (below the level of injury). The prominent N2P2 waveforms recorded following contact heat stimulation are highlighted. In contrast to C4 and T2, C8 perception to contact heat stimulation applied with the same temperature parameters was not perceived and an evoked potential could not be recorded (baseline = 35°C and peak temperature = 54°C). Therefore, the neurophysiological findings were in agreement with the clinical pinprick testing results. However, further investigation of this individual’s C8 dermatome did reveal evidence of afferent sparing (i.e. a CHEP could be recorded from C8 and the subject reported perceiving the contact heat stimulation) when a more intense stimuli was applied then what was employed for C4 and T2 (see Chapter Five for methodology describing how the intensity of stimulation was increased).
**2.3. Methods**

### 2.3.1. European Multi-Center Study about Spinal Cord Injury database

Individuals in this ongoing prospective study (started 2001; current enrollment = 1890) have sustained a traumatic SCI and are being recruited from 18 European centers for the observation of neurological (i.e. ISNCSCI) and functional (e.g. WISCI, SCIM) outcomes during the first year after SCI, while receiving current standards of clinical care. Participants undergo an initial examination during the very acute period (~1 week) after SCI, with follow-up assessments targeted at 4, 12, 24, and 48 weeks after SCI. Other findings from this database have been previously published (van Hedel and Curt, 2006; Curt, *et al.*, 2008; Spiess, *et al.*, 2008; Wirth, *et al.*, 2008b; Jakob, *et al.*, 2009; Spiess, *et al.*, 2008).
2009; van Hedel, 2009; van Hedel and Dietz, 2009). Trained examiners with at least one year of experience perform the ISNCSCI evaluations. The purpose of this ongoing collection of data is to evaluate the normal course of spontaneous neurological recovery (Curt, et al., 2008). For additional information, see www.emsci.org.

2.3.2. Light touch and pinprick testing

Briefly, the patient’s perception to light touch and pinprick stimuli is scored on a three-point scale (normal, impaired, or absent), based on a comparison to their perception of “normal” sensation from the face (i.e. trigeminal innervation unaffected by SCI). Light touch sensation is tested with a tapered wisp of cotton stroked once across the key sensory point (~1 cm). Pinprick testing is performed with a safety pin, applying the pointed end for a sharp stimulus and the rounded end as a dull stimulus. In order to be considered normal, the patient must correctly discriminate between sharp and dull stimuli. Pinprick sensation is scored as impaired in cases where the patient can accurately discriminate sharp and dull stimuli, but perceives sensation as being less intense compared to the face. For both light touch and pinprick, testing is performed with eyes closed. The sensory level, as defined by ISNCSCI, is the most caudal spinal segment with normal sensory perception (2/2, pinprick and light touch), with all rostral cord segments also being normal. Light touch and pinprick scores were assessed using the key sensory stimulation points within the respective dermatome, as outlined by ISNCSCI (shown in Figure 2.4) (Ditunno, Jr., et al., 1994).
2.3.3. Inclusion/exclusion criteria

Patterns of sensory preservation after SCI were examined by analyzing the ISNCSCI light touch and pinprick data from individuals with cervical SCI. Individuals with C4 or C5 SCI (sensorimotor complete and incomplete, AIS-A – D) according to sensory level (defined above) at one-week after injury were specifically included. These levels of tetraplegia were included to match the general SCI characteristics of the case study and evaluate sensory preservation in as many cervical dermatomes as possible before the transition to upper thoracic dermatomes. While C2 and C3 injuries would also be appropriate to examine lower cervical and upper thoracic sensation, such levels of injury are generally rare. Individuals with a C6, C7, or C8 level of SCI would, by definition, have at least some sensory preservation within the caudal cervical cord, and were therefore excluded.

2.3.4. Statistical analysis

For the descriptive analysis of sensory patterns, light touch and pinprick sensation was classified as being either “preserved” (i.e. normal or impaired) or “absent”. This dichotomous classification of sensation was used to derive the proportion of individuals with preserved or normal light touch and/or pinprick sensation in each dermatome at one week after SCI, without making any judgment as to the quality of the sensation (i.e. impaired or normal). A descriptive analysis was also performed on the proportion of individuals with normal sensation.

To investigate the potential functional significance of the discontinuous patterns of segmental sensory preservation, spontaneous motor recovery (total motor score) and AIS
grade conversion were examined (AIS grades are described in the General Introduction, see Table 1.4). Motor recovery was examined between 1 and 24 weeks. Motor score changes in two groups, differentiated on the basis of the initial pattern of sensory preservation (one week after SCI), was analyzed using an independent sample t-test (p<0.05). 24 weeks was selected rather than the 48 week time-point to measure motor recovery because a greater number of individuals were available for analysis (higher drop-out at 48 weeks, presumably related to the time of discharge from the hospital). Sensorimotor complete AIS-grade conversion was examined at 4, 12, and 24 weeks after SCI. The odds of spontaneous AIS-grade conversion based on patterns of sensory preservation were statistically examined using logistical regression (p<0.05). All statistical procedures were performed in SPSS.
Figure 2.4 Dermatomal boundaries of the International Standards for the Neurological Classification of Spinal Cord Injury
The distance of stimulation site from the midline (0cm) is shown for one male subject (178cm tall).
2.4. Results

2.4.1. Subject characteristics

280 individuals with C4/C5 cervical SCI at one week after injury in the EM-SCI database were analyzed. At the time of their cervical injury, the mean age was 46.9±18.6 years. 73% of individuals were male.

2.4.2. Patterns of sensory preservation one week after cervical spinal cord injury

The proportion of individuals with preserved (impaired or normal) pinprick sensation in dermatomes caudal to C4/C5 cervical SCI at one-week post injury is illustrated in Figure 2.5. The number of individuals with preservation of light touch and pinprick sensation gradually decreased from C6 to C8 (i.e. increasing caudal distance from the level of injury, 81% to 66% and 65% to 50% for light touch and pinprick, respectively). In the same group of individuals with C4/C5 SCI, the frequencies of light touch and pinprick sensation increased in upper thoracic dermatomes (peak values of 72% in T3 and 60% in T2, respectively). Approximately one-third of individuals with absent sensation in C8 had preserved light touch (n=33/96, 34%) and/or pinprick sensation (n=50/142, 35%) in one of T1, T2, T3, or T4 segments. 15 of the 33 individuals with light touch preservation in upper thoracic dermatomes but not in lower cervical dermatomes were clinically complete injuries (AIS-A). 14 of the 50 individuals that demonstrated discontinuous sensory preservation in the upper thoracic dermatomes according to pinprick were clinically complete (AIS-A).
Caudal to T4, sensory preservation decreased for each caudal spinal segment (T4 - L2). As expected based on the topography of the spinal cord, a higher proportion of individuals had preserved light touch and pinprick sensation in the sacral segments compared to more rostral lumbar segments. Absent light touch or pinprick sensation in S1 (n=122 or 172, light touch and pinprick, respectively) coupled with preserved sensation in more caudal spinal segments (at least one of S2, S3, or S4S5) was observed in 9% (light touch) and 11% (pinprick) of individuals with C4/C5 SCI.

Figure 2.5 Preserved pinprick sensation
The proportion (%) of C4/C5 individuals with pinprick preservation (impaired or normal) in cervical (C6-C8), thoracic (T1-T12), and lumbosacral (L1-S4S5) dermatomes one week after cervical (C4 or C5) SCI (n=280). Light touch sensation (not shown) demonstrated the same pattern.
If we examined the proportion of the individuals with C4/C5 cervical SCI that had normal (intact) sensation in dermatomes caudal to the level of injury (normal light touch or pinprick sensation), rather than any preserved sensation (impaired or normal), the emerging pattern of sensory preservation was very similar (Figure 2.6). Normal sensation (light touch and pinprick) was more frequently observed in upper thoracic and lower sacral spinal segments relative to more rostral spinal segments (C8 and L4-S1, respectively).

Figure 2.6 Normal pinprick sensation
The proportion (%) of C4/C5 individuals with normal pinprick preservation in cervical (C6-C8), thoracic (T1-T12), and lumbosacral (L1-S4SS) dermatomes one week after cervical (C4 or C5) SCI (n=280).
2.4.3. Motor recovery and grade conversion after cervical spinal cord injury: Patterns of sensory preservation

To investigate if there was any functional significance of sensory preservation from upper thoracic dermatomes when there was no sensation preserved from lower cervical dermatomes, the change in total motor score and AIS grade conversion was examined over the first year after cervical SCI. Specifically, we used the following two initial patterns of pinprick sensation for the subsequent analysis of motor recovery and AIS grade conversion: 1) initial assessment (one week after SCI) showed no pinprick sensation from C8 – T12 dermatomes (pattern 1 – continuous, Figure 2.7), or 2) pinprick sensation initially preserved (one week after SCI) in at least one of T1, T2, T3 or T4 dermatomes but absent in C8 and absent between T5 – T12 (pattern 2 – discontinuous, Figure 2.7).
Figure 2.7 Patterns of pinprick preservation
Illustration of 1) continuous and 2) discontinuous patterns of sensory preservation after cervical spinal cord injury (one week post injury). In the case of continuous sensation, once light touch or pinprick sensation becomes absent, it does not reemerge in more caudal dermatomes. Dark shading represents dermatomes with absent sensation.

The mean change in total motor score between 1 and 24 weeks post injury was significantly different depending on the pattern of pinprick preservation (pattern 1, n=72, mean Δ=14.6; pattern 2, n=21, mean Δ=31.0 motor points; t=3.2, p=0.002, Figure 2.8). Only at 4 weeks after sensorimotor complete cervical SCI were the odds of spontaneous AIS-grade conversion significantly different based on the initial pattern of sensory preservation. 45% (5/11) of individuals with the discontinuous sensory preservation in the upper thoracic segments (pattern 2 – discontinuous) spontaneously converted at least one AIS grade by 4 weeks (B=3, C=1, D=1). All these individuals were initially sensorimotor complete (AIS-A) at one week after SCI (i.e. no sensation in S4-S5, no voluntary anal contraction). In comparison, only 14% (8/58) of cervical AIS-A (i.e. sensorimotor
complete) individuals with no (i.e. absent) C8 or T1-T4 sensation (pattern 1 – continuous) spontaneously converted an AIS grade by 4 weeks (B=5, C=2, D=1). According to the logistical regression analysis, an individual with no preservation of C8 or upper thoracic sensation (pattern 1 – continuous) was 5.2 times (p=0.021) more likely to remain AIS-A sensorimotor complete compared to an individual with discontinuous sensory preservation within the upper thoracic segments (pattern 2).

![Figure 2.8 Spontaneous motor score recovery](image)

**Figure 2.8 Spontaneous motor score recovery**
Motor recovery between one and twenty-four weeks after cervical spinal cord injury based on different patterns of pinprick preservation at one week (n=72 and n=21 for patterns 1 and 2, respectively). X-axis represents the different patterns of pinprick preservation. Error bars, 95% confidence intervals. *, p<0.05

### 2.5. Discussion

Based on our analysis individuals with C4/C5 SCI in the EM-SCI database, discontinuous afferent sparing is not limited to sacral spinal segments. In fact, discontinuous sensory preservation was even more frequently observed at the transition of lower cervical (C6-C8) and upper thoracic segments (T1-T4) than in sacral segments.
Similar to those studies which have investigated sacral sparing (Folman and el, 1989; Waters, et al., 1991; Katoh and El Masry, 1995; Weiss, et al., 1996; Curt and Dietz, 1997; Curt, et al., 1997; Curt, et al., 1998; Curt and Dietz, 1999; Oleson, et al., 2005; van Middendorp, et al., 2011), discontinuous preservation of sensation in upper thoracic dermatomes was shown to be functionally meaningful with regards to motor recovery and AIS grade conversion over the first year after cervical injury. Interestingly, the case study indicates that afferent sparing may actually be present in C8 among those individuals where clinical pinprick testing is absent, but that a standardized, higher intensity stimulus is required for this to be detected (e.g. CHEPs).

2.5.1. Anatomical afferent sparing

In contrast to discontinuous sacral sparing (i.e. preserved S4S5 sensation in the absence of sensation in more rostral segments), it seems rather unlikely that “highly intermingled” lower cervical and upper thoracic afferents could be selectively spared in the dorsal column by the primary or secondary sequelae of SCI (Kakulas, 1984; Smith and Deacon, 1984; Hayes and Kakulas, 1997). Less detail regarding the topographical organization of the spinothalamic tract in humans is available. Generally speaking, more rostral segments are thought to project medially to more sacral afferents. Therefore, discontinuous pinprick sensation in the sacral as well as in the upper thoracic segments, which would consequently be more lateral than cervical afferents, could be a function of the neuropathology of SCI sparing these more lateral afferents. However, discontinuous pinprick sensation in the upper thoracic dermatomes was observed in individuals with no sensation in the lower extremities (i.e. individuals with sensorimotor complete SCI). In
these cases (n=14), it is difficult to understand how the neuropathology of traumatic SCI would preserve cervical and sacral afferents but spare upper thoracic afferents (i.e. wedged between cervical and sacral spinal segments). Small diameter afferents ascending in the tract of Lissauer, also potentially spared in the rim of spared white matter, may also account for patterns of discontinuous sensory preservation close to the level of lesion. However, in primates the primary projections of the tract of Lissauer ascend rostrally only one segment (LaMotte, 1977). In order to account of upper thoracic preservation after C4/C5 SCI, afferents would be required to ascend at least five to six segments (T2/T2).

### 2.5.2. Dermatomal overlap

In Foerster’s landmark study in humans that outlined dermatomal boundaries, on which the ISNCSCI is based (Ditunno, 2010), he reported considerable overlap between upper cervical and upper thoracic dermatomes (Foerster, 1933). Therefore, it could be argued that dermatomal overlap between T1 and C5 or T2/T3 and C4 led an examiner to inadvertently test a more rostral cervical segment to no fault of the examiner, and consequently to report that upper thoracic dermatomes were preserved in the absence of lower cervical dermatomes. Although, we were careful in performing the neurophysiological examination (independent of EM-SCI), the same testing error could also have led to the neurophysiological outcomes in the case report presented above. However, if discontinuous preservation of upper thoracic afferents were a measurement artifact, such a pattern would not be expected to have an impact on spontaneous recovery. Indeed, we noted a significantly greater number of motor points recovered between 1 and 24 weeks after cervical SCI in those individuals having preserved upper thoracic sensation.
(Figure 2.8, p<0.05), and, when we examined AIS grade conversion in a subset of individuals (i.e. sensorimotor complete at one week, AIS-A), discontinuous upper thoracic sensory preservation resulted in a higher proportion spontaneously converting to incomplete (AIS-B or better at 4 week after SCI) compared to a group with no upper thoracic sensation. Therefore, we conclude that sensory preservation from upper thoracic dermatomes when there is no preserved sensation in the lower cervical segments (e.g. C8) is not the result of an inaccurate assessment (e.g. dermatomal overlap), but is a genuine reflection of residual tissue sparing in the ascending pathways from these spinal segments.

2.5.3. All dermatomes are not created equal

To perform the sensory examination of the ISNCSCI, all dermatomes, whether they innervate cervical, thoracic, or lumbosacral spinal cord segments, are tested using similar manually applied stimuli. Based on a comparison with an unaffected dermatome rostral to the level of injury (e.g. face), sensation is graded as normal, impaired, or absent by the patient. Therefore, the ISNCSCI operates on an assumption that all dermatomes share a similar capacity to detect afferent input with the same general level of sensitivity. This may not be a valid assumption. Firstly, various QST parameters have been shown to vary significantly by peripheral stimulation site in neurologically intact subjects (described below) and secondly, subtle differences in the perception of light touch or pinprick inputs across different dermatomes may be unmasked after damage to the spinal cord.

Of the variables that affect sensory perception, including skin type and thickness, changes in the peripheral conduction distance may be the most difficult to control while performing a clinical neurological exam. Except for the first thoracic dermatomes (T1 and
T2), all other thoracic dermatomes are positioned relatively equidistant and close to the midline (T3 to T12, Figure 2.4). However, this is not the case for dermatomes within the upper and lower extremities, which are located at varying distances from their relevant spinal cord segments in order to avoid dermatomal overlap (Figure 2.4). More distal sites have been reported to have decreased sensitivity for pinprick sensation (Agostino, et al., 2000; Truini, et al., 2005) and, to a lesser extent light touch sensation (lower limbs only) (Halar, et al., 1987). One study also found a significant cranial to caudal relationship for touch sensation along the midline (Haanpaa, et al., 1999). In a comprehensive series of studies by Rolke and colleagues (Rolke, et al., 2006a; Rolke, et al., 2006b), the sensation from the face (i.e. proximal site) was compared to sensory perception from the hands and feet (i.e. distal), and the face was shown to have a lower threshold for the perception of touch and pain stimuli.

Two mechanisms have been proposed to contribute to the reduced sensitivity of sensory input from dermatomes located at a greater distance from the spinal cord: 1) a graded decrease (proximal to distal) in the density of cutaneous afferent receptors (McArthur, et al., 1998), and 2) greater temporal dispersion of the afferent volley (Cruccu, et al., 1999; Truini, et al., 2005). A higher density of afferent receptors in a proximal dermatome would mediate greater transduction of sensory stimuli at the stimulation site, whereas a shorter conduction distance synchronizes the afferent volley. In neurologically healthy subjects the same stimulus would then be perceived as being more intense when applied to a proximal versus distal dermatome. This is the case for LEPs and CHEPs, which yield larger amplitude cortical responses following proximal compared to distal
stimulation at the same intensity in neurologically healthy subjects (Truini, *et al.*, 2005) (also see *Chapter Four* and *Five*).

The net effect of a proximal-distal sensitivity gradient and uniformly applying afferent stimuli on sites caudal to a SCI may be that dermatomes positioned closer to the midline are biased towards preserved sensation (impaired or normal), whereas distal dermatomes are biased towards absent sensation. This would explain why discontinuous sensory preservation was prominently observed at the transition between lower cervical segments examined on distal stimulation sites and upper thoracic segments examined on proximal stimulation sites (Figure 2.4). Our findings would also suggest that this phenomenon occurs more frequently for pinprick (50/280 compared to 33/280 for light touch), coinciding with more robust evidence that these differences exist for protopathic stimuli. Finally, in the case report, a more intense sensory stimulus enabled the subject to perceive the contact heat stimulus on the C8 dermatome. This suggests that applying a higher intensity stimulus in distal dermatomes may be necessary to detect afferent sparing in C8 for some individuals with discontinuous upper thoracic sensation.

### 2.5.4. Study strengths and limitations

After cervical SCI, sensory preservation within the upper thoracic segments but no preservation of sensation within the caudal cervical segments may cause an examiner to think the assessment is highly anomalous or erroneous. The only way to convincingly examine such a specific phenomenon is through the use of a large dataset (i.e. EM-SCI). A large sample size was perhaps most important for determining if discontinuous upper
thoracic sensory preservation after SCI was functionally meaningful in terms of spontaneous motor recovery or AIS grade conversion.

The primary limitation of our study is that our conclusions are based primarily on clinical sensory testing and supported by the neurophysiological findings from only a single case. To further elucidate the mechanisms underlying discontinuous sensory preservation, ruling out peripheral nerve impairment in lower cervical segments would also be important. In particular, brachial plexus injuries after cervical SCI, specifically those that affect the ulnar nerve and the C8 distribution, may also contribute to the appearance of discontinuous upper thoracic sensory sparing. Nerve conduction studies of upper limb mixed nerves are warranted. With regards to our analysis of spontaneous motor recovery in the subset of patients with cervical SCI, we explored whether upper thoracic afferent sparing, in the absence of sensation from lower cervical segments, might have functional implications for motor recovery. It does not exclude that other variables may also be as important as sensory preservation within upper thoracic segments. Furthermore, it would be valuable to examine neuroimaging findings to better understand how the neuropathology of SCI may account for the emergence of discontinuous upper thoracic preservation (e.g., rostral-caudal extent of injury).

2.5.5. Clinical implications and conclusions

After cervical SCI, a patient may report having sensation within upper thoracic dermatomes (e.g., T2/T3), but no sensation in dermatomes of the hand (e.g., C8). Rather than being considered a measurement error in the clinical assessment of light touch and pinprick, we propose that this pattern of sensation be considered evidence of relevant
afferent sparing after cervical SCI. Furthermore, we postulate that differences arising from examining sites at varying distances in the upper and lower extremities biases clinical testing methods to detect afferent sensory preservation within proximal dermatomes. The case report also highlights the benefits of employing standardized methods of sensory testing that can be varied to accurately detect afferent sparing after SCI.
3. Dermatomal somatosensory evoked potentials and electrical perception thresholds during recovery from cervical spinal cord injury

Based on the findings from the previous study it is clear that more advanced methods need to be employed to accurately determine the extent of afferent sparing in individual dermatomes after traumatic SCI. This chapter focuses on the application of cervical dsSEPs to track changes during spontaneous recovery in individuals with tetraplegia (acute to sub-acute and chronic SCI). The responsiveness of EPT was examined in conjunction with dsSEPs. The findings from this study indicate that dsSEPs, but not EPT, are responsive to changes in individual spinal segments during the first year after SCI.

3.1. Introduction

The neurophysiological evaluation of SSEPs has long been known to complement the neurological assessment of SCI and improve the accuracy of the diagnosis and prediction of functional outcome (Rowed, et al., 1978). Following electrical stimulation of a large mixed peripheral nerve (e.g. tibial or median), afferent impulses ascend across multiple spinal levels and cross through the epicenter of any spinal cord pathology in the dorsal column to reach the somatosensory cortex (described in detail above, General Introduction, see Figure 1.6). Consequently, the measured SSEPs after SCI reflect the full extent of afferent spinal cord damage (caudal to rostral) and cannot assess the involvement of each spinal segment to distinct sensory impairments. Furthermore, tracking subtle changes in conduction based on conventional SSEP outcomes (e.g. latency) is limited to
only a subset of individuals with incomplete SCI (Spiess, et al., 2008). SSEPs can be refined in order to examine individual dermatomes (dSSEPs, see Figure 1.8). dSSEPs have been employed sparingly to examine traumatic SCI (Date, et al., 1988; Cheliout-Heraut, et al., 1998; Shields, et al., 2006; Kramer, et al., 2008).

The aim of the present study was to determine the reliability and responsiveness of dSSEPs and EPT after sensorimotor complete or incomplete cervical SCI during spontaneous recovery, and demonstrate the stability of these measures in chronic SCI. Initial and follow-up combined recordings of dSSEP and EPT from C4 through C8 dermatomes were performed during the acute and sub-acute/chronic stages in individuals with tetraplegia. Within normal uninjured spinal segments (e.g. above level of SCI), the reliability of segmental sensory responses (dSSEPs and EPTs) was hypothesized to remain unchanged from acute to chronic examinations. Conversely, it was hypothesized that dSSEP latencies and EPTs from spinal segments exhibiting initially impaired responses (e.g. at or below the level of injury), indicative of spinal cord damage, should return toward normal latencies and perception thresholds, respectively, before stabilizing in chronic SCI. Finally, the return of a dSSEP in a dermatome, which initially had no measurable cortical response, should be accompanied by the emergence of an EPT. The alternative hypothesis was that temporal changes in sensory conductivity, as assessed by the latency of dSSEPs, would not change during spontaneous recovery and be unrelated to the subjective perception of sensation (i.e. EPT).
3.2. **Methods**

A retrospective review of an acute patient cohort with cervical SCI was performed. These patients were examined during their acute hospitalization at Vancouver General Hospital (VGH) following their injury and as out-patients in the Spine Clinic while under the care of Dr. Armin Curt. A cohort of chronic subjects was also recruited prospectively to this study.

3.2.1. **Inclusion/exclusion criteria**

Patients with traumatic tetraplegia (incomplete/complete) examined at VGH with an initial assessment of cervical dSSEP and EPT (C4-C8) performed during the first 6 months after injury and later on follow-up as outpatients were included specifically to determine responsiveness (n=29). A cohort of subjects (n=10) with initial and follow-up recording performed after 6 months from the time of their injury, no longer as patients in the retrospective review but as research participants at the Blusson Spinal Cord Center, were included to assess the reliability of dSSEP and EPT in the sub-acute/chronic phases of injury. 8 of 10 of these SCI subjects were also identified in the acute patient cohort (i.e. were previously patients at Vancouver acute and had been examined clinically by Dr. Curt). Therefore, in 8 subjects, we had acute and multiple follow-up examinations to track responsiveness during spontaneous recovery and chronic stability.

3.2.2. **Neurophysiology and quantitative sensory testing**

Right and left cervical EPTs and dSSEPs were recorded. Electrode placements and recording configuration were identical and performed by the same trained and qualified
The stimulation points were based on key sensory stimulation points for the upper limb (C4 to C8), as defined by the ISNCSCI (illustrated in Figure 2.4). Based on the experience of the technician, the total time of assessment (including setup and signal acquisition) was approximately 1.5 hours to complete C4-C8 dermatomes in patients with cervical SCI.

3.2.3. Dermatomal somatosensory evoked potentials

dSSEPs were elicited by repetitive, square wave (0.5ms) electrical pulses (at 3Hz) from standard clinical surface gel electrodes (20mm) on key cervical sensory points using a Keypoint electrophysiological stimulating and recording device (bandpass = 2Hz – 2kHz) (Medtronic, Mississauga, Ontario, CAN). dSSEPs were collected at a stimulus intensity well above perception threshold (i.e. at the highest level that could be maximally tolerated for a 10-minute duration, but not exceeding 40mA).

All subjects were lying in a supine position, with eyes closed, and instructed to remain relaxed and quiet during testing. AIS sensory testing sites (cervical dermatomes) were thoroughly prepared with NuPrep (D.O. Weaver & Co., Colorado, USA) and disposable stimulating electrodes were placed on the clean, dry skin. Silver-silver chloride disc recording electrodes (10mm, 60” lead wires) were placed on the scalp after preparation with NuPrep and fixed with Elefix paste (Nihon Kohden, Tokyo, Japan). The ground strap electrode was secured with even contact around the forearm for all recordings. In all dermatomes, 2 complete recording runs were undertaken during each session with averages of 250 – 1200 cortical responses from scalp surface recording electrodes (C3’-C4’ in a ten-twenty electrode configuration, illustrated in Figure 3.1) of the contralateral scalp.
to the C4 – C8 dermatomes being stimulated. C3’-C4’ were employed to measure the activity of S1. The impedance of ground and scalp electrodes were maintained <5 kΩ. The first negative (N1) and positive (P1) peak latencies (ms) were determined based on the mean of the two consecutive runs (Figure 3.2). Clinical interpretation of dSSEP N1 and P1 markers was reviewed by a study neurologist.

Figure 3.1 Electrode placement for the acquisition of dermatomal somatosensory evoked potentials
Active (red) and reference (blue) for right and left dSSEP acquisition. The active electrode was always contralateral to the stimulating side.

3.2.4. Electrical perception threshold

Methods to record EPT were adapted from previous studies and have been previously described (Kramer, et al., 2008). Briefly, the skin area stimulated within each dermatome was thoroughly cleaned (as described above for dSSEP recordings) to ensure comparable skin conditions at initial and follow-up examinations. Briefly, EPT was
determined for each cervical dermatome by gradually increasing the intensity of the electrical stimulus from 0mA until the individual verbally reported perception of the first sensation (method of limits), or until 40mA was achieved (without a report of any perceived sensation). The electrical stimulation parameters (0.5ms, square wave, 3Hz) were maintained from dSSEP acquisition. All subjects were blinded to the perceived rate of increase of stimulation intensity. The EPT was then re-examined in a similar manner. The EPT for each cervical dermatome was determined as the mean electrical stimulation intensity of the 2 repeated trials.

3.2.5. Analysis

dsSEPs were interpreted based on the latency of the prominent onset/N1 peak. Normative values of cervical dSEPs established in our laboratory in 29 neurologically healthy subjects (Figure 3.3). The N1 peak potential has been previously used to quantify pathological dSSEP recordings in SCI patients (Shields, et al., 2006; Kramer, et al., 2008). Averaged dSEPs were visually inspected for N1 and P1 latencies and qualified as: 1) having a normal (unaffected) N1 latency (within +2.5 standard deviations, SD) of mean values), 2) having a delayed N1 latency (≥+2.5 SD, of mean control values), or 3) having an abolished N1P1 (i.e. no measureable peaks). dSEPs were examined at a high recording gain (0.2µV/division). Waveform configuration (N1P1 interpeak interval and amplitude) was examined to confirm the presence of a dSSEP. In averaged recordings where a prominent N1 was not clear, the visual inspection of two separate runs was further analyzed to identify and accurately mark the onset latency.
The interpretation of EPT was based on previous recordings from neurologically intact control subjects in our laboratory (Kramer, et al., 2008). EPTs from C4-C8 dermatomes in these uninjured subjects are usually perceived at a threshold of 1mA to 2mA. The determination of an impaired EPT was made when a stimulus intensity of 2.5mA or greater was required for perception. The EPT was considered to be absent in dermatomes when there was no patient perception with a stimulus at 40mA.
Figure 3.2 Dermatomal somatosensory evoked potentials in a neurologically healthy subject
N1 and P1 peaks are marked. Two consecutive runs built on the average of 500-1000 consecutive stimulations are considered for the interpretation of peak latency (N1). The agreement between these runs is essential for accurate determination of peaks. If there is no agreement or poor agreement, the dSSEP is considered abolished.
Figure 3.3 Normative values of cervical dermatomal somatosensory evoked potentials in neurologically healthy subjects
Error bars represent 2.5 SD (n=29, neurologically healthy subjects). Values that exceed the upper error bars are interpreted as delayed.

3.2.6. Statistics

dSSEPs were grouped according to the initial interpretation of N1 latency (unaffected, delayed, or abolished). The initial and follow-up N1 latencies and EPTs of dermatomes with unaffected and delayed dSSEPs were examined separately by paired Student’s t-test (p<0.05). The test retest reliability of N1 latency and EPT was examined in unaffected (acute and chronic) and delayed (chronic cohort only) dermatomes by ICC. The relationship between initial dSSEP N1 latency and EPT was examined by Pearson’s correlation coefficient (p<0.05). Those dSSEPs that were initially abolished (i.e. not measurable, see above description), but then subsequently recorded at follow-up, were examined for corresponding changes in EPT.
3.3. **Results**

3.3.1. **Spinal cord injury characteristics and examination details**

The age (years), time of examination after SCI (days), and time between initial and follow-up examinations (days) of the 29 patients with acute (<6 months) and 10 with chronic (≥6 months) traumatic tetraplegia included in the analyses are summarized in Table 3.1.

<table>
<thead>
<tr>
<th>SCI Cohort</th>
<th>Acute (n=29)</th>
<th>Chronic (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age at time of SCI (years)</td>
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<td>17.42</td>
</tr>
<tr>
<td>Time of initial examination</td>
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<td>33.74</td>
</tr>
<tr>
<td>(days post SCI)</td>
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<tr>
<td>Time elapsed between</td>
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<td>154.79</td>
</tr>
<tr>
<td>examinations (days)</td>
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</tbody>
</table>

Table 3.1 Spinal cord injury demographics and examination details

3.3.2. **Acute spinal cord injury: Responsiveness**

There were a total of 197 acute cervical dSSEPs included in the analysis (C4=44, C5=43, C6=37, C7=38, and C8=35). 45 dSSEPs were initially interpreted as delayed (C4=8, C5=10, C6=10, C7=9, and C8=8) and 46 were interpreted as abolished (C4=5, C5=14, C6=7, C7=8, C8=12). The remainder (n=106) were unaffected by cervical SCI (C4=31, C5=19,
C6=20, C7=21, and C8=15). Initially impaired (>2.5mA) or absent EPT (>40mA) corresponded with delayed and abolished dSSEPs in 33.0% and 80.0% of dermatomes, respectively. 4.7% of dermatomes with normal dSSEPs had an impaired EPT; none had absent EPT. One dermatome with absent EPT had a recordable, but delayed dSSEP. In all other dermatomes with absent EPT, dSSEPs were abolished. The mean stimulus intensity for the EPT in dermatomes with unaffected, delayed, and abolished initial dSSEP interpretations was 1.38±SD1.02mA, 3.59±6.42mA, and 18.63±16.87mA, respectively. The longer the initial N1 delay of the initial dSSEP from normative values, the higher EPT that was reported for that dermatome (ρ=0.511, p<0.01, Pearson correlation; Figure 3.4).

Representative initial (acute) and follow-up (sub-acute) dSSEPs from an individual with cervical SCI are illustrated in Figure 3.5. The ICC of unaffected dSSEP N1 latencies and corresponding EPT values was 0.957 (p<0.001) and 0.500 (p<0.001), respectively. Follow-up examination of initially unaffected dSSEPs revealed no significant change in N1 latency (Δ=-0.17±1.79ms, t=0.992, p=0.323). 91.5% of initially unaffected dSSEPs remained unaffected on follow-up, compared to 8.5% that deteriorated (delayed, 5.7%; abolished, 2.8%). Initially delayed dSSEPs showed a significantly decreased N1 latency on follow-up examination (Δ=3.12±3.43ms, t=5.60, p<0.01; Figure 3.6). 40.0% of initially delayed dSSEPs recovered to normal latency on follow-up, compared to 4.4% that deteriorated (i.e. abolished on follow-up). 54.4% of initially abolished dSSEPs recovered to either delayed (43.5%) or normal (10.9%) latency. There was no mean change of EPT on follow-up examination for dermatomes with initially unaffected (Δ0.12±0.87mA, t=1.46, p=0.147) or delayed dSSEPs (Δ=0.54±5.07mA, t=0.688, p=0.495).
Figure 3.4 Relationship between the initial electrical perception threshold and N1 latency
Higher EPT values are associated with more delayed N1 latencies.

In dSSEPs that recovered from abolished to recordable (delayed or unaffected), there was a significant decrease in EPT ($\Delta=7.38\pm11.55\text{mA}$, $t=3.13$, $p=0.005$). Comparatively, in dermatomes not recovering a delayed or unaffected dSSEP on follow-up, there was no significant change in EPT ($\Delta=0.48 \pm 14.19\text{mA}$, $t=0.150$, $p=0.883$). In dermatomes that deteriorated on follow-up (i.e. initially normal or delayed dSSEP to delayed/abolished), there was also no significant change in EPT ($\Delta=-0.49 \pm 1.88\text{mA}$, $t=1.04$, $p=0.315$).
Figure 3.5 Representative initial and follow-up dermatomal somatosensory evoked potentials during spontaneous recovery after spinal cord injury

Representative initial acute (i) and follow-up sub-acute/chronic (f) dSSEPs in an individual with cervical SCI (runs 1 and 2 displayed). The peak latencies are marked with a dotted line. The N1 peak in dermatomes initially unaffected remains stable during spontaneous recovery (C4, top panel), whereas the N1 peak in dermatomes initially delayed recovers towards normal values (C7, middle panel, and C8, bottom panel).
3.3.3. Chronic spinal cord injury: Stability

The acute responsiveness and chronic stability of an unaffected and delayed dSSEP are illustrated in Figure 3.7. There was a total of 79 dSSEPs (abolished=6, delayed=25, and unaffected=48) recorded initially and on follow-up in patients with chronic SCI (>6 months post injury, n=10). The ICC of dermatomes with unaffected and delayed dSSEPs in patients with chronic cervical SCI was 0.881 (p<0.001) and 0.680 (p<0.001). Only one dSSEP was recordable on follow-up that could not be recorded initially, and no dSSEP could be recorded initially but not on follow-up. 68.0% and 89.6% of initially delayed and unaffected dSSEPs, respectively remained unchanged on follow-up. The N1 latency of initially delayed dSSEPs did not significantly change on follow-up (Δ=0.03 ±7.18ms, t=0.019, p=0.985). The ICC of EPT in dermatomes with initially unaffected and delayed dSSEPs was 0.699.
(p<0.001) and 0.638 (p<0.001). The EPT of dermatomes with initially delayed dSSEPs did not significantly change on follow-up ($\Delta=-0.99 \pm 3.21, t=1.55, p=0.135$).

Figure 3.7 Representative initial and follow-up dermatomal somatosensory evoked potentials during spontaneous recovery after spinal cord injury
Compared to the unaffected N1 latency of C4, C5 N1 latency (initially delayed) shifts towards normative values during recovery and remains stable in chronic SCI. A change in amplitude may also be observed (C4) but this does not affect the N1 latency.

3.4. Discussion

The present study demonstrates that dSSEPs and EPTs are useful to monitor changes in afferent conduction in individual cervical segments after tetraplegia during a
period of spontaneous recovery. Comparisons can be made between dSSEP latency (neurophysiology) and sensory perception (patient-reported function), which complement conventional neurological (i.e. ISNCSCI) and functional assessments. Current sensory assessments after SCI have limited sensitivity (e.g. AIS sensory score) to detect a MCID (Furlan, et al., 2008), and/or lack the refinement (e.g. SSEP) to specify the location of spinal segment(s) underlying a change stimulated by a therapeutic intervention. dSSEPs have the potential to address the limitations of conventional SSEPs. Specifically, dSSEPs enable the close monitoring of neurophysiological changes in spinal segments, adjacent to the level of SCI, and can detect either sensory detriments or benefits (i.e. ascending loss of a normal dermatome or recovery of a pathological dermatome).

3.4.1. Reliability and responsiveness of dermatomal somatosensory evoked potentials

Despite electrical stimulation well above perception and close to pain threshold, the latency for the first recorded dSSEP response measured at the cortex, observed in both neurologically intact (uninjured) control subjects and subjects with cervical SCI (normal and delayed responses), was consistent with activation from large diameter Aβ fibers and afferent conduction in the dorsal column. This does not rule out the possible co-activation of small diameter or thinly myelinated sensory afferent fibers and conduction along other ascending sensory pathways (e.g. via the spinothalamic tract), but rather that the first measured evoked potential reflects conduction within tactile sensory pathways associated with the sensation of light touch. The neurophysiological examination of the spinothalamic tract may be better examined using radiant heat and thermal stimulation modalities.
The finding that initial dSSEP latency is reliable in dermatomes unaffected by SCI during recovery and in unaffected dermatomes in chronic SCI, is an important step in validating this method for clinical application in SCI. During recovery from SCI, monitoring the neurophysiology of an unaffected (normal) and stable spinal segment, in conjunction with pathological dermatomes, provides a within-subject control condition to compare and contrast changes, while confirming the consistency of the dSSEP recording setup and data acquisition for each individual.

In this study, there was a general trend for the recovery of dSSEPs (i.e. significantly decreased N1 latency of delayed dSSEPs, and abolished to delayed/normal and delayed to normal dSSEPs), with only a limited number of patients demonstrating neurophysiological evidence of deterioration (i.e. normal to delayed/abolished and delayed to abolished dSSEPs). The recovery of initially abolished dSSEP did occur predominantly in dermatomes where there was some initial preservation of sensation (i.e. EPT but no recordable dSSEP). The prevailing pattern of dSSEP responsiveness (i.e. the reliability of normal versus the responsiveness of affected dermatomes) is in line with those clinical observations made during spontaneous recovery from SCI (i.e. stable or minor improvements within dermatomes adjacent to the SCI, Ditunno, Jr., et al., 2000a). Importantly, the spontaneous improvement of sensation and/or motor function may yield a caudal shift in the level of injury, and be of clinically meaningful benefit to individuals with tetraplegia, who stand to gain greater independence with improving hand function.

The present findings demonstrate a significant mean decrease in the N1 latency of initially delayed dSSEPs in cervical dermatomes during spontaneous recovery. This is
generally in agreement with a subset of individuals with incomplete SCI based on conventional SSEPs (Spiess, et al., 2008). An advantage of dSSEPs is that such an observation can be made in individuals with complete and incomplete injuries (i.e. conventional SSEPs can only be recorded in incomplete SCI). However, in both cases, regression towards the mean (RTM) could explain why initially delayed latencies of SSEPs (i.e. outliers) return closer to normal values (i.e. mean). The impact of RTM on neurophysiological outcomes should be further examined.

3.4.2. Reliability and responsiveness of electrical perception threshold

A proposed advantage of EPT and other QST methods is that continuous scaled measurements will provide a sensitive and reliable outcome to more objectively assess impaired dermatomes, thus improving upon the ordinal rating of the AIS sensory scale (2=normal, 1=impaired, 0=absent) (Steeves, et al., 2007). This study supports that EPT is reliable during chronic SCI (i.e. does not significantly change over time in unaffected dermatomes above the level of injury). Furthermore, the higher the EPT, the more pronounced were the neurophysiological deficits for an individual dermatome. However, a robust change in EPT was not observed in the same dermatomes exhibiting decreased N1 latency. A possible explanation is that an improvement in EPT may require the magnitude of the neurophysiological change (e.g. decreased N1 latency) to first exceed a certain threshold (i.e. more closely approximate the normal waveform configuration, latency and amplitude). This suggestion is supported by the emergence of EPT when a dSSEP recording (at follow-up) changed from abolished-to-recordable. This emphasizes the potential functional importance of changes in neurophysiology after SCI. Nevertheless, the
importance of subtle neurophysiological changes (e.g. 2-5ms latency shifts) should not be underestimated, given it may represent a primary mechanism of recovery that could be targeted for further improvement with an appropriate therapy within an optimal window of intervention.

The ICC of EPT in unaffected dermatomes in the present study (0.500, p<0.001) was slightly less than previously described for SCI subjects (King, et al., 2009) and markedly less than the N1 latency of dSSEPs for the same dermatomes reported here. The majority of acute SCI patients in this study were initially examined during the first month after injury, representing a more homogenous acute assessment time-point than King et al. (2009) (1-72 months post SCI). The reliability of any QST outcome is dependent on a standardized protocol, the sensory modality, the recording environment (i.e. room temperature), skin quality, and the cooperation of the subject (i.e. attention and motivation) (Chong and Cros, 2004); all of which may be particularly difficult to fully control during acute care (e.g. a pain medication can alter attention and motivation). Given the importance for the reliability of any initial SCI assessment or measurement on subsequent comparisons during the course of spontaneous recovery, the incorporation of neurophysiology with QST to track changes may be warranted.

3.4.3. Study limitations

The chronic reliability dSSEP and EPT stability findings from the current study are limited by a relatively small sample size (n=10). Furthermore, prospectively monitoring dSSEP and EPT changes in serial assessments during rehabilitation at planned time-points (i.e. 1, 3, 6, and 12 months post injury) may provide additional insight into possible
mechanisms underlying changes (e.g. axonal sprouting, resolution of conduction failure, etc.) (Cafferty, et al., 2008). The interpretation of dSSEP latencies in this study was performed by visual inspection of at least two separate recording averages (from each session); thus, it remains a semi-quantitative measure. While the study focused on the examination of N1 latency, other changes in dSSEP parameters may also characterize functional aspects of recovery. The meaning of changes in waveform configuration (i.e. emerging late potentials) remains less clear than the reported moderate changes in N1 latency, and would require further study. To improve the overall understanding of the relation between changes in neurophysiological recordings (latencies and amplitudes) and functional outcomes, several modalities of QST may have to be examined in conjunction with the appropriate neurophysiological test (Hayes, et al., 2002). Ideally, a longitudinally designed study should also include an independent functional outcome measure (i.e. SCIM). Lastly, dSSEPs are a measure of large diameter fibers and, thus the dorsal column. The objective assessment of the spinothalamic tract should be a priority of future study in SCI to complement pinprick testing. A variety of methods may be useful for this, including CHEPs. This avenue of research may be particularly important with regards to the study of neuropathic pain after SCI (Wydenkeller, et al., 2008; Wydenkeller, et al., 2009).

3.4.4. Conclusion

The present findings demonstrate that the responsiveness of dSSEP N1 latency is similar to that reported for initially delayed conventional tibial SSEPs (P40) during spontaneous recovery (Spiess, et al., 2008). However, because dSSEPs can be employed to examine conduction deficits in individual spinal segments after complete and incomplete
injury, segmental neurophysiological outcomes may be more useful in a broader SCI population. The relationship between dSSEP and EPT suggests a minimum threshold for changes in neurophysiology before the detection of improved sensation.
4. Test-retest reliability of contact heat evoked potentials from cervical dermatomes: Implications for spinal cord injury

The findings from the previous study demonstrate the potential for segmental neurophysiological approaches to detect subtle changes after traumatic cervical SCI (complete and incomplete). As pointed out in the study limitations, the evaluation of conduction based on dSSEPs is restricted to the dorsal columns. Therefore, this chapter focuses on the use of CHEPs to examine conduction in the spinothalamic tract. Specifically, the test-retest reliability of cervical CHEPs is demonstrated in neurologically healthy subjects and individuals with chronic tetraplegia.

4.1. Introduction

The examination of the spinothalamic tract can be achieved by activating type II A-fiber mechano-heat nociceptors using thermal or radiant heat stimuli (Bromm and Treede, 1984; Cruccu, et al., 2000; Chen, et al., 2001; Valeriani, et al., 2002; Granovsky, et al., 2005; Iannetti, et al., 2006; Valeriani, et al., 2007; Wydenkeller, et al., 2008; Granovsky, et al., 2008; Warbrick, et al., 2009; Wang, et al., 2010). The majority of clinical studies to date have focused on the investigation of the N2P2 of contact heat (i.e. thermal) and laser (i.e. radiant) evoked potentials (CHEPs and LEPs, respectively) recorded from vertex scalp electrodes (Iannetti, et al., 2001; Quante, et al., 2007) (for review, see(Cruccu, et al., 2008) (see Figure 1.9). The N2P2 of CHEPs and LEPs is thought to primary reflect the activation of the ACC; a region in the pain matrix integrally involved in the emotional-cognitive aspect of
processing pain (e.g. attention), receiving afferent projections from the medial thalamic nuclei (Lenz, et al., 1998; Valeriani, et al., 2002; Valeriani, et al., 2007; Mobascher, et al., 2009).

The validity of a clinical tool to provide meaningful diagnostic and prognostic information depends, in part, on the test-retest reliability of the primary outcome. Thus, an understanding of the normal variability of evoked potential parameters (i.e. amplitude and latencies of prominent negative and positive peaks), within healthy subjects in a repeated measures study, is fundamental to appropriately tracking meaningful changes in patient populations over time (i.e. responsiveness). The reproducibility of LEPs has only been examined following trigeminal nerve stimulation (i.e. forehead) (Kazarians, et al., 1995), and, in a more recent study, after stimulation of the foot over multiple experimental sessions in the same day (Bachmann, et al., 2010). The reproducibility of CHEPs following stimulation of the volar forearm has also been demonstrated in a feasibility study (Roberts, et al., 2008a), although the elapsed time between examinations was not specified.

To our knowledge, there has been no comprehensive statistical review of CHEP or LEP test-retest reliability. Therefore, the primary aim of this study was to investigate the test-retest reliability of CHEPs following thermal stimulation of cervical dermatomes (C4-C6 and C8) in neurologically healthy subjects using statistical methods previously described (Bland and Altman, 1986; Shrout, 1998). For comparison purposes, the test-retest reliability of dSSEPs was also examined. The re-test reliability of cervical CHEPs was further examined for a cohort (n=11) of individuals with chronic traumatic tetraplegia.
4.2. Methods

Neurologically healthy subjects and individuals with chronic (≥6 months) cervical SCI participated in this study. All subjects provided informed consent. None of the participants (neurologically healthy or individuals with SCI) reported experiencing any acute or chronic pain at either of the measurement time-points (i.e. test or retest). Healthy subjects reported no history of peripheral and/or central neurological disease. All SCI subjects had an injury in the cervical cord between C5 and C8 according to their light touch and pinprick testing (ISNCSCI).

4.2.1. Contact heat evoked potentials

According to the protocol described below, healthy subjects and individuals with SCI underwent two sessions of cervical CHEPs (n=17 and n=11, respectively) recordings. In the neurologically healthy subject group there were 12 males and 5 females, with a mean age (±SD) of 28.88 ±4.91 years. The time between initial and follow-up examinations ranged from 1 to 136 days (mean ± SD = 38 ± 41 days). In the SCI group, there were 2 females (9 males) and the average age was 42.5 ±16.4 years. All individuals with cervical SCI were at least 6 months from their day of injury. Right and left recordings were performed in individuals with SCI. The mean time between examinations for individuals with SCI (test and retest) was 104.7 ±4103.6 (range, 5 to 370 days). All testing was performed while subjects were lying quietly in the supine position.

Scalp recording sites were thoroughly prepared with Nuprep (D. O. Weaver & Co, Aurora, CO). Silver-silver disc active (Cz) and reference (A1-A2) surface recording electrodes
(10 mm) were placed firmly on the scalp with Elefix (Nihon Kohden, Tokyo, Japan), according to the 10-20 electrode configuration (Figure 4.1).

![Figure 4.1 Electrode placement for the acquisition of contact heat evoked potentials](image)

**Figure 4.1 Electrode placement for the acquisition of contact heat evoked potentials**  
Active (red) and linked references (blue).

A ground strap electrode was secured with even contact around the forearm on the stimulating side. The impedance of ground and scalp electrodes was maintained at <5 kΩ. Testing sessions lasted approximately 1-1.5 hours, including set-up, the provision of instructions, and acquisition of evoked potentials. The boundaries of the cervical dermatomes being examined (C4-C8) were defined according to clinical standards set by the ISNCSCI (illustrated in Figure 2.4). The C7 dermatome (middle finger) was not examined with CHEPs because the thermode could not be variably repositioned without moving into an adjacent dermatome (C6 or C8).
Heat stimuli were delivered using a contact heat stimulator (PATHWAY Pain & Sensory Evaluation System, Medoc, Ramat Yishai, Israel). Briefly, this device is capable of generating a rapid pulse of heat (↑70°C/s, ↓40°C/s) up to a maximum of 55°C at the thermode surface from an actively controlled baseline (30-45°C) using a thermofoil (↑) or Peltier element (↓). Two thermocouples embedded in the thermode provide continuous, online monitoring of the temperature at the skin-thermode interface.

In the present study, the baseline temperature of the thermode was maintained at 35°C. 35°C or greater has been frequently adopted as a baseline temperature in previous CHEP studies (Granovsky, et al., 2005; Iannetti, et al., 2006; Chao, et al., 2007; Chao, et al., 2008; Wydenkeller, et al., 2008). For individuals with SCI, the stimulus intensity increased by 1°C/stimulation until the first report of a change in sensation (thermal perception threshold). To ensure safety and stimulus tolerability during the acquisition of CHEPs, all subjects were stimulated up to a maximum of 54°C, depending on individual tolerance, with an offset temperature of 2°C. If a subject was unable to tolerate 54°C on a given dermatome, the stimulus intensity was incrementally decreased from 54°C to a temperature they could more readily tolerate. The order of cervical spinal cord dermatomes examined was randomized in neurologically healthy subjects, however, in individuals with SCI, CHEPs were always first recorded from a dermatome with a normal thermal perception threshold. The inter-pulse interval of heat stimuli was randomly varied between 8 and 10 seconds. The parameters used to acquire the evoked potential on initial examination were also applied on follow-up for each subject. Before beginning the experimental procedures (described below), three contact heat stimulations at 46°C (2°C
offset temperature) on the volar surface of the forearm were provided to familiarize subjects to the stimulus modality.

For the acquisition of CHEPs, subjects were instructed to keep their eyes open in a fixed, neutral position for at least 2 seconds after perception to the stimulus (i.e. after the conclusion of the recording epoch). Eyes remained open to avoid alpha artifact contamination (Cruccu, *et al.*, 2008). To ensure that the subject remained alert and attentive to the stimulation, subjects were instructed to rate the intensity of stimuli using a numerical rating scale (NRS, 0-10) approximately two seconds after perception according to the scale illustrated in Figure 4.2. Twenty stimulations were applied to each dermatome. The thermode was slightly repositioned after each stimulus to avoid habituation, though always remained within the confined boundary of the targeted dermatome. C6 and C8 dermatomes were examined on the dorsum aspect of the hand.

![Numerical rating scale](image)

**Figure 4.2 Numerical rating scale**

CHEPs were sampled at 2kHz using a single channel pre-amplifier (20,000x, bandpass filter 0.25-300Hz, ALEA Solutions, Zurich, Switzerland). A 200ms pre-trigger preceded signal acquisition (≥1000ms). The visually detected N2P2 (inverted and
bandpass filtered: 0.5-30Hz) was based on an average of the first 15 of 20 stimulations that were perceived and uncontaminated by artifact. Missed stimulations were only observed when the thermode was not in position at the onset of a stimulus in healthy subjects. All signals were recorded in a LabView (National Instruments, Austin, TX, USA) based software package (ALEA Solutions, Zurich, Switzerland).

4.2.2. Dermatomal somatosensory evoked potentials

17 neurologically healthy subjects with a mean age of 30.84±9.25 (12 males, 5 females) were recruited to undergo test-retest analysis of dSSEPs. The methods used to acquire dSSEPs have been previously described in Chapter Three and will not be repeated here.

4.2.3. Statistics

Any differences (follow-up – initial) in the initial average rating of intensity, peak latency (N2 and P2) and peak-to-peak amplitude (N2P2) of CHEPs were examined by repeated measures analysis of variance (by cervical dermatome). Pair-wise comparisons were Bonferroni corrected. Test-retest reliability of dSSEPs and CHEPs were examined separately for each dermatomal stimulation site by ICC (single measures, two-way random effect model). ICC values were characterized based on recommendations in Shrout, 1998 . Briefly, “fair”, “moderate”, and “substantial” ICC were considered for ranges of 0.41-0.60, 0.61-0.80 and 0.81-1.00, respectively. To examine if the mean difference between the test-retest examinations was significantly greater than zero, a one-sample t-test was employed. A coefficient of repeatability (i.e. 2 x SD of mean difference between initial and follow-up, or
95% confidence) for latency and peak-to-peak amplitude parameters was determined for combined dermatomes whose mean difference between examinations was not statistically significant (Bland and Altman, 1986). Statistical significance was set at α<0.05. All statistical tests were performed in SPSS (V.16).

4.3. Results

4.3.1. Neurologically healthy subjects

The mean (± SD) CHEP (n=17) peak latencies (N2 and P2) and peak-to-peak amplitude (N2P2) from the initial examination of cervical dermatomes are shown in Table 4.1. The cervical dermatome examined had a significant main effect on subject perception as denoted by NRS (F=14.25, df=3, p<0.001), N2P2 amplitude (F=13.31, df=3, p<0.001), P2 latency (F=21.1, df=3, p<0.001), and N2 latency; P2 (F=7.4, df=3, p=0.009). Distal dermatomes (C6 and C8) had significantly smaller mean N2P2 amplitude, longer N2 and P2 latencies, and lower average NRS than proximally stimulated dermatomes (C4 and C5, p<0.05). No significant differences between proximal (C4 and C5) or distal dermatomes (C6 and C8) for measures of latency (N2 and P2) or peak-to-peak amplitude were found (p>0.05). Representative test-retest cervical (C4-C6 and C8) CHEPs from one neurologically healthy subject are shown in Figure 4.3.
<table>
<thead>
<tr>
<th>Cervical Dermatome</th>
<th>Latency (ms)</th>
<th>Amplitude</th>
<th>NRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N2</td>
<td>P2</td>
<td>N2P2</td>
</tr>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
</tr>
<tr>
<td>4</td>
<td>372.12 (37.38)</td>
<td>496.68 (30.55)</td>
<td>34.18 (12.35)</td>
</tr>
<tr>
<td>5</td>
<td>369.32 (40.49)</td>
<td>505.65 (31.24)</td>
<td>38.96 (15.55)</td>
</tr>
<tr>
<td>6</td>
<td>409.18 (45.37)</td>
<td>540.97 (35.73)</td>
<td>29.84 (14.19)</td>
</tr>
<tr>
<td>8</td>
<td>414.12 (53.80)</td>
<td>544.19 (39.79)</td>
<td>24.65 (12.06)</td>
</tr>
</tbody>
</table>

Table 4.1 Initial cervical contact heat evoked potential latencies and amplitudes

Figure 4.3 Representative cervical contact heat evoked potentials
Initial (black) and follow-up (grey, thin) recordings in a neurologically healthy subject.
The ICC values for measures of NRS and CHEP peak latency and peak-to-peak amplitude (N2 and P2, and N2P2, respectively) reported in Table 4.2 were significant for all cervical dermatomes examined (p<0.05). The overall coefficient of repeatability (i.e. Bland-Altman plots) for NRS, N2 latency, and N2P2 amplitude was derived from all cervical dermatomes (Figure 4.4). This grouping was possible because the mean differences between examinations were not significantly different from zero, for any dermatome.

<table>
<thead>
<tr>
<th>Cervical dermatome</th>
<th>Parameter</th>
<th>Bland-Altman confidence</th>
<th>Intra-class correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean Δ</td>
<td>(±2SD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>N2 latency (ms)</td>
<td>-12.44</td>
<td>(58.78)</td>
</tr>
<tr>
<td></td>
<td>P2 latency (ms)</td>
<td>9.31</td>
<td>(35.68)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>-.43</td>
<td>(14.52)</td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>.08</td>
<td>(2.58)</td>
</tr>
<tr>
<td>C5</td>
<td>N2 latency (ms)</td>
<td>7.71</td>
<td>(68.32)</td>
</tr>
<tr>
<td></td>
<td>P2 latency (ms)</td>
<td>1.68</td>
<td>(66.86)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>-1.49</td>
<td>(15.16)</td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>.19</td>
<td>(2.40)</td>
</tr>
<tr>
<td>C6</td>
<td>N2 latency (ms)</td>
<td>-12.26</td>
<td>(84.74)</td>
</tr>
<tr>
<td></td>
<td>P2 latency (ms)</td>
<td>-2.09</td>
<td>(27.70)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>-2.36</td>
<td>(13.62)</td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>-.18</td>
<td>(3.28)</td>
</tr>
<tr>
<td>C8</td>
<td>N2 latency (ms)</td>
<td>8.47</td>
<td>(85.78)</td>
</tr>
<tr>
<td></td>
<td>P2 latency (ms)</td>
<td>17.06*</td>
<td>(52.80)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>2.47</td>
<td>(16.06)</td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>-.20</td>
<td>(1.76)</td>
</tr>
</tbody>
</table>

*p<0.05, independent samples t-test
Δ, follow-up – initial

Table 4.2 Summary of contact heat evoked potential reliability
In the case of P2 latency, however, the mean difference between examinations for the C8 dermatome CHEPs was significantly different from zero (p<0.05). Therefore, the overall coefficient of repeatability for P2 latency excluded C8 CHEPs. A representative example of the variability of P2 latency in C8 CHEPs is shown in Figure 4.5.

**Figure 4.4 Bland-Altman plots**
The combined cervical dermatome analysis of CHEP parameters in neurologically healthy subjects (A. N2 and P2 latency, top and bottom, respectively, and B. N2P2 amplitude and NRS, top and bottom respectively). The mean change between initial and follow-up examination are shown for each parameter with 95% confidence intervals.
Figure 4.5 Representative variability of contract heat evoked potential N2P2
An example of the difference in test-retest reliability of the P2 potential of the N2P2 in C8 compared to C5. Neurologically healthy subject. (Black, initial and grey, follow-up recordings)

The ICC values for cervical dSSEPs are shown in Table 4.3 (all values, p<0.05). The mean difference in N1 latency between examinations was not significantly different from zero for any of the cervical dSSEPs. The N1 latency coefficient of repeatability (2 x SD), derived from all of the cervical dermatomes examined (for the same reason as described above for CHEPs NRS, N2 latency, and N2P2 amplitude), was ±2.16ms. The mean difference in N1P1 amplitude of cervical dSSEPs between examinations was significantly different from zero for C6 (-0.25µV) and C8 (-0.18µV) (p<0.05). The coefficient of N1P1 amplitude repeatability, excluding the peak-to-peak amplitudes of C6 and C8 (for the same reason as described for CHEPs P2 latency), was ±1.2µV.
<table>
<thead>
<tr>
<th>Cervical dermatome</th>
<th>Parameter</th>
<th>Bland-Altman confidence</th>
<th>Intra-class correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean Δ (±2SD)</td>
<td>Single measures (95% Confidence interval)</td>
</tr>
<tr>
<td>C4</td>
<td>N1 latency (ms)</td>
<td>.03 (3.10)</td>
<td>.397 (Lower -.088 Upper .730)</td>
</tr>
<tr>
<td></td>
<td>N1P1 amplitude (µV)</td>
<td>.03 (1.40)</td>
<td>.590 (Lower .168 Upper .829)</td>
</tr>
<tr>
<td>C5</td>
<td>N1 latency (ms)</td>
<td>.27 (1.98)</td>
<td>.671 (Lower .296 Upper .867)</td>
</tr>
<tr>
<td></td>
<td>N1P1 amplitude (µV)</td>
<td>.12 (.98)</td>
<td>.762 (Lower .457 Upper .907)</td>
</tr>
<tr>
<td>C6</td>
<td>N1 latency (ms)</td>
<td>.02 (1.92)</td>
<td>.798 (Lower .527 Upper .922)</td>
</tr>
<tr>
<td></td>
<td>N1P1 amplitude (µV)</td>
<td>-.25* (.82)</td>
<td>.878 (Lower .697 Upper .954)</td>
</tr>
<tr>
<td>C8</td>
<td>N1 latency (ms)</td>
<td>.02 (1.44)</td>
<td>.818 (Lower .568 Upper .930)</td>
</tr>
<tr>
<td></td>
<td>N1P1 amplitude (µV)</td>
<td>-.18* (.50)</td>
<td>.942 (Lower .847 Upper .978)</td>
</tr>
</tbody>
</table>

*p<0.05, independent samples t-test
Δ, follow-up – initial

Table 4.3 Summary of dermatomal somatosensory evoked potential reliability

4.3.2. Individuals with spinal cord injury

The test-retest reliability statistics from cervical dermatome CHEPs examined in individuals with tetraplegia are shown in Table 4.4. A representative test-retest set of CHEPs from an individual with SCI are illustrated in Figure 4.6. In one individual with cervical SCI, C6 and C8 CHEPs were unrecordable at the initial examination but were recorded on follow-up. In two other individuals, this observation was limited only to C8. C8 was only recordable in 3 individuals with cervical SCI. All of C5 CHEPs that were abolished initially were also abolished on follow-up. All C4 CHEPs were recordable on both initial and follow-up examination.
<table>
<thead>
<tr>
<th>Cervical dermatome</th>
<th>Parameter</th>
<th>Bland-Altman confidence</th>
<th>Intra-class correlation coefficients</th>
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<tr>
<td></td>
<td></td>
<td>Mean Δ</td>
<td>(±2SD)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>N2 latency (ms)</td>
<td>4.45</td>
<td>(36.76)</td>
</tr>
<tr>
<td></td>
<td>P2 latency (ms)</td>
<td>-2.82</td>
<td>(50.60)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>-0.04</td>
<td>(20.04)</td>
</tr>
<tr>
<td></td>
<td>N2 latency (ms)</td>
<td>-2.27</td>
<td>(110.90)</td>
</tr>
<tr>
<td>C5</td>
<td>P2 latency (ms)</td>
<td>2.07</td>
<td>(73.90)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>-.186</td>
<td>(14.72)</td>
</tr>
<tr>
<td></td>
<td>N2 latency (ms)</td>
<td>45.40</td>
<td>(390.20)</td>
</tr>
<tr>
<td>C6</td>
<td>P2 latency (ms)</td>
<td>5.56</td>
<td>(29.16)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>-1.63</td>
<td>(12.54)</td>
</tr>
<tr>
<td></td>
<td>N2 latency (ms)</td>
<td>8.00</td>
<td>(92.40)</td>
</tr>
<tr>
<td>C8</td>
<td>P2 latency (ms)</td>
<td>5.33</td>
<td>(23.24)</td>
</tr>
<tr>
<td></td>
<td>N2P2 amplitude (µV)</td>
<td>1.32</td>
<td>(15.00)</td>
</tr>
</tbody>
</table>

Δ, follow-up – initial

Table 4.4 Summary of contact heat evoked potential reliability in individuals with spinal cord injury
Figure 4.6 Representative cervical contact heat evoked potentials in an individual with spinal cord injury
C4 (top left panel, unaffected), C5 (top right, unaffected), C6 (bottom left, impaired), and C8 (bottom right, abolished). The N2 (first, negative deflection) and P2 (first, positive deflection) are marked (dotted lines) where applicable.
4.4. Discussion

To date, those studies which have demonstrated that thermal and radiant heat evoked potentials (CHEPs and LEPs, respectively) are reliable have been limited by: 1) the lack of appropriate test-retest statistical analysis, 2) a single stimulation site examined per session, and 3) the reacquisition of CHEPs within a short period of time after the initial examination (i.e. hours) (Kazarians, et al., 1995; Roberts, et al., 2008a; Bachmann, et al., 2010). Additionally, none of the aforementioned studies have examined the test-retest reliability of CHEPs in a patient population (i.e. individuals with SCI). Based on these findings alone, there is limited evidence to conclude CHEPs or LEPs are robustly reliable for most clinical applications. In general, the findings from the present study do confirm that CHEPs can be reliably examined from cervical dermatomes (C4-C6 and C8). According to statistical guidelines (Shrout, 1998), the ICC values reported here range from “fair” (0.41-0.61) to “substantial” (0.81-1.00) for measures of CHEP latency and peak-to-peak amplitude. Importantly, we have also provided preliminary evidence of test-retest reliability in individuals with impaired sensation due to SCI.

4.4.1. Clinical implications

Contact heat represents a stimulus modality suitable for the activation of mechano-heat receptors (i.e. Type II) and the examination of conduction in the spinothalamic tract based on shared cortical activation as LEPs (Chen, et al., 2001; Valeriani, et al., 2002; Iannetti, et al., 2006; Wydenkeller, et al., 2008; Roberts, et al., 2008a; Bachmann, et al., 2010). Based on the coefficients of repeatability (2 x SD of the mean difference), P2 latency
(±46.80ms) is a more reliable measure of conduction in the spinothalamic tract than is N2 (±95.06ms). Expressed as a percentage of mean latency, the P2 coefficient of repeatability (46.80ms/515.82ms, 9.1%) is similar to that of the N1 latency of dSSEPs (=2.16ms/19.30ms, 11.2%).

The P2 potential of LEPs and CHEPs has been proposed for clinical investigation because it reflects “genuine” activation of the ACC, an integral brain region involved in the pain matrix (Mobascher, et al., 2009), compared to the N2 potential (Valeriani, et al., 2002; Valeriani, et al., 2007). Our findings indicate two additional advantages: 1) P2 is subject to less variability between examinations than N2, and 2) P2 can be measured in conjunction with the N1 latency of dSSEPs to resolve a similar change in latency. However, the mean difference between examinations in P2 latency of C8 CHEPs suggests that dermatomal characteristics, including: receptor density, skin thickness, and/or peripheral conduction properties can influence test-retest reliability (Cruccu, et al., 2000; Granovsky, et al., 2005; Iannetti, et al., 2006; Valeriani, et al., 2007; Granovsky, et al., 2008). Any of these factors, as they relate to the C8 dermatome, in part may explain why C8 CHEPs were smaller in peak-to-peak amplitude and stimulation was perceived as being less intense than the proximal dermatomes examined (C4 and C5). Whereas subjects and dermatomes that perceive contact heat stimulation as being more intense may be less affected by peripheral sensory habituation (Greffrath, et al., 2007) and/or changes in attention, these factors may introduce a greater source of N2P2 variability in dermatomes that perceive contact heat stimulation as less intense (i.e. C8). To investigate if the changes in the rating of intensity or peak-to-peak amplitude contributed to the difference in P2 latency between examinations, a post-hoc linear regression analysis was performed for C8 CHEPs. P2 latency changes
were significantly correlated with a change in NRS (adjusted $R^2=0.33$, $p=0.012$). Based on this finding, using stimulation temperatures that elicit a more intense sensation would improve the reliability of measuring the P2 latency of C8 CHEPs.

Since latency represents a less variable measure of spinal conduction (for review see Cruccu, et al., 2008), amplitude is largely considered a secondary parameter in the evaluation of sensory evoked potentials. Based on all cervical dermatomes, ±14.96µV, or 47.4% of the mean CHEPs peak-to-peak amplitude (31.57µV), represents a threshold for detecting a statistically meaningful change (95% confidence). The use of amplitude to measure conduction in the dorsal column represents a challenge for dSSEPs, particularly since the mean N1P1 difference between examinations was significantly different from zero for both C6 and C8 dermatomes. Even for C4 and C5 dermatomes, the coefficient of repeatability (±1.2µV) accounts for 95.2% of the mean N1P1 amplitude. N1P1 amplitude of dSSEPs are subject to other forms of within-subject variability (i.e. right-left differences, Slimp, et al., 1992). By way of comparison with dSSEPs, CHEPs may be better suited to track changes in amplitude.

It was important to address the reliability of CHEPs in individuals with SCI separately from neurologically healthy subjects in order to determine whether damage in the spinothalamic tract renders latency and amplitude measures more variable over time (i.e. test-retest). In general, the same parameters that were most reliable on test-retest analysis of cervical CHEPs (e.g. P2 latency and N2P2 amplitude) in neurologically healthy subjects also appear reliable in individuals with SCI. The N2 latency of C6 was highly variable among individuals with SCI (±390ms), likely owing to difficulty interpreting the N2 in the presence of a low signal to noise ratio signal. Similar to neurologically healthy
subjects, problems recording CHEPs from C6 and C8 were encountered. Most notably, CHEPs could only be recorded at one examination time-point in a number of subjects. The clinical significance of the variability observed in neurologically healthy subjects among individuals with reduced thermal sensation due to SCI is illustrated by this finding.

4.4.2. Study limitations

A larger sample of individuals with SCI will be required to make a definitive conclusion with regards to the test-retest reliability of the N2P2 in affected dermatomes caudal to their level of injury, particularly if CHEPs are going to be used to measure changes over time (i.e. longitudinal study). Previous studies that used LEPs to characterize spinal cord lesions have stimulated the dorsum of the back in order to reduce the variable effects on N2P2 amplitude associated with examining different peripheral dermatomes (i.e. increasing peripheral conduction distance and differences in receptor density or skin thickness) (Iannetti, et al., 2001; Wydenkeller, et al., 2008). The presently adopted stimulating method is likely more accurate at isolating specific cervical spinal segments, since there is greater dermatomal overlap observed at the midline of the back (Foerster, 1933). A challenge of the current contact heat technologies is that peak stimulation could not be adjusted to overcome the changes in perception related to increasing peripheral distance and differences in the skin (i.e. thickness and/or receptor), in order to match a maximum perceived rating of intensity across all cervical dermatomes. Indeed, neurologically healthy subjects rarely reported that maximum thermal stimulus intensity at C8 was comparable to that well tolerated and perceived at C4-C6. Future studies should
focus on implementing novel approaches to overcome decreased sensitivity due to increased peripheral distance.

Lastly, the acquisition of the shorter latency contralateral CHEPs/LEP N1 potential reflects a more direct somatosensory response to thermal and radiant heat, and may be preferred for some clinical applications (Valeriani, et al., 2007; Cruccu, et al., 2008). However, given that N1 may not be recordable even in healthy subjects, most clinical studies using CHEPs and LEPs have focused on the analysis of N2P2 (Cruccu, et al., 2008). Recently, N2P2 of LEPs was suggested as characterizing the saliency of the stimulation rather than the primary processing of the pain stimulus (Wang, et al., 2010). Although the vertex recorded N2P2 may reflect secondary processing of the ascending stimulus unspecific to the sensory modality per se, a segment-by-segment detection of spinal lesions based on the absence or change in latency and amplitude may be useful for monitoring clinical outcomes.

4.4.3. Conclusions

The validity of N2P2 CHEPs to examine the spinothalamic tract from cervical dermatomes is supported by the test-retest findings reported here for neurologically healthy subjects. CHEPs represent a reliable alternative and/or complementary approach to dSSEPs, and may be better suited to measure peak-to-peak amplitude. Based on the normal test-retest variability of CHEPs and dSSEPs latency parameters, subtle changes in latency can be similarly detected in the two major ascending sensory pathways (i.e. spinothalamic tract and dorsal column, respectively). However, methods should be
developed to improve the reliable acquisition of CHEPs in individuals with spinal cord injury.
5. Increased baseline temperature improves the acquisition of contact heat evoked potentials: Implications for spinal cord injury

The findings from the previous chapter demonstrated the test-rest reliability of CHEPs in neurologically healthy subjects and individuals with SCI. However, it was a concern that lower stimulation intensity may reduce the reliability of CHEPs, which could result in a misleading diagnosis in individuals with SCI. Therefore, in the present chapter, novel methods to improve the acquisition of CHEPs were developed. Specifically, this focused on a method to increase the intensity of CHEPs by increasing the baseline temperature from 35°C to 42-45°C. The mechanism underlying changes in the intensity by adopting different baseline temperatures was investigated in neurologically healthy subjects and individuals with SCI.

5.1. Introduction

A central neurophysiological readout of small diameter, thinly myelinated afferents can be achieved by recording cortical sensory evoked potentials (SEPs) following noxious stimulation in the periphery. To date, the majority of clinical studies, including those investigating sensory impairments due to lesions in the spinal cord (Kakigi, et al., 1991; Treede, et al., 1991; Iannetti, et al., 2001; Quante, et al., 2007; Hatem, et al., 2010), have focused on radiant heat stimulation and the acquisition of LEPs. Based on similar estimates of conduction velocity (Cruccu, et al., 2000; Iannetti, et al., 2003; Wydenkeller, et al., 2008) and shared patterns of cortical activation (Valeriani, et al., 2000; Valeriani, et al., 2002),
CHEPs have emerged as a viable alternative to LEPs. Although CHEP outcomes have been extensively characterized in neurologically healthy subjects (Chen, et al., 2001; Chen, et al., 2002; Le, et al., 2002; Valeriani, et al., 2002; Granovsky, et al., 2005; Chen, et al., 2006; Iannetti, et al., 2006; Chao, et al., 2007; Greffrath, et al., 2007; Truini, et al., 2007; Granovsky, et al., 2008; Roberts, et al., 2008a; Warbrick, et al., 2009), only recently have they been applied to clinical populations (Atherton, et al., 2007; Chao, et al., 2008; Schestatsky, et al., 2008; Wydenkeller, et al., 2009; Xu, et al., 2009; Chao, et al., 2010; Casanova-Molla, et al., 2011; Suttrup, et al., 2011).

The relationship between noxious radiant and thermal heat stimulation parameters and objective measures of pain have been extensively studied. Pertovaara and colleagues (1999) applied contact heat stimulation of the skin surface in animals or humans to a peak temperature of 54°C at a rate of 10°C/s from different “adapting” or baseline temperatures (25°C, 30°C, and 35°C) (Pertovaara, 1999). In animals, the number of impulses recorded in dorsal horn neurons significantly increased in response to 54°C stimulus when a higher baseline temperature was applied. These findings were largely supported in humans; higher baseline temperatures had the primary effect of increasing the rating of perceived intensity to the same peak temperature. These results were also in agreement with an earlier study, which had shown that increasing skin temperature reduced pain thresholds to radiant heat stimulation (Wertheimer and Ward, 1952). Technically limited by the slow rate of contact heat stimulation (10°C/s), neurophysiological evidence in humans that an increased baseline temperature might also have an effect on CHEPs was not provided (Pertovaara, 1999). However, LEP amplitude is reduced by interfering noxious warming and cooling (Kakigi and Shibasaki, 1992; Watanabe, et al., 1996), and innocuous cooling
(Nahra and Plaghki, 2005), presumably via descending control mechanisms (i.e. diffuse noxious inhibitory control). Recently, the rate of contact heat stimulation has been increased (up to 70°C/s), and the effects of increasing the starting baseline temperature can now be easily studied from a neurophysiological perspective.

According to the European Federation of Neurological Societies, CHEPs are not currently “supported by evidence-based studies that demonstrate their diagnostic value” (Cruccu, et al., 2010). Therefore, there is an emphasis on performing studies that validate contact heat stimulation and optimize the parameters for stimulation in clinical populations. The primary aim of this study was to systematically investigate the effects of increasing the baseline skin temperature for CHEP stimulation, including the parameters (i.e. latency and amplitude) of the prominent evoked vertex potential, N2P2. We hypothesized that increasing the baseline temperature would increase the subject rating of perceived intensity, as well as the peak-to-peak amplitude of CHEPs by increasing the target skin temperature and/or synchronizing the afferent volley (Kakigi and Shibasaki, 1992; Magerl and Treede, 1996; Iannetti, et al., 2004; Baumgartner, et al., 2005). Conventional and increased baseline CHEPs were also examined in individuals with chronic SCI in order to determine whether a change in the baseline temperature influenced the reliability for perceiving contact heat stimulation and/or altered evoked vertex potentials in dermatomes with diminished sensation.

5.2. Methods

8 neurologically intact subjects and 8 subjects with chronic, traumatic cervical SCI participated in this study. None of the healthy participants reported experiencing acute or
chronic pain; nor did they have any history of peripheral and/or central neurological disease or chronic pain. None of the SCI subjects reported at or below level neuropathic pain in the dermatome(s) examined at the time of CHEP acquisition.

5.2.1. Contact heat evoked potentials

According to the protocol described previously (Chapter Four), subjects underwent examination of CHEPs. Briefly, all testing was performed while subjects were lying quietly in the supine position. Scalp recording sites were thoroughly prepared with Nuprep (D. O. Weaver & Co, Aurora, CO) and silver-silver disc active (Cz) and reference (A1-A2) surface recording electrodes (10 mm) were placed firmly on the scalp with Elefix (Nihon Kohden, Tokyo, Japan), according to the standard 10-20 electrode configuration (Figure 4.1). A ground strap electrode was secured with even contact around the forearm on the stimulating side. The impedance of ground and scalp electrodes was maintained at <5 kΩ.

In the present study, three stimulation conditions were tested in neurologically healthy subjects: 1) 35°C baseline and 70°C/s contact heat stimulation, 2) 42°C baseline and 70°C/s contact heat stimulation, and 3) 42°C baseline with decreased rate (41°C/s) of the contact heat stimulation to match the duration to reach the peak temperature of condition 1 (illustrated in Figure 5.1). In preliminary testing, 49°C was tolerable to all subjects using 35°C or 42°C as the baseline temperature.
Figure 5.1 Contact heat stimulation conditions
The contact heat stimulation conditions employed in the present study. 1) Conventional baseline (35°C) and 70°C/s (red, dotted), 2) increased baseline (42°C) and 70°C/s (green, dotted), and 3) increased baseline (42°C) and decreased rate of stimulation (41°C/s) (blue, solid). Note: The stimulus duration of condition 1 and 3 is approximately the same and the rate of increase of condition 1 and 2 are the same.

Contact heat pulses were delivered to proximal and distal skin dermatome sites (C5 and C6, respectively) according to the ISNCSCI dermatomal map of key segmental sensory points (Figure 2.4). The order of the three conditions performed in neurologically healthy subjects described above was randomized for both sites. However, stimulation of C5 always preceded stimulation of C6. The inter-pulse interval between single heat pulses randomly varied from 8 to 10 seconds.

SCI subjects were examined with condition 1 and 2 only. In SCI subjects, the cervical dermatome examined depended on the neurological level of injury. Where possible, a dermatome rostral to the neurological level of injury (i.e. unaffected by SCI) was examined in conjunction with a dermatome caudal to the level of injury. The dermatome rostral to the
level of injury (i.e. unaffected) was always examined first. In dermatomes caudal to SCI, contact heat pulses (up to a maximum of 52°C) were delivered from baseline temperature of 35°C or from an increased baseline skin temperature of 45°C. SCI subjects were also examined for light touch and pinprick sensation according to the ISNCSCI (methods described above).

Similar to Chapter Four, during the acquisition of CHEPs, subjects were instructed to keep their eyes open in a fixed, neutral position for at least 2 seconds after perception to the stimulus (i.e. after the conclusion of the recording epoch). Subjects were instructed to rate the intensity of each contact heat pulse using a NRS approximately two seconds after perception to the stimulus (see Figure 4.2). Each recording trial consisted of 10-15 heat pulses. To avoid habituation (Greffrath, et al., 2007), the thermode was slightly repositioned after each stimulus, though always remained within the confined boundary of the targeted dermatome. C6 and C8 were examined on the dorsum aspect of the hand. Testing sessions lasted approximately 1 hour, including set-up, instructions, and acquisition of evoked vertex potentials.

CHEPs were sampled at 2kHz using a single channel pre-amplifier (20,000x, bandpass filter 0.25-300Hz, ALEA Solutions, Zurich, Switzerland) using the same recording device previously described in Chapter Four. Briefly, a 200ms pre-trigger period preceded signal acquisition (≥1000ms). Single trials that were not perceived (pain rating=0 or missing) or contaminated by artifact were excluded from subsequent off-line analysis. The N2P2 was visually detected based on an average CHEP for each dermatome, comprising the first 10 bandpass filtered (0.5-30Hz) recordings uncontaminated by artifact. All signals
were recorded in a LabView (National Instruments, Austin, TX, USA) based software package (ALEA Solutions, Zurich, Switzerland).

5.2.2. Statistics

The means (±SD) of the N2 and P2 latency (ms), as well as the N2P2 amplitude (µV) were determined for each stimulation condition in neurologically intact and SCI subjects. A linear mixed model was used to examine the main effect of the different stimulation conditions and interaction effect between the stimulation condition and site. Random subject effects were included in this model. N2 and P2 latencies from condition 2 were also standardized to condition 1 in order to control for the change in stimulation duration that accompanies increased baseline. 100ms (i.e. the theoretical difference in duration between conditions 1 and condition 2 measured at the skin-thermode interface) was added to N2 and P2 latencies of condition 2 CHEPs, and standardized N2 and P2 latency were reexamined for statistical differences between conditions. For SCI subjects, N2 and P2 latency and N2P2 amplitude of CHEPs recorded in both conditions (i.e. conventional and increased baseline) were examined using a paired t-test. Alpha was set at 0.05 and multiple comparisons were Bonferroni corrected.

5.3. Results

Neurologically intact subjects were on average 30.4±5.4 years of age (5 males, 3 females). The injury characteristics and demographics of the subjects with SCI (n=8) are shown in Table 5.1. The average age of subjects with SCI was 39.3±16.0 years.
5.3.1. Neurologically intact subjects

Representative CHEPs from each of the three conditions are illustrated in Figure 5.2. C5 CHEPs were recordable (i.e. N2P2 visually detected) in all subjects regardless of the stimulation condition. However, only when using the 42°C baseline and 70°C/s contact heat stimulation (i.e. condition 2) were C6 CHEPs recorded from all neurologically intact subjects. Although all subjects perceived conditions 1 (35°C baseline, 70°C/s) and 3 (42°C baseline, 41°C/s) contact heat stimulation, the C6 N2P2 was not always detected (condition 1, n=2; condition 3, n=1). The mean (±SD) latencies (N2 and P2) and amplitude (N2P2) of CHEPs from healthy subjects for the three experimental conditions are shown in Table 5.2.

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Gender</th>
<th>Etiology of SCI</th>
<th>Neurological Level of SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>M</td>
<td>MVA</td>
<td>C4</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>M</td>
<td>Fall</td>
<td>C5</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>M</td>
<td>Bicycle</td>
<td>C7</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>F</td>
<td>MVA</td>
<td>C5</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>M</td>
<td>Fall</td>
<td>C6</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>F</td>
<td>MVA</td>
<td>C6</td>
</tr>
<tr>
<td>7</td>
<td>45</td>
<td>M</td>
<td>Fall</td>
<td>C7</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>M</td>
<td>MVA</td>
<td>C7</td>
</tr>
</tbody>
</table>

Table 5.1 Spinal cord injury characteristics and demographics
Figure 5.2 Representative contact heat evoked potentials

10 superimposed single C6 CHEPs from a neurologically intact female subject. Condition 2 (42°C baseline and 70°C/s) was rated the highest (NRS=7.2), followed by conditions 3 (42°C baseline and 41°C/s, NRS=7.0) and 1 (35°C baseline and 70°C, NRS=6.8). The prominent N2 and P2 potentials are marked for each condition. Black line marks the end of the pre-trigger period and the beginning of the recording epoch.
Table 5.2 Summary of contact heat evoked potential findings in neurologically intact subjects

<table>
<thead>
<tr>
<th>CHEPs parameter</th>
<th>35°C, 70°C/s (Condition 1)</th>
<th>42°C, 70°C/s (Condition 2)</th>
<th>42°C, 41°C/s (Condition 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C5</td>
<td>C6</td>
<td>C5</td>
</tr>
<tr>
<td>NRS</td>
<td>Mean</td>
<td>5.60</td>
<td>4.39</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.44</td>
<td>2.11</td>
</tr>
<tr>
<td>N2 latency (ms)</td>
<td>Mean</td>
<td>401.62</td>
<td>432.90</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>56.02</td>
<td>104.03</td>
</tr>
<tr>
<td>P2 latency (ms)</td>
<td>Mean</td>
<td>519.69</td>
<td>540.90</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>32.71</td>
<td>82.93</td>
</tr>
<tr>
<td>N2P2 amplitude (µV)</td>
<td>Mean</td>
<td>35.88</td>
<td>27.86</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>11.70</td>
<td>19.91</td>
</tr>
</tbody>
</table>

NRS, numerical rating scale

5.3.1.1. Main effects

5.3.1.1.1. Latency

There was a significant main effect of stimulation condition on unstandardized N2 and P2 latency (F=29.30, p<0.001 and F=47.78, p<0.001). Pair-wise comparisons were significantly different for the second condition (42°C baseline, 70°C/s) compared to the first (35°C baseline, 70°C/s, p<0.001) and third conditions (42°C baseline, 41°C/s, p<0.01). However, there was no significant main effect when N2 and P2 latencies from condition 2 were standardized to condition 1 (F=1.11, p=0.344 and F=3.01, p=0.065; Figure 5.3).

5.3.1.1.2. Amplitude and numerical rating

There was also a significant main effect of stimulation condition on N2P2 amplitude (F=31.12, p=0.001) and rating of perceived intensity (F=14.54, p<0.001). All pair-wise comparisons of N2P2 amplitude for stimulation condition were significant (condition 2 > condition 1, p<0.001; condition 2 > condition 3, p<0.001; condition 1 > condition 3,
p<0.032; Figure 5.3). Conditions 2 and 3 were rated significantly higher than condition 1 (p<0.001 and p=0.01, respectively; Figure 5.3).

**Figure 5.3 Contact heat evoked potential latency, amplitude and rating of perceived intensity by stimulation condition**

Standardized N2 and P2 latency, N2P2 amplitude and NRS for the three contact heat stimulation conditions in neurologically intact subjects (C5, black; C6, grey). Significant pair-wise comparisons are marked. Error bars represent ± SD.

### 5.3.1.2. Interaction effects

There were no significant interaction effects between the stimulation site and stimulation condition for any of the evoked potential parameters (standardized and unstandardized N2 and P2 latency, and N2P2 amplitude) or rating of perceived intensity.
5.3.2. Individuals with spinal cord injury

5.3.2.1. Dermatomes unaffected by spinal cord injury

The CHEP outcomes from SCI subjects are shown in Table 5.3. 7/8 SCI subjects had CHEPs recorded in both conditions in dermatomes with normal pinprick sensation at or caudal to the level of injury, and thus were available for statistical comparison. Similar to the neurologically intact subjects, with increased baseline contact heat stimulation N2 and P2 latency significantly decreased (t=9.53, p<0.001 and t=5.67, p=0.001, respectively), and N2P2 amplitude and the rating of perceived intensity significantly increased (t=2.72, p=0.03 and t=2.81, p=0.031, respectively). Standardized N2 and P2 latency was not significantly different between the two conditions (p=0.089 and p=0.352, respectively).

5.3.2.2. Dermatomes affected by spinal cord injury

There were three outcomes from examining conventional and increased baseline conditions in dermatomes affected by SCI, caudal to the injury. First, contact heat stimulation delivered from either baseline condition was not perceived and CHEPs were not recorded (Subjects 1, 5, 6, and 7, Subject 1 shown in Figure 5.4). Second, contact heat stimulation was only perceived when the baseline temperature was increased from 35°C to 45°C (Subjects 2 and 4). In both Subjects, CHEPs could only be recorded when the baseline temperature was increased (Subject 2 shown in Figure 5.4). Thirdly, contact heat stimulation at both baseline temperatures was perceived; however CHEPs could only be recorded when the baseline temperature was increased (Subjects 3 and 8, Subject 3 shown in Figure 5.4).
<table>
<thead>
<tr>
<th>SCI ID</th>
<th>Site</th>
<th>PP</th>
<th>Baseline temperature</th>
<th>35°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NRS</td>
<td>Amplitude (µV)</td>
<td>Latency (ms)</td>
</tr>
<tr>
<td>1</td>
<td>C5</td>
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<td>0.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
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<td>2.0</td>
<td>31.1</td>
<td>491.5</td>
</tr>
<tr>
<td></td>
<td>C6</td>
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<td>0.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>C8</td>
<td>0</td>
<td>0.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td></td>
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<td>C5</td>
<td>2</td>
<td>4.8</td>
<td>17.7</td>
<td>455.0</td>
</tr>
<tr>
<td></td>
<td>C8</td>
<td>1</td>
<td>2.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

PP, pinprick (0-absent, 1-impaired, 2-normal)
-, not recordable (abolished)

Table 5.3 Contact heat evoked potentials in individuals with spinal cord injury
Figure 5.4 Contact heat evoked potentials in individuals with spinal cord injury
Conventional and increased baseline conditions (35°C baseline and 70°C, and 45°C baseline and 70°C/s, respectively). N2P2 is outlined (dotted line) where it could be visually detected. Refer to Table 5.3 for rating of perceived intensity. Black line marks the end of the pre-trigger period and the beginning of the recording epoch.
5.4. **Discussion**

Increasing the baseline temperature of contact heat stimulation enhances the acquisition of cortical evoked potentials (i.e. larger N2P2, peak-to-peak amplitude). By adopting an increased baseline temperature in subjects with SCI, contact heat stimulation generated reliable cortical evoked potentials in some dermatomes that were otherwise insensitive to pinprick stimuli (ISNCSCI) or contact heat stimulation from the conventional baseline temperature of 35°C. Therefore, adjusting the baseline temperature of contact heat stimulation may improve the sensitivity of CHEPs to detect afferent sparing in nociceptive pathways after SCI (e.g. spinothalamic tract).

5.4.1. **Mechanisms underlying the effect of increased baseline contact heat evoked potentials**

The influence of skin temperature on pinprick thresholds in response to radiant heat stimulation has long been established (Wertheimer and Ward, 1952; Pertovaara, 1999) and has also been more recently been investigated by examining LEPs (Nahra and Plaghki, 2005). Since increasing the skin temperature is expected to reduce the pain threshold, it is not surprising that adjusting the baseline temperature from 35°C to 42°C, thereby increasing the skin temperature, increased the rating of perceived intensity and amplitude of CHEPs to the same peak temperature (49°C). The findings from the present study suggest that this may be explained by a combination of two factors. Firstly, the temperature gradient at skin-thermode interface and nociceptors may be affected by an increased baseline temperature. Only a fraction of the nominal peak contact heat stimulation measured at the skin-thermode interface (i.e. 49°C in the present study) has
been reported to ultimately reach the level of the nociceptors (Magerl and Treede, 1996; Baumgartner, et al., 2005). Therefore, delivering contact heat stimulation from a 35°C baseline likely only achieved a peak temperature equivalent to the baseline temperature of conditions 2 and 3 (i.e. 42°C), whereas conditions 2 and 3 would presumably result in much higher peak temperatures. Increasing the baseline temperature would then have the end result of activating a greater number of noxious afferents in the periphery, which in turn increases the rating of perceived intensity (i.e. both conditions 2 and 3) and yields larger cortical evoked potentials (i.e. condition 2 only). Adjusting the baseline temperature from 35°C to 42-45°C would then be analogous to increasing the peak temperature to obtain a higher rating of perceived stimulation intensity.

Secondly, shortening the stimulus duration of contact heat stimulation may have led to improved spatial and temporal summation of nociceptive afferent impulses, and thus generated a more synchronized afferent volley. Shorter duration laser stimulation (20 compared to 2ms) has been postulated to decrease activation times of nociceptors and result in shorter latency and larger amplitude evoked potentials (Iannetti, et al., 2004). Interestingly, a significant effect was not reported for LEP N2P2 amplitude in this study, which was perhaps undetected because of the relatively small change in stimulus duration (i.e. 18ms compared to 100ms in the present study). We attempted to differentiate between the effects of reaching higher peak skin temperature and synchronizing the afferent volley using a shorter stimulus duration by comparing the conventional (35°C) and increased baseline (42°C) conditions to a distinct third condition (Figure 5.1). Condition 3 was designed to match the baseline temperature of condition 2 (42°C), but have the contact heat stimulation duration of condition 1. This was achieved by decreasing the rate of
temperature change from 70°C/s to 41°C/s. Although both increased baseline conditions (2 and 3) resulted in higher ratings of perceived intensity compared to the conventional baseline stimulation (35°C, condition 1), only the N2P2 amplitude of condition 2 CHEPs (42°C, 70°C/s) significantly increased. Therefore, the effects on N2P2 amplitude appear dependent on the stimulus duration being shortened (~100ms) by the increased baseline. Since the slope of the temperature ramp remained the same between conditions 1 (35°C, 70°C/s) and 2 (42°C, 70°C/s), increasing the baseline may have had the effect of conditioning the test stimulus. Kakigi and Shibasaka (1992) postulated that increased amplitude of LEPs during noxious cooling could be explained by an increase in “the excitability of the appropriate dorsal horn neurons and then that particular phenomenon might have the effects of increasing the synchronicity of the volley” (Kakigi and Shibasaki, 1992). A similar phenomenon may account for our observations during innocuous warming, particularly since the same afferents that were activated with the baseline warming (42°C) were also activated with the test stimulus (49°C).

In general, these findings are in accordance with previous studies demonstrating that higher baseline temperatures increase the rating of perceived intensity (Pertovaara, 1999). However, Pertovaara and colleagues (1999) also reported decreased latency of evoked responses in neurons in the dorsal horn with increasing baseline contact heat stimulation, even after correcting for the change in stimulus duration. This is not confirmed by examining vertex CHEPs in humans. Rather, the decrease in N2 and P2 latency following contact heat stimulation from an increased baseline does not appear any greater than what can be explained by the shortening the stimulus duration. This suggests that the decreased
N2 and P2 latency accompanying increased baseline temperature contact heat stimulation is chiefly related to nociceptor afferents reaching their activation threshold faster.

### 5.4.2. Contact heat evoked potentials and spinal cord injury

Neurophysiological measurements have been proposed to improve the sensitivity of clinical sensory testing methods (e.g. light touch and pinprick) after SCI by: 1) detecting subtle deficits in “normal” dermatomes and afferent sparing in “absent” dermatomes and 2) differentiating between dermatomes with “impaired” sensation (Steeves, et al., 2007). In the present study, the combination of using a conventional and increased baseline protocol was useful to highlight the potential of CHEPs to achieve these goals (Figure 5.5). Perhaps most importantly, increasing the baseline temperature revealed neurophysiological evidence of sensory sparing from dermatomes that would have been judged to be completely de-afferented using conventional baseline contact heat stimulation or examining pinprick sensation (e.g. subject 2). The most parsimonious explanation for why an increased baseline skin temperature facilitated the acquisition CHEPs after SCI is that a larger number of nociceptive afferents were recruited, which was sufficient to activate the residual spared fibers within central pathways (e.g. spinothalamic tract).
Figure 5.5 Flow chart of clinical and contact heat evoked potential outcomes
The sensory profiles are based on clinical pinprick testing and the preservation or abolishment of CHEPs.

5.4.3. Study limitations and future directions

It is important to consider that this study also highlights a known limitation of contact heat stimulation, namely that the peak stimulus temperature, measured at the thermode-skin interface, does not necessarily reflect the temperature achieved at the level of the skin (Magerl and Treede, 1996; Baumgartner, et al., 2005), let alone at the nociceptors located some 150μm below the skin’s surface (Tillman, et al., 1995). This discrepancy between the peak temperature delivered and the peak temperature achieved at the various levels (i.e. skin and nociceptors) is particularly problematic when faster rates of stimulation are applied (i.e. those used to record cortical evoked potentials, 70°C/s) (Magerl and Treede, 1996; Baumgartner, et al., 2005). Although we did not measure the peak temperature of the skin during contact heat stimulation in the present study, we suggest that increased baseline temperature contact heat stimulation facilitates the skin
reaching a higher critical threshold for the activation of small diameter afferents, which is closer to the theoretical target temperature measured at the skin-thermode interface.

This study has focused on the effect of increasing baseline temperature on the vertex N2P2 of CHEPs, rather than the earlier contralateral N1 component. As was previously discussed in Chapter Four, a number of recent studies indicate that the N2P2 is not a direct measure of pain, but rather of stimulation saliency (Iannetti, et al., 2008; Mouraux and Iannetti, 2009; Wang, et al., 2010). In the context of examining the level and severity of SCI in an alert patient, this distinction may not be as important, primarily because CHEPs are being used to measure the severity of damage in the spinothalamic tract, not as a direct measure of pain per se. Regardless, future pain studies should consider the value of increasing the skin baseline temperature for CHEPs recordings, when acquisition of the earlier N1 component is important.

Ultimately, the discussion of which nociceptive parameters are most appropriate for clinical applications, including whether laser or contact heat stimulation is more suitable for the acquisition of evoked potentials, will depend on the relationships established with other functional (perceptual or behavioral) correlates of disease. To our knowledge, CHEPs and LEPs have only been directly examined in the same patient population and study once (Casanova-Molla, et al., 2011). Casanova-Molla and colleagues (2011) reported in patients with painful distal neuropathy that CHEPs were more likely to be preserved and more closely related to epidermal nerve fiber density than LEPs (Casanova-Molla, et al., 2011). Future studies should continue to address differences between noxious stimulation parameters in clinical populations in order to validate different approaches for a variety of sensory deficits.
5.4.4. Conclusion

In summary, we have provided evidence that: 1) increasing the baseline skin temperature for contact heat stimulation enhances the acquisition of cortical evoked potentials, and that this effect is, in part, attributed to shortening the stimulus duration, and 2) variations in the baseline skin temperature can improve the resolution of CHEPs to more accurately measure sensory deficits or sensory sparing in patient populations with abnormal or diminished sensation due to damage in the spinal cord.

The focus of the previous chapters has been on the examination of sensory function (ISNCSCI light touch and pinprick, dSSEPs/EPT, and CHEPs, Chapters Two, Three, and Four/Five, respectively). To conclude the thesis, this research chapter turns to the evaluation of motor function after cervical sensorimotor complete SCI (ISNCSCI). Motor function is important to consider as it can be most readily correlated with performance of ADL. Two large databases, including the EM-SCI which was described in Chapter Two, were examined, with a specific interest in describing the effect of motor score recovery on changes in motor level. An important component of this chapter links functional independence (i.e. SCIM) to changes in motor scores and motor levels.

6.1. Introduction

The International Campaign for Cures of spinal cord Injury Paralysis recently provided support to a panel tasked with reviewing past SCI clinical studies and making recommendations on the conduct of future trials. To date, four integrated SCI guidelines papers have been published addressing: spontaneous neurological recovery (Fawcett, et al., 2007), clinical trial outcome measures (Steeves, et al., 2007), inclusion/exclusion and ethical criteria (Tuszynski, et al., 2007), and clinical trial design (Lammertse, et al., 2007b). Of primary interest in early phase SCI studies are the selection of appropriate participants and the use of sensitive outcomes to track changing sensorimotor function. The latter is
particularly important for individuals with sensorimotor complete cervical SCI, who represent a priority population for early phase trials given the high incidence for such spinal injuries and considerable injury-related disability.

With additional support of the Spinal Cord Outcomes Partnership Endeavour, the present analysis focused on changes in spontaneous motor activity during the first year after sensorimotor complete (AIS-A) cervical (C4-C7) SCI. In collaboration with members of the EM-SCI, the original data from the Sygen multi-centre trial (Geisler, et al., 2001b) was re-analyzed in conjunction with the more recent data from the EM-SCI study (www.emsci.org). The purpose of this retrospective analysis of the Sygen and EM-SCI databases was to build on the existing knowledge of spontaneous recovery after SCI (Mange, et al., 1990; Waters, et al., 1993; Waters, et al., 1994; Marino, et al., 1999; Ditunno, Jr., et al., 2000a; Fawcett, et al., 2007; Curt, et al., 2008) by examining: 1) the extent of AIS grade conversions, 2) recovery of UEMS, 3) changes in cervical motor level, and 4) the degree of association between these measures. Additionally, using the EM-SCI database only, changes in SCIM during spontaneous recovery were examined relative to changes in motor level and UEMS (note: SCIM not available for the Sygen database). This data provides an opportunity for a more in-depth evaluation of UEMS and/or motor level as effective clinical endpoints in early phase SCI studies.

6.2. Methods

Using the Sygen and EM-SCI databases, a retrospective analysis was undertaken of AIS grade, upper extremity motor score (UEMS), and motor level during spontaneous recovery after sensorimotor complete cervical (C4-C7) SCI according to the revised 2002
ISNCSCI assessment guidelines. Briefly, AIS grade and motor scores from the key muscles for the upper extremities (C5-T1) were determined at baseline (within 1-week after SCI) and for all subsequent AIS examinations performed over the first year after SCI. Since the original analysis of the Sygen data, there have been revisions to the ISNCSCI assessment for determining AIS grade and motor level. To facilitate comparisons with the more recent EM-SCI data, the original data from the Sygen study was re-scored. For a definition of motor level and examples of how changes in cervical motor score impacts motor level determination during recovery, see Table 6.1. SCIM scores were also examined during spontaneous recovery in the EM-SCI database.

<table>
<thead>
<tr>
<th>Change in Motor Level</th>
<th>Initial motor scores and motor level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C5</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Examples of subsequent motor scores that could change motor level (by definition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>-1</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>+1</td>
</tr>
<tr>
<td>+2</td>
</tr>
</tbody>
</table>

*, motor level is defined as most caudal spinal level as indexed by the key muscle group for that segment having a muscle strength of 3/5 or above while key muscle for spinal segment, immediately above, is normal (5/5). In these examples, the initial sensory level is C5 and does not change.

Table 6.1 Example of motor level changes after cervical spinal cord injury
6.2.1. Original sources of data

6.2.1.1. European Multi-Center Study about Spinal Cord Injury

For a description of the EM-SCI database, and to avoid repetition here, please refer to Chapter Two.

6.2.1.2. Sygen

The Sygen database (initial enrollment = 797, followed = 760) comprises traumatic SCI individuals enrolled between 1992 and 1997 in a randomized, double-blind, multi-center study to examine the therapeutic benefits of monosialotetrahexosylganglioside (GM-1) therapy (Geisler, et al., 2001b). To measure primary efficacy, the proportion of individuals recovering two Modified Benzel Grades (i.e. “marked recovery”) in the GM-1 treatment groups (low and high dose) were compared to the placebo control group. Initial assessments were conducted within 72 hours of SCI to a clinical endpoint of 26 weeks after SCI. Interim and follow-up AIS examinations were performed at 4, 8, 16 and 52 weeks after SCI. There was no significant difference in “marked recovery” in either of the GM-1 treatment groups at 26 or 52 weeks when compared to the placebo control group, and thus failed to reach the primary endpoint.

6.2.2. Statistical analysis

6.2.2.1. Inclusion criteria

All individuals in the Sygen and EM-SCI databases with sensorimotor complete (AIS-A) SCI and a motor level of C4-C7 at the baseline assessment (~1 week from SCI) were included in the present analysis. C4 motor level SCI was determined based on the normal
preservation of sensory function on the C4 dermatome. Individuals with an initial motor level of C8 or T1 were not included a priori since recovery of multiple motor levels could not be tracked. Since the recovery of UEMS and motor level were not primary endpoints in the Sygen study, a preliminary statistical analysis (described below) was performed to assess possible differences between treatment groups and the placebo control. No significant differences in the recovery of UEMS or the proportion of individuals (C5-C7) changing motor levels from baseline to any of the follow-up time-points was observed in the treatment groups compared to the placebo control group. Individuals with SCIM II and III scores were included in the EM-SCI database in order to determine the relationship between functional changes and motor (score and level) recovery. The Sygen database does not include SCIM scores.

**6.2.2.2. Statistical procedures**

For each initial motor level of SCI (C4, C5, C6, and C7), the statistical analysis of AIS grade, motor level (right and left side), UEMS (right, left, and sum of both sides), and SCIM (total and self-care subscore) was performed, separately for each database. An analysis of UEMS and motor level changes was also performed for the combined group of C5-C7 SCI patients; once again for each database and from baseline to different time-points over the first year after SCI. Individuals with C4 motor level SCI were analyzed separately for motor level and UEMS changes since there is no key muscle delineating the C4 spinal segment and it is difficult to reliably track deterioration of an initial C4 motor level. Individuals with C4 motor level SCI were however included in the analysis of combined motor level AIS grade
conversion from baseline. All statistical analyses were conducted in SPSS (SPSS, Inc., v. 16) and R (R foundation for Statistical Computing, v. 2.10.1).

Comparisons between databases for the proportion of individuals converting an AIS grade or changing motor levels were performed at 4 weeks (EM-SCI and Sygen), 8 (Sygen) and 12 weeks (EM-SCI), 12 (EM-SCI) and 16 weeks (Sygen), 24 (EM-SCI) and 26 weeks (Sygen), and 50 (EM-SCI and 52 (Sygen) weeks after cervical SCI (chi-square test, $X^2$). The distribution of UEMS at baseline and at follow-up time-points was examined for each initial cervical motor level of SCI. The normality of the UEMS distribution appeared to some extent dependent on the initial motor level of SCI and/or the time-point examined. First, UEMS at early time-points ($\leq$12 weeks) was less normally distributed than at later assessment times. Second, initial C4 and C7 motor levels of SCI were also less normally distributed than baseline C5 and C6 motor levels of SCI.

Linear mixed effects models account for fixed and random subject effects by describing both the behavior of the entire sample related to a specific “fixed” variable (e.g. time), and those “random” subject variables (e.g. total UEMS) specifically associated with an individual (Pinheiro and Bates, 2000). In the present study, linear mixed effects models were used to analyze repeated measures of UEMS within each database and to compare spontaneous UEMS recovery between databases. In both statistical analyses, the main effect on UEMS (time) was modeled separately for each initial motor level (C4 to C7). Random subject effects were modeled with repeated measure correlated variables (i.e. diagonal repeated measures covariance structure). To compare spontaneous recovery between databases, UEMS was modeled with the natural-log of time. This process
transforms an exponential (i.e. motor recovery after SCI) to a linear function, thereby facilitating linear modeling. The slopes and intercepts of linear UEMS recovery were then compared between the same initial motor levels across databases. The slopes of UEMS recovery were further analyzed within databases, between different initial levels of cervical SCI. To account for the ordinal ranking of UEMS and non-normal distribution at early time-points and within different motor levels, UEMS in the first year after SCI was further examined by non-parametric statistics (between databases, Mann-Whitney U; within databases, Friedman test and post-hoc Wilcoxon signed rank sum test).

The effect of initial motor level, AIS grade conversion and motor level changes on UEMS recovery at one year after SCI were examined by step-wise linear regression using combined datasets and combined cervical motor levels (C5-C7). Furthermore, the effect of AIS grade conversion on motor level changes was examined by ordinal regression (logit link function). SCIM total and SCIM self-care changes between the 1 and 50 week time-point in the EM-SCI database were compared for different numbers of motor levels recovered (0, 1, or ≥2) (Kruskal-Wallis test). Alpha (α) was set at 0.05, and all multiple pair-wise comparisons were Bonferroni corrected (including multiple comparisons of time within each database for the analysis of initial motor level within a database).

6.3. Results

6.3.1. Subject demographics and spinal cord injury characteristics

While the ratio of males to females was similar in both databases (Sygen, M=79.5; EM-SCI, M=78.8%), EM-SCI individuals were older (43.7±18.7 years) at the time of injury compared to individuals in the Sygen database (31.2±12.5 years). The total number of
individuals from both databases with cervical AIS-A SCI (C4-C8) at baseline was 426. The number of individuals for each initial cervical motor level, at each assessment time-point, is shown in Table 6.2.

6.3.2. Grade conversion

The proportion of individuals converting from sensorimotor complete cervical SCI (C4-C7, AIS A) to incomplete SCI (≥AIS B) was generally similar between databases, and only significantly different for the 8 (Sygen) and 12 week (EM-SCI) comparisons ($X^2=8.34$, $p=0.003$, see Table 6.3). In addition, at 16 weeks after SCI and only in the Sygen database was the proportion of individuals spontaneously converting from complete to incomplete SCI different between initial motor levels of SCI ($X^2=15.24$, $p=0.002$). At this same time-point, 58.8% of (10 of 17) individuals with a C7 motor level SCI had converted to an incomplete AIS impairment grade, which is in contrast to the 15.2% of C4, 23.1% of C5, and 28.6% for C6 motor levels.
### 6.3.3. Motor level recovery

The initial cervical motor level of SCI (C5-C7) indicated no preference for the right-side (R) or left-side (L) of the cord and was symmetrical in 79.6% and 76.1% of individuals in the EM-SCI and Sygen databases, respectively. The proportion of individuals with C5-C7 AIS-A SCI deteriorating one or more motor levels (≤-1), having a stable motor level (0), or recovering motor levels (+1 or ≥+2) on the right side is shown in Figure 6.1.

<table>
<thead>
<tr>
<th>Initial right-side motor level</th>
<th>Estimated marginal</th>
<th>Sygen (n=286)</th>
<th>EM-SCI (n=140)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Time after SCI (weeks)</td>
<td>Time after SCI (weeks)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td>2.9</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>65</td>
<td>53</td>
</tr>
<tr>
<td>C5</td>
<td>Mean</td>
<td>14.7</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>154</td>
<td>140</td>
</tr>
<tr>
<td>C6</td>
<td>Mean</td>
<td>23.0</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>48</td>
<td>42</td>
</tr>
<tr>
<td>C7</td>
<td>Mean</td>
<td>32.9</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

SE, standard error

**Table 6.2 Upper extremity motor score during spontaneous recovery from sensorimotor complete cervical spinal cord injury**

Note: Repeated measures, linear mixed model findings.
### Table 6.3 Percentage of individuals converted from sensorimotor complete to incomplete spinal cord injury

<table>
<thead>
<tr>
<th>Time after SCI (weeks)</th>
<th>Sygen %</th>
<th>EM-SCI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (Sygen/EM-SCI)</td>
<td>14.5</td>
<td>19.2</td>
</tr>
<tr>
<td>8/12 (Sygen/EM-SCI)</td>
<td>20.7</td>
<td>32.8*</td>
</tr>
<tr>
<td>12/16 (EM-SCI/Sygen)</td>
<td>26.3**</td>
<td>32.8</td>
</tr>
<tr>
<td>24/26 (EM-SCI/Sygen)</td>
<td>30.4</td>
<td>34.7</td>
</tr>
<tr>
<td>50/52 (EM-SCI/Sygen)</td>
<td>30.2</td>
<td>32.9</td>
</tr>
</tbody>
</table>

*p=0.003, between databases

**p=0.002, between initial motor levels of (C4-C7), Sygen database only

As a function of the initial cervical motor level of SCI, the proportion of individuals remaining stable or spontaneously changing motor levels is shown in Table 6.4. In only the Sygen database, and only at 4 ($X^2=46.25, p<0.001$) and 8 weeks ($X^2=32.97, p<0.001$) after cervical SCI, was the proportion of individuals changing motor levels dependent on the initial motor level. This significant difference was transient and not present at later time-points. The proportion of individuals with an initial C4 motor level who subsequently change motor levels was not significantly different between databases at any time-point after SCI.
Figure 6.1 Proportion of individuals spontaneously deteriorating, remaining stable, or recovering a motor level

Initial C5-C7 motor level (right-side) spontaneously deteriorating, remaining stable, or gaining motor levels from baseline to different time-points over the first year after cervical sensorimotor complete (AIS-A) SCI. The percentage of individuals in each category of motor level change or stability at one year after SCI is displayed on the right. (A: Sygen; B: EM-SCI).
<table>
<thead>
<tr>
<th>Motor level changes</th>
<th>Time after SCI (weeks)</th>
<th>Sygen</th>
<th>EM-SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial right-side motor level</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>C5</td>
<td>C6</td>
</tr>
<tr>
<td>≤-1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>77.2</td>
<td>69.2</td>
<td>38.1</td>
</tr>
<tr>
<td>1</td>
<td>22.8</td>
<td>17.5</td>
<td>9.5</td>
</tr>
<tr>
<td>≥2</td>
<td>.0</td>
<td>7.0</td>
<td>7.1</td>
</tr>
<tr>
<td>≤-1 0</td>
<td>60.8</td>
<td>54.3</td>
<td>47.5</td>
</tr>
<tr>
<td>0</td>
<td>31.4</td>
<td>31.4</td>
<td>17.5</td>
</tr>
<tr>
<td>1</td>
<td>7.8</td>
<td>10.7</td>
<td>7.5</td>
</tr>
<tr>
<td>≥2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤-1 0</td>
<td>47.9</td>
<td>38.2</td>
<td>28.9</td>
</tr>
<tr>
<td>0</td>
<td>37.5</td>
<td>41.2</td>
<td>36.8</td>
</tr>
<tr>
<td>1</td>
<td>14.6</td>
<td>16.2</td>
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<tr>
<td>≥2</td>
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<tr>
<td>≤-1 0</td>
<td>37.3</td>
<td>30.2</td>
<td>43.6</td>
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<tr>
<td>0</td>
<td>51.0</td>
<td>42.4</td>
<td>23.1</td>
</tr>
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<td>11.8</td>
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<td>17.9</td>
</tr>
<tr>
<td>≥2</td>
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<td></td>
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<tr>
<td>≤-1 0</td>
<td>29.3</td>
<td>19.7</td>
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<td>0</td>
<td>43.9</td>
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</tr>
<tr>
<td>1</td>
<td>26.8</td>
<td>32.6</td>
<td>25.0</td>
</tr>
<tr>
<td>≥2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05, between initial motor levels of SCI (C5-C7), Sygen database only

Table 6.4 Proportion of individuals with spinal cord injury remaining stable or changing motor levels from baseline time-point
6.3.4. Upper extremity motor score

Based on the slopes and intercepts of the linear mixed models, there were no significant differences between databases in UEMS recovery for the same initial cervical motor level (right side motor level; see Table 6.5 for model parameters). In neither database was there a significant difference in the rate of UEMS recovery (slope of the linear model) based on the initial cervical motor level of SCI (all comparisons, p>0.05). For all of the comparative time-points, only UEMS recovery (combined C5-C7) between 1 and 12 weeks (EM-SCI) was significantly greater than 1 and 8 weeks (Sygen) (Mann-Whitney U, p=0.003). Similarly, UEMS recovery was significantly greater between 1 and 12 weeks in the EM-SCI database compared to 1 and 8 weeks in the Sygen database for individuals with C4 motor level SCI (Mann-Whitney U, p<0.01).

For each cervical motor level (C4, C5, C6 and C7), UEMS significantly increased from initial baseline to one year after SCI (p<0.001, Figure 6.2). In both databases, all pair-wise comparisons of UEMS (C5-C7) from baseline to later time-points were significant at each successive time-point up to 26 weeks (p<0.05; Table 6.6). However, only in the Sygen database did UEMS significantly increase between 26 and 52 weeks after SCI (p<0.001). UEMS significantly recovered from one week after SCI to approximately one year in both databases for all motor levels of SCI (Friedman’s test, p<0.01). For combined cervical motor levels (C5-C7) in both databases, UEMS significantly increased at each successive time-point up to one year after SCI (Wilcoxon rank, p<0.001).
<table>
<thead>
<tr>
<th>Initial right-side motor level</th>
<th>Sygen</th>
<th>EM-SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>slope $x + \text{intercept}$ (SE) (SE)</td>
<td>slope $x + \text{intercept}$ (SE) (SE)</td>
</tr>
<tr>
<td>C4</td>
<td>$2.78x + 1.91$ (0.16) (1.60)</td>
<td>$2.92x + 2.15$ (0.30) (1.78)</td>
</tr>
<tr>
<td>C5</td>
<td>$2.73x + 14.07$ (0.11) (0.94)</td>
<td>$3.07x + 13.35$ (0.27) (1.66)</td>
</tr>
<tr>
<td>C6</td>
<td>$2.13x + 21.40$ (0.21) (1.41)</td>
<td>$2.66x + 23.83$ (0.35) (2.15)</td>
</tr>
<tr>
<td>C7</td>
<td>$2.73x + 32.22$ (0.24) (1.62)</td>
<td>$2.98x + 31.09$ (0.46) (2.32)</td>
</tr>
</tbody>
</table>

$x$, natural log of time after SCI (weeks)

**Table 6.5 Linear mixed effects models of spontaneous upper extremity motor score recovery**
Figure 6.2 Upper extremity motor score spontaneous recovery
EM-SCI (grey) Sygen (black). Error bars, 95% confidence intervals.

<table>
<thead>
<tr>
<th>Database</th>
<th>Time after SCI (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Sygen</td>
<td>Mean Δ</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>EM-SCI</td>
<td>Mean Δ</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
</tbody>
</table>

SE, standard error

Table 6.6 Upper extremity motor score change from baseline to time-points during spontaneous recovery
Note: Combined C4-C7 motor levels
6.3.5. Relationship between outcomes

The motor score and motor level determination were completed independently for each side of the cervical spinal cord. Unilateral values from only the same side of the cord were used to investigate relationships between UEMS and motor level. First, the initial cervical motor level was not a significant factor in UEMS recovery \( (p=0.237) \) and thus was automatically excluded by the step-wise regression model. Second, the UEMS change from baseline to approximately one year after SCI was associated with a motor level change \( (p<0.001; \text{Figure 6.3}) \). However, the relationship was only moderate \( (r^2=0.30) \). Including AIS conversion in the step-wise regression model only improved \( r^2 \) to 0.33 \( (p=0.006; \text{Figure 6.3}) \). According to ordinal regression analysis, the initial cervical motor level \( (\text{C5-C7, } p>0.08) \) or AIS grade conversion \( (p=0.321) \) were not significant predictors of motor level changes.
Figure 6.3 Upper extremity motor score change
A) Changes in right side UEMS by the number of motor levels recovered and B) changes in total UEMS by AIS grade conversion from baseline to approximately one year after SCI. (Combined databases).
6.3.6. Spinal cord independence measure relationship with motor level changes

71 individuals in the EM-SCI database with C4 (n=26), C5 (n=29), C6 (n=11), or C7 (n=6) sensorimotor complete (AIS-A) SCI had the SCIM and ISNCSCI motor exam performed at the 1 and 50 week time-point after SCI. The SCIM total and self-care recovery between 1 and 50 weeks was significantly dependent on the initial motor level of SCI (p<0.01). Individuals with C4 AIS-A SCI at 1 week after SCI recovered significantly fewer SCIM self-care points (2.77±5.82) than all other levels of injury (all comparisons, p<0.001; C5=6.45±5.62, C6=10.73±5.76 and C7=11.67±3.88). There was no significant difference between other initial motor levels after correction for multiple comparisons. SCIM total and self-care scores significantly increased with the number of motor levels recovered (grouped C4-C7 AIS-A SCI, p<0.005). A significant increase was noted for each comparison (i.e. between 0 and 1, 0 and 2, as well as between 1 and 2, p<0.05; Figure 6.4).
Figure 6.4 Relationship between the motor level and SCIM self-care recovery
Between the 1 and 50 week time-point in the EM-SCI database.

6.4. Discussion

The purpose of this study was to examine the extent of spontaneous motor recovery in the upper extremities of individuals after traumatic sensorimotor complete (AIS-A) cervical SCI. Similar to previous reports (Ditunno, Jr., et al., 2000a), the majority of C4-C7 AIS-A individuals regained at least one motor level in the injured cervical cord within one year after SCI (Sygen=73.2%, EM-SCI=57.6%). Of these individuals, a smaller proportion (~30%) recovered two or more motor levels, which is a novel finding that was confirmed in both datasets. In all cases, the percentage recovering one or two motor levels was lower at intermediate time-points (e.g. 18% for two motor levels at 16 weeks). Motor level
deterioration, although relatively rare in both databases (combined total, n=11, 4.6%), was more prevalent after C6 or C7 SCI, but only at early intermediate time-points (4 to 8 weeks).

The spontaneous recovery of UEMS at one year after SCI (~10-11 motor points bilaterally), was similar to previously reported estimates of total motor score change (Waters, et al., 1993; Marino, et al., 1999; Geisler, et al., 2001a; Steeves, et al., 2007; Fawcett, et al., 2007). Interestingly, an individual with an initial C7 motor level SCI spontaneously recovers a similar number of motor points as an individual with an initial C5 motor level, despite having a fewer number of available cervical segments to record a change, and differences in inter-rater reliability of cervical myotomes (Savic, et al., 2007). Collectively, this suggests that spontaneous UEMS recovery: 1) occurs predominantly within the initial motor level segment (C4-C7) and/or within two spinal segments caudal to the initial motor level, and 2) is sufficiently robust to track changes in all cervical myotomes (C4-C7), irrespective of differences in psychometric properties.

The rapid increase in motor scores from one week to 6 months, followed by a slower rate of change from 6 months to one year characterizes the typical pattern of spontaneous motor recovery after sensorimotor complete SCI (Marino, et al., 1999; Ditunno, Jr., et al., 2000a; Fawcett, et al., 2007; Steeves, et al., 2007; Curt, et al., 2008). It was notable that even a small increase in UEMS (~2 points) from 6 months to one year was accompanied by a marked increase in the proportion of individuals recovering one or more motor levels.

The greatest proportion of spontaneous AIS grade conversions was evident at earlier time-points (12 and 16 weeks, EM-SCI and Sygen, respectively) compared to the
time-course for UEMS and motor level changes. By 12 months after sensorimotor complete SCI, similar rates of AIS impairment grade conversion (20% to 30%) were reported as in previous analyses of the EM-SCI and Sygen databases (Geisler, et al., 2001a; Spiess, et al., 2009). The relatively large percentage (~59%) of Sygen subjects with an initial C7 motor level SCI that undergo a conversion from AIS-A to incomplete SCI (≥ AIS-B) may be a random effect due to the small sample size (at baseline n=17). This large conversion from AIS-A grade did not occur when there was a larger sample size (e.g. initial motor level of C5 or C6).

In general, the conversion of the impairment grade from sensorimotor complete (AIS-A) to an incomplete status (≥ AIS-B) is dependent on a number of variables, including: experience of the examiner, patient self-reports (e.g. sensory perception), measurement procedures (e.g. anal sensory and/or motor assessment), and SCI co-morbidities (e.g. brain injury), all of which can collectively influence the accurate classification of SCI severity during the acute phase (Burns, et al., 2003). Electrophysiological outcomes (e.g. evoked potentials) and other novel strategies, such as biomarkers of SCI that correlate with injury severity (Kwon, et al., 2010b), may help to accurately confirm the completeness of SCI (Note: both may still be subject to the deleterious effects of brain injury).

In the present study, AIS grade conversion was only weakly related to an increase in UEMS at one year after SCI and not accompanied by greater motor level recovery. This moderate relationship was likely the result of most individuals converting from cervical AIS-A to AIS-B (anal sensory recovery) or to AIS-C (anal motor recovery only) (Steeves, et al., 2007; Spiess, et al., 2009). It was not the intention of this paper to undertake a detailed
analysis of changes in lower body motor recovery as they relate to specific AIS impairment grades. Nevertheless, our present findings parallels previous study findings, which found AIS grade conversion was not directly associated with an increase in the “number of muscles graded 3 or more” (i.e. a functional muscle score, Spiess, et al., 2009). Collectively, this suggests that the return of S4-S5 sensation (minimal criterion for incomplete AIS-B SCI) and the recovery of motor activity within individual spinal segments may not be mediated by the same underlying neural mechanisms.

An increase in UEMS with successive increments of motor level recovered (i.e. -1, 0, 1, and 2) was not a surprising observation, given that cervical motor level is a function of the motor score in the upper extremities. However, it is difficult predict a change in motor level based on the number of motor points recovered, in particular for those individuals recovering 1 or 2 motor levels. This study has also highlighted the relationship between changes in motor levels and the recovery of functional independence (i.e. SCIM). Although all levels of cervical SCI recovered a similar number of upper extremity motor points, and this ultimately led to approximately the same number of individuals recovering motor levels (no significant differences between initial motor levels), C4 individuals recovered significantly fewer SCIM self-care points than all other motor levels. Since these significant differences persist even between individuals with C4 and C5 SCI, a minimum recovery of C6 (i.e. wrist extensors, of which approximately 20% of individuals with C4 will recover and 70% of individuals with C5 SCI will recover) may be a threshold for improving functional self-care independence among individuals initially with C4 sensorimotor complete SCI.
It was also interesting to note that while there was a small mean motor point difference between individuals recovering 1 and 2 motor levels (1 and 50 week time point, 6.18±3.58 to 8.36±4.48 unilaterally, 1 and 2 motor level recovery groups, respectively), individuals recovering 2 motor level recovered significantly greater SCIM total and self-care score (25.30±17.07 to 38.32±22.31 and 5.79±5.80 to 10.53±5.92, respectively). The implications of this finding are two-fold: 1) recovering a small number of motor points may still be functionally meaningful, and 2) where motor recovery occurs may be as important as the total number of motor points recovered. For example, an individual with C4 AIS-A SCI recovering 8 motor points unilaterally spared across all 5 myotomes in the upper limbs may be less functionally independent than an individual with the same level of injury recovering a similar number of motor points (or fewer) but restricted to the first two caudal spinal segments that ultimately shifts the motor level caudally (e.g. C5 and C6).

6.4.1. Comparison of databases

As reported previously (Fawcett, et al., 2007), using preliminary data from the EM-SCI database, the similarities in motor changes between the EM-SCI and Sygen studies indicates upper extremity motor outcomes are reproducible measures of spontaneous recovery after cervical sensorimotor complete SCI (AIS-A). At the intermediate time-points of 8, 12, and 16 weeks after SCI, the differences in the proportion of individuals changing motor levels or converting AIS grade is most likely a function of the disparate examination times (the 12 week EM-SCI data being intermediate between the Sygen data at 8 and 16 weeks). This suggestion is supported by the similar data obtained from both databases at congruent assessment time-points (e.g. 4 weeks, 24/26 weeks, and 50/52 weeks). The
overall similarities are apparent even though EM-SCI has less rigorous inclusion/exclusion criteria, including: age, surgery, variability in the time of assessments, and more subjects dropping out at later assessment times (24 and 50 weeks after SCI). Therefore, although the EM-SCI protocol differs from the strict criteria of a RCT, such as the Sygen trial, the similar patterns of motor recovery after cervical AIS-A SCI would appear to underscore the reliability of the recovery patterns and overcome any data collection differences.

Given the approximate 10 year difference in the data collected by the two databases, it may seem disappointing that greater spontaneous recovery was not observed within the EM-SCI database. Although more intensive surgical and rehabilitation efforts have been implemented in the interim, there has been little success in improving the neurological recovery after cervical AIS-A SCI. Since the current prevailing viewpoint is that recent treatment advances have facilitated recovery after incomplete SCI, further analysis should be undertaken to determine if upper and lower extremity motor recovery patterns following AIS-B – D cervical SCI are different between databases.

### 6.4.2. Implications for clinical trials

A fundamental question regarding clinical trials is which outcome measure will provide a sensitive and reliable endpoint for accurately measuring safety and detecting a treatment effect. It is difficult to develop optimal clinical trial protocols and clinical endpoints for a disorder, like SCI, that has yet to validate a treatment with a clear clinical benefit. Furthermore, since many neuroprotective and regenerative therapeutic studies will recruit individuals during the acute or sub-acute stage of SCI, the safety and efficacy of a therapeutic must be shown during a period of spontaneous neurological change.
Therefore, achievable clinical endpoints can only be established if the natural history of neurological outcomes over the first year after SCI is known.

For therapies applied locally to the site of spinal injury, detecting proximal changes in adjacent spinal segments may be more sensitive than measuring an aggregate neurological change (i.e. AIS grade conversion), especially for early phase clinical trials. AIS grade conversion is acceptable as a clinical endpoint, but only when the treatment effect is expected to be large (Blight and Tuszynski, 2006). After cervical AIS-A SCI, our analysis indicates that change in AIS impairment grade is not accompanied by a consistent or predictable improvement in UEMS or motor level. The use of motor score subscales, i.e. UEMS, has been suggested to improve the relationship of motor scores with functional outcomes (Marino, et al., 1999). Thus for individuals with sensorimotor complete cervical SCI, an improvement in UEMS or motor level may more readily detect a subtle therapeutic effect (e.g. improvement in hand function).

Individuals with complete thoracic level injuries are often suggested as initial participants for early phase SCI studies since any deleterious intervention is less likely to compromise functional capacity (van Hedel and Curt, 2006). Since thoracic motor change is not measured by the ISNCSCI, the determination of a therapeutic gain or loss is difficult and is limited to a change in thoracic sensory dermatomes. Changes in sensory level during spontaneous recovery after thoracic sensorimotor complete SCI have only recently been investigated (Harrop, et al., 2009; Zariffa, et al., 2011). Based on the findings from these studies during spontaneous recovery, motor level changes would provide a more sensitive indication of therapeutic benefit or detriment than sensory level.
For a treatment to be validated in later phase clinical trials (i.e. pivotal phase III) an outcome measure must be directly related to an improved functional capacity. Although UEMS is related to the independent capacity for ADL (Rudhe and van Hedel, 2009), motor level improvement is a more direct index of enhanced activities of daily living (Bromley, 2006; van Hedel and Curt, 2006). For example, an increase in non-functional muscle strength (motor score of 1-2) across a large number of key muscles (representing specific cord segments) would not be as beneficial as an increase in functional muscle strength (motor score $\geq 3$) across a smaller number of contiguous cervical cord segments.

**6.4.3. Study limitations**

Given that the standards of care after SCI depend on a variety of other factors (e.g. country, institution), the lack of treatment uniformity in both the Sygen and EM-SCI database limits the true understanding of “spontaneous” neurological recovery. Indeed, since a majority of individuals with AIS-A, C5-C7 motor level SCI in the Sygen database underwent surgical intervention (82.9%), there is only a small group of non-randomized C5-C7 individuals sufficient for such an analysis. Furthermore, rehabilitation after SCI is not well standardized, and should be expected to greatly impact spontaneous recovery. Future studies should be designed to address these specific research questions.

**6.4.4. Sample size calculations**

We have used the present data to generate illustrative sample calculations of the number of participants that would have to be enrolled to a clinical study using UEMS or change in motor levels as clinical endpoints. Sample size calculations can be accomplished
using a myriad of different formulae. Briefly, we used tests of equality for a two-sample, parallel-designed study; including a test of proportions (Shao, et al., 2008b) and comparison of means (Shao, et al., 2008a). Sample sizes were calculated for individuals with C5-C7 SCI, based on the recovery of either mean UEMS or two motor levels from baseline to approximately one year after SCI (50 and 52 weeks, EM-SCI and Sygen, respectively). We used 80% power (β=0.2) for detecting a clinically meaningful difference and α=0.05. The numbers of subjects required to be enrolled in the treatment and control groups (equal allocation) for varying treatment effect sizes are shown in Figure 6.5. Nevertheless, there are a number of factors that can influence power calculations, including the heterogeneity of the study population and the size of the postulated treatment effect (Tuszynski et al, 2007). The disparity between the number of subjects required to demonstrate a significant effect using motor score compared to motor levels may be, in part, be explained by the demand for motor level changes to have functional improvement of motor points (≥3/5) over contiguous spinal segments (described above) as well as differences in statistical testing methodology (Snapinn and Jiang, 2007).

6.4.5. Conclusion

Over the entire course of a clinical trial program, complete validation of a therapeutic as safe and beneficial is likely to require a comprehensive “toolbox” of outcome measures, including: electrophysiological, neurological, neuroimaging, autonomic, pain, functional outcome, and quality of life tools (Steeves, et al., 2007). Many proposed therapeutic interventions are likely to involve the local infusion of a drug or transplantation of cells at or near the site of SCI. It is reasonable to suggest that the
principal activities of these types of treatments, however modest, will be most readily apparent in spinal segments adjacent to the administered therapeutic agent and facilitating an endogenous neural substrate of repair or recovery. Thus in early phase studies, careful tracking of motor scores in the upper limbs may provide the necessary sensitivity and accuracy to reliably detect a subtle, but statistically significant treatment effect after cervical SCI. Likewise, the derivation of motor levels, from motor scores, might provide a surrogate clinical endpoint that is linked to functional capacity and predict a clinically meaningful important difference in a pivotal phase III trial (Ditunno, 2010).
Figure 6.5 Sample size calculations
Example of sample size calculations to demonstrate a significant change in either UEMS (A) or recovery of two motor levels (B) from baseline to approximately one year after SCI (β=0.2, α=0.05, equal allocation of subjects to treatment and control groups). Note: Effect size based on the mean spontaneous UEMS increase or two motor level improvement approximately one year after SCI in combined databases and cervical motor levels (C5-C7).
7. General discussion

The discussion that follows provides an outline of how the knowledge acquired during the course of this doctoral thesis could be applied to clinical trials for the treatment of the neurological consequences of traumatic SCI. A description of future study directions, as well as the strengths and limitations of this thesis are highlighted throughout the discussion.

7.1. Research chapter summaries

The primary objective of this thesis was to address the need to improve the sensitivity of outcomes for the assessment of neurological recovery after traumatic SCI. Five original research studies were undertaken employing clinical sensory and motor testing, as well as neurophysiological approaches to evaluate the injured spinal cord segmentally.

7.1.1. Discontinuous segmental afferent sparing after spinal cord injury is not limited to sacral segments

Sensation in upper thoracic segments is frequently observed in the absence of sensation in lower cervical segments after tetraplegia (i.e. discontinuous sensory preservation). Based on spontaneous recovery of sensorimotor function, discontinuous cervico-thoracic sensation is a real indication of afferent sparing caudal to the level of cervical SCI. The case report illustrates that C8 may actually retain some function in a subset of these individuals; however that a higher stimulus intensity is required for this to be detected. While characteristically similar to anatomical sacral sparing in the absence of
more rostral spinal segment sparing, discontinuous cervico-thoracic sensation may be attributable to other mechanisms, including that the C8 dermatome (i.e. distal) may be inherently less sensitive to afferent stimuli than upper thoracic dermatomes (i.e. proximal). Standardized afferent stimuli (e.g. QST or neurophysiology) could address the limitations of clinical sensory testing methods to accurately detect afferent sparing after SCI.

7.1.2. Dermatomal somatosensory evoked potentials and electrical perception thresholds during recovery from cervical spinal cord injury

During the transition from acute to sub-acute and chronic SCI, the prominent first negative peak (N1) of dSSEPs was found to recover towards normal latencies. EPT in dermatomes with recovering dSSEP N1 latency were not found to significantly change over the same time period. Repeated measurements of N1 latency during sub-acute or chronic time-points remained unchanged, which confirmed test-retest reliability of dSSEPs. The validity of dSSEPs as a measure of dorsal column conduction is supported by the ability of N1 latency to track changing neurophysiology during spontaneous recovery (i.e. a period of robust change) and to remain stable in chronic SCI (i.e. a period of unchanging neurophysiology). While EPT was reliable in unaffected dermatomes and remained stable during chronic SCI, the responsiveness of EPT to measure spontaneous recovery appears limited.
7.1.3. Test-retest reliability of contact heat evoked potentials from cervical dermatomes: Implications for spinal cord injury

Neurophysiological techniques to examine the spinothalamic tract had until now not been thoroughly investigated in individuals with SCI. In general, the test-retest reliability of cervical CHEPs (i.e. N2P2) in neurologically intact subjects is supported by our findings. The intensity of contact heat stimulation, which is restricted to a maximum peak temperature of 54°C (presumably to prevent skin burns), may however limit the reliable acquisition of CHEPs in distal dermatomes (e.g. C6 and C8). CHEPs were also generally reliable among individuals with SCI rostral and caudal to the level of injury. Insufficient intensity of contact heat stimulation noted in neurologically intact subjects may be problematic after SCI due to impaired thermal sensation, which may have led to CHEPs that were recordable at only one of the initial or follow-up assessments. Studies that aim to improve the acquisition of CHEPs in dermatomes affected by SCI are warranted.

7.1.4. Increased baseline temperature improves the acquisition of contact heat evoked potentials: Implications for spinal cord injury

In neurologically intact subjects, adjusting the baseline temperature from 35°C to 42-45°C increases the intensity of contact heat stimulation and increases the N2P2 amplitude of CHEPs to the same delivered peak temperature (52-54°C). Although increasing the baseline temperature (42°C) and reducing the rate of stimulation (70°C/s to 41°C/s, in order to match the duration of contact heat stimulation from a 35°C baseline as described for condition 3) also has the effect of increasing the perceived intensity, the N2P2 amplitude was not significantly increased. This suggests that by increasing the
baseline stimulation of CHEPs and maintaining the rate of stimulation (70°C/s), the synchronization of the afferent volley is improved. In individuals with SCI, increasing the baseline of contact heat stimulation improved the assessment of CHEPs by further dissociating sensory deficits and detecting afferent sparing.

7.1.5. Extent of spontaneous motor recovery after traumatic cervical sensorimotor complete spinal cord injury: Functional implications of two motor level recovery

Independent of the initial level of sensorimotor complete (AIS-A) cervical SCI (C4-C7), approximately 10 motor points will be recovered bilaterally and 70% of individuals with spontaneously recover at least one motor level during the transition from acute to chronic SCI (e.g. 70% of individuals initially with C4 motor level tetraplegia will be at least C5 motor level tetraplegia by approximately one year after injury). 20% of those individuals recovering a motor level will also recover a second motor level. The recovery of functional independence during this same time (1 week to ~ one year) according to the SCIM was found to be dependent on the initial motor level of cervical injury. Most notably, individuals with C4 AIS-A SCI recover fewer SCIM points than more caudal levels of injury (C5-C7). Two motor level recovery was accompanied by a significant increase in functional independence compared to both zero and one motor level recovery.

7.2. Improving the neurological scoring for clinical studies of spinal cord injury

For the purposes of diagnosis and prognosis, the adoption of standardized sensory and motor testing (i.e. ISNCSCI) represents arguably the most important achievement of
the clinical SCI community in the last three decades. However, in light of the failure of recent clinical trials to demonstrate efficacy in humans and because of concerns that this failure may be related, in part, to the insensitivity of the outcomes employed to measure changes (e.g. AIS grades and total motor scores), improving the classification of the neurological deficits associated with SCI for research purposes is a high priority. Two prominent strategies can be employed in order to meet the needs of research: 1) reconsider the relevant sensory and motor outcomes of the ISNCSCI, and 2) develop and implement advanced techniques more suitable to track subtle changes in neurology (e.g. dSSEPs and CHEPs).

### 7.2.1. Phase I, II, and IIa studies

The objective of a phase I interventional study is to demonstrate safety. To meet this objective, serious adverse events (SAE) during the study period will be closely monitored and reported. While a treatment may be well tolerated and safe based on rare occurrence of SAEs (e.g. respiratory, urinary tract infections), the neurological safety and tolerance should be considered. Individuals with complete SCI have been postulated for “first-in-man” studies (e.g. embryonic stem cells), in part because it is thought that worsening of the neurological condition, at least insofar as making the lesion “more complete”, will not result in functional deterioration. The most alarming deleterious neurological change in the case of individuals with sensorimotor complete SCI is that which would cause a rostral ascent of the level of injury. Compared to individuals with thoracic SCI, losing a neurological level in the cervical cord would be expected to negatively impact functional outcomes (van Hedel and Curt, 2006; Spiess, et al., 2008). Given that nearly 70% of
individuals with cervical sensorimotor complete SCI are expected to recover at least one motor level in the first year after injury (Chapter Six), an acute therapeutic or rehabilitation intervention that substantially increases the proportion deteriorating a motor level (≤-1) or not recovering a motor level (i.e. unchanging motor level, 0 = SAE) should be considered, caeteris paribus (i.e. other meaningful benefits are not observed that outweigh losing a motor level), unsafe.

Sensory levels are subject to greater variability than are motor levels during spontaneous recovery. After thoracic SCI, only the deterioration of three or more sensory levels has been postulated as being rare enough to warrant concern that a therapeutic intervention in an early phase acute SCI clinical trial may be unsafe (Zariffa, et al., 2011). The variability regarding the number of individuals recovering sensory levels after sensorimotor complete cervical SCI is similar (unpublished findings based on the review of the EM-SCI database, which revealed that as many individuals recover a sensory level as those that deteriorate a sensory level, and up to 50% do not change sensory levels during spontaneous recovery). Furthermore, afferent sparing may not be accurately measured by clinical testing methods, as was shown in the case report in Chapter Two. The difficulty of accurately detecting afferent sparing and tracking changes in sensory level employing standard light touch and pinprick warrants that other sensory testing methods be considered to assess safety. dSSEPs and CHEPs represent two potential methods suitable for this purpose. Based on the findings in Chapters Three, Four, and Five, employing dSSEPs and CHEPs (conventional and increased baseline) in an acute early phase I study at the level of injury may be helpful to ensure that the lesion area is not expanding to rostral segments and affecting conduction in the dorsal columns and spinothalamic tract,
respectively. The most alarming neurophysiological change with regards to monitoring safety would be the complete abolishment of a cortical evoked potential that was clearly present (i.e. large amplitude, normal latency) pre-treatment. Increased dSSEP N1 (>2ms) and CHEP P2 (>46ms) latency may also be employed as indicators of deleterious changes in spinal conduction (*Chapter Four*).

The variables that predict the course of neurological recovery for individuals with sensorimotor complete cervical SCI are largely unknown. According to communications with Professor Armin Curt and Dr. Marc Bolliger at Balgrist Hospital (University of Zurich), based on an ongoing prospective review of the EM-SCI database, there are no acute injury characteristics or subject demographics (e.g. age at injury) that, at one week, can accurately distinguish those individuals with tetraplegia spontaneously converting to incomplete at one year (~30%) from those individuals who will remain sensorimotor complete (unpublished findings, personal communication). Although limited to 4 weeks after injury, evidence of afferent sparing in upper thoracic segments (T1-T4) was associated with higher rates of conversion from complete to incomplete (*Chapter Two*). Since spontaneous AIS grade conversion also influences the recovery of muscle strength in the upper extremities (*Chapter Six*), upper thoracic sensation should be considered when evaluating preliminary efficacy based on changes in UEMS. In order to avoid the variability introduced by AIS grade conversions during spontaneous recovery, interventions specifically focused on improving upper limb function should likely focus on impact of motor score changes on motor levels (i.e. in *Chapter Six*, changes in motor level were found not to be affected by spontaneous conversion). To further improve the prediction of sensorimotor complete spontaneous converters beyond upper thoracic sensory preservation, other factors may
need to be considered, including the etiology of injury (e.g. injury was high or low impact), and findings from diagnostic neuroimaging (e.g. signal intensity change).

Increasing the baseline temperature of CHEPs was shown to be effective for the purpose of confirming the absence of sensation in individual spinal segments (Chapter Five). Confirming the completeness of injury using increased baseline CHEPs, as was done for a single segment (C8) in Chapter Two, may reduce some of the heterogeneity of the subjects enrolled in an early phase study. This raises an important issue for future study of segmental outcomes, that the “completeness” of injury be determined in the context of the upper extremities. While the distinction between complete and incomplete SCI (AIS grades) has long been valuable for diagnosis and prognosis, most individuals with sensorimotor complete (AIS-A) tetraplegia will have some sensation (as was illustrated in Chapter Two) and/or motor muscle strength preserved in a ZPP in segments adjacent to their level of cervical injury, which is to say that these individuals will be “segmentally incomplete” in the upper extremities. Facilitating the recovery of muscle strength in these segments, partially affected by SCI and already ongoing spontaneous repair processes (Chapters Three and Six), may be a realistic goal for early studies aimed at improving performance of ADL.

Preserved sensation after SCI has previously been shown to be predictive of the recovery of an individual spinal segments (Browne, et al., 1993). Although not specifically addressed in the present thesis, dSSEPs and CHEPs may provide additional prognostic information with regards to the spontaneous recovery in individual spinal segments. Specifically, based on neurophysiological evidence of sparing in the upper extremities (e.g. dSSEPs and/or CHEPs), the prediction of motor level changes may be improved.
Additionally, Chapter Three findings support that segmental neurophysiological techniques may serve as surrogate outcomes to detect subtle changes in spinal conduction. This could be important in a phase II study to demonstrate the biological activity of a therapeutic (e.g. remyelination). At this early phase of study, demonstrating that a therapeutic improves conduction but does not change functional outcomes remains important for planning later phases of study (e.g. dosing). Future study should also focus on employing other neurophysiological techniques in order to examine the integrity of the alpha motor neuron (e.g. H-reflex) in order to exclude subjects based on evidence of lower motor neuron damage, which may prevent the recovery of a myotome.

7.2.2. Pivotal phase III study

At this point in the clinical trial program, an intervention has proven to be safe (phase I), based on the lack of SAEs related to the treatment and limited evidence of neurological deterioration, and there is some indication of a preliminary efficacy and biological activity (phase I/IIa or II). While the emphasis in a phase III pivotal study is detecting a functional benefit, continuing to demonstrate safety remains important. For this purpose, assessing neurological and neurophysiological deterioration according to changes in motor levels and evoked potentials (dSSEPs and CHEPs) may remain valuable. Provided that there are few concerns regarding the safety of the intervention, the costs associated with neurophysiological techniques (e.g. expensive, training required for examiners) may, however, be too high for the inclusion of CHEPs/dSSEPs in multi-center phase III studies. Regardless, carefully monitoring the proportion of individuals not recovering a motor level
or deteriorating a motor level will remain important, particularly at interim time-points to decide if the study should be continued (i.e. safe) or halted (i.e. unsafe).

Based on Chapter Six findings, there is a strong argument for the use of ‘two motor level recovery’ as a clinical trial endpoint in a phase III study. Perhaps most importantly for meeting the goal of phase III study, recovery of two motor levels represents a functionally meaningful change with respect to both zero and one motor level recovery. In essence, examining motor level recovery addresses concerns that motor score changes may not constitute a meaningful functional improvement (Walker, 1991). Recovering two motor levels can be distinguished from one, which is also associated with greater functional improvements compared to not recovering a motor level, on the basis that: 1) two motor levels is likely functionally meaningful regardless of the initial cervical motor level, and 2) since one motor level is expected spontaneously in up to 70% of individuals after sensorimotor complete cervical SCI, in a study designed to measure, for example, a change of 30% between the control and the treatment group (which based on the power calculations performed in Chapter Six would require fewer than 50 subjects per group), detecting a statistical effect if, in practice 75% of the control group was found to recover one motor level would be impossible. This later point illustrates a ceiling effect for one motor level recovery as a clinical trial endpoint. The same problem would be unlikely to affect the proportion of individuals recovering two motor levels (i.e. lower starting point, approximately 20-30%).

While the relationship between the number of motor levels recovered and the recovery of motor score is obvious, Chapter Six also illustrates that recovering muscle
strength in segments adjacent to the level of injury may be more important than recovering a greater or equal number of motor points across multiple segments. An example from the EM-SCI database is illustrated in Table 7.1. Both of these individuals (assessed at different centers) were C4 during the very acute stage of injury (<2 weeks), demonstrate considerable recovery of upper extremity muscle strength by chronic time-points (9 motor points, unilaterally). The key difference between subjects 180001 and 5294 is how the recovery of muscle strength is distributed over the upper limb segments (C5-T1). The recovery of motor points across multiple segments (180001) was less meaningful with regards to SCIM self-care score, even though all three segments regain functionality (>3), compared to if the majority of recovery was concentrated within the segments immediately adjacent to the level of injury, shifting the motor level caudally 2 segments (5294).
<table>
<thead>
<tr>
<th>Right myotome</th>
<th>EM-SCI ID#</th>
<th>180001</th>
<th>5294</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute</td>
<td>Chronic</td>
<td>Acute</td>
</tr>
<tr>
<td>C5</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>C6</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>C7</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>C8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UEMS</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>SCIM self-care</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* denotes the motor level

Table 7.1 Example of motor score changes in the European Multi-Center Study about Spinal Cord Injury database
7.3. Future directions

A number of remaining questions related to the outcomes proposed for clinical trial applications not addressed in the present thesis are listed below:

- What other mechanisms may account for the emergence of patterns of sensory preservation after SCI (e.g. remapping of dermatomes in the periphery due to cortical reorganization)?

- Do changes in conduction occur in spinothalamic tract? Could they mediate the recovery of thermal/pinprick sensation after acute SCI?

- Is the neurophysiological examination of the dorsal column AND the spinothalamic tract necessary or can the outcome from one be used to predict the other?

- How does segmental neurophysiology (e.g. latency and amplitude of evoked potentials) correlate with the performance of functional tasks (e.g. GRASSP)?

- Is the preservation of thermal or electrical sensation after SCI in individual dermatomes meaningful for the recovery of muscle strength in the corresponding myotome?

- Are improvements in muscle strength adjacent to the level of injury more important for the performance of functional tasks (e.g. GRASSP) than recovering “functional muscle strength” in multiple myotomes (as was illustrated in Table 7.1 according to SCIM self-care)?

- Could CHEPs be useful to assess other neurological deficits associated with SCI (e.g. autonomic dysreflexia)?

Many of these questions are currently being addressed with collaborators at the University of Zurich. Preliminary evidence indicates that CHEPs are more sensitive than dSSEPs to detect segmental spinal cord damage (personal communication, Dr. Armin Curt). This is in agreement with previous studies that have compared conventional mixed nerve SSEPs and LEPs (i.e. LEPs are more sensitive to a variety of neurological impairments) (Bromm and
Treede, 1991; Kakigi, et al., 1991). An analysis of spontaneous recovery after cervical incomplete SCI is also underway to determine if two motor level recovery is a suitable clinical trial endpoint for studies that will also include these individuals (AIS-B – D).

The question remains as to whether QST and neurophysiology can distinguish different types of sensory impairments. Segmental neurophysiology (i.e. dSSEPs and CHEPs) and QST should be employed to objectively measure at- and below level neuropathic pain. The longstanding debate as to whether partial damage in the spinothalamic tract is a requisite for the onset of neuropathic pain would benefit from such an investigation (Defrin, et al., 2002; Finnerup, et al., 2007; Wasner, et al., 2008; Hatem, et al., 2010). Furthermore, assessing the wind-up phenomenon (i.e. increased reporting of pain to the same intensity of noxious stimulation) and paradoxical heat sensation (i.e. reporting warm sensation to cooling of the skin) during spontaneous recovery may elucidate other temporal changes in sensory processing that are otherwise not examined by standard afferent stimulation methods. A priority of neurophysiologists should also be to evaluate how conduction deficits relate to findings from advanced neuroimaging.

### 7.4. General strengths and limitations

The specific strengths and limitations of each study were highlighted in the research chapters and will not be repeated here. A number of the major findings, including patterns of afferent preservation (Chapter Two) and motor level recovery (Chapter Six), can be generalized to the larger population on account of the statistical power afforded by the EM-SCI. Chapter Three involved a relatively large number of subjects for a study that involved acquiring acute neurophysiological recordings (n≈30). The magnitude of change (i.e.
increased peak-to-peak amplitude, N2P2) and statistical significance observed by increasing the baseline temperature of contact heat stimulation in a small sample (n=8, *Chapter Five*) indicates the robustness of an effect. Overall, the primary strength of this thesis is that the segmental outcomes devised during the course of study can be readily adopted for clinical purposes. Indeed, the normative segmental dSSEP/CHEP values collected during the course of study (*Chapters Three and Four*) and the methods developed for the improved acquisition of CHEPs (increased baseline temperature, *Chapter Five*) have been adopted in clinical practice and are undergoing further study with our collaborators in Zurich at the Spinal Cord Injury Center, Balgrist Hospital (personal communication, Dr. Armin Curt).

In general, the outcomes proposed for use in clinical trials, particularly segmental sensory evoked potential approaches (i.e. dSSEPs/CHEPs), may be useful for assessing changes in conduction that occur within spinal segments adjacent to the level of injury. As mentioned in the introduction, meaningful changes in spinal and cortical circuitry (i.e. those that ultimately will impact functional outcomes) may occur without changes in conduction through the lesion epicenter or within individual spinal segments (e.g. activity dependent plasticity in the lower limbs). Other neuroimaging and neurophysiological outcomes, including the blood oxygen level dependent response (i.e. fMRI, Jurkiewicz, *et al.*, 2007) and EMG activity (e.g. Brain Motor Control Assessment, Sherwood, *et al.*, 1996; McKay, *et al.*, 2011), may be important with regards to objectively tracking these changes. Furthermore, differentiating between repair mechanisms in the spinal cord (i.e. regeneration vs. repair vs. remyelination vs. sprouting) is not possible in humans per se, but rather will be based on preclinical findings. The present interpretation of the
mechanism underlying changes in sensation after cervical SCI has also been limited to conduction in damaged afferent pathways. It is clear that lost descending control, including serotonergic modulation of dorsal horn neurons (Messing and Lytle, 1977; Lopez-Garcia, 2006) may also be important after SCI, in particular with regards to the perception of noxious stimuli (Chapters 4 and 5, i.e. contact heat stimulation).

A limitation of this thesis is that only segmental outcomes for individuals with tetraplegia have been considered. Although dSSEPs and CHEPs may be useful for measuring safety and preliminary efficacy in individuals with paraplegia, especially in order to account for the lack of key muscles in the thoracic segments, motor levels, as defined by the ISNCSCI, have limited value except perhaps after lower thoracic SCI (T10-T12), where changes in muscle strength may occur into the lower extremities (unpublished observations, Dr. José Zariffa). The inclusion of individuals with paraplegia into clinical studies will likely require that other methods, including objective neurophysiological techniques (e.g. MEPs to thoracic paravertebral myotomes, Cariga, et al., 2002), be developed to measure segmental changes. The retrospective nature of this thesis (Chapters Two, Three and Six) represents a general limitation. Although all were hypothesis generated studies, there are a number of pitfalls to retrospective analyses, including that other important information may be missing. In the case of the responsiveness of dSSEP N1 latency and EPT (Chapter Three), the ISNCSCI light touch and pinprick findings were rarely available and, where available, the accuracy of the assessment was unknown (e.g. training of examiners). Overall, the primary limitation of this thesis is that proposed outcomes, like those outcomes previously employed (e.g. AIS grade and total motor score), have not been validated to measure a therapeutic effect. The segmental approach to assessing SCI still
faces all other limitations of translating preclinical findings from the bench to the bedside, including that the MCID is unknown.

7.5. Concluding remarks: Segmental approaches and future clinical trials

The proposed applications of segmental neurological outcomes for clinical trials based on the findings from the present doctoral thesis are summarized in Table 7.2. The outcomes associated with traumatic SCI with regards to mortality and morbidity, as well as long term QoL have vastly improved over the span of 60 years. The focus in the next 10 years will be to successfully translate promising preclinical findings in order to improve neurological and functional outcomes of individuals with SCI in pivotal phase III RCTs. To meet this challenge, validating and implementing novel outcome measures and revising existing International Standards (i.e. ISNCSCI) to assess neurological safety, biological activity, and efficacy of a therapeutic is of the utmost importance. The proposed evaluation of segmental neurological outcomes to replace “global” measures as primary endpoints in different phases of study (e.g. AIS grade, total motor score, conventional SSEPs) would represent progress towards addressing the priority of individuals with tetraplegia to improve arm and hand function (Anderson, 2004).
<table>
<thead>
<tr>
<th>Phase</th>
<th>Tool</th>
<th>Primary outcome</th>
<th>Purpose</th>
<th>Thesis Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>ISNCSCI</td>
<td>Motor level: % of individuals with unchanging/deteriorating motor levels</td>
<td>Monitor ascending neurological deficits to determine safety</td>
<td>Six</td>
</tr>
<tr>
<td></td>
<td>Neurophys.</td>
<td>SEPs: Unaffected dSSEPs/CHEPs in dermatomes adjacent to the level of injury</td>
<td></td>
<td>Three and Four/Five</td>
</tr>
<tr>
<td>II/IIa</td>
<td>ISNCSCI</td>
<td>Sensory scores: Preserved upper thoracic sensation</td>
<td>Prediction of motor score and spontaneous AIS grade conversion</td>
<td>Two</td>
</tr>
<tr>
<td></td>
<td>Neurophys.</td>
<td>Motor Level: % of individuals recovering motor levels</td>
<td>Preliminary efficacy (unrelated to AIS grade conversion)</td>
<td>Six</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEPs: Improvements in segmental spinal conduction</td>
<td>Proof of mechanism (e.g. remyelination)</td>
<td>Three</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Examine segments with absent clinical LT and/or PP sensation</td>
<td>Confirm the completeness of injury in individual spinal segments</td>
<td>Five</td>
</tr>
<tr>
<td>III</td>
<td>ISNCSCI</td>
<td>Motor levels: % of individuals with unchanging/deteriorating motor levels</td>
<td>Monitor ascending neurological deficits to determine if the study should continue</td>
<td>Six</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% of individuals recovering two motor levels</td>
<td>Measure treatment efficacy</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.2 Summary of proposed application of the International Standards and neurophysiology in acute traumatic cervical spinal cord injury clinical trials
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