Doctoral Thesis

Sensory Plasticity in Cervical Spinal Cord Injury

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SENSORY PLASTICITY IN CERVICAL SPINAL CORD INJURY

A thesis submitted to attain the degree of

DOCTOR OF SCIENCES of ETH ZURICH

(Dr. sc. ETH Zurich)

Presented by

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-2015-
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Summary
The integration of sensory input from the periphery provides essential awareness about the external environment and body state, as well as modifying motor output patterns. As a consequence of a spinal cord injury (SCI), afferent feedback can be completely abolished or severely compromised due to disruption of nerve fiber bundles that convey ascending sensory information. Damage to sensory afferents is typically hallmarked by symptoms localized below the level of injury, including abolished or altered responses to stimuli of pain and temperature, vibration, and proprioception. Additionally, trauma-induced alterations of different sensory modalities above the level of injury have been revealed and in fact, some of these alterations are even suspected to be involved in the initiation and maintenance of sensory complications, such as neuropathic pain. Neuropathic pain represents a major secondary complication for people living with spinal cord injury (SCI), which unfortunately is largely refractory to treatment. While preclinical models are well suited to address mechanisms, translational studies in humans are vital to determine shared pathophysiological and anatomical substrates of sensory impairments and complications.

The employment of clinical pain models (e.g., acute and chronic pain) in healthy control individuals serves as an intermediate step to gain knowledge regarding mechanism underlying the modulation of noxious input. As such, the first study (Chapter 2) in the present thesis took advantage of clinical pain models in order to investigate the modulation of noxious input (i.e., thermal stimuli) in the presence of altered nociception (i.e., capsaicin-induced sensitization). Under normal physiological condition, the capacity of the sensory system to modulate acute noxious contact heat was not altered with additional neuromodulation (i.e., no pain relief due to transcutaneous electrical nerve stimulation, TENS). In contrast, capsaicin-induced sensitization unmasked the analgesic effect of conventional TENS on perception to noxious contact heat stimuli. Taken together with the fact that TENS also effectively relieved the capsaicin-evoked pain, these findings indicate that TENS modulates distinct aspects of sensitization, which in turn results in analgesia to thermal stimulation. The differential modulation of Aδ fiber (i.e., acute, short pain) and C fiber activation (i.e., prolonged, dull pain) mediated by large-diameter excitation highlights the importance of the interactions between the sensory systems in the pain modulation process. In line with previous studies and observations in clinical settings, the variability in pain rating and cortical responses in this first study was striking, but a plausible explanation was not at hand. Thus, a further study of the thesis (Chapter 4) employing structural magnetic resonance imaging (MRI) and neurophysiological assessments (i.e., contact heat evoked potentials) aimed at disclosing traits of cortical structures explaining the variability in pain processing in healthy control individuals. Indeed, the variability of pain ratings and amplitudes of evoked potentials is not uniquely related to measurement artifacts (i.e., differences in the interpretation of pain rating scales between individuals), but rather attributable to anatomical differences between individuals. Translating this information from studies in
healthy control individuals, the modulation of afferent input after a spinal cord injury and in presence of neuropathic pain was examined. Focusing on changes above the level of lesion, deficient modulation of afferent noxious contact heat was found in individuals with SCI suffering from neuropathic pain compared to healthy control individuals and pain-free individuals with SCI (Chapter 3). Moreover, reduced perception of both, short (i.e., contact heat) and prolonged pain (i.e., capsaicin) was further observed among those with neuropathic pain. These findings collectively suggest that the presence of neuropathic pain after SCI is associated with the changes in the capacity to modulate noxious afferent input, while deafferentation alone has no impact on modulation. In conjunction with Chapters 6 and 7, this is interesting, especially since both MRI studies revealed changes in structure and function related to deafferentation, which could be discerned from pain-associated alterations. Importantly, not only the brain adapts to the insult to the central nervous system (CNS), but also the spinal cord. Crucially, pain was associated with changes along the entire neuroaxis (i.e., brain and spinal cord). In concert with previous studies, the present studies emphasize a pivotal role of structural and functional adaption of the CNS in the initiation and maintenance of neuropathic pain. The majority of the previous studies favor the maladaptive plasticity theory, which states that these changes in structure and function are linked with pain. In contrast, the results of this thesis indicate that the lack of adaption, namely preserved structure and function, is associated with pain. Lastly, a systematic review of the literature was undertaken to assess the robustness of evidence in support of “maladaptive plasticity” as well as “preserved structure and function” emerging from applications of advanced functional and structural MRI in humans.

In conclusion, deafferentation and the presence of neuropathic pain are strongly associated with plasticity of the sensory system. The employment of a multi-methodological approach comprising neurophysiological and imaging techniques turned out to be a promising strategy to detect the plasticity along the neuroaxis, which is important in sight of the development of therapeutics to ameliorate sensory deficits.
Zusammenfassung


Unter physiologischen Bedingungen hat die transkutane elektrische Nervenstimulation (TENS) von stark myelinisierten Aβ-Fasern keine modulierende Wirkung auf die Kapazität des sensorischen Systems, um Hitzestimulationen zu modulieren. Nach der Applikation von Capsaicin Creme hat TENS jedoch eine schmerzlindernde Wirkung auf die Hitzestimulationen. Wenn man zusätzlich berücksichtigt, dass TENS auch auf den Capsaicin-Schmerz lindernd wirkte, kann daraus geschlossen werden, dass TENS der capsaicininduzierten Sensitivierung entgegenwirkt und dadurch auch eine Linderung des durch die Hitzestimulationen induzierten Schmerzes resultiert. Die unterschiedliche Modulation von dünn myelinisierten Aδ-Fasern (d.h. akuter Schmerz) und nicht myelinisierten C Fasern (d.h. anhaltender Schmerz) durch die Anregung von stark myelinisierten Aβ-Fasern hebt die Wichtigkeit der Interaktionen zwischen den Fasertypen in der Modulation von afferenten Informationen deutlich hervor. Sowohl, in dieser Studie, als auch in früheren Studien und im klinischen Alltag ist die Variabilität der Schmerzempfindung sowie die dazugehörige kortikale Verarbeitung (d.h. evozierte Potentiale) auffällig hoch. Daher wurde eine weitere Studie (Kapitel 4) an gesunden Probanden durchgeführt, in welcher...
neurophysiologische Untersuchungen und Magnetresonanztomographie (MRT) angewandt wurden, um nach strukturellen Merkmalen im Gehirn zu suchen, welche die erwähnte Variabilität der Schmerzempfindung und –verarbeitung erklären könnten. In der Tat wurden strukturellen Merkmalen in Hirnarealen gefunden, welche zu einem gewissen Grad die Diskrepanz zwischen der Schmerzempfindung und –verarbeitung erklären.


Zusammenfassend kann gesagt werden, dass sowohl die Rückenmarksverletzung als auch der neuropathische Schmerz mit der Plastizität des sensorischen Systems assoziiert sind. Die Kombination von verschiedenen Untersuchungsmethoden, wie zum Beispiel neurophysiologische Untersuchungen und MRT, ermöglichte den Nachweis der Plastizität im ZNS, welcher wiederum wichtig für die Entwicklung neuer Therapieansätze ist.
General Introduction
1 General Introduction
1.1 The anatomical organization of the spinal cord

The mammalian spinal cord – the vital link between brain and body – extends from the brainstem where it
continuous to the level of the first or second lumbar vertebrae. Three layers of meninges (dura mater, the
arachnoid, and pia mater) and cerebrospinal fluid (CSF) protect the spinal cord that is contained
within the spinal column. As illustrated in Figure 1.1, the spinal cord is
organized into two
anatomically and
functionally different
regions denominated
according to their gray and
white appearance\(^1\). The
central gray matter is
primarily comprised of
neuron cell bodies and chiefly responsible for integrating and relaying sensory and motor stimuli. The
white matter, surrounding the central gray matter, contains sensory, motor, and propriospinal axons.
Axons with the related functional features typically travel together in “tracts” that are located in particular
regions of the white matter (Figure 1.1). The main ascending and descending white matter tracts in
humans are described in Table 1.1. Active (e.g., saltatory conduction velocity) and passive properties
(e.g., diameter, myelination state) of axons in the white matter tracts convey the action potentials to their
intended synaptic target (Table 1.2). Analogous to cortical and sub-cortical structures, the central gray and
white matter of the spinal cord is somatotopically organized (Figure 1.1). In the dorsal columns, afferents
from cervical and upper thoracic segments are located in the cuneate fasciculus, lateral to the most caudal
afferents from lumbosacral and lower thoracic segments in the graciles fasciculus. In the spinothalamic
tract, afferents from rostral segments ascend medial to more caudal spinal segments. Importantly, the
topographical organization of the spinal cord, likewise the relative cross-sectional area of gray matter to
white matter, varies depending on the rostral-caudal level of the spinal cord. Spinal cord is segmentally
organized according to 31 pairs of nerves bilaterally exiting the cord (8 cervical, 12 thoracic, 5 lumbar, 5
sacral, and 1 coccygeal) in order to innervate a group of muscles (i.e., myotome) and a given area of skin
(i.e., dermatome) via the ventral and dorsal horn, respectively.

![Spinal cord cross-section](image)

**Figure 1.1: Spinal cord cross-section:** The corticospinal tract (lateral and anterior, orange), the dorsal columns (red), and the spinothalamic tract (blue) are shown. The segmental organization of each pathway is marked.
### Table 1.1: Major ascending and descending white matter tracts in humans

<table>
<thead>
<tr>
<th>Direction</th>
<th>Name</th>
<th>Funiculus</th>
<th>Primary functional significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascending</strong></td>
<td><strong>Dorsal column – medial lemniscal system</strong></td>
<td>Dorsal, Dorsal-lateral</td>
<td>Kinesthesia and discriminative touch, Proprioception</td>
</tr>
<tr>
<td></td>
<td><strong>Posterior spinocerebellar</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Anterior-lateral system</strong></td>
<td>Lateral</td>
<td>Pain and temperature</td>
</tr>
<tr>
<td></td>
<td><em>Lateral spinothalamic</em></td>
<td>Lateral</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Anterior spinothalamic</em></td>
<td>Ventral</td>
<td>Non-discriminative pain</td>
</tr>
<tr>
<td><strong>Descending</strong></td>
<td><strong>Corticospinal</strong></td>
<td>Anterior, Lateral</td>
<td>Precise movements (e.g., hands and feet)</td>
</tr>
<tr>
<td></td>
<td><strong>Rubrospinal</strong></td>
<td>Lateral</td>
<td>Voluntary movement (e.g., large extensors and flexors)</td>
</tr>
<tr>
<td></td>
<td><strong>Lateral vestibulospinal</strong></td>
<td>Ventral, Lateral</td>
<td>Balance</td>
</tr>
<tr>
<td></td>
<td><strong>Medial vestibulospinal</strong></td>
<td>Ventral</td>
<td>Head position</td>
</tr>
<tr>
<td></td>
<td><strong>Reticulospinal</strong></td>
<td>Ventral</td>
<td>Movement, respiration, inhibitory effects on transmission of sensory stimuli</td>
</tr>
</tbody>
</table>

Adapted from Goshgarian, et al., 2010

### Table 1.2: Axonal conduction properties

<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><strong>Myelinated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>I</td>
<td>A</td>
<td>12-20</td>
<td>72-120</td>
</tr>
<tr>
<td>Medium</td>
<td>II</td>
<td>A</td>
<td>6-12</td>
<td>36-72</td>
</tr>
<tr>
<td>Small</td>
<td>III</td>
<td>A</td>
<td>1-6</td>
<td>4-36</td>
</tr>
<tr>
<td><strong>Unmyelinated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>C</td>
<td>0.2-1.5</td>
<td>0.4-2</td>
</tr>
</tbody>
</table>

Adapted from Gardner, et al., 2000
1.2 Spinal cord injury

1.2.1 Epidemiology of traumatic spinal cord injury
Spinal cord injury (SCI) represents a devastating condition with an incidence varying between 10.4 and 83 per million inhabitants per year world-wide\(^2\). In Switzerland, two thirds of the affected individuals are male, one third female. The age at the time of injury is bi-modally distributed with a first peak between 10 and 24 years of age and a second in adults between 45 and 49 years. Comparable to other first world countries, traffic accidents involving motor vehicle, bicycles, and pedestrians account for the greatest number of traumatic SCI (up to 50%) in Switzerland. Other causes of traumatic SCI include falls (25%) and sports accidents (33%). Lastly, the incidence of tetraplegic and paraplegic SCI is of comparable frequency. While improvements in emergency and primary treatment\(^3\) have substantially lowered the mortality as a consequence of SCI, the average lifespan of individuals living with SCI reside shorter compared to that of the general population\(^4, 5\). The lifetime impact of SCI on society is immense and attributable to the costs associated with acute hospitalization and rehabilitation, but also to treat ongoing secondary health challenges over the chronic stages of living with SCI\(^6, 7\). However, the direct costs to health care of traumatic SCI fail to consider the high indirect costs to society (e.g., lost productivity) and the deleterious impact on the individual’s quality of life.

1.2.2 Primary and secondary spinal cord injury
Spinal cord injuries arise from either traumatic (e.g., fall, car accident, gun shot, sports accident) or non-traumatic damage (e.g., vertebral spondylosis, tumorous compression, vascular ischemia, congenital disease). Irrespective of the cause, the consequential pathology of traumatic SCI is a multiple-step process involving primary and secondary injury mechanisms\(^8, 9\). The primary mechanism comprehends the mechanical injury due to deformation of the vertebral column that, in turn, mechanically disrupts the spinal cord parenchyma. The initial impact leads to disruption of cell membranes, along with hemorrhagic necrosis, anoxia, and the release of neurotoxic cellular constituents from within disrupted cells, all mechanisms that, in turn, initiate a complex secondary injury cascade of biochemical and cellular processes culminating in a progressive degenerative injury to the spinal cord\(^8-11\). Subsequent Wallerian degeneration rostral and caudal to the ‘epicenter of injury’, demyelination, the formation of a glial scar, and central cavitation within days and weeks after acute primary traumatic SCI are presumed to vastly contribute to long-term neurological impairment as well as hinder regeneration and remyelination\(^12\). Nonetheless, neither primary nor secondary mechanisms of injury typically cause a complete transection of the spinal cord (i.e., anatomical complete disruption), but spare an intact residual of white matter\(^13, 14\).

Spinal shock
The term “spinal shock” describes the clinical state in patients suffering from acute SCI with absent or pathological tendon reflexes (e.g., delayed plantar response), paralyzed muscles, and flaccid muscle tone.
caudal to the lesion site\textsuperscript{15, 16}. Resolution of paralysis, appearance of pathological reflexes and reemergence of absent reflexes typically occurs between one and three days after injury and is often followed by progressive development of a “spastic syndrome” with hyperreflexia, increased muscle tone, and spasticity. Several physiological processes have been suspected to account for the distinct phases of spinal shock. Paralysis and areflexia have been largely attributed to acute neuronal hyperpolarization, absent supraspinal background neural excitation, and disturbances in presynaptic inhibition\textsuperscript{16}. The recovery of abolished reflexes and hyperreflexia has been associated with increased neuronal signaling as a resultant of denervation supersensitivity by receptors to neurotransmitters and the unmasking of new synaptic linkages within the central nervous system (CNS)\textsuperscript{16}. Diminished motor axon excitability of peripheral nerves in both, upper and lower extremities during the initial period of SCI may also contribute to the emergence of spinal shock symptoms\textsuperscript{17}.

1.2.3 Spontaneous recovery and chronic spinal cord injury
The degree of neurological impairments associated with acute traumatic SCI greatly depends on the severity of disruption of the major ascending and descending white matter tracts. Regardless of its level and completeness of SCI, plasticity can occur throughout the neuraxis\textsuperscript{18} rendering possible spontaneous neurological recovery to a greater or lesser extent within the first year post injury\textsuperscript{19-23}. Several mechanisms have been suggested to underlie the spontaneous neurological recovery, including alterations in the properties of spared neuronal circuitries (compensatory strategies), intact or lesioned axon collateral sprouting (neural plasticity, regeneration), and synaptic rearrangements (neural repair). However, the degree of spontaneous axonal regeneration (plasticity) within the injured spinal cord is rather limited\textsuperscript{24} probably owing to an actively inhibiting environment (CNS myelin and astrogliosis) that is unsupportive of regeneration\textsuperscript{12, 25}. Neural plasticity caudal (i.e., spinal structures) and rostral (i.e., supraspinal structures) to the level of injury has been reported with regards to locomotion. Caudal to the lesion level, activity dependent plasticity arising from afferent feedback to the lower limb central pattern generators has been suggested as a potential mechanism enabling functional recovery\textsuperscript{26-28}. Neural plasticity has also been found rostral to the level of injury in supraspinal structures in response to treadmill training\textsuperscript{29}, but likewise during spontaneous neurological recovery\textsuperscript{30}.

In the chronic phase of SCI, acute pathophysiologic processes resolve and neurological impairment becomes relatively stable. Late neurological recovery or secondary deteriorations, such as post-traumatic syringomyelia, rarely occur (in ~5% of all cases)\textsuperscript{31, 32}. Depending on completeness and level of injury, individuals with traumatic SCI experience not only deficits in sensorimotor functions, but also a variety of secondary complications, including dysfunction of autonomic system (e.g., neurogenic lower urinary tract dysfunction, sexual dysfunction), upper respiratory tract infections, and neuropathic pain\textsuperscript{33, 34}.
1.2.4 Sensory plasticity following SCI

Sensory impairments

Functional recovery is dependent on concomitant improvements in both motor and sensory systems function. As the name implicates, the sensory system is responsible for processing sensory information providing essential awareness about the external environment and body state (e.g., proprioception), but also the integration of sensory feedback during locomotion is crucial for modulating motor output patterns\(^{35}\). Aside from the ascending part of afferent input that mediates the conscious perception of movement, many afferent inputs also assemble local synapses at the level of the spinal cord\(^{36}\). Needless to say, sensory feedback can be completely abolished or severely compromised after disruption of nerve fiber bundles that convey ascending sensory information. Damage to sensory afferents causes an abolished or altered response to pain and temperature stimuli, two-point discrimination, vibration, and proprioception. Furthermore, spinal circuitries compromising of complex system of neural networks are altered and in turn the regulation of motor output via their influence on spinal motor neurons is impaired. Sensory dysfunctions are likely to emerge during the first hours to days after injury. Various phenotypes with different sensory deficits and complications are reported, but a profound understanding of these alterations and underlying mechanisms is lacking.

Sensory complications: neuropathic pain

A large proportion (up to 80\%) of individuals sustaining an acute spinal cord injury (SCI) reports neuropathic pain in the initial days to weeks after injury, which is often refractory to treatment and, consequently, persists for a lifetime\(^{37-40}\). The collective burden to the individual (i.e., financial, quality of life, loss of function) and society (i.e., financial) is enormous\(^{41, 42}\). Regardless of considerable progress in understanding the cellular and molecular alterations that occur in response of a SCI, many of which are suspected to contribute to the development of neuropathic pain, individuals with SCI continue to suffer from neuropathic pain. Conventionally, neuropathic pain is divided into at-level (i.e., anywhere within the neurological level and two dermatomes below this level) and below-level neuropathic pain (i.e., three segments or more below the level of injury) referring to the location of appearance. Neuropathic pain may be spontaneous, either ongoing or intermittent, or stimulus-evoked\(^{43}\). The qualities of spontaneous pain include tingling, shooting, and aching, while stimulus-evoked pain encompasses allodynia (i.e., a non-painful stimulus is perceived as noxious) and hyperalgesia (i.e., augmented response to noxious stimulus). Other features associated with neuropathic pain are changes in the endogenous pain modulation as well as alterations in nociception at\(^{44-46}\), below\(^{47-49}\), and above the SCI level\(^{45, 46, 50}\) reflecting several pathophysiological processes. The mechanisms that contribute to the emergence and maintenance of neuropathic pain are not well understood, yet several are proposed including abnormal spinothalamic function\(^{51, 52}\), imbalance between dorsal column and spinothalamic tract\(^{53}\), thalamic dysfunction\(^{54, 55}\), or central sensitization\(^{56-58}\). Therapeutic interventions to treat neuropathic pain are limited and ‘clinically
meaningful analgesia’ (i.e., reductions in pain intensity >30%) is only achieved in a subpopulation of individuals with SCI\textsuperscript{59,60}. An incomplete understanding of mechanisms underlying neuropathic pain is central to the lack of more effective interventions (e.g., pharmacological and non-pharmacological)\textsuperscript{61}.

1.2.5 Classification and assessments of spinal cord injury

**International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)**

Conventionally, severity (i.e., complete vs. incomplete) and lesion level of injury are determined according to the "International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)" established by the American Spinal Injury Association (ASIA)\textsuperscript{62-64}. A complete spinal cord injury (cSCI) refers to the total loss of sensory and motor function in the lowest sacral segments (i.e., S4-S5), while an incomplete SCI indicates partial disruption of spinal neural pathways with preserved sensory and/ or motor function below the neurological level including sacral segments S4-S5\textsuperscript{62}. The degree of impairment is graded as A through E (Table 1.3). The neurological examination is performed according to the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) published by the American Spinal Injury Association (ASIA)\textsuperscript{62-64}. Briefly, sensory, motor, and neurological levels of injury are identified allowing characterization of sensory/ motor functioning as well as determination of the completeness of injury. Sensory levels are assessed by testing two aspects of sensation, light touch and pin prick (sharp-dull discrimination), of a keypoint in each dermatome (C4-S4-5, bilateral). Motor function assessment comprised testing key muscle functions corresponding to 10 paired myotomes (C5-T1 and L2-S1).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Functional Impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = Complete</td>
<td>No sensory and motor function is preserved in the sacral segments S4-S5.</td>
</tr>
<tr>
<td>B = Incomplete</td>
<td>Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5.</td>
</tr>
<tr>
<td>C = Incomplete</td>
<td>Motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle graded less than 3 (Grades 0-2).</td>
</tr>
<tr>
<td>D = Incomplete</td>
<td>Motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade greater than or equal to 3.</td>
</tr>
<tr>
<td>E = Normal</td>
<td>Sensory and motor function is normal.</td>
</tr>
</tbody>
</table>

Adapted from Marino et al., 2003

**Neurophysiological assessments**

Performed in conjunction with the ISNCSCI, the employment of detailed electrophysiological approaches aims at more detailed understanding of the neurological deficits related to SCI that are not fully assessed by the ISNCSCI\textsuperscript{65,66}. Combining these assessments may provide a more objective measure of spinal cord...
function and subsequently improve diagnosis and prognosis of SCI. These methods encompass multiple techniques, such as evoked potentials (sensory and motor), nerve conduction studies (sensory and motor), and sympathetic skin response (e.g., autonomic function). In theory, implementing electrophysiological 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).

Motor evoked potentials (MEPs) are generally elicited in the motor cortex employing transcranial magnetic stimulation, and the resultant compound muscle action potential (CMAP) is recorded using electromyography (EMG) at the target muscle. The CMAP is believed to arise from efferent impulses conveyed in the corticospinal tract, however due to the lack of specificity of magnetic stimulation the contribution of other descending pathways (e.g., reticulospinal, rubrospinal or vestibulospinal tracts) is likely. Conventional somatosensory evoked potentials (SSEP) provide information regarding conduction in large diameter fibers that ascend in the dorsal column, while contact heat evoked potentials (CHEPs) have emerged as the neurophysiological assessment of conduction in small diameter fibers ascending in the spinothalamic tract. Conventional SSEPs are examined by peripheral electrical stimulation of the median (arm) and tibial (leg) nerves with electrical pulses and recording electroencephalography (EEG) from scalp electrodes. However, it is

<table>
<thead>
<tr>
<th>Time [ms]</th>
<th>Contact heat evoked potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>100</td>
<td>Impaired (amplitude)</td>
</tr>
<tr>
<td>200</td>
<td>Impaired (latency and amplitude)</td>
</tr>
<tr>
<td>300</td>
<td>Abolished</td>
</tr>
</tbody>
</table>

Figure 1.3: Contact heat evoked potentials under physiological and pathological conditions. Depending on the severity and completeness of the lesion CHEPS can be normal, impaired (reduced amplitude, prolonged latency, or both), or completely abolished.
difficult to precisely determine the level of SCI derived from findings of conventional SSEPs. For this reason, electrical stimulation of distinct individual dermatomes using similar EEG methodology as employed for conventional SSEPs allows a segmental neurophysiological assessment of posterior spinal cord innervation (dorsal root entry and ascending dorsal column conduction). Originally described in 1976\textsuperscript{81}, laser evoked potentials (LEPs) were used to apply painful heat stimuli for the segmental assessment of the spinothalamic tract. However, due to the practical limitations of acquiring LEPs in a clinical setting (e.g., skin burns, safety precautions), CHEPs emerged to the method of choice\textsuperscript{71}. The cortical structures involved in processing CHEPs are comparable to those for LEPs, and include the secondary somatosensory cortex, dorsal and anterior insular cortex and anterior cingulate cortex. Depending on the severity of pathology, the latency and amplitude of CHEPs may be impaired (i.e., delayed or reduced amplitude) or completely abolished (as illustrated in Figure 1.3), similar to SSEPs and MEPs. A dermatomal neurophysiological approach shares the advantages of the ISNCSCI to assess segment-by-segment sensory outcomes above, at, and below the level of lesion. Unfortunately, current techniques do not enable segmental myotomal MEPs (i.e., the activation of individual muscles) using surface stimulation of the cranium overlying multiple regions of the motor cortex.

**Imaging assessments**

For the evaluation of the injured spinal cord, magnetic resonance imaging (MRI) has been described as the “imaging modality of choice”, supposedly due to high-resolution images of the damage in the spinal cord compared to computer tomography\textsuperscript{82}. Particularly in acute or sub-acute SCI, objective MRI measures of parenchymal hemorrhage or contusion, edema, and spinal cord disruption provide further information of severity of injury crucial for prognosis of neurological improvement. During the chronic stage of SCI, MRI is highly relevant in tracking progressive deterioration that may arise (e.g., syringomyelia, atrophic changes of spinal cord and brain)\textsuperscript{83, 84}. The employment of advanced neuroimaging techniques, including diffusion tensor tractography\textsuperscript{85}, functional MRI (fMRI) of the spinal cord\textsuperscript{86}, and tensor-based morphometry (TBM)\textsuperscript{87}, aims at improving the assessment of the injured spinal cord. Admittedly, most of these techniques remain investigational due to several limitations, including low cross-sectional resolution to detect damage in specific white matter tracts in the spinal cord, limited information regarding specific correlations between functional deficits and anatomical damage within the spinal cord, and difficulty imaging the spinal cord close to surgical instrumentation. Investigating the spinal cord atrophy rostral to the lesion level may constitute a valuable tool to address the latter limitation\textsuperscript{88, 89}.
1.3 Thesis Objectives

The overall objective of this doctoral dissertation was to investigate segmental sensory plasticity following spinal cord injury (SCI) by employing neurophysiological and imaging techniques applied in healthy control individuals, and individuals with chronic SCI (i.e., tetraplegia and paraplegia). Five original research studies (Chapters 2-4 and 6-7) and one literature review (Chapter 5) were undertaken between October 2011 and February 2015. The objective of the first study reported in Chapter 2 was two-fold: firstly, to examine the modulating effect of non-noxious conventional transcutaneous electrical nerve stimulation (TENS) in healthy control individuals, investigating the potential for interactions between large and small diameter afferents in the spinal cord, and secondly, to address how sensitization (i.e., induced by capsaicin) might alter the effectiveness and specificity of TENS to modulate small diameter, but not large diameter afferents. In the second study (Chapter 3), procedural information gained from the first study (Chapter 2) was applied in order to examine how SCI and neuropathic pain influence endogenous capacity to modulate noxious thermal stimuli and prolonged capsaicin application. Furthermore, structural magnetic resonance imaging (MRI) has highlighted an important relationship between normal brain anatomy and sensory function. Thus, the third study (Chapter 4) intended to address the question whether between-subject variability in cortical structure (i.e., gray and white matter volumes and cortical thickness) could account for differences in responses to contact heat stimulation in healthy individuals. Chapter 5 (systematic literature review) serves as introduction for two subsequent studies. The primary objective of this review was to systematically examine studies that have addressed the relationship between reorganization in the brain after deafferentation and chronic pain using advanced functional and anatomical MRI. Lastly, the objectives of study four (Chapter 6) and five (Chapter 7) were to investigate the trauma-induced and pain-associated alterations in brain anatomy and function, respectively, by incorporating elaborative imaging and neurophysiological protocols.
Study 1

Effectiveness of high frequency electrical stimulation following sensitization with capsaicin
Catherine R. Jutzeler, Armin Curt, and John L.K. Kramer

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~ We would like to thank all of the individuals participating in our study, and Corinne Diggelmann, Sophie Egger, and Jenny Haefeli for their support in collecting data.~
2.1 Abstract

Although non-noxious, high frequency electrical stimulation applied segmentally (i.e., conventional transcutaneous electrical nerve stimulation, TENS) has been proposed to modulate pain, mechanisms underlying analgesia remain poorly understood. To further elucidate how TENS modulates pain, we examined evoked responses to noxious thermal stimuli after the induction of sensitization using capsaicin in healthy volunteers. We hypothesized that sensitization caused by capsaicin application would unmask TENS analgesia, which could not be detected in the absence of sensitization. Forty-nine healthy individuals took part in a series of experiments. The experiments comprised the application of topical capsaicin (0.075%) on the left hand in the C6 dermatome, varying the location of TENS (segmental, left C6 dermatome; versus extra-segmental, right shoulder), and assessing rating of perception (numeric rating scale: 0-10) and evoked potentials to noxious contact heat stimuli. The extra-segmental site was included as a control condition, since previous studies indicate no analgesic effect to remote conventional TENS. Conventional TENS had no significant effect on rating or sensory evoked potentials in individuals untreated with capsaicin. However, segmental TENS applied in conjunction with capsaicin significantly reduced sensation to noxious thermal stimuli following a 60-minute period of sensitization. The study indicates that sensitization with capsaicin unmasksthe analgesic effect of conventional TENS on perception to noxious contact heat stimuli. Our findings indicate that TENS may be interacting segmentally to modulate distinct aspects of sensitization, which in turn results in analgesia to thermal stimulation.
2.2 Introduction

In healthy individuals, modulatory effects of transcutaneous electrical nerve stimulation (TENS) on experimental measures of pain (e.g., thresholds) have been extensively investigated\(^\text{90-96}\). While strongly supporting an analgesic effect on pressure pain thresholds\(^\text{96-98}\), evidence of thermal analgesia is more limited\(^\text{99, 100}\). Owing to the specificity of segmental stimulation delivered at non-noxious intensities, the analgesic effect of conventional TENS has been historically attributed to inhibition of pain signaling at the level of the spinal cord (i.e., gate control theory of pain)\(^\text{101-103}\). Compared to noxious counter-irritation and recruitment of supraspinal structures involved in diffuse noxious inhibitory control (DNIC), the use of non-noxious high frequency TENS represents a distinctly different approach to modulate pain through recruitment of large diameter afferents. Although studies have confirmed the requisite anatomy and physiology to facilitate spinal interactions between A-beta and A-delta/C-fibers (e.g., wide dynamic range neurons), other putative mechanisms of conventional TENS have been proposed to explain the effectiveness of TENS in chronic pain states. Prominently among these is that electrical conditioning stimulation modulates neuropeptides and neurotransmitters involved in central and peripheral sensitization\(^\text{104-106}\).

Activating transient receptor potential cation channel subfamily V member 1 (TrpV1) receptors, capsaicin represents a well-known model of peripheral and central sensitization\(^\text{107-109}\). Following capsaicin application, animal studies have demonstrated up-regulation of various neurotransmitters and neuropeptides in the dorsal horn, including those shown to be modulated by TENS\(^\text{110-112}\). Behaviorally in humans, responses to capsaicin application are consistent with sensitization, in that sensitivity to noxious stimuli is increased (i.e., pain thresholds are reduced)\(^\text{107, 113-115}\). While capsaicin has been routinely applied in humans as an experimental model of pain\(^\text{109, 115, 116}\), translational studies examining the effect of conventional TENS after the induction of sensitization are currently lacking. The use of capsaicin to induce sensitization recognizes that TENS is typically not therapeutically applied in individuals with normal sensation, and that modulation may depend on factors underlying the generation of pain symptoms – that is, TENS may behave differently in patients than in the healthy individuals. Pre-treatment with capsaicin represents one step towards modeling chronic pain conditions, including those characterized by peripheral and central sensitization\(^\text{109, 116, 117}\).

The primary objectives of the proposed study were two-fold. First, building on previous studies, we aimed to further assess the effectiveness of conventional TENS to reduce perception to noxious heat stimulation. More specifically, we were interested to examine the modulating effect of non-noxious conventional TENS, investigating the potential for interactions between large and small diameter afferents in the spinal cord. For this purpose, responses to brief pulses of noxious contact heat stimulation were examined in healthy individuals before and after segmentally applied TENS (100Hz at non-noxious intensities). Second, we sought to address how sensitization might alter the effectiveness of conventional TENS to
modulate perception to contact heat stimulation. Following the topical application of capsaicin (60 minutes), responses to contact heat and non-noxious electrical stimulation were examined before and after segmental and extra-segmental TENS. The goal of testing different stimulation modalities (i.e., contact heat and electrical stimuli) was to examine the specificity of TENS to modulate small diameter, but not large diameter afferents. The working hypothesis was that if conventional TENS modulated sensitization, capsaicin would increase the effectiveness to alter perception to thermal stimulation.

### 2.3 Material and Methods

#### Individuals
Forty-nine neurologically healthy individuals were included in the study (31 women, 18 men; mean age 28.9 ± 6.6 years; range 20-52 years). All individuals gave their written informed consent. The protocol was in accordance with the Declaration of Helsinki and approved by the local Ethics committee (ref. number: EK-04/2006).

#### Experimental design

**Study 1: Effects of conventional TENS on thermally and electrically evoked pain**

![Figure 2.1: Study design.](image)

(A) In experiment 1, subjects underwent sessions of contact heat and electrical stimulations at 0, 5, 10, 30, and 60 minutes, and again following 10 minutes of conventional TENS. TENS was applied in the area tested with contact heat stimulation. (B) Contact heat stimulation and electrical stimulation was examined during 60 minutes of topical application of capsaicin (baseline, 10, 30, and 60 minutes). After the final removal of capsaicin, 10 minutes of segmental (Experiment 2, dorsum of left hand) or extra-segmental sham TENS (Experiment 3, shoulder) were followed by the final examination of contact heat and electrical stimulations.

**CHEPs:** Contact heat evoked potentials  
**SSEPs:** Somatosensory evoked potentials  
**TENS:** Transcutaneous electrical nerve stimulation
Individuals (n=10) were examined with contact heat and electrical stimulation on the dorsum of the hand at the base of the thumb at 0, 10, 30, and 60 minutes, and again after 10 minutes of conventional TENS. The purpose of repeatedly stimulating the hand before applying TENS was to ensure that the effect of TENS, applied at 60 minutes, was examined from a stable baseline (i.e., reduce the potential effect of habituation on perception). To specifically address segmental effects (Figure 2.1A), TENS was applied in the area tested with contact heat and electrical stimulation (left hand, base of the thumb, within the innervation of the 6th cervical spinal segment). TENS (100Hz, 0.2ms, continuous) was delivered from a Keypoint Electrodiagnostic Device (Medtronic, Mississauga, Ontario, Canada) at intensities adjusted to each individual. Individuals were instructed to indicate a threshold that caused a non-painful tingling sensation. Electrical intensities were adjusted manually from 0mA at 0.1mA intervals until the individual identified an intensity that was clearly perceived, but not painful. All individuals reported this intensity to be between 5 and 7mA. Ten minutes of conventional TENS was selected based on a previous study that demonstrated significant effects on pain intensity in response to laser stimulation in healthy individuals.

**Study 2: Effects of conventional TENS on thermally and electrically evoked pain following sensitization with capsaicin**

In a separate study, the modulating effect of conventional TENS (100Hz, 0.2ms, continuous) on perception to contact heat and electrical stimulation was examined following the topical application of capsaicin (n=39). Individuals were randomly assigned to receive 60 minutes of capsaicin followed by 10 minutes of conventional segmental TENS (n=24, same procedures as performed in Study 1 but now with capsaicin), or 60 minutes of capsaicin followed by 10 minutes of extra-segmentally applied TENS (n=15). One mL of capsaicin cream (0.075%, Haenseler AG, Switzerland) was applied to the left dorsum of the hand, within the C6 dermatome (i.e., contact heat and electrical stimulation test site). Previous studies have used similar concentrations of capsaicin to sensitize peripheral afferents. Prior to application, an area 4x4cm area was marked as a boundary for capsaicin (Figure 2.1B). Capsaicin cream was temporarily removed during testing with contact heat and electrical stimulation. This removal was required to perform testing with the contact heat stimulator, as well as to adhere electrodes for electrical stimulation. For removal, capsaicin cream was gently rubbed clean with a wet cloth. Immediately following testing with contact heat and electrical stimuli, capsaicin was re-applied to the area. Since previous studies indicate no analgesic effect to remote conventional TENS, extra-segmental conditioning comprised of TENS (non-noxious, 100Hz, pulse width 0.2ms) applied on the contralateral shoulder. The extra-segmental site was included primarily as a sham control condition.
Afferent stimulation

Contact heat stimulation

In both Study 1 and 2, responses to noxious stimuli were examined using a contact heat stimulator (Pathway, Medoc, Ramat Yishai, Israel). The thermode surface (diameter: 27 mm) consists of a heating thermo-foil covered with a layer of thermo conductive plastic. The nominal heating rate of this device is 70°C/s (thermo-foil), with a cooling rate of 40°C/s (peltier element). All measurements of perception to contact heat stimulation were made from a baseline temperature of 35°C. A total of 10 stimuli were applied with an inter-stimulus time interval that randomly varied between 8 and 12 s. In line with previous studies, inter-pulse intervals varied randomly to increase stimulation saliency (i.e., individual was unaware when the next stimulation would come), and improve the acquisition of evoked potentials. Individuals rated each stimulus using an 11-point numeric rating scale (NRS; 0 = no pain, 10 = worst pain imaginable). To reduce receptor fatigue or sensitization by overheating of the skin, the thermode was slightly repositioned after each stimulus within a squared area of approximately 4 x 4 cm.

Electrical stimulation

In conjunction with contact heat stimulation in both studies, perception to non-noxious electrical stimulation was also examined. Electrical stimulation was examined to control for potential changes in arousal and attention throughout the duration of the experiment. Specifically, electrical stimulation was applied to account for the potential generalized effects of TENS on sensation. Electrical stimuli were comprised of 10 single, square wave (0.5ms) pulses, delivered at 0.1 Hz using surface gel electrodes. Stimuli were delivered using a Keypoint electro-diagnostic device (Medtronic, Mississauga, Ontario, Canada; bandpass = 2 Hz-2 kHz). Stimulation intensities were adjusted for each individual, using the same procedures described for TENS (i.e., non-noxious tingling sensation, between 5-7mA). Individuals were asked to rate their level of unpleasantness to successive stimulation (NRS=0-10).

Sensory evoked potential recordings

The validity and reliability of contact heat and somatosensory evoked potentials (CHEPs and SSEPs, respectively) for the assessment of the spinothalamic and dorsal column pathways, respectively, has been shown in previous studies. CHEPs and SSEPs were recorded as a surrogate measure of perception to contact heat and electrical stimulation. Sensory evoked potentials were recorded with eyes open, while individuals were lying in a supine position. Individuals were asked to fix on a point on the ceiling, and to remain relaxed and quiet during testing. CHEPs and SSEPs were recorded with 9mm Ag/AgCl surface disc electrodes filled with conductive adhesive gel. Scalp recording sites were prepared with Nuprep (D.O. Weaver & Co. Aurora, CO) and alcohol. Silver-silver disc recording electrodes were positioned according to the 10-20 system, with the active electrode at Cz and referenced to the linked earlobes (A1-A2). The rationale for a reduced electrode set up was that consistent negative and positive
potentials (i.e., N2P2) can be detected at Cz\textsuperscript{potentials}. CHEPs were sampled at 2000Hz using a preamplifier (20000x, bandpass filter 0.25-300Hz, ALEA Solutions, Zurich, Switzerland). Data were recorded with 100ms pre-trigger and a two second post-trigger in a LabView based program (V1.43 CHEP, ALEA Solutions, Zurich, Switzerland). SSEPs were recorded using a Keypoint electrophysiological stimulating and recording device (bandpass = 2 Hz-2 kHz; Medtronic, Mississauga, Ontario, Canada). Electrode impedance was maintained below 5kΩ, and verified prior to the initiation of each recording. All CHEPs and SSEPs were bandpass filtered (1-30Hz). The N2P2 waveforms were visually detected based on the average of the 20-recorded trials.

**Sensory outcomes: Determining stimulation intensities**

As a measure of changes in thermally evoked pain, an adjusted peak temperature protocol was employed for both Study 1 and 2. Based on initial ratings to contact heat and electrical stimulation, the peak temperature of contact heat stimulation and intensity of electrical stimulation, respectively, was adjusted to maintain ‘baseline’ ratings. At the baseline measurement, all individuals were asked to rate contact heat stimulation delivered from a 35°C baseline to 52°C. Likewise, individuals were asked to rate the unpleasantness of electrical stimulation delivered at self-selected intensity (i.e., 5-7mA). Three consecutive stimuli (i.e., contact heat and electrical) were provided to ensure a stable rating and allow the individual to become familiar with stimulation. After 10 minutes, individuals were asked to rate the same stimulation as applied at baseline (i.e., 35°C baseline temperature to 52°C and self-selected electrical intensity). Depending on whether individuals reported stimuli as stronger or weaker on average, the peak temperature and intensity of electrical stimulation (mA) was adjusted accordingly (i.e., decreased or increased, respectively). For adjustments of contact heat stimulation, peak temperature was modified by 1°C. This procedure was repeated until the peak temperature and intensity of electrical stimulation elicited a rating similar to the previous test stimuli. The entire procedure was repeated at 30 and 60 minutes, with temperatures and electrical intensities adjusted for each individual relative to individual changes in perception. Adopting such a protocol, decreases in peak temperature and electrical intensity reflect increased perception, and increases in peak temperature and electrical intensity represent decreased perception. The rationale for using this protocol was primary related to contact heat stimulation. First, using a fixed peak temperature, we were concerned that not all individuals could tolerate contact heat stimulation after 60 minutes of capsaicin application. Previous studies have demonstrated that capsaicin increases numeric rating to evoked thermal sensations\textsuperscript{115, 116, 132}. Second, adjusting the peak temperature meant that individual perception to contact heat stimulation in the presence of capsaicin would not effectively change. Thus, changes in perception related to TENS stimulation would not be a function of simply increasing the rating of intensity, from which analgesia could be easier to detect.
Over 60 minutes, capsaicin is known to gradually induce symptoms like itching pain, burning sensation, as well as thermal and mechanical hypersensitivity in an area of primary hyperalgesia\textsuperscript{109, 115, 133, 134}. Therefore, in Study 2, individuals were asked to rate pain every five minutes for 60 minutes using a numeric rating scale (0 = no pain, 10 = worst pain imaginable). None of the individuals reported any painful sensations prior to conditioning with capsaicin.

**Statistical Analysis**

Statistical analyses were performed using IBM’s Statistical Package for the Social Sciences (SPSS) version 19.0 (Armonk, New York, U.S.). All data were tested for normal distribution using the Kolmogorov–Smirnov test.

**Experiment 1**

Linear mixed model analyses were performed to analyze thermally evoked pain (NRS), as well as CHEPs parameters (N2P2 amplitude, N2- and P2-latencies) over time. Individuals were included as random factor, while age and gender were treated as covariates. Main and interaction effects were assessed with rating (NRS) as the dependent variable, and all time-points (baseline, pre-TENS, and post-TENS) as fixed effects. In case of significance, post-hoc paired t-tests were employed to identify significant differences between time-points. Multiple comparisons were Bonferroni corrected. Statistical significance was set at $\alpha = 0.05$. All $p$ values reported are corrected for multiple comparisons.

**Experiment 2**

Linear mixed models were also employed to analyze capsaicin-evoked pain (NRS) and thermally evoked pain (stimulation peak temperature), as well as CHEPs parameters (N2P2 amplitude, N2- and P2-latencies). In all models, individuals were included as random factor while age and gender were treated as covariates. To analyze the temporal progression of the capsaicin-evoked pain, 13 different time-points (5, 10, 15, 25, 30, 35, 40, 45, 50, 55, 60 minutes (during capsaicin-application) and 65, 70 minutes (post-capsaicin)) were tested. Main and interaction effects were determined by using the rating (NRS) as the dependent variable and all time-points were set as fixed effects. Thermally and electrically evoked pain was assessed at baseline, 10, 30, 60 (pre-TENS), and 70 minutes (post-TENS). The dependent variable was stimulation peak temperature and electrical stimulation intensity (n=39). CHEP and SSEP parameters, namely N2 latency, P2 latency, and N2P2 amplitude, were also considered as dependent variables. Fixed effects included time-point, the dermatomal location of the TENS, as well as the presence/absence of capsaicin. In case of significant effects, post-hoc paired and unpaired sample t-tests were performed to identify significant differences between time-points and dermatomal location of TENS, respectively. Bonferroni correction was used to account for multiple comparisons. Statistical significance was set at $\alpha = 0.05$. All $p$ values reported are corrected for multiple comparisons.
2.4 Results

Overall, there were no differences between groups with regards to age (F=0.125, df: 39, p=0.869), gender (F=0.66, df: 39, p=0.799), and baseline ratings to contact heat stimulation (F=0.176, df: 39, p=0.678) (Table 2.1). The values of skewness and kurtosis were for all parameters tested approximate to zero as required for a normal distribution. Furthermore, the Kolmogorov-Smirnov test yielded significance values greater than 0.05, suggesting normally distributed data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1 No capsaicin</th>
<th>Experiment 2 Segmental TENS</th>
<th>Experiment 2 Extra-segmental TENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>Gender [m:f]</td>
<td>3:7</td>
<td>9:15</td>
<td>6:9</td>
</tr>
<tr>
<td>Age [yrs]</td>
<td>31.9 ± 9.9</td>
<td>27.7 ± 6.5</td>
<td>29.2 ± 8.1</td>
</tr>
<tr>
<td>N2 Latency [ms]</td>
<td>350.1 ± 45.0</td>
<td>368 ± 49.3</td>
<td>352 ± 47.4</td>
</tr>
<tr>
<td>P2 Latency [ms]</td>
<td>472.8 ± 44.0</td>
<td>489 ± 56.4</td>
<td>457 ± 58.1</td>
</tr>
<tr>
<td>N2P2 Amplitude [µV]</td>
<td>42.0 ± 9.5</td>
<td>34.9 ± 17.5</td>
<td>38.8 ± 15.6</td>
</tr>
<tr>
<td>Pain rating (NRS)</td>
<td>5.6 ± 2.0</td>
<td>4.2 ± 1.5</td>
<td>4.0 ± 1.9</td>
</tr>
</tbody>
</table>

*: Baseline measurement, prior to capsaicin application

Study 1: Effect of conventional TENS on thermally and electrically evoked pain and potentials

In unconditioned individuals (i.e., absence of capsaicin), contact heat stimulation delivered at a peak temperature of 52°C elicited constant in NRS at 0, 10, 30, and 60 minutes. Furthermore, segmental TENS had no modulatory effect on perception to contact heat stimulation (Figure 2.2, NRS$_\text{pre}$=5.0 and Temp$_\text{pre}$=52°C; NRS$_\text{post}$=4.8 Temp$_\text{post}$=52°C). Similarly, perception to non-noxious electrical stimulation was unaltered by TENS (NRS$_\text{pre}$=1.9 and Intensity$_\text{pre}$=6.0mA; NRS$_\text{post}$=1.8 and Intensity$_\text{post}$=6.0mA). While CHEP and SSEP amplitudes significantly decreased from baseline to 60 minutes (habitation following...
repetitive stimulations), TENS had no further modulatory effect (Figure 2.3). The results from this study are summarized in Table 2.2.

Table 2.2: Effect of repetitive electrical and thermal stimulations on evoked potentials and perception: Summary of N2 and P2 latencies, N2P2 amplitude, pain rating, and stimulation intensity

<table>
<thead>
<tr>
<th>Time-point of Stimulation [min]</th>
<th>Significant pair-wise comparisons (p&lt;0.05)*</th>
<th>Time [min]</th>
<th>N2P2 amplitude [µV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t10</td>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>t30</td>
<td></td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>t60</td>
<td></td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>t70</td>
<td></td>
<td>70</td>
<td>70</td>
</tr>
</tbody>
</table>

Results are displayed as mean ± standard deviation.

- tB: Baseline measurement
- t10: Time after baseline (in minutes)
- †: Measurement after 10 minutes of HFES
- ‡: Bonferroni corrected
- *: Significant differences between pre- and post-HFES
- NRS: Numeric Rating Scale

Figure 2.3: Changes in CHEPs and SSEPs amplitude over time. In absence of capsaicin, CHEP (A) and SSEP (B) amplitudes undergo a habituation towards smaller amplitudes due to repetitive stimulations. The pain ratings remained stable over time. Grand averages of CHEPs and SSEPs are shown at baseline and 10, 30, 60 min of capsaicin application, and after 10 min segmental TENS.
Study 2

Effects of conventional TENS on capsaicin pain

Except one, all individuals tolerated capsaicin for the entire 60 minutes application period. In general, capsaicin was reported as mildly to moderately painful (NRS = 4.81 ± 2.28). As reported by others, capsaicin progressively increased pain ratings from baseline to 60 minutes (Figure 2.4). Pain ratings to capsaicin were not different between the two study groups (F=0.03; df: 37.7; p>0.05). Independent of the location, conventional TENS (i.e., segmental or extra-segmental) significantly reduced the capsaicin induced ongoing pain (average ratings pre- and post-TENS were NRS = 4.81 ± 2.2 and 1.82 ± 2.0, respectively). However, compared to extra-segmental, segmental TENS was significantly more effective relieving capsaicin pain (∆NRS\textsubscript{Segmental} = -3.5 ± 0.4 versus ∆NRS\textsubscript{Extra-segmental} = -2.2 ± 0.9; F=36.1; df:37.1; p=0.043).

Effect of TENS on perception to contact heat and electrical stimulation

In order to maintain the perception at comparable baseline levels, peak contact heat stimulation temperature was significantly decreased at 30 (F=31.4; df: 38; p<0.001) and 60 minutes (F=12.9; df: 38; p=0.001) after topical application of capsaicin (Figure 2.5). Following pretreatment with capsaicin and segmental application of conventional TENS, peak temperature to elicit ‘pre-capsaicin’ ratings were significantly increased (F=28.8; df: 38; p<0.05). In contrast, extra-segmental sham TENS peak temperature had to be decreased over the course of 60 minutes capsaicin application. Segmental TENS application allowed significant increase of the stimulation peak temperature (i.e., pain relief) while the extra-segmental sham TENS required further decrease in temperature (i.e., pain increase).
resulted in no detectable analgesia – that is, peak contact heat temperatures either remained similar to 60-minute ‘post-capsaicin’ values or required further reductions to maintain baseline NRS (Figure 2.5). Direct comparison between the segmental TENS and extra-segmental sham TENS yielded superior effectiveness of segmental TENS over extra-segmental TENS to relieve pain (i.e., change in peak temperature) (F=70.6, df: 38, p<0.001).

**Sensory evoked potentials**

The effect of capsaicin on the CHEP and SSEP N2P2 amplitudes are summarized in Table 2.3. Similar to effects observed without capsaicin, adjusting the stimulation peak temperature yielded significant reductions in N2P2 amplitudes (SSEPs and CHEPs) between 0 and 60 minutes (i.e., before TENS). TENS (all conditions, p>0.05) induced no discernible changes in CHEP or SSEP amplitudes (Table 2.4).

<table>
<thead>
<tr>
<th>Table 2.3: The effect of capsaicin on contact heat and somatosensory evoked potentials: Summary of pain ratings, N2 and P2 latencies, N2P2 amplitude, pain rating, and stimulus intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contact heat evoked potentials</strong></td>
</tr>
<tr>
<td><strong>Time-point of Stimulation [min]</strong></td>
</tr>
<tr>
<td>Peak Temperature [°C]</td>
</tr>
<tr>
<td>N2 Latency [ms]</td>
</tr>
<tr>
<td>P2 Latency [ms]</td>
</tr>
<tr>
<td>N2P2 Amplitude [µV]</td>
</tr>
<tr>
<td>Pain rating (NRS)</td>
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<table>
<thead>
<tr>
<th><strong>Somatosensory evoked potentials</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity [mA]</td>
</tr>
<tr>
<td>N2 Latency [ms]</td>
</tr>
<tr>
<td>P2 Latency [ms]</td>
</tr>
<tr>
<td>N2P2 Amplitude [µV]</td>
</tr>
<tr>
<td>Pain rating (NRS)</td>
</tr>
</tbody>
</table>

Results are displayed as mean ± standard deviation.

\( t_{0} \): Time of capsaicin application (in minutes)

\( t_{10} \): Baseline measurement prior to capsaicin application

\( t_{60} \): Bonferroni corrected

NRS: Numeric Rating Scale
Table 2.4: The effect of segmental and extrasegmental sham TENS on contact heat and somatosensory evoked potentials: Summary of peak temperature/stimulation intensity, N2 and P2 latencies, N2P2 amplitude, and pain rating

<table>
<thead>
<tr>
<th>Segmental TENS</th>
<th>Time-point of Stimulation</th>
<th>( t_{\text{pre}} )</th>
<th>( t_{\text{post}} )</th>
<th>( t_{\text{pre}} - t_{\text{post}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact heat evoked potentials</td>
<td>( \text{Peak Temperature [}^\circ \text{C]} )</td>
<td>46.9 ± 3.2</td>
<td>50.9 ± 1.9</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>( \text{N2 Latency [ms]} )</td>
<td>318.3 ± 41.9</td>
<td>368.7 ± 67.8</td>
<td>p=0.020</td>
</tr>
<tr>
<td></td>
<td>( \text{P2 Latency [ms]} )</td>
<td>424.0 ± 46.7</td>
<td>469.6 ± 63.2</td>
<td>p=0.033</td>
</tr>
<tr>
<td></td>
<td>( \text{N2P2 Amplitude [} \mu \text{V]} )</td>
<td>26.9 ± 14.0</td>
<td>23.0 ± 10.5</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Pain rating (NRS)</td>
<td>4.6 ± 1.8</td>
<td>4.5 ± 1.7</td>
<td>ns</td>
</tr>
<tr>
<td>Somatosensory evoked potentials</td>
<td>( \text{Intensity [mA]} )</td>
<td>6.2 ± 0.7</td>
<td>6.0 ± 0.5</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>( \text{N2 Latency [ms]} )</td>
<td>132.8 ± 21.6</td>
<td>133.0 ± 23.6</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>( \text{P2 Latency [ms]} )</td>
<td>216.9 ± 39.2</td>
<td>201.0 ± 29.2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>( \text{N2P2 Amplitude [} \mu \text{V]} )</td>
<td>23.0 ± 16.1</td>
<td>16.6 ± 5.9</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Pain rating (NRS)</td>
<td>2.1 ± 1.5</td>
<td>1.9 ± 1.4</td>
<td>ns</td>
</tr>
<tr>
<td>Extrasegmental sham TENS</td>
<td>Contact heat evoked potentials</td>
<td>( \text{Peak Temperature [}^\circ \text{C]} )</td>
<td>46.9 ± 3.2</td>
<td>44.1 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>( \text{N2 Latency [ms]} )</td>
<td>318.3 ± 41.9</td>
<td>301.1 ± 34.4</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>( \text{P2 Latency [ms]} )</td>
<td>424.0 ± 46.7</td>
<td>411.0 ± 54.4</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>( \text{N2P2 Amplitude [} \mu \text{V]} )</td>
<td>26.9 ± 14.0</td>
<td>19.7 ± 10.0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Pain rating (NRS)</td>
<td>4.6 ± 1.8</td>
<td>3.8 ± 2.2</td>
<td>ns</td>
</tr>
<tr>
<td>Somatosensory evoked potentials</td>
<td>( \text{Intensity [mA]} )</td>
<td>5.9 ± 0.5</td>
<td>6.0 ± 0.4</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>( \text{N2 Latency [ms]} )</td>
<td>142.5 ± 39.5</td>
<td>132.4 ± 12.2</td>
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</tr>
<tr>
<td></td>
<td>( \text{P2 Latency [ms]} )</td>
<td>215.1 ± 40.8</td>
<td>200.2 ± 32.2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>( \text{N2P2 Amplitude [} \mu \text{V]} )</td>
<td>27.1 ± 11.6</td>
<td>24.9 ± 18.9</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Pain rating (NRS)</td>
<td>1.9 ± 1.1</td>
<td>1.9 ± 1.4</td>
<td>ns</td>
</tr>
</tbody>
</table>

Results are displayed as mean ± standard deviation.

\( t_{\text{pre}} \): Pre segmental or extrasegmental TENS
\( t_{\text{post}} \): Post segmental or extrasegmental TENS
\( t_{\text{pre}} - t_{\text{post}} \): Bonferroni corrected
NRS: Numeric Rating Scale
2.5 Discussion

In the presence of capsaicin, conventional TENS (high-frequency, delivered at non-noxious intensity) resulted in robust modulation of perception to contact heat stimulation. In contrast, there was no evidence of thermal analgesia in the absence of capsaicin-induced sensitization (i.e., Study #1), or in response to extra-segmental TENS following capsaicin sensitization (i.e., control condition, Study #2). Overall, these findings suggest that the analgesic effect of TENS may be mediated through interactions with sensitized small diameter afferents.

Capsaicin model to examine the effectiveness of TENS

The aim of applying capsaicin in the current study was to examine the effectiveness of conventional TENS in a model of sensitization, whilst exploring alternative mechanisms of analgesia. We hypothesized that if TENS demonstrated greater efficacy in reducing perception to noxious stimuli in the presence of capsaicin, modulation would be related, in part, to factors underlying sensitization (e.g., up-regulation of neuropeptides and neurotransmitters in the spinal cord). Importantly, in the present study we controlled for potential: 1) placebo effects using a sham, extra-segmental TENS condition applied on the shoulder, and 2) changes in pain ratings due to capsaicin application (i.e., adjusted peak temperature protocol). In order to assess the unique effect of sensitization on conventional TENS, controlling for pain ratings was considered an integral component of the study. Had a fixed peak temperature been employed, changes in perception related to physiological aspects of sensitization (e.g., up-regulation of neurotransmitters and neuropeptides in the spinal segment, such as glutamate, aspartate, and substance P) would be difficult to distinguish from behavioral changes in pain ratings caused by capsaicin. In line with pain ratings using an adjusted peak temperature, CHEP amplitudes also did not change after TENS. Likewise, conventional TENS had no effect on SSEPs. In principle, no change in CHEP/SSEP amplitudes supports that cortical processing of afferent stimuli were not obviously affected by TENS, and that contact heat and electrical stimulation was perceived similarly before and after conditioning. This is important, since the aim of adjusting the peak temperature was to maintain baseline perception. Therefore, CHEPs and SSEPs provide confirmatory evidence that peak temperature was accurately adjusted, and that changes in peak temperature reflect meaningful changes in perception.

Following conventional TENS, the complete reversal of thermal hyperalgesia was achieved, with some healthy individuals even able to tolerate baseline, ‘pre-capsaicin’ temperatures of contact heat stimulation (i.e., 52°C). Comparatively, extra-segmental TENS had no pain relieving effect. Indeed, a number of individuals required that the temperature of the contact heat thermode be further decreased after 10 minutes of extra-segmental TENS, indicating a lingering effect of TRPV-1 activation after removal of capsaicin from the skin. Generally speaking, these findings support a therapeutic effect of conventional TENS in humans beyond spinal afferent gating. Despite emerging evidence in animals,
to our knowledge, few studies have provided evidence for alternative mechanisms underlying the effectiveness of conventional TENS in humans. Examining thermal thresholds, Leonard and colleagues demonstrated a role for endogenous opioids in TENS analgesia. A similar mechanism may underlie the effects of conventional TENS on noxious contact heat stimulation following pre-treatment with capsaicin. For example, TENS may up-regulate endogenous opioids, inhibiting neuropeptides and neurotransmitters involved in sensitization (i.e., released in response to capsaicin), which in turn returns “normal” perception to contact heat stimulation. Preclinical and human studies support that: 1) peripheral applications of capsaicin up-regulate the concentration of neurotransmitters (e.g., glutamate, aspartate) and neuropeptides (substance P and calcitonin-related gene peptide) in the dorsal horn, 2) the efficacy of...
TENS to induce analgesia is dependent on endogenous opioids\textsuperscript{104}, and 3) activation of spinal opioid receptors inhibits neurotransmitters and neuropeptides to unfold their action\textsuperscript{104-106}. In the present study, 60 minutes pretreatment with capsaicin may have caused marked up-regulation of various neurotransmitters as well as neuropeptides involved in the nociception, which was then effectively inhibited by opioid release in response to 10 minutes of TENS (Figure 2.6). Interestingly, conditioning electrical stimulation is then not directly inducing analgesia, but rather mediates reduced sensitivity to noxious stimuli through modulating the activity of capsaicin. Our observations also potentially highlight differential modulation of sharp pain (i.e., contact heat; linked with $\alpha$ fiber activation) and dull, spontaneous like pain (i.e., capsaicin; linked with $\gamma$ fiber activation) by TENS-released opioids\textsuperscript{106}. Regardless of the exact mechanism (e.g., endogenous opioids), our findings suggest that TENS may not directly induce thermal analgesia. Rather, TENS appears to be modulating sensitivity to noxious stimuli via interactions with exogenous factors – such as those related to sensitization\textsuperscript{140}. Interestingly, TENS and extra-segmental sham stimulation both relieved ongoing pain due to capsaicin. Since neither TENS nor sham electrical stimulation on the shoulder was delivered at noxious intensities, DNIC would be an unlikely candidate mechanism for this observation\textsuperscript{95, 141}. A more parsimonious explanation is that electrical stimulation modulated ongoing heat pain through non-specific bottom-up and top-down processes, such as distraction and/or placebo. The potential for a strong placebo effect to reduce reported spontaneous pain highlights a major problem with measuring TENS efficacy in clinical populations, as well as a need to complement assessment with quantitative measures.

**Limitation and future directions**

A limitation of our study is that the effects of TENS on perception to contact heat stimulation were not demonstrated in the same individuals across all conditions. The optimal study design would be to fully randomize each individual to receive conventional TENS applied segmentally and extra-segmentally, with and without capsaicin (i.e., a total of 4 conditions, examined on separate days, with sufficient time for washout of capsaicin). Additionally, blinding of individuals and raters may be important. Collectively, the design could have led to biases and an overestimation of the effect of TENS on thermal sensitivity after capsaicin application, including potential placebo effects.

A smaller sample size of individuals undergoing segmental TENS in the absence of capsaicin (i.e., Study 1, n=10) may also be problematic, in that subtle changes in perception may have been overlooked. Assuming similar variability as segmental TENS with capsaicin (SD\textsuperscript{+/-}1.7), a post-hoc analysis (i.e., mean difference from a constant of 0, performed in G\textsuperscript{*}Power 3.1)\textsuperscript{142, 143} indicated that our statistical power to detect the smallest change in temperature (i.e., $1\degree$C) with 10 individuals was approximately 0.52. Furthermore, since the peak temperature of contact heat stimulation can only reasonably be adjusted in $1\degree$C increments, changes in perception based on increases/decreases in peak temperature may also be somewhat limited to measure small effects. Overall, the approach to measure changes in perception to
thermal stimuli represents a modification from existing quantitative sensory testing techniques, such as thermal thresholds. As such, the psychometric properties, including reliability, are currently unknown. In spite of these limitations, the robustness of the findings supports that sensitization unmasks analgesia due to conventional TENS application, which is at least more difficult to detect in the absence of capsaicin.

Outside the scope of the current study, we did not test the effect of conventional TENS on sensation to afferent stimuli in the secondary area of hyperalgesia caused by topical capsaicin application. Addressing if TENS is preferentially alleviating primary and/or secondary hyperalgesia represents an intriguing future line of investigation. Additionally, we only examined the analgesic effect of conventional TENS on thermal sensitivity after 10 minutes of stimulation. Future studies should investigate how long the analgesic effects persist after TENS is turned off.

**Conclusion**

Our results indicate that the induction of sensitization with capsaicin provides greater sensitivity to detect the neuromodulatory properties of TENS on thermally evoked pain. This novel observation suggests that thermal sensation may be modulated through interactions with specific features of sensitization, such as the up-regulation of pain neuropeptides and neurotransmitters.

**Acknowledgment**

We would like to thank all of the individuals participating in our study, and Corinne Diggelmann, Sophie Egger, and Jenny Haefeli (University Hospital Balgrist, Zurich, Switzerland) for their support in collecting data. The study was supported by the Swiss National Science Foundation (SNF) and the Clinical Research Priority Program “Neurorehab” of the University of Zurich, Switzerland. John Kramer was supported by a Michael Smith Foundation for Health Research and Rick Hansen Institute Scholar award.

**Authors Contribution**

Catherine Jutzeler contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Furthermore, she drafted the research article. Armin Curt made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content. John Kramer contributed substantially to the conception and design of the study, data interpretation and drafted the research article.
Study 2

Distinct spinal and supraspinal effects on modulation of pain after spinal cord injury

Catherine R. Jutzeler, Marina Freitag, Armin Curt, and John L.K. Kramer

In preparation

~ We would like to thank all of the individuals participating in our study. ~
3.1 Abstract

Accompanying spontaneous and evoked neuropathic pain (NP) symptoms in dermatomes at and below the level of spinal cord injury (SCI), impaired sensation has been reported in otherwise intact dermatomes above the level of injury. The present study aimed to extend these observations, as well as address other potential mechanisms of NP after SCI (i.e., less efficient modulation). In a crossover-designed study, 33 individuals with SCI (19 with NP) and 15 healthy control individuals underwent two experimental sessions. Topical capsaicin (0.075%) was applied for 30 minutes on the left hand in the C6 dermatome followed by 10 minutes of either ipsilateral (left C6) or contralateral (right C6) transcutaneous electrical nerve stimulation (TENS). Thermal and electrical detection and pain thresholds, rating of perception to capsaicin, and contact heat pulses and electrical stimuli were examined before, after 30 minutes of capsaicin application (pre TENS), and post-TENS. In individuals with SCI and NP, behavioral responses to capsaicin and contact heat were significantly lower compared to pain-free individuals. Individuals with SCI and NP also demonstrated reduced habituation to contact heat stimulation, and were unable to modulate perception to capsaicin and contact heat stimulation in response to contralateral TENS. The extent of modulation achieved through contralateral TENS correlated with the intensity of ongoing NP symptoms. The presence of NP after SCI was associated with impaired habituation and reduced perception to noxious stimuli above the level of injury. The novel finding was that NP was associated with less efficient modulation through conditioning electrical stimulation.
3.2 Introduction

Negatively impacting quality of life, neuropathic pain (NP) represents a major secondary health problem for people currently living with spinal cord injury (SCI). Accompanying spontaneous and evoked NP symptoms in dermatomes at and below the level of injury (e.g., continuous burning and allodynia, respectively), reduced sensation to afferent stimuli has been reported in otherwise intact dermatomes (i.e., above the level of injury). Suggesting that the presence of NP “gives rise to recruitment of endogenous pain suppression”, initial studies using thermal quantitative sensory testing (QST) demonstrated increased pain and perception thresholds (i.e., decreased sensation) above the level of injury. Following a series of studies that failed to replicate these seminal findings, Kumru and colleagues proposed that individuals with SCI suffering from NP (SCI-NP) were associated with a global state of central sensitization, evidenced by decreased thermal pain thresholds (i.e., increased sensation), as well as hyperalgesia and reduced habituation to noxious contact heat stimulation. While central to both explanations of why changes in sensation occur above the level of injury, few studies have directly addressed the impact of SCI on the capacity to modulate noxious afferent input.

Mechanisms of conventional, high frequency, non-noxious transcutaneous electrical nerve stimulation (TENS) have been extensively examined in preclinical animal models and healthy individuals. Among leading candidate theories underlying the effectiveness of TENS, recruitment of large diameter afferents is thought to reduce perception to noxious stimuli by “closing the gate” (i.e., gate control theory of pain). While the majority of studies suggest that conventional high frequency electrical stimulation is most effective when applied segmentally (i.e., within the painful site), there is also evidence in support of contralateral analgesia. From a mechanistic perspective, contralateral effects have been attributed to supraspinal descending modulation in the rostroventral medulla (RVM).

The primary objective of the study was to examine the impact of SCI and NP on endogenous capacity to modulate noxious thermal stimuli above the lesion level. We were specifically interested in applying TENS as a conditioning stimulus, utilizing segmental homotopic (i.e., ipsilateral) and heterotopic (i.e., contralateral) stimulation locations to differentiate spinal and supraspinal modulation. Individuals with SCI (with and without NP) and healthy control individuals (without NP) participated in this crossover-designed study, and were examined using a variety of non-noxious and noxious afferent stimuli, before and after ipsi- and contralateral TENS. Our primary hypothesis was that individuals with SCI and NP would demonstrate a reduced capacity to modulate perception to noxious afferent stimuli above the level of injury, evidenced through reduced efficacy of TENS to relieve thermal hyperalgesia.
3.3 Material and Methods

Participants
Thirty-four individuals with SCI were recruited for the study. Inclusion criteria were chronic traumatic SCI, neurological lesion level below the 6th cervical vertebrae (C6, i.e., normal sensation at the testing site), presence/absence of below-level neuropathic pain, and age between 18-60 years. Additionally, 15 neurologically healthy individuals were enrolled. Exclusion criteria for all participants were presence of any other neurological disorder beside SCI, depression, the intake of analgesics (e.g., opioids, NSAIDs) on days of examination, and pregnancy. All participants provided written informed consent and all procedures described below were in accordance with the Declaration of Helsinki and approved by the local ethics board (ref. number: EK-04/2006).

Clinical assessments
Prior to the measurements, all participants were interviewed to determine the existence of pain and to measure pain catastrophizing using the German versions of the European Multicenter Study about SCI (EMSCI) pain questionnaire (V4.2, http://www.emsci.org/) and the pain catastrophizing scale (PCS) 162, respectively. The pain questionnaire examines various aspects of pain (e.g., duration, maximal and average pain intensity) as well as pain associated psychosocial factors. Accordingly, pain can be grouped into nociceptive (e.g., musculoskeletal or visceral) or neuropathic pain (e.g., at or below the lesion). To be classified as below-neuropathic pain, symptoms (e.g., burning, cold, tingling) reported had to be located three or more segments below the neurological level of lesion. The PCS investigates individuals catastrophic thinking related to painful experiences. The overall reliability and validity of the PCS was demonstrated in various studies 163-166. In individuals with SCI, the neurological level of injury was assessed using the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) published by the American Spinal Injury Association (ASIA) 62, 63, 167.

Experimental design
The modulating effect of conventional TENS on perception to contact heat and electrical stimulation was examined following the topical application of capsaicin. Figure 3.1 illustrates the experimental design. The study consisted of two sessions at least seven days apart and only differed in the location of TENS application (i.e., segmental ipsilateral or segmental contralateral). All participants randomly completed both sessions. In both sessions, participants received 30 minutes of continuous topical capsaicin on the left hand (i.e., C6), and TENS was applied ipsilateral within the same area (segmental homotopic TENS) or contralaterally in the right C6 dermatome (segmental heterotopic TENS, session 2). Electrical stimulation (100Hz, 0.2ms) was delivered with stimulus intensity adjusted to each participant (ranging from 5 to 7mA). Stimulation was intended to cause a non-painful tingling sensation for a period of 10 minutes 90, 91. Participants were examined with contact heat and electrical stimulation (see parameters below) on the
dorsum of the hand at the base of the thumb at baseline (pre-capsaicin), after 30 minutes of capsaicin application, and again after 10 minutes following conventional TENS.

Figure 3.1: Cross-over study design. Prior to the study, all participants were interviewed to determine the existence of pain and to measure pain catastrophizing using the EMSCI pain questionnaire and pain catastrophizing scale (PCS), respectively. Participants underwent sessions of thresholding (cold (CDT) and warm detection thresholds (WDT) as well as cold (CPT) and warm pain thresholds (WPT), and electrical perception (EPerT) and pain thresholds (EPT)) and contact heat and electrical stimulations before and after 30 minutes of capsaicin application. Participants were asked to rate their perception of capsaicin every five minutes using a numeric rating scale (NRS). After the removal of capsaicin, 10 minutes of ipsilateral (dorsum of left hand) or contralateral (dorsum of right hand) transcutaneous electrical nerve stimulation (TENS) were followed by the final examination of contact heat and electrical stimulations. The order of the TENS location was randomized between the two measurement sessions being seven days apart. Included participants completed both sessions.

EMSCI: European Multicenter Study about Spinal Cord Injury

Sensory assessments
The sensory assessments were performed at pre-capsaicin (baseline) and post-capsaicin application (pre-TENS), and after 10 minutes of TENS. Sensory assessment comprised two parts: (a) thresholding and (b) sensory stimulation applying noxious heat and non-noxious electrical stimulation. Cold (CDT) and warm detection thresholds (WDT), as well as cold (CPT) and warm pain thresholds (WPT) were determined using the method of limits using a 30 x 30 mm ATS thermode (Pathway, Medoc, RamatYishai, Israel). Briefly, stimulus temperature increased or decreased with a constant rate of change (8 °C/s) starting from a preset temperature (i.e., 32°C). As soon as warm or cold sensation (CDT and WDT) was perceived, the stimulus was halted by the examiner. The same procedure was employed for the CPT and WPT but the stimulation was stopped when participant perceived the stimulation as painful (on the numeric rating scale.
(NRS) between 6-7). Similarly, electrical detection and pain thresholds (i.e., EDT and EPT, respectively) were assessed by applying single, square wave (0.5 ms) electrical pulses (at 0.1 Hz) from standard clinical surface gel electrodes (20 mm) overlying the marked 4 x 4 cm testing area on the left C6 dermatome using a Keypoint electrophysiological stimulating and recording device (Medtronic, Mississauga, Ontario, Canada). Each threshold was determined three times.

A contact heat stimulator was employed (Pathway, Medoc, RamatYishai, Israel) to deliver noxious heat pulses. The thermode surface (diameter: 27 mm) consists of a heating thermo-foil covered with a layer of thermo conductive plastic. The device is capable of generating a heating rate of up to 70°C/ s (thermo-foil) and a cooling rate of 40°C/ s (peltier element). For all measurements, contact heat stimuli were delivered from a 35°C baseline to a peak temperature of 52°C at a fixed, nominal rate of 70°C/ s. In total, 10 stimuli were applied with an inter-stimulus time interval randomly varying between 8 and 12 s. The thermode was slightly different positioned after each stimulus within the marked area (size 4 x 4 cm, Figure 3.1) within left C6 dermatome in order to minimize receptor fatigue or sensitization by overheating of the skin. All participants were examined lying in a supine position with eyes open. Participants were advised to rate the perceived sensation of the single stimuli (thermal and electrical) on a numeric rating scale (NRS) ranging from 0-10 (i.e., 0 = no pain, 10 = worst pain imaginable).

**Capsaicin application**

In order to induce peripheral sensitization in tested area, capsaicin cream (1 mL, 0.075%, Haenseler AG, Switzerland) was applied to the left dorsum of the hand, in the distribution of C6. Prior to application, the application area sizing 4 x 4 cm was marked (Figure 3.1).

**Pain outcomes**

Acute application of capsaicin gradually evokes symptoms like itching pain, burning sensation, as well as thermal and mechanical hypersensitivity. Thus, participants were prompted to rate the capsaicin-induced sensory discomfort every five minutes over a 30-minute period (i.e., capsaicin application) using a numeric rating scale (0 = no pain, 10 = worst pain imaginable). Participants rated the perceived pain sensation (thermal and electrical) on a NRS ranging from 0-10 (i.e., 0 = no pain, 10 = worst pain imaginable). Participants were advised to rate the perceived perception approximately two seconds after the stimulus presentation.

**Statistical Analysis**

Statistical analyses were performed using IBM’s Statistical Package for the SocialSciences (SPSS) version 19.0 (Armonk, New York, U.S.). Non-parametric tests (Mann-Whitney-U and Kruksal-Wallis) were employed to determine significant differences in clinical parameters between the three groups. Generalized nonparametric mixed effect models were employed to analyze capsaicin-evoked pain, thermally/electrically evoked pain as well as thresholds (i.e., CDT, WDT, CPT, WPT, EDT, and EPT). To
analyze the temporal progression of the capsaicin-evoked pain, 6 different time-points (5, 10, 15, 25, 30 minutes (during capsaicin-application) and 40 minutes (post-capsaicin) were tested. Main and interaction effects were determined by using the rating (NRS) as the dependent variable and all time-points were set as fixed effects. Evoked pain was assessed at pre-capsaicin (baseline), and post-capsaicin, and post-TENS. The dependent variable was pain rating to contact heat and electrical stimulation (NRS). In case of significance, post-hoc Mann-Whitney-U tests were employed to identify significant differences between time-points. Multiple comparisons were Bonferroni correction. Statistical significance was set at $\alpha = 0.05$. All $p$ values reported are corrected for multiple comparisons.

Additionally, a post-hoc multiple linear regression analysis was planned to examine the relationship between sensory impairments (i.e., significant changes in sensory function relative to healthy control individuals and pain-free individuals with SCI) and the intensity of NP. The goal was to examine all factors in a single statistical model, accounting for age, sex, and pain catastrophizing. This post-hoc multiple linear regression analysis was also performed in SPSS.

### 3.4 Results

Overall, no significant inter-group differences were found regarding age ($p=0.677$), gender ($p=0.936$), and PCS ($p=0.193$). Moreover, no differences regarding lesion level ($p=0.177$) and severity ($p=0.439$) were measured between individuals with SCI (i.e., with and without NP). However, time since injury was significantly longer in individuals with SCI suffering from NP compared to their pain-free counterparts ($p=0.008$)(Table 3.1).

Table 3.1: Demographic and clinical details of the sample

<table>
<thead>
<tr>
<th>Parameter</th>
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</tr>
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<td>15</td>
</tr>
<tr>
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</tr>
<tr>
<td>Age [yrs]</td>
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<tr>
<td>PCS Score†</td>
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<tr>
<td>Lesion level [c:th:l]†</td>
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<tr>
<td>Severity [cSCI : iSCI]</td>
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<tr>
<td>Time since injury [yrs]</td>
<td>11.0 ± 7.9</td>
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<tr>
<td>Mean pain intensity [NRS]‡</td>
<td>4.2 ± 2.2</td>
</tr>
<tr>
<td>Duration of pain</td>
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</tr>
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</table>

Results are displayed as mean ± standard deviation.

†: Level of lesion: c=cervical, t=thoracic, l=lumbar SCI
‡: German version of the 13-item pain catastrophizing scale (PCS) including a 5-point scale ranging from 0 (not at all) to 4 (all the time)
†: EMSCI pain questionnaire with incorporated visual analogue scale ranging from 0 (no pain) to 10 (worst pain imaginable)
*: Significant difference between pain-free SCI and SCI-NP

cSCI: Complete spinal cord injury, iSCI: incomplete spinal cord injury, c: cervical, th: thoracic, l: lumbar

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Demographics and clinical assessment
In total, we enrolled 15 healthy control individuals (mean (SD) 46.07 (9.5) years; gender: 1 female, 14 male) and 34 individuals with traumatic SCI (47.0 (11.0) years; gender: 2 female, 27 male). Two individuals with SCI had to be excluded due to incomplete dataset (i.e., drop-out). Lesion level included cervical (n=5), thoracic (n=22), and lumbar injuries (n=5). According to the ISNCSCI impairment classification, 16 of 32 individuals with SCI had complete (1 tetraplegic, 15 paraplegic) and 16 incomplete (4 tetraplegic, 12 paraplegic) injury of the spinal cord. From the individuals with SCI, 19 individuals (18 with paraplegia, 1 with tetraplegia) suffered from neuropathic pain (SCI-NP) (Table 3.2). The mean and

Table 3.2: Demographics of individuals

<table>
<thead>
<tr>
<th>ID</th>
<th>Age [yrs]</th>
<th>Gender</th>
<th>Time since Injury [yrs]</th>
<th>Level of Lesion</th>
<th>AIS*</th>
<th>Drug (indication)</th>
<th>PCS†</th>
<th>Duration of pain‡</th>
<th>Mean pain intensity§</th>
<th>Max pain intensity§</th>
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<td>Th7</td>
<td>A</td>
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<td>4</td>
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<td>C7</td>
<td>D</td>
<td>Gabapentin, baclofen (pain)</td>
<td>25</td>
<td>7</td>
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<td>Th4</td>
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<tr>
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<td>f</td>
<td>22</td>
<td>Th11</td>
<td>D</td>
<td>Gabapentin, baclofen (pain)</td>
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<td>Gabapentin, baclofen (pain)</td>
<td>25</td>
<td>7</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

* ASIA impairment scale: A, no sensory or motor function is preserved; B, sensory function is preserved below the level of the injury; C, motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade of <3; D, motor function is preserved below the neurological level, and at least half of the key muscles below the neurological level have a muscle grade of >3.

† EMSCI pain questionnaire (V4.2, http://www.emsci.org/)
‡ Pain Catastrophizing Scale: A 13-item self-report scale to measure pain catastrophizing. Each item is rated on a 0 – 5 point scale: 0 (Not at all) to 5 (all the time). (Sullivan et al, 1995)
§ ASIA impairment scale: A, no sensory or motor function is preserved; B, sensory function is preserved below the level of the injury; C, motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade of <3; D, motor function is preserved below the neurological level, and at least half of the key muscles below the neurological level have a muscle grade of >3.

A: Study 2: Spinal and supraspinal effects on pain modulation

Sensory Plasticity in Cervical Spinal Cord Injury
maximal pain intensities were 4.2 ± 2.2 and 6.5 ± 1.9, respectively, and the duration of ongoing pain ranged from 3 to 35 years (mean 10.5 +/-7.8 years).

**Sensory thresholds and modulation of repetitive noxious thermal stimuli**
Baseline thermal (i.e., cold and warm) and electrical perception as well as pain thresholds were not significantly different across groups (all p>0.05) (Table 3.3 and Figure 3.2). Healthy control individuals and pain-free individuals with SCI demonstrated a progressive decrease in pain rating to repetitive contact heat stimulation from initial to final stimulation (NRS\textsubscript{controls}=6.8±1.9, ΔNRS\textsubscript{controls}= -1.7±0.3; NRS\textsubscript{pain-freeSCI}=7.0±1.4, ΔNRS\textsubscript{pain-freeSCI} = 1.6±0.9). In comparison, individuals with SCI-NP exhibited significantly lower rating to the initial stimulus (NRS\textsubscript{SCI-NP} = 5.2±2.2), as well as no significant decline of rating from the first to last stimulus (ΔNRS\textsubscript{SCI-NP} =0.7±0.6). Figure 3.3 depicts the differences in rating (i.e., perception) and changes of rating over time in respect to the first stimulus (i.e., habituation).

![Figure 3.2: Cold and warm perception and pain thresholds pre- and post-capsaicin application as well as post-TENS (ipsilateral or contralateral).](image)

Contrary, warm pain threshold significantly decreased following 30min of capsaicin application. Following TENS (i.e., ipsi- and contralateral), elevated warm pain thresholds were observed again. Overall no group differences were observed. Each threshold measurement was initiated at a baseline temperature of 32°C (dashed line). All participants completed two measurement sessions receiving capsaicin application followed by TENS (i.e., randomized order of ipsi- or contralateral).
Effect of Capsaicin

All participants tolerated capsaicin for the entire 30-minute application period. As reported by others\textsuperscript{116, 135}, spontaneous pain progressively increased from baseline to 30 minutes. In general, capsaicin was reported as mildly to moderately painful (NRS = 4.81 ± 2.28). As illustrated in Figure 3.4, healthy control individuals and pain-free individuals with SCI reported significantly higher ratings (NRS\textsubscript{controls} = 4.9±2.1; NRS\textsubscript{pain-freeSCI} = 4.7±2.6) than individuals with SCI-NP (NRS\textsubscript{SCI-NP} = 3.7±1.8). The effect of capsaicin on thresholds and thermally evoked pain is summarized in Table 3.3. Capsaicin had no effect on electrical and cold detection and pain thresholds, as well as warm detection threshold (all p>0.05) (Figure 3.2). However, warm pain thresholds were reduced following capsaicin in all groups (healthy control individuals: F=110.0, df: 38.2, p=; pain-free SCI: F=130.6, df: 30.1, p<0.001; SCI-NP: F=126.2, df: 36.1, p<0.001). The post-capsaicin pain ratings in response to contact heat stimulation were significantly
elevated compared to baseline in all three groups (NRS_control = 6.8, F=3.7, df:28, p=0.02; NRS_pain-freeSCI = 7.0, F=4.03, df:24, p=0.049; NRS_SCI-NP = 6.2, F=, df:38, p=0.017).

**Effect of transcutaneous electrical nerve stimulation (TENS)**

**WDT, WPT, CDT, CPT, and EPT**

Overall, TENS had no effect on electrical, cold detection and pain thresholds, as well as warm detection threshold (Figure 3.2 and Table 3.3). However, warm pain threshold was elevated post-TENS, independent of the group (i.e., SCI with and without pain, healthy control individuals) and stimulation site (i.e., ipsi- or contralateral TENS) (all p<0.05).

![Figure 3.5: The effect of TENS stimulation on capsaicin evoked pain.](image)

(A) **Ipsilateral TENS**

(B) **Contralateral TENS**

(C) **Contralateral TENS in SCI-NP**

**Capsaicin and contact heat stimulation**

The effect of TENS on capsaicin-evoked and thermally evoked pain is summarized in Table 3.3. Ipsilateral TENS significantly reduced capsaicin-evoked pain in all individuals with SCI and healthy individuals (all p<0.001). Pain-free individuals and healthy control individuals also experienced pain-relief in response to TENS applied contralaterally. In comparison, individuals with SCI-NP did not demonstrate significant modulation in response to contralateral TENS (p=0.053). As shown in Figure 3.5, the reduction of capsaicin-evoked pain was correlated with the intensity of reported neuropathic pain.
Ipsilateral TENS resulted in significant thermally evoked pain relief in the area of capsaicin application across all three groups (all p<0.001). Notably, the NRS was decreased relative to pre-TENS values (i.e., after 30 minutes of capsaicin). Similarly, segmental contralateral TENS had modulatory effects on thermally evoked pain in healthy control individuals and pain-free individuals with SCI (all p<0.001). In contrast, contralateral TENS resulted in no significant thermally evoked pain relief in individuals with SCI suffering from NP (p=0.59). All results are summarized in Table 3.3.
Table 3.3: Summary of thermal and electrical thresholds as well as rating of capsaicin and thermally-induced pain

<table>
<thead>
<tr>
<th>Group</th>
<th>Time-point of Stimulation [min]</th>
<th>Significant pairwise comparisons (p&lt;0.05&lt;sup&gt;‡&lt;/sup&gt;)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Healthy Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cold detection threshold [°C]</td>
<td>30.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Warm detection threshold [°C]</td>
<td>34.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Cold pain threshold [°C]</td>
<td>3.9 ± 3.8</td>
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<tr>
<td></td>
<td>Warm pain threshold [°C]</td>
<td>48.6 ± 3.2</td>
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<tr>
<td></td>
<td>Electrical detection threshold [mA]</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Electrical pain threshold [mA]</td>
<td>16.8 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>Pain rating heat (NRS)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>5.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Pain rating capsaicin (NRS)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Pain-free SCI</td>
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</tr>
<tr>
<td></td>
<td>Cold detection threshold [°C]</td>
<td>29.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Warm detection threshold [°C]</td>
<td>34.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Cold pain threshold [°C]</td>
<td>2.8 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Warm pain threshold [°C]</td>
<td>48.3 ± 2.0</td>
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<tr>
<td></td>
<td>Electrical detection threshold [mA]</td>
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<td></td>
<td>Electrical pain threshold [mA]</td>
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<tr>
<td></td>
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<td>SCI with NP</td>
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<tr>
<td></td>
<td>Cold detection threshold [°C]</td>
<td>29.7 ± 1.0</td>
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<td>Pain rating capsaicin (NRS)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Results are displayed as mean ± standard deviation.

T<sub>1</sub>: Baseline, T<sub>2</sub>: After 30min capsaicin application, T<sub>3a</sub>: Following 10min ipsilateral TENS, T<sub>3b</sub>: 10min after contralateral TENS

<sup>†</sup>: Numeric Rating Scale ranging from 0 (no pain) to 10 (worst pain imaginable)

<sup>‡</sup>: Bonferroni corrected
3.5 Discussion

After a period of sensitization with capsaicin, pain-free individuals with SCI and healthy control individuals robustly modulated thermal hyperalgesia following a short bout (10 minutes) of ipsi- and contralaterally applied TENS. In contrast, evidenced by a lack of change in response to contralateral TENS, individuals with SCI-NP demonstrated impaired modulation of noxious afferent input. Suggesting an association with clinical pain symptoms, the extent of endogenous modulation of capsaicin evoked pain significantly correlated with the rating of ongoing NP. Collectively, the present findings suggest that chronic NP may be related to impaired endogenous modulation after SCI, which could be mediated through altered supraspinal processes.

Spinal cord injury and impaired endogenous modulation of noxious afferent stimuli

To our knowledge, only one recent study has reported changes in “endogenous modulation” to noxious afferent stimuli in individuals with SCI-NP\textsuperscript{154}. Using a conditioned pain modulation (CPM) paradigm, noxious stimulation applied contralaterally to the test location (i.e., cold pressor test) failed to modulate responses to tonic heat stimulation among those with NP, while robustly reducing sensation in healthy individuals and pain-free individuals with SCI. Based on established CPM mechanisms, impaired modulation was considered evidence of reduced descending control of noxious afferent input from supraspinal structures\textsuperscript{154}. Evidence of impaired CPM in individuals with SCI-NP builds on an extensive number of studies investigating other chronic pain conditions\textsuperscript{141, 154, 169-175}.

We have further demonstrated impaired endogenous modulation, adopting a different approach (i.e., ipsi- and contralateral TENS in the presence of capsaicin) in a larger sample of individuals with SCI-NP (n=19), highlighting modulatory deficits in response using other noxious modalities (i.e., contact heat stimulation and capsaicin). In addition, our results suggest that other forms of endogenous modulation are unaffected by SCI-NP – most notably, there remains a robust capacity to “turn down” noxious input (i.e., contact heat and capsaicin) in response to ipsilateral TENS.

Mechanisms of failed endogenous modulation after spinal cord injury

Following ipsilateral TENS, modulation of perception to noxious stimuli is mediated via recruitment of large diameter afferents and subsequent “spinal gating” in the dorsal horn\textsuperscript{102, 176, 177}. By in large, the balance between large and small diameter afferents proposed in the gate-control theory of pain appears to be unaffected by SCI. However, individuals with NP, but not healthy controls or pain-free individuals with SCI, failed to modulate contact heat or capsaicin pain following contralateral TENS. Further demonstrating the association between NP and contralateral TENS modulation, the intensity of clinical pain symptoms was significantly correlated with changes in capsaicin-evoked pain rating. The dissociation between ipsi- and contralateral effects in individuals with NP may be related to distinct mechanisms underlying TENS-analgesia. One potential difference is that contralateral TENS analgesia is more reliant
on the release of endogenous opioids in supraspinal structures (e.g., RVM), which in turn inhibits activity at the spinal level in dorsal horn neurons\textsuperscript{159}. Changes in structure and function in key brain areas involved in descending modulation may play a pivotal role in reduced modulation of noxious stimuli after SCI (e.g., anterior cingulate cortex)\textsuperscript{44, 178}.

**Reduced sensation and decreased habituation to evoked contact heat stimulation above the level of injury in individuals with SCI-NP**

In the present study, significant differences in thermal sensitivity above the level of injury in individuals with SCI-NP were observed in response to contact heat stimulation, including decreased ratings and reduced habituation to repetitive stimuli. In agreement with other studies adopting a QST approach\textsuperscript{154}, no differences in thermal thresholds (i.e., pain and perception) were measured between healthy controls, pain-free individuals with SCI, and individuals with SCI-NP (Figure 3.2). Although potentially suggesting improved diagnostic sensitivity and specificity to detect afferent deficits rostral to the lesion site, recent studies using contact heat stimulation reported no differences\textsuperscript{154} or the opposite outcome (i.e., individuals with SCI-NP demonstrate increased behavioral ratings in response to contact heat)\textsuperscript{50}. An earlier study applying topical capsaicin above the level of injury also reported no differences in sensation in individuals with and without NP over a 30-min time-period\textsuperscript{45}. While these discrepancies are difficult to reconcile, hypoalgesia compared to hyperalgesia may be related to a number of factors, including the testing location. Previous work in our laboratory has found that perception to noxious stimulation depends considerably on the site examined\textsuperscript{179}. Given inherent variability related to location, sensation above the level of injury may be differentially affected by the presence of NP. Regardless, the lack of consistency between studies, suggests that absolute responses to thermal stimuli have limited utility to assess NP after SCI.

**Clinical implications, limitations, and future directions**

In a previous study (Chapter 2) in our laboratory, we demonstrated the advantage of inducing sensitization with capsaicin to examine the effects of TENS on thermal hyperalgesia. Importantly, the modulating effects of TENS were not observed in the absence of sensitization (i.e., without capsaicin). Adopting the same approach, in the current study we have revealed evidence of impaired modulation among individuals with NP. In general, the multi-modal approach we applied (i.e., modeling sensitization and examining dynamic QST) demonstrates the complexity of examining NP after SCI. To our knowledge, few studies have identified clinical correlates of NP after SCI using QST. In contrast to other sensory deficits (i.e., reduced perception to contact heat stimulation and capsaicin), our findings demonstrate that NP is strongly associated with impaired modulation.

In the present study, dynamic QST outcomes (i.e., changes in perception to repetitive contact heat stimulation, responses to capsaicin, and modulation of perception using a conditioning stimulation)
revealed subtle evidence of afferent deficits in individuals with NP. In contrast, conventional QST (e.g., thermal thresholds) demonstrated normal sensation among individuals with SCI, with and without NP.

A primary limitation of our study is that we have not included a “sham” TENS condition or stimulation site that was not effective in modulating. In our earlier study (Chapter 2), we demonstrated that contralateral shoulder stimulation had no effect on responses to contact heat stimulation applied on the hand. Outside the scope of the current study, we did not test the effect of conventional TENS on sensation to afferent stimuli in the secondary area of hyperalgesia caused by topical capsaicin application. Due to the cross-sectional nature of the study and inclusion criteria for individuals with SCI (i.e., chronic SCI), conclusions regarding the development of the observed impairments remain speculative.

**Conclusion**

Our results indicate that the presence of NP selectively alters the supraspinal modulation of above-level nociception mirrored as reduced ability to habituate to repetitive presentation of identical stimuli and reduced perception to capsaicin-evoked sensory discomfort. Interestingly, individuals with SCI (with and without neuropathic pain) retained a normal capacity to modulate noxious stimuli in response to ipsilateral conditioning. Pain-free individuals (i.e., SCI and healthy controls) experienced a significant pain-relief through contralateral conditioning, however in individuals with SCI-NP the contralateral conditioning had overall no appreciable effect on sensation. Importantly, among individuals with SCI-NP, the extent of modulation achieved through contralateral TENS correlated with the intensity of ongoing NP symptoms. Crucially, for the first time we could show that NP was associated with deficient modulation of noxious afferent through conditioning electrical stimulation.

**Acknowledgement**

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**Author’s contribution**

Catherine R. Jutzeler contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Furthermore, she drafted the research article. Marina Freitag made substantial contribution to the data acquisition and revising the research article. Armin Curt made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content. John L. Kramer contributed substantially to the conception and design of the study, the data acquisition, analysis, interpretation and revision of the research article.
Study 3

Discrepancy between perceived pain and cortical processing: A voxel-based morphometry and contact heat evoked potential study

John L.K. Kramer, Catherine R. Jutzeler, Jenny Haefeli, Armin Curt, and Patrick Freund

Clinical Neurophysiology (2015)

~ We are thankful for the technical support of Dr. Roger Luechinger. Further, we would like to thank all of the individuals participating in our study. ~
4.1 Abstract

The purpose of this study was to determine if local gray and white matter volume variations between individuals (i.e., inter-subject variability) could account for variability in responses to CHEP stimulation. Structural magnetic resonance imaging was used to perform voxel-based morphometry (VBM) of gray and white matter in 30 neurologically healthy individuals. Contact heat stimulation was performed on the dorsum of the right hand at the base of the thumb. Evoked potentials were acquired from a vertex-recording electrode referenced to linked ears. Controlling for age, total intracranial volume, and skull/scalp thickness, CHEP amplitude and pain rating were not significantly correlated between individuals. A VBM region of interest approach demonstrated a significant interaction between pain rating and N2 amplitude in the right insular cortex (p<0.05, family-wise error corrected, FWE). In white matter, a significant interaction was localized in the right inferior frontal occipital fasciculus (IFOF, p<0.05 FWE). Accounting for gray matter volume in the right insular cortex, resulted in a significant relationship between CHEP amplitude and pain rating. This finding suggests that the discrepancy between pain ratings and the amplitude of evoked potentials is not solely related to measurement artifact (i.e., differences in the interpretation of pain rating scales between individuals), but rather attributable, in part, to anatomical differences between individuals.
4.2 Introduction

At present, self-report (i.e., description and intensity) represents a clinical standard for the evaluation of pain. To detect subtle changes in somatosensory function, an objective assessment of pain is desirable. Electrophysiological analogues of conventional somatosensory evoked potentials acquired in response to electrical stimulation and recruitment of large diameter afferents in the periphery and central conduction in the dorsal columns, laser and contact heat evoked potentials (LEPs and CHEPs) represent objective methods to assess the integrity of small diameter afferents conveying temperature and pain sensation to the brain in the spinothalamic tract. However, ratings as well as the amplitude of prominent cortical waveforms (e.g., N2P2) typically demonstrate high between-subject variability. Furthermore, while significant positive relationships between amplitude of evoked potentials and pain ratings have been reported this too is highly variable – some individuals rating low intensity to stimulation but demonstrating large amplitude cortical potentials, and vice versa. The dissociation between pain rating and amplitude of prominent cortical potentials is also evident within an individual, shown in response to repeated and predictable stimulation. On one hand, these observations may be related, in part, to inherent difficulties rating noxious stimuli. For example, two individuals subjected to the same stimulation may lead to comparable EP amplitudes, but due to differences in their interpretation of scales used to rate pain intensity (e.g., 0-10), one individual reports an appreciably higher rating than the other. In such a case, the mismatch between CHEP/LEP amplitude and rating is prominently a function of measurement artifact, unrelated to differences in anatomy and physiology. However, central pain patients with thermal hyperalgesia demonstrate high prevalence of a similar phenomenon, often reporting high ratings coupled with small amplitude evoked potentials. Collectively, these observations in healthy individuals and central pain patients raise the question whether a neural substrate underlies the discrepancy between ratings and evoked potential amplitudes.

By accounting for differences in sensitivity to noxious thermal stimuli, ability to modulate pain, as well as the amplitude of evoked cortical responses to non-noxious afferent stimuli, structural magnetic resonance imaging (MRI) has highlighted an important relationship between normal brain anatomy and sensory function. Based on this knowledge, we intended to address the question whether between-subject variability in cortical structure could account for differences in responses to contact heat stimulation in healthy individuals. Specifically, we were interested in determining if estimates of gray and white matter volume explained, in part, why an individual perceived stimulation as low/high but generated an evoked cortical potential that was large/small. To this end, voxel-based morphometry (VBM) was used to explore associations between pain rating and CHEP amplitude in gray and white matter.
4.3 Material and Methods

Individuals
Thirty neurologically healthy individuals participated in this study (13 females, 17 males). All individuals were prescreened for MRI contraindications and reported no acute or chronic pain at the time of examination. Individuals provided written informed consent and all procedures described below were in accordance with the Declaration of Helsinki, and approved by research ethics board at the University of Zurich (ref. number: EK-04/2006).

Study Protocol

Acquisition of pain rating and contact heat evoked potentials (CHEPs)
CHEPs and pain rating to contact heat stimuli were recorded following stimulation of the dorsal surface of the C6 dermatome at the base of the right thumb using the Pathway Pain and Sensory Evaluation System (Medoc Advanced Medical Systems®). Ten contact heat stimuli were delivered from a baseline temperature of 35°C to a peak temperature of 52°C, at an inter-pulse interval of 8-12 seconds. To familiarize individuals and limit the startle effect, individuals were exposed to contact heat stimuli on an untested site (e.g., forearm) before acquisition of evoked potentials. The contact heat stimulation thermode was repositioned (i.e., variable stimulation protocol) after each stimulus to reduce receptor fatigue. In response to an audio cue presented 2 seconds after contact heat stimulation, individuals rated perceived intensity according to a 0-10 numerical rating scale (NRS, 0 = no pain, 10 = worst pain imaginable). Individuals were instructed not to blink in response to the stimulation, and withhold from blinking until hearing the audio cue. N2P2 was acquired from an active vertex-recording electrode (Cz) referenced to both earlobes (A1-A2). Previous studies have adopted a similar electrode configuration (i.e., single recording channel) for the acquisition of N2P2\(^{73, 126, 199}\), reporting significant intra-subject (i.e., test-retest) reliability for N2P2 amplitude\(^{76, 200}\). A ground strap electrode was secured on the upper arm of the stimulating side. A pre-trigger period of 100ms preceded each recording, followed by a 1500ms post-trigger, for a total 2000ms epoch. All signals were sampled at 2,000Hz and amplified (20,000x). Each stimulus was manually reviewed for artifact.

Automated detection of N2P2 amplitude
To enhance the signal-to-noise ratio of CHEPs, vertex recordings were bandpass (1-30Hz) and wavelet filtered for automated detection of N2 and P2 amplitudes. The aim of wavelet filtering is to improve the signal to noise ratio, so as to allow automated detection of peak waveforms. Based on wavelet filtered CHEPs, single trial averaged N2P2, N2, and P2 amplitudes were determined. The advantage of single trial averaging compared to across trial averaging is that latency jitter does not affect the amplitude of responses. Rather, a measure of amplitude is extracted for each stimulus, and averaged across the total number of stimulations. In order to perform an unbiased single trial analysis of N2P2 amplitude from
wavelet filtered CHEPs, an automated approach utilizing multiple linear regression with a dispersion term was performed in MatLab (Mathworks)\textsuperscript{201,202}. We applied the same techniques as described previously for CHEPs\textsuperscript{203}.

\textbf{Magnetic resonance image sequence}

Using a Philips 3T Ingenia, a 3D-GRE T1-weighted (T1w) sequence was used to acquire a whole-brain, structural scan optimized for simultaneous assessment of the brain and spinal cord\textsuperscript{204}. The imaging parameters were: isotropic 1mm\(^3\) resolution, field of view 256 x 256mm\(^2\), matrix 256 x 256, 180 sagittal partitions, repetition time = 7.15 ms, echo time = 3.29 ms, inversion time = 858.65ms, flip angle 8°, fat saturation, bandwidth 250 Hz/pixel and a scan time of 6min 31s. Prior to VBM analysis, MRI data from each individual was visually screened for movement artifacts.

\textbf{Voxel-based morphometry (VBM)}

To assess voxel-wise associations of gray and white matter volumes with N2, P2, and N2P2 amplitudes and pain ratings, VBM was performed in the framework of Statistical Parametric Mapping 8 (SPM8, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), applied on the acquired T1w 3D volumetric MRI data\textsuperscript{205}. First, a unified model inversion (unified segmentation) was used for bias correction and segmentation of MRI data into gray and white matter, and cerebrospinal fluid. Then Dartel was used to warp the gray and white matter segments into an optimal (average) space\textsuperscript{206}. The resulting gray and white matter images were modulated and affine transformed to Montreal Neurological Institute (MNI) space and smoothed using an isotropic Gaussian kernel with 6 mm full width at half-maximum. After each post-processing step, the alignment of images (i.e., gray and white matter) was verified to ensure the accuracy of fully automated post-processing in SPM.

\textbf{Analysis and Statistics}

To determine the relationship between CHEP parameters and rating, partial correlations were examined. This analysis was performed in IBM SPSS Statistics (V.19.0), and included controlling for age, skull/scalp thickness, as well as total intracranial volume (TIV). Two-tailed significance was determined at p<0.05.
General linear models in SPM were utilized to assess associations between the covariates of interest (i.e., automated single trial averaged N2, P2, and N2P2 amplitudes and corresponding pain rating to contact heat stimulation) and local gray and white matter volumes. This is a routine procedure within the SPM framework, and operates irrespectively of whether data is derived from functional or anatomical imaging. To account for possible non-specific effects on regional gray and white matter volumes, TIV, skull/scalp thickness, and age were included as nuisance variables. Skull/scalp thickness was estimated at the vertex from anatomical T1w scans. Using Gaussian random field theory, F-contrasts were examined to address the interaction between CHEP amplitude and pain rating in gray and white matter. In each model, a CHEP amplitude parameter (N2P2, N2, or P2), pain rating, and all nuisance variables (i.e., age, TIV, skull/scalp thickness) were included. Initially, a whole brain analysis of gray and white matter was performed. For a more directed approach of gray matter, regions of interest (ROIs) were then examined. The ROIs included the primary and secondary somatosensory cortices, anterior and mid cingulate gyrus, and insular cortex – that is, areas previously shown to be associated with noxious contact heat stimulation\textsuperscript{207-209}. ROIs and the white matter mask were created using the Pick Atlas in SPM\textsuperscript{8210, 211}. The John Hopkins University (JHU) White-Matter Tractography Atlas was used to identify the location of significant peak voxels in the white matter\textsuperscript{212}. Associated p-values were corrected for multiple comparisons across the whole brain, and in each individual ROI (Family Wise-Error, FWE). Significance was set at p<0.05 (FWE-corrected). To determine if estimates of gray or white matter volume improved the relationship between CHEP amplitude and pain rating, partial correlations were again examined in SPSS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>33.00 ± 11.57</td>
</tr>
<tr>
<td>Skull/scalp thickness (mm)</td>
<td>7.79 ± 0.80</td>
</tr>
<tr>
<td>Total intracranial volume (L)</td>
<td>1.55 ± 0.16</td>
</tr>
<tr>
<td>Pain rating</td>
<td>4.65 ± 2.09</td>
</tr>
<tr>
<td>CHEP amplitude (µV)</td>
<td></td>
</tr>
<tr>
<td>N2P2</td>
<td>30.16 ± 13.95</td>
</tr>
<tr>
<td>N2</td>
<td>15.99 ± 8.04</td>
</tr>
<tr>
<td>P2</td>
<td>14.17 ± 6.90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rating</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2P2</td>
<td>0.265</td>
<td>0.158</td>
</tr>
<tr>
<td>N2</td>
<td>0.243</td>
<td>0.196</td>
</tr>
<tr>
<td>P2</td>
<td>0.241</td>
<td>0.199</td>
</tr>
</tbody>
</table>

Table 4.1: Summary demographics and CHEP outcomes
In addition to age, TIV, and skull/scalp thickness, gray and white matter contrast estimates extracted from significant peak voxels were included in this analysis.

4.4 Results

**Correlations between CHEP amplitude and pain rating: Absolute and relative relationship**

A representative example of CHEPs (single trial and grand average for one individual) is shown in Figure 4.1. Partial correlations of CHEP amplitude parameters and pain rating, controlling for age, TIV, and skull/scalp thickness, are shown in Table 4.1. While positive, all correlations between CHEP amplitude (N2, P2 or N2P2) and rating were insignificant (p>0.05). Further highlighting the poor correlation between CHEP amplitude and pain rating, among individuals demonstrating higher than average amplitudes (+1SD, n=5), only one individual also reported a similar trend in pain rating (i.e., NRS was +1SD greater than the average). Of those individuals with CHEP amplitudes less than average (-1SD, n=6), only two also reported ratings below average. In total, only 50% of individuals demonstrated the same relative relationship between CHEP amplitude and pain rating (i.e., low/average/high rating, small/average/large amplitudes, see Table 4.2).

**Voxel-based morphometry**

At the whole brain level analysis in gray matter, no significant associations for N2P2, N2, or P2 amplitude with pain rating were identified. However, ipsilateral to contact heat stimulation, a significant interaction between N2 amplitude and pain rating (p=0.034, F=14.35, z=3.78, Kₑ=18; Figure 4.2A) was observed in the right insular cortex (MNI: x=48, y=7, z=6). Put simply, increased gray matter volume in the insular cortex was associated with larger N2 amplitudes and lower pain ratings. In white matter, also ipsilateral to contact heat stimulation, there was a significant association between pain rating and N2P2 amplitude (p=0.025, F=29.43, z=4.96, Kₑ=414) in the inferior frontal-occipital fasciculus (IFOF; MNI: x=33, y=-35, z=9; Figure 4.2B). The association was only significant for N2P2 amplitude (positive, such that larger amplitudes were associated with greater white matter volume).

**Table 4.2: Relative CHEP amplitude (N2P2) and pain rating (n=30)**

<table>
<thead>
<tr>
<th>Rating (n=)</th>
<th>Average</th>
<th>CHEPs (n=)</th>
<th>Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>12</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Higher</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lower</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

No difference, +/- 1SD
Higher, >1SD
Lower, <1SD

*relative CHEPs and pain rating are in agreement*
Effect of controlling for gray/white matter volume on correlations between pain rating and CHEP amplitude

Accounting for age, skull/scalp thickness, TIV, and contrast estimates of gray matter volume in the right insular resulted in a significant relationship between N2 amplitude and rating (Figure 4.3). In contrast, controlling for age, skull/scalp thickness, TIV, and estimates of white matter volume in the IFOF did not improve the correlation between N2P2 amplitude and pain rating.

4.5 Discussion

In the present study, individuals with higher volumetric estimates of gray matter in the right insular cortex and white matter in the right IFOF were found to be associated with larger amplitude CHEPs (N2 and N2P2, respectively, Figure 4.2). Furthermore, gray matter estimates in the right insular cortex, ipsilateral to contact heat stimulation, improved between individual associations for CHEP amplitude and pain rating, such that higher volumes were associated with larger N2 amplitudes and lower pain ratings. Building on previous studies\cite{189,191}, these findings collectively support that structural differences in the brain may contribute to variability in physiological responses to noxious stimulation.

Role of the insular cortex in the generation of CHEPs and pain

There is strong rationale for including the insular cortex in our analysis. Based on neuroimaging studies (e.g., fMRI), the operculoinsular cortex has been proposed as fundamentally involved in the encoding of the intensity of perceived pain\cite{213}. To our knowledge, we are the first to demonstrate that structure in the insular cortex may account for the apparent discrepancy between the amplitude of sensory evoked potentials to noxious stimuli and rating of perceived intensity – that is, rating stimulation as high/low and CHEPs that are small/large. Wu and colleagues proposed an imbalance in medial (i.e., sensory-affective) and lateral pain systems (i.e., sensory-discriminative) underlying the dissociation between perceived intensity and LEP amplitude\cite{188}. This explanation fits with our anatomical findings, as the insular cortex is a prominent structure in the medial pain system.
**Lateralized effects in the right hemisphere**

In agreement with gray matter findings in an *a priori* region of interest, significant associations between CHEP amplitude and white matter were lateralized to the right hemisphere, localized in the IFOF. The IFOF represents associative nerve fibers connecting occipital, temporal, parietal, and frontal cortical areas\(^{214, 215}\). Comparatively, the relationship in white matter appears much stronger than in gray matter – significant on the whole-brain level (i.e., bilateral white matter hemispheres). However, associations were only evident with regards to CHEPs (i.e., N2P2), and did not improve the relationship between amplitude and rating (Figure 4.3). In general, anatomical variations in the right IFOF, as well as the right insular cortex may contribute to individual differences in CHEP amplitudes through a mechanism non-specific to nociception (e.g., attention and arousal)\(^{182, 183}\). Indeed, both are prominent white and gray matter structures in the “ventral attention network”\(^{216-218}\) – a group of temporal-parietal and ventral frontal areas lateralized to the right side of the brain, which play pivotal roles in saliency detection. Although speculative, higher volumes in areas in the ventral attention network may provide individuals an advantage to perceive afferent stimuli as more salient, in turn generating larger amplitude CHEPs compared to individuals with smaller volumes. Several recent studies have highlighted the importance of stimulation saliency in the generation of LEPs – demonstrating a unique capacity to dissociate amplitudes and ratings when stimuli are delivered at short, fixed inter-pulse intervals\(^{184}\).

**Limitations**

Important to note in the current study are potential limitations. Based on *a priori* hypotheses, we employed a ROI approach to examine gray matter volume. From the many brain areas involved in the processing of noxious stimuli, areas were selected based on overlap with prominent structures active in response to noxious contact heat stimulation\(^{207}\). Despite a relatively small sample size (n=30), significant
associations in a gray matter region (i.e., insular) were detected. While these effects are meaningful, we cannot rule out that other gray matter areas also underlie the dissociation between CHEPs and perceived intensity and that we did not have sufficient power to detect these relationships.

An additional limitation of our study is that only the amplitude of the vertex N2P2 was considered in examining associations between CHEPs and gray/white matter volume, and only in response to noxious stimulation. At present, N2P2 represents the most clinically viable waveform to assess the integrity of spinothalamic tract conduction and cortical processing – the earlier, lateralized N1 component difficult to interpret following conventional contact heat stimulating techniques (i.e., low signal to noise ratio)\textsuperscript{203}. While N1 may be less subject to cognitive factors, there is still considerable between-individual variability, and the correlation with pain rating remains low (i.e., is a poor objective measure of pain). Associations with cortical anatomy may be more readily detected by examining N1 amplitudes, including in the primary somatosensory cortex\textsuperscript{219}.

The present study also did not account for other factors that may potentially contribute to variability in responses to pain, including levels of attention and arousal\textsuperscript{220-222}. Interestingly, reductions in arousal have been shown to lead to dissociation of behavioral and physiological responses, evidenced by reduced LEP amplitudes and higher pain ratings following sleep restriction\textsuperscript{223}. However, it is also important to consider that, from a statistical perspective, a confounding variable must be associated with not only the explanatory variable of interest (e.g., pain), but also the outcome variable of interest (e.g., brain volume). While several factors that we did not control for may affect individual responses to noxious stimuli, if these are not also associated with brain volume, they will not materially affect statistical significance.

**Conclusion**

In conclusion, our findings demonstrate a significant association between pain rating and CHEP amplitude in local gray and white matter volume. These findings provide an anatomical substrate to explain the discrepancy between the amplitude of cortical evoked potentials in response to noxious stimulation, and pain rating. In a broader context, our findings provide further evidence that between-subject differences in brain anatomy can account for variations in response to sensory stimuli.

**Acknowledgements**

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Author’s contribution
John L. Kramer contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Furthermore, he drafted the research article. Catherine R. Jutzeler contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Jenny Haefeli made substantial contribution to the data acquisition and revising the research article. Armin Curt made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content. Patrick Freund made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content.
Systematic Literature Review

Relationship between chronic neuropathic pain and brain reorganization after deafferentation: A systematic review of MRI findings

Catherine R. Jutzeler, Armin Curt, and John L.K. Kramer

*Journal of Pain (submitted)*

~ We would like to thank Prof. Dr. Volker Dietz for his insightful comments regarding the manuscript.~
5.1 Abstract
Mechanisms underlying the development of phantom limb pain and neuropathic pain after limb amputation and spinal cord injury, respectively, are poorly understood. In support of a role for “maladaptive plasticity”, studies have demonstrated a correlation between the amount of cortical reorganization in primary sensorimotor areas and the severity of neuropathic pain. The goal of this systematic review was to assess the robustness of evidence in support of “maladaptive plasticity” emerging from applications of advanced functional and structural magnetic resonance imaging (MRI) in humans. Using MeSH heading search terms in PubMed and SCOPUS, a systematic review was performed querying published manuscripts. From 145 candidate publications, 13 were identified as meeting the inclusion criteria. Of 13 included publications, 5 examined structural outcomes (diffusion tensor imaging and voxel based morphometry, DTI and VBM, respectively), 7 applied functional MRI (fMRI), and 1 included VBM and fMRI. Six studies were specifically designed to assess the relationship between reorganization and chronic pain (i.e., healthy control group, patient population with and without chronic pain). Overall, fMRI investigations provide some support for maladaptive cortical plasticity. However, based on fMRI and VBM findings, two studies report the opposite relationship – that is, chronic pain is preserving cortical function and structure. Despite support for the maladaptive plasticity model, we identified studies that demonstrate a limited relationship between pain intensity and measures of functional and structural reorganization (i.e., no group level differences), or even the inverse relationship – that is, greater pain intensity associated with less reorganization. The review demonstrates the need for additional neuroimaging studies to clarify the relationship between neuropathic pain and reorganization.
5.2 Introduction

From seminal studies in individuals with limb amputations\textsuperscript{224, 225} and spinal cord injuries (SCI)\textsuperscript{226-229}, neurophysiological evidence supporting sensory and motor plasticity in the adult central nervous system (CNS) began to emerge more than two decades ago. While initially demonstrating a unique potential for change in the CNS, efforts quickly turned to understanding the effects of central plasticity on functional outcomes. In terms of detrimental effects, Flor and colleagues were among the first to show that cortical reorganization was associated with phantom limb pain – pioneering the maladaptive plasticity model using magnetoencephalography (MEG)\textsuperscript{230}. The central proposal of this model is that larger shifts in somatotopically organized brain areas (e.g., S1 and M1) are associated with more severe pain symptoms. Similar observations have since been extended to other chronic pain conditions\textsuperscript{231, 232}. Based in large part on this knowledge, rehabilitation practices to relieve chronic pain have been developed to target maladaptive cortical organization\textsuperscript{233-235}. The past twenty years has also seen considerable advances in the field of neuroimaging, including quantifiable functional and structural magnetic resonance imaging (MRI). In addition to technological developments in MR scanners, preprocessing in standardized (and widely available) platforms (e.g., SPM, FreeSurfer), as well as statistical analysis of neuroimaging data (e.g., correcting for multiple comparisons) have been vastly improved\textsuperscript{236}. This raises the question as to what extent the original concept of maladaptive plasticity has been supported by recent investigations using advanced functional and structural MRI techniques.

The primary objective of this review was to systematically examine studies that have addressed the relationship between reorganization in the brain after deafferentation and chronic pain. In addition to amputation and phantom limb pain, we also examined the strength of this relationship in individuals with spinal cord injury (SCI). The specific aim was to identify the level of evidence in support for the original concept of “maladaptive plasticity”\textsuperscript{230}. To address these aims, our systematic review focused on findings from quantifiable functional MRI (fMRI) and structural imaging techniques (e.g., voxel based morphometry and diffusion tensor imaging, VBM and DTI).

5.3 Material and Methods

Search methods for identification of studies

Pubmed and SCOPUS were searched using the time range from their individual inception dates 1977 and 1960, respectively, to the 31st of October 2014. The PubMed search was conducted using the methodological individuals heading (MeSH) keywords ‘spinal cord injury’ along with ‘neuropathic pain’ and ‘magnetic resonance imaging’ for SCI related pain, as well as ‘amputation’ along with ‘phantom limb pain’ and ‘magnetic resonance imaging’ for amputation-associated pain. Similarly, SCOPUS search included the same combination of keywords used for PubMed and also different combinations of...
keywords (e.g., phantom limb pain and magnetic resonance imaging). To identify additional studies that may have been overlooked, bibliographies of identified studies were hand searched.

**Selection of studies**

One author (JLK) carried out an initial screening of retrieved articles and applied inclusion criteria. Subsequently, a second reviewer (CRJ) independently reviewed all the studies in order to assure the publications met all inclusion criteria. All disagreements were discussed and resolved at a consensus meeting with a third reviewer (AC).

**Inclusion and exclusion criteria**

All original English language studies using quantifiable imaging techniques to investigate neuropathic pain and phantom limb pain following SCI or amputation, respectively, were included. The imaging techniques of primary interest comprised of fMRI, VBM (gray and white matter volumes), and diffusion tensor imaging. A “quantifiable” technique was considered as an MRI outcome derived from automated analysis of gray (function and anatomy) or white matter, performed in a neuroimaging platform (e.g., Statistical Parametric Mapping (SPM), FSL, and FreeSurfer). Included studies must have performed a statistical analysis specifically focusing on pain and reorganization. Preclinical studies in species other than humans (e.g., rodents, and monkeys) were excluded. Also excluded were pediatric studies, case studies, and review articles.

**Outcomes**

The specific outcomes extracted from each study included: 1) individuals’ characteristics (i.e., age and sex, and time since deafferentation), 2) pain rating (converted to 0-10 if necessary), 3) number of individuals with amputations or SCI with and without pain, 4) number of healthy individuals, 5) type of MRI technique applied (e.g., fMRI versus VBM), 6) imaging parameters (i.e., echo and repetition time), 7) regions of interest examined, 8) statistical approach (i.e., correction for multiple comparisons), and 9) type of pain assessment. For outcomes that could be summarized across studies, grand-weighted averages (by the number of individuals) were calculated (+/- standard deviation, SD). For fMRI studies, we also considered the type of task performed while in the scanner (e.g., executed movement versus imagery), as well as methods used to analyze differences in patterns of BOLD activation.

**Levels of evidence**

Studies were divided into two levels of evidence. 1st level evidence comprised all studies that included a healthy control group, as well as patients (i.e., SCI and amputation) with and without neuropathic or phantom limb pain. Additionally, 1st level evidence was required to explicitly qualify examining the correlation between pain intensity and a measure of cortical reorganization. 2nd level evidence included studies that did not incorporate a healthy control condition and/or individuals that were ‘pain-free’, and thus less well suited to address the concept of maladaptive plasticity. 2nd level evidence studies examined...
the correlation between pain intensity and reorganization, or group level comparisons without considering the correlation between pain intensity and cortical reorganization. For each publication, the direction of the relationship between pain and reorganization was made based on the task performed (fMRI only), and the area of the brain examined. Based on this information, we determined whether the results supported (or opposed) the concept of maladaptive plasticity.

**Quality assessment rating**
Based on 10 criteria relevant to the objectives of the review (adapted from Campbell)\textsuperscript{237}, the outcomes extracted from each study were considered in a descriptive analysis. CJ and JK independently performed the quality assessment. Disagreements of ratings were discussed and final scores for each publication were determined.

### 5.4 Results

**Included/Excluded studies**
145 candidate publications (54 for SCI, 91 for amputation) were first identified, of which 13 (5 SCI, 8 amputation) were suitable for review\textsuperscript{233, 234, 238-248}. The reasons for the exclusion of studies (n=134, 49 SCI, 85 amputation) are highlighted in Figure 5.1.

![Figure 5.1: Diagram of the review procedure.](image)
Study details and characteristics

Pain assessment and imaging acquisition parameters for each study are shown in Table 5.1. The results from the quality assessment are shown in Table 5.2. Further, Figure 5.2 illustrates the mean (+/−SD) age, time since deafferentation, and pain rating for each study, and the grand weighted average for each parameter.

Table 5.1: Characteristics of included studies

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study population</th>
<th>Type of pain (n)</th>
<th>Structural/Functional Imaging</th>
<th>Echo time (TE)</th>
<th>Repetition time (TR)</th>
<th>Regions of interest</th>
<th>Statistical correction</th>
<th>Pain Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith et al., (2010)</td>
<td>16 upper arm amputees (14 traumatic, 2 malignant tumors), 8 healthy controls</td>
<td>Painful</td>
<td>Functional (SPM)</td>
<td>13 T1, 5 T2</td>
<td>5.55, 165.0</td>
<td>NA</td>
<td>Uncorrected, p&lt;0.05</td>
<td>Not reported</td>
</tr>
<tr>
<td>Desk et al., (2010)</td>
<td>14 unilateral upper limb amputees (13 traumatic, 1 vascular disease), 6 healthy controls</td>
<td>Painful</td>
<td>Functional (SPM)</td>
<td>11 T1, 5 T2</td>
<td>36.0, 2333.0</td>
<td>S1, S2, M1, SMA</td>
<td>Correction for multiple comparisons using FDR</td>
<td>German version of the modified phantom pain inventory, modified to separate phantom arm and residual limb pain</td>
</tr>
<tr>
<td>Diagnesi et al., (2009)</td>
<td>28 unilateral upper limb amputees (17 traumatic, 5 non-traumatic), 20 age and gender-matched healthy controls</td>
<td>Painful</td>
<td>Structural (SPM)</td>
<td>11 T1</td>
<td>36.0, 2333.0</td>
<td>NA</td>
<td>Correction for multiple comparisons using FWE</td>
<td>Visual analog scale (VAS) from 0 to 10, 2 representing no pain and 10 the worst possible pain</td>
</tr>
<tr>
<td>Flodqvist et al., (2014)</td>
<td>16 unilateral upper limb amputees (15 traumatic, 3 vascular disease), 3 healthy controls</td>
<td>Painful</td>
<td>Functional (SPM)</td>
<td>11 T1</td>
<td>36.0, 2333.0</td>
<td>M1 in patients and M1 in controls</td>
<td>Correction for multiple comparisons using FWE</td>
<td>Multidimensional Phantom Limb Pain Inventory Scale (Range 1-6)</td>
</tr>
<tr>
<td>Gustin et al., (2013)</td>
<td>14 unilateral upper limb amputees (7 traumatic, 7 non-traumatic), 10 patients (5 traumatic, 5 non-traumatic), 20 age and gender-matched healthy controls</td>
<td>Painful</td>
<td>Structural (SPM)</td>
<td>11 T1</td>
<td>36.0, 2333.0</td>
<td>NA</td>
<td>Correction for multiple comparisons using FWE</td>
<td>Visual analog scale (VAS) from 0 to 10, 2 representing no pain and 10 the worst possible pain</td>
</tr>
<tr>
<td>Hoxie et al., (2009)</td>
<td>13 unilateral upper limb amputees (12 traumatic, 1 vascular disease), 5 healthy controls</td>
<td>Painful</td>
<td>Structural (SPM)</td>
<td>13 T1</td>
<td>36.0, 2333.0</td>
<td>p&lt;0.05, cluster-level corrected</td>
<td>Phantom limb pain questionnaire (Koopman et al., 2003), numeric rating scale (0 = no pain to 10 = worst pain ever imagined)</td>
<td></td>
</tr>
<tr>
<td>Miletin et al., (2014)</td>
<td>21 unilateral upper limb amputees (19 traumatic, 2 non-traumatic), 10 patients (5 traumatic, 5 non-traumatic), 20 age and gender-matched healthy controls</td>
<td>Painful</td>
<td>Functional (SPM)</td>
<td>17 T1</td>
<td>36.0, 2333.0</td>
<td>Hand area</td>
<td>Correction for multiple comparisons using FWE</td>
<td>Visual Analog Scale (VAS) with 0 being no pain and 100 the worst imaginable pain</td>
</tr>
<tr>
<td>Prussler et al., (2008)</td>
<td>20 unilateral upper limb amputees (18 traumatic, 2 non-traumatic), 10 patients (5 traumatic, 5 non-traumatic)</td>
<td>Painful</td>
<td>Functional (SPM)</td>
<td>17 T1</td>
<td>36.0, 2333.0</td>
<td>Dorsolateral frontal cortex, thalamus, and left posterior parietal cortex, basal ganglia, thalamus, cerebellum</td>
<td>Correction for multiple comparisons using FWE</td>
<td>Visual analog scale from 0 (no pain) to 10 (the most pain imaginable)</td>
</tr>
<tr>
<td>Rock et al., (2010)</td>
<td>11 patients (11 complete thoracic lesion, 2 complete cervical lesion)</td>
<td>Painful</td>
<td>Functional (SPM)</td>
<td>15 T1</td>
<td>36.0, 2333.0</td>
<td>NA</td>
<td>Correction for multiple comparisons using FDR</td>
<td>Pain diary was completed for one week prior to scanning (0 cm = &quot;no pain&quot;) to 10 cm = &quot;worst pain imaginable&quot;) three times a day</td>
</tr>
<tr>
<td>Rock et al., (2010)</td>
<td>25 patients (11 complete thoracic lesion, 1 complete cervical lesion, 3 complete thoracic lesion, 6 healthy controls, 14 healthy controls)</td>
<td>Painful</td>
<td>Structural (SIT)</td>
<td>12 T1</td>
<td>36.0, 2333.0</td>
<td>NA</td>
<td>Uncorrected, p&lt;0.05, minimum cluster-size 20</td>
<td>Pain diary was completed for one week prior to scanning (0 cm = &quot;no pain&quot;) to 10 cm = &quot;worst pain imaginable&quot;) three times a day</td>
</tr>
<tr>
<td>Rock et al., (2010)</td>
<td>20 patients (10 complete thoracic lesion, 10 complete cervical lesion), 20 age and gender-matched healthy controls</td>
<td>Painful</td>
<td>Structural (SIT)</td>
<td>18 T1</td>
<td>36.0, 2333.0</td>
<td>M1 and S1 of leg area, thalamus, left posterior parietal cortex, right insula</td>
<td>Correction for multiple comparisons using FWE</td>
<td>Visual analog scale (VAS) with 0 (no pain) to 10 (the most pain imaginable)</td>
</tr>
<tr>
<td>Rock et al., (2010)</td>
<td>20 patients (10 complete thoracic lesion, 10 complete cervical lesion), 20 age and gender-matched healthy controls</td>
<td>Painful</td>
<td>Structural (SIT)</td>
<td>15 T1</td>
<td>36.0, 2333.0</td>
<td>NA</td>
<td>Correction for multiple comparisons using FDR</td>
<td>International Association for the Study of Pain (ISI) Pain Taxonomy. A pain diary was completed for one week prior to scanning (0 cm = &quot;no pain&quot;) to 10 cm = &quot;worst pain imaginable&quot;) three times a day</td>
</tr>
<tr>
<td>Rock et al., (2010)</td>
<td>10 patients (5 multilevel thoracic spinal cord lesions, 5 multilevel cervical spinal cord lesions)</td>
<td>Painful</td>
<td>Functional and structural (PET, SPM5 and SIT)</td>
<td>12 T1</td>
<td>36.0, 2333.0</td>
<td>NA</td>
<td>Uncorrected, p&lt;0.05</td>
<td>11-point NRS scale (P= no pain, 10 = the most intense imaginable pain)</td>
</tr>
</tbody>
</table>

Notes: 1D=diffusion tensor imaging (DTI); 2D=deoxy-Hb imaging; 3D=functional magnetic resonance imaging (fMRI); 4D=functional connectivity; 5D=positron emission tomography (PET); 6D=statical parametric mapping (SPM); 7D=spinal cord injury; 8D=primary motor cortex; 9D=primary sensory cortex; 10D=secondary motor cortex; 11D=secondary sensory cortex; 12D=spinal cord slice; 13D=spinal cord level; 14D=spinal cord segment; 15D=spinal cord level.
**Table 5.2: Quality assessment of included studies**

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Design</th>
<th>Pathology</th>
<th>Scoring Criteria for quality assessment</th>
<th>Score %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dettmers C.</td>
<td>2001</td>
<td>Cross-sectional</td>
<td>Amputation</td>
<td>1 Y N N Y Y Y Y N N N</td>
<td>40</td>
</tr>
<tr>
<td>Diers M.</td>
<td>2010</td>
<td>Cross-sectional</td>
<td>Amputation</td>
<td>1 N N Y Y Y Y Y Y Y</td>
<td>70</td>
</tr>
<tr>
<td>Dragan G.</td>
<td>2006</td>
<td>Cross-sectional</td>
<td>Amputation</td>
<td>1 Y N N Y Y Y Y Y Y N N N</td>
<td>40</td>
</tr>
<tr>
<td>Goell J.</td>
<td>2014</td>
<td>Longitudinal</td>
<td>Amputation</td>
<td>1 Y N N Y Y Y N Y Y Y Y</td>
<td>70</td>
</tr>
<tr>
<td>Lotze M.</td>
<td>2001</td>
<td>Cross-sectional</td>
<td>Amputation</td>
<td>1 N N N Y Y Y Y N N N N</td>
<td>40</td>
</tr>
<tr>
<td>MacIver K.</td>
<td>2008</td>
<td>Longitudinal</td>
<td>Amputation</td>
<td>1 Y N N Y Y Y N Y Y Y Y</td>
<td>70</td>
</tr>
<tr>
<td>Makin T.</td>
<td>2013</td>
<td>Cross-sectional</td>
<td>Amputation</td>
<td>1 Y N N Y Y Y Y Y Y Y N</td>
<td>70</td>
</tr>
<tr>
<td>Preissler S.</td>
<td>2013</td>
<td>Cross-sectional</td>
<td>Amputation</td>
<td>1 Y N N Y Y Y N N Y Y Y N</td>
<td>80</td>
</tr>
<tr>
<td>Totals (%) Amputation</td>
<td></td>
<td></td>
<td></td>
<td>75 12.5 12.5 100 87.5 100 62.5 62.5 87.5 50</td>
<td>65</td>
</tr>
<tr>
<td>Gustin S.*</td>
<td>2010</td>
<td>Cross-sectional</td>
<td>SCI</td>
<td>1 Y Y Y Y Y Y Y Y Y Y Y Y</td>
<td>90</td>
</tr>
<tr>
<td>Gustin S.†</td>
<td>2010</td>
<td>Longitudinal</td>
<td>SCI</td>
<td>1 Y N N Y Y Y Y N Y Y Y N</td>
<td>40</td>
</tr>
<tr>
<td>Mole T.</td>
<td>2014</td>
<td>Cross-sectional</td>
<td>SCI</td>
<td>1 Y Y Y N Y N Y Y Y Y Y Y</td>
<td>85</td>
</tr>
<tr>
<td>Wrigley P.</td>
<td>2009</td>
<td>Cross-sectional</td>
<td>SCI</td>
<td>1 Y N N Y Y Y Y Y Y Y N</td>
<td>70</td>
</tr>
<tr>
<td>Yoon E.</td>
<td>2013</td>
<td>Cross-sectional</td>
<td>SCI</td>
<td>1 Y Y Y Y Y Y N N Y Y N</td>
<td>80</td>
</tr>
<tr>
<td>Totals (%) SCI</td>
<td></td>
<td></td>
<td></td>
<td>100 60 40 90 100 100 60 60 100 60</td>
<td>77</td>
</tr>
<tr>
<td>Overall Totals %</td>
<td></td>
<td></td>
<td></td>
<td>84.6 30.8 30.8 96.2 92.3 100 61.5 61.5 92.3 53.8</td>
<td>69.6</td>
</tr>
</tbody>
</table>

Quality assessment criteria questions

1) Does the study have a clear defined research objective?
2) Does the study adequately describe the inclusion criteria?
3) Does the study adequately describe the exclusion criteria?
4) Does the study report on the population parameters/demographics?
5) Does the study report details on assessment of pain?
6) Does the study provide details of imaging protocol?
7) Does the study provide a proper control group?
8) Does the study apply proper statistical analysis? Correction for multiple comparisons?
9) Does the study adequately report on the strength of the results (e.g., ways of calculating effect sizes, reporting of confidence intervals’ standard deviation)?
10) Do the authors report on the limitations of their study?

Y= yes, N=no,
* Published in Cerebral Cortex, † published in PAIN
Summary of key 1st level evidence: fMRI

According to the criteria for assessing the relationship between pain and reorganization, four studies were identified as 1st level evidence. Included in fMRI studies (findings summarized in Table 5.3), two were identified as clearly supporting the theory that chronic pain was associated with maladaptive plasticity. Studies fundamentally demonstrated maladaptive plasticity based on single subject shifts in topography, group level shifts in topography, and group level differences in activity. All studies specifically examined reorganization in the primary somatosensory and motor cortices. Evidence of maladaptive plasticity from group level differences was derived from the observation of increased BOLD in a brain area that should otherwise NOT be active during a motor task (e.g., lip M1 area during imagined hand movements). While not observing group level differences between activation in response to brushing, single subject shifts in S1 topography were greater in individuals with pain, and correlated positively with pain rating. A third study observed no significant differences in patterns of BOLD activation between individuals with and without neuropathic pain, but reported a significant negative correlation between pain rating and BOLD activation in the hand area during movement of the intact hand. In principle, this finding is also in agreement with the concept of maladaptive plasticity (i.e., decreased activity in an area that should otherwise be active). In contrast, Makin and colleagues demonstrated that individuals with congenital missing upper limbs and without phantom limb pain showed

<table>
<thead>
<tr>
<th>Publication</th>
<th>Type</th>
<th>Task</th>
<th>n</th>
<th>Summary of findings</th>
<th>Quality Score* (/10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diers et al., 2010</td>
<td>Amputation</td>
<td>Intact hand movement, imagined movement (phantom hand)</td>
<td>7</td>
<td>No significant changes in BOLD activation in those with or without phantom limb pain compared to healthy controls during ‘executed’ (i.e., intact hand) or imagined movements (i.e., phantom hand). Significant negative correlation between BOLD activity and pain rating during intact hand movement.</td>
<td>7</td>
</tr>
<tr>
<td>Makin et al., 2013</td>
<td>Amputation</td>
<td>Lip movement, executed movement (phantom hand)</td>
<td>17</td>
<td>No significant differences in activation during lip movements in the M1 hand area between individuals with or without phantom limb pain compared to healthy subjects. BOLD activation in the M1 hand area significantly greater in individuals with phantom limb pain and healthy controls compared to amputees without pain; positively correlated with pain rating during executed movement of the phantom hand.</td>
<td>7</td>
</tr>
<tr>
<td>Lotze et al., 2001</td>
<td>Amputation</td>
<td>Lip and intact hand movement, imagined movement (phantom hand)</td>
<td>7</td>
<td>Individuals with phantom limb pain have a medial shift of the lip into the deafferented hand area, enlarged representation of the mouth, and greater S1 and M1 BOLD activation during lip movement compared to amputees without neuropathic pain and healthy controls. Additionally, individuals with phantom limb pain demonstrated increased M1/S1 BOLD activation in the mouth area during imagined hand movements of the phantom limb.</td>
<td>5</td>
</tr>
<tr>
<td>Wrigley et al., 2009</td>
<td>SCI</td>
<td>Brushing of the hand at lip and hand locations</td>
<td>10</td>
<td>No significant differences in patterns of BOLD activation between individuals with and without neuropathic pain compared to healthy controls. Significant medial shifts in location of BOLD activity in S1, correlated with the intensity of below-level neuropathic pain.</td>
<td>7</td>
</tr>
</tbody>
</table>

n, number of subjects with pain; SCI, spinal cord injury; BOLD, blood oxygen level–dependent; M1, primary motor cortex; S1, primary sensory cortex
* Quality assessment criteria and single ratings are listed in the Table 5.2.
less activation in M1 compared to healthy individuals and individuals with phantom limb pain during executed movement of the phantom hand\textsuperscript{244}. Moreover, there was a positive correlation between pain rating and BOLD activation in M1, such that individuals with higher pain ratings yielded responses more similar to healthy control individuals than individuals without pain.

**Table 5.4:** Anatomical MRI studies meeting the inclusion criteria and adequately designed to assess the relationship between cortical reorganization and neuropathic pain (first level evidence)

<table>
<thead>
<tr>
<th>Publication</th>
<th>Type</th>
<th>Technique</th>
<th>n</th>
<th>Summary of findings</th>
<th>Quality Score* (/10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gustin et al., 2010</td>
<td>SCI</td>
<td>DTI</td>
<td>12</td>
<td>Positive correlation between mean diffusivity and pain intensity in dorsolateral prefrontal cortex, posterior parietal cortex, anterior insular, and premotor cortex; negative correlation in the amygdala, ventroposterior thalamus</td>
<td>9</td>
</tr>
<tr>
<td>Makin et al., 2013</td>
<td>Amputation</td>
<td>VBM</td>
<td>18</td>
<td>Gray matter volume in the M1 hand area was positively correlated with pain intensity.</td>
<td>7</td>
</tr>
<tr>
<td>Mole et al., 2014</td>
<td>SCI</td>
<td>VBM</td>
<td>18</td>
<td>Individuals without neuropathic pain showed increased gray matter volume in S1. No difference in S1 volume was found between individuals with pain compared to healthy controls. However, S1 volume in individuals with neuropathic pain was negatively correlated with pain intensity.</td>
<td>8.5</td>
</tr>
</tbody>
</table>

* n, number of subjects with pain; SCI, spinal cord injury; M1, primary motor cortex; S1, primary sensory cortex; VBM, voxel-based morphometry; DTI, diffusion tensor imaging

*: Quality assessment criteria and single ratings are listed in the Table 5.2.

**Summary of key 1\textsuperscript{st} level evidence: Structural MRI**

Of the three anatomical MRI studies that meet 1\textsuperscript{st} level evidence, two employed the same technique (i.e., VBM, see Table 5.4). In contrast to the maladaptive plasticity model, Mole and colleagues provide group level evidence that pain preserves cortical structure in primary somatosensory and motor cortices, in that only individuals without neuropathic pain after SCI demonstrate significant differences from healthy individuals\textsuperscript{245}. Interestingly, GM volume was, however, negatively correlated with NP intensity among individuals with SCI. Similar to their fMRI findings, Makin and colleagues reported a positive correlation between pain and gray matter volume in the M1 hand area, suggesting that pain preserves cortical anatomy\textsuperscript{244}.

**Summary of key 2\textsuperscript{nd} level evidence: fMRI and structural MRI**

Functional MRI studies meeting 2\textsuperscript{nd} level evidence (Table 5.5) all lacked one or more control conditions (i.e., healthy individuals and/or individuals without pain)\textsuperscript{234, 238, 240, 243}. However, three studies were designed longitudinally to assess the impact of changes in pain rating on functional measures of reorganization\textsuperscript{234, 240, 243}. Based on increased activity in brain areas that should NOT be active during a...
motor task, two studies demonstrate evidence supporting maladaptive plasticity. Mental imagery training in individuals with amputations resulted in reductions in pain rating, which were correlated with decreases in the amount of activity in the M1 hand area following lip movement\textsuperscript{243}. Similarly, Foell and colleagues

Table 5.5: fMRI studies meeting the inclusion criteria and adequately designed to assess the relationship between cortical reorganization and neuropathic pain (second level evidence)

<table>
<thead>
<tr>
<th>Publication</th>
<th>Type</th>
<th>Task</th>
<th>n</th>
<th>Summary of findings</th>
<th>Quality Score* (/10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dettmers et al., 2001</td>
<td>Amputation</td>
<td>Finger tapping with the intact hand, repetitive eye closing, anteflexion of stump or intact shoulder</td>
<td>8</td>
<td>Increased likelihood for BOLD activation in SMA in individuals with phantom limb pain (not compared to healthy subjects) during anteflexion of the stump.</td>
<td>4</td>
</tr>
<tr>
<td>Foell et al., 2013</td>
<td>Amputation</td>
<td>Lip movement</td>
<td>11</td>
<td>No comparison with a healthy control group or individuals without phantom limb pain. Correlation analysis performed between pain intensity and BOLD activation not reported.</td>
<td>7</td>
</tr>
<tr>
<td>Gustin et al., 2010</td>
<td>SCI</td>
<td>Imagined leg movement</td>
<td>11</td>
<td>Significant BOLD activation in M1 leg area during imaginary leg movement. No correlation performed or comparison with individuals without pain.</td>
<td>6</td>
</tr>
<tr>
<td>MacIver et al., 2008</td>
<td>Amputation</td>
<td>Lip and intact hand movement, imagined movement (intact and phantom)</td>
<td>13</td>
<td>Significant positive correlation between BOLD activation during lip and intact hand movement in M1 hand area and pain rating. No correlation between BOLD activation during imagined phantom hand movement and pain rating. Individuals with amputation but without phantom limb pain were not included.</td>
<td>7</td>
</tr>
</tbody>
</table>

n, number of subjects with pain; SCI, spinal cord injury; BOLD, blood oxygen level–dependent; M1, primary motor cortex; SMA, supplementary motor area

*: Quality assessment criteria and single ratings are listed in the supplementary Table 5.2.

Table 5.6: Anatomical MRI studies meeting the inclusion criteria and adequately designed to assess the relationship between cortical reorganization and neuropathic pain (second level evidence)

<table>
<thead>
<tr>
<th>Publication</th>
<th>Type</th>
<th>Technique</th>
<th>n</th>
<th>Summary of findings</th>
<th>Quality Score* (/10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Draganski et al., 2006</td>
<td>SCI</td>
<td>VBM</td>
<td>28</td>
<td>No relationship between pain and gray matter volume</td>
<td>7</td>
</tr>
<tr>
<td>Preissler et al., 2013</td>
<td>Amputation</td>
<td>VBM</td>
<td>21</td>
<td>Positive correlation between gray matter volume in the caudal anterior cingulate cortex and pain rating, negative correlation in the insular gray matter</td>
<td>8</td>
</tr>
<tr>
<td>Yoon et al., 2013</td>
<td>SCI</td>
<td>DTI and VBM</td>
<td>10</td>
<td>No correlation reported</td>
<td>8</td>
</tr>
</tbody>
</table>

n, number of subjects with pain; SCI, spinal cord injury; M1, primary motor cortex; S1, primary sensory cortex; VBM, voxel-based morphometry; DTI, diffusion tensor imaging

*: Quality assessment criteria and single ratings are listed in the supplementary Table 5.2.
adopted mirror-training therapy to relieve phantom limb pain, demonstrating a positive correlation in the extent pain was relieved and a shift in peak activity\textsuperscript{234}. There were no significant changes in BOLD activity in primary somatosensory or motor cortices reported in response to imagined foot movements (and increases in neuropathic pain) with SCI\textsuperscript{240}. Two of the three 2\textsuperscript{nd} level evidence anatomical MRI studies reviewed examined the correlation between pain rating gray matter volume\textsuperscript{239, 248}. Neither reported significant associations between pain rating and gray matter volume in either the primary sensory or motor cortices (Table 5.6).

5.5 Discussion
The primary goal of this review was to assess the strength of the relationship between deafferentation, neuropathic pain, and brain reorganization using advanced functional and structural neuroimaging techniques. Our systematic review identified 13 eligible published studies. To date, cross-sectional and longitudinal fMRI investigations demonstrate at least some evidence in support for maladaptive plasticity. However, differences in terms of the approach employed to assess functional reorganization (illustrated in Figure 5.3) as well as inconsistencies in group-level findings, reveal some incongruences of the relation of neuropathic pain and cortical reorganization (i.e. strength of the association between pain and reorganization after deafferentation).

<table>
<thead>
<tr>
<th>(A) Single subject level</th>
<th>(B) Group level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Δ Topography</strong></td>
<td><strong>Δ Topography</strong></td>
</tr>
<tr>
<td>Anatomical Marker</td>
<td>Anatomical Marker</td>
</tr>
<tr>
<td>(0 -35 70)</td>
<td>(0 -35 70)</td>
</tr>
<tr>
<td>Euclidean Distance</td>
<td>Euclidean Distance</td>
</tr>
</tbody>
</table>

Figure 5.3: Methods of analysis of cortical reorganization. (A) Single subject analysis of cortical reorganization is conventionally performed using the Euclidean distance (ED). The ED is calculated for each individual (C1 and P1) between an anatomical marker (i.e., point at which the central sulcus meets the longitudinal fissure at the dorsal aspect of the brain) and the maximally activated voxel in task-specific ROI. (B) On the group-level, cortical reorganization can be assessed by looking at changes in topography (left panel) or differences of overall activity (right panel).

C: Healthy control individuals, P: Patients

Functional reorganization: Maladaptive plasticity or preserved function?
While the concept of maladaptive cortical plasticity is nearly 20 years old, and significant advances in field of neuroimaging have been achieved during this time, relatively few studies have adopted MRI to assess the relationship between pain and cortical reorganization after deafferentation. The original support
for maladaptive plasticity using fMRI originates from Lotze and colleagues, which demonstrated group-level increases in BOLD activity during executed and imaginary movement tasks between individuals with phantom limb pain compared to healthy control individuals. Importantly, the same comparisons were not significantly different between healthy individuals and individuals without phantom limb pain. Since this seminal publication, subsequent studies have not replicated large-scale evidence of maladaptive plasticity after deafferentation (i.e., no group level differences between individuals with pain compared to healthy individuals during any of the functional tasks examined). However, more subtle evidence of maladaptive plasticity (e.g., shifts in S1 topography correlating with pain intensity) has been reported.

In direct contrast to these findings, supported by group level differences, a recent study suggests the inverse relationship to maladaptive plasticity – that is, neuropathic pain preserves functional organization in the brain. At present, this is the only fMRI evidence that directly opposes the concept of maladaptive plasticity.

**Longitudinal studies and cortical reorganization**

From a theoretical perspective, the most convincing evidence in support of the maladaptive plasticity model comes from longitudinal studies assessing pain-modulating interventions. These studies offer the unique potential to examine the relationship between cortical reorganization and pain, as well as evaluate how the relationship may change as result of an intervention. Unfortunately, at the present time, all longitudinal studies consist of second level evidence – that is, lack adequate control conditions. Due to the lack of adequate control groups, no longitudinal study can conclude that reorganization is specific to reductions in pain, and does not likewise occur in individuals without pain (or healthy control individuals) in response to intervention. Thus, it remains possible that cortical organization due to an intervention is related to general changes in the brain, or may be a function of other factors (e.g., regression towards the mean).

**Structural changes and reorganization**

Based on first level evidence, current VBM findings further raise the possibility that structural reorganization in S1 and M1 may be protective against the development of chronic pain. In general, these findings fit with the longstanding concept that emerging neural plasticity in the central nervous system is an adaptive process, involved in the recovery of beneficial outcomes and likewise might rather be preventive of pain. However, the interpretation of anatomical changes in the brain related to deafferentation and pain is, at present, rather difficult. Reorganization in humans has been historically considered in the context of underlying improved functional outcomes, few studies reconciling the relationship between functional and structural alterations after deafferentation. In addition, there is little understanding how functional cortical reorganization in response to deafferentation should overlap with anatomical changes. Accordingly the relation of changes in cortical anatomy (i.e., gray matter...
volume) to changes of neuronal and parenchymal (like glia, vascularization etc.) brain structure is less established.

**Discrepancies between studies: Now and then**

A number of factors may explain the discrepancy between earlier findings and more recent investigations, and a lack of consistent outcomes. Given that fMRI was in the early stages of development in 2001, more advanced data acquisition and processing could conceivably be leading to a more comprehensive picture, necessitating more refined approaches to assess reorganization after deafferentation. For example, BOLD sensitivity in fMRI is highly dependent on acquisition factors including voxel size, echo time, and acquisition bandwidth, which have been dramatically improved in recent years.

Regardless of technological differences, comparison between neuroimaging studies is very difficult due to other methodological considerations. Notably, sensory and motor tasks performed during fMRI to assess reorganization vary considerably. For example, Makin and colleagues adopted a different motor task than used previously by others. However, the difficulty comparing between studies is, perhaps in larger part, related to the fact that few investigators adopt the same definition of reorganization or report consistent outcomes. For example, increased activity in areas of the brain that should otherwise NOT be active, as well as decreased activity in areas that should be active are both considered evidence of maladaptive plasticity. At present, it is difficult to conclude what outcome constitutes the strongest (valid and reliable) measure of cortical reorganization.

**Age, time since deafferentation, and pain rating**

Of notable concern, the majority of neuroimaging studies to date have examined individuals well after deafferentation (i.e., >10 years, See Figure 5.2). Therefore, very little is known regarding the early stages of deafferentation and the impact of short- versus long-term pain on cortical reorganization. Subject age also varies considerably between studies, and is heterogeneous within individual studies (i.e., large SD). While large variability within studies is not necessary problematic (with age-matched controls), at present, a statistical analysis of the relationship between reorganization and pain for different age categories is limited due to low subject numbers.

**Areas of future research**

There are several potential lines of future investigation. First, studies could be improved by consensus with regards to a more standardized approach to assess reorganization. This requires determining methods that are valid and reliable, as well as sensitive to subtle changes in function and structure. To our knowledge, no study to date has performed a test-rest reliability analysis of measures of cortical reorganization after deafferentation. The development of standardized tasks (e.g., lip movement) and methods of assessment across studies would facilitate pooling of results and a future meta-analysis. Adopting a standardized approach, additional cross-sectional studies are needed, further clarifying the
direction of the relationship between pain and deafferentation. An important aim of future cross-sectional studies should also be to include a larger, more representative sample of individuals with SCI and/or amputations. Larger sample sizes could be helpful in addressing questions related to the effect of age and time since deafferentation. In terms of longitudinally designed studies, there is a considerable need to include patient populations without pain, as well as healthy controls, to determine the specificity of reversing reorganization to relieve pain. Given the potential importance of developing novel strategies targeting mechanisms underlying chronic pain, these areas of study are a priority.

**Limitations of this review**

The most notable limitation of this review is that we did not consider other techniques that have been used to examine cortical reorganization after SCI and amputation, such as EEG, MEG, and TMS. As such, we cannot make conclusions on the overall level of evidence, but only in the context of advanced functional and anatomical imaging. Different underlying principles of brain activation (i.e., BOLD versus electrical activity, stimulation of the motor cortex versus recording of the motor cortex) may render some functional techniques more suitable than others to assess cortical reorganization. However, it is interesting to note that the controversy with regards to the direction of the relationship between pain, deafferentation, and reorganization has also recently emerged using MEG.

**Potential for publication bias**

The limited number of studies reporting no association between neuropathic pain and cortical organization speaks to a high probability of publication bias. We identified two studies that reported the presence and intensity of phantom pain in the methods, but did not plan, perform, and/or report findings from a “pain analysis” (i.e., examining relationship between pain intensity and imaging outcomes). Since a considerable number of studies using a variety of neuroimaging techniques have addressed reorganization in cortical structures after deafferenation unrelated to pain, difficulty publishing negative results (i.e., no pain specific differences) may contribute to a publication bias.

**Conclusion**

There is evidence supporting the concept of reorganization after SCI and limb amputation, and that the extent of reorganization may depend on the presence and intensity of neuropathic pain (i.e., below-level and phantom limb pain). However, current findings from functional and anatomical MRI are inconsistent, even proposing the complete opposite to maladaptive plasticity (i.e., preserved function associated with neuropathic pain). There is an urgent need for additional studies appropriately designed (i.e., including requisite control groups, large and representative samples) to better address the most likely rather complex relationship between reorganization and pain after deafferentation. Future studies should also consider a standardized approach to assess reorganization, which will allow for a meta-analysis of pooled investigations.
Acknowledgement
We would like to thank Prof. Dr. Volker Dietz for his insightful comments regarding the manuscript. The study was supported by the Swiss National Science Foundation (SNF) and the Clinical Research Priority Program “Neurorehab” of the University of Zurich, Switzerland. John Kramer is supported by a Michael Smith Foundation for Health Research and Rick Hansen Institute Scholar award.

Author’s Contribution
Catherine R. Jutzeler contributed substantially to the data acquisition, analysis (i.e., quality assessment), and interpretation. Furthermore, she was involved in drafting the review article. Armin Curt made substantial contributions to data analysis (i.e., quality assessment) and participated in revising the review article critically for important intellectual content. John L.K. Kramer contributed substantially to data acquisition, analysis (i.e., quality assessment), and interpretation. Furthermore, he drafted the review article.
Study 4

Association of pain and CNS structural changes after spinal cord injury

Catherine R. Jutzeler, Eveline Huber, Martina F. Callaghan, Roger Luechinger, Armin Curt, John L.K. Kramer, and Patrick Freund

*Journal of Neurology, Neurosurgery, and Psychiatry (under review)*

~ We would like to thank all of the individuals participating in our study, and Jenny Haefeli and Alexandra Schaettin for their support in collecting data.~
6.1 Abstract

Traumatic spinal cord injury (SCI) has been shown to trigger remote and widespread structural atrophic changes within the spinal cord and brain. However, the relationship between structural changes and magnitude of below-level neuropathic pain (NP) remains incompletely understood. Voxel-wise analysis of anatomical magnetic resonance imaging (MRI) data provided information on cross-sectional cervical cord area, volumetric brain, and cortical thickness changes in 30 individuals with chronic traumatic SCI and 31 healthy controls. Participants were clinically assessed including a full neurological examination and pain questionnaire. Regression analyses assessed associations between structural changes and clinical indices of pain. Compared to controls, individuals with SCI exhibited decreased cord area (16.5% paraplegic SCI; 28.2% tetraplegic SCI), reduced gray matter (GM) volumes in anterior cingulate cortex (ACC), left insula, left secondary somatosensory cortex, bilateral thalamus (all p<0.05), and decreased white matter volumes in the pyramids and the left internal capsule (all p<0.05). The presence of NP was related with smaller cord area (p=0.002, -11.6%), increased GM in the left ACC and right M1, as well as decreased GM in right primary somatosensory cortex and thalamus (all p<0.05). Greater GM volume in M1 was associated with amount of NP (r=0.637; p=0.001). Below-level NP associated structural changes in the spinal cord and brain can be discerned from trauma-induced consequences of SCI. Crucially, the directionality of these relationships reveals specific changes across the neuroaxis (i.e., atrophic changes versus increases in volume) and may provide substrates of underlying neural mechanisms in the development of below-level NP.
6.2 Introduction

Traumatic spinal cord injury (SCI) is thought to drive structural changes - both degeneration and repair - across the entire neuroaxis (brain and spinal cord)\textsuperscript{264, 265}. The magnitude of these changes is associated with sensorimotor impairments and recovery\textsuperscript{83, 88}. A major limiting factor of the recovery is the development of below-level neuropathic pain (NP) of which the majority of the individuals with SCI suffer from\textsuperscript{56, 145, 146, 266}. Commonly, the development of below-level NP in SCI has been attributed to maladaptive plasticity in brain areas encoding sensory stimuli (i.e., noxious and innocuous sensation) including primary and sensory cortices (S1 and S2), thalamus, and anterior cingulate cortex (ACC)\textsuperscript{245, 246}. Cortical reorganization of primary somatosensory cortex (S1) was shown to be associated with the intensity and duration of ongoing NP following SCI\textsuperscript{241, 246}. These findings prompt the assumption that persistent NP is related to alterations in neuronal activity that translate into long-term structural changes in the brain\textsuperscript{227, 241}. Gray and white matter volume changes were detected in SCI individuals suffering from NP when compared to pain-free counterparts\textsuperscript{245}. Trauma-induced cord atrophy rostral to the injury is frequently reported\textsuperscript{83, 88}, however less is known about the pathological relationship between cord atrophy and below-level NP.

The primary objective was to investigate the impact of traumatic SCI and NP on central nervous system (CNS) structure. We used cross-sectional cord area measurement to assess cord atrophy and voxel-based morphometry/thickness to assess gray and white matter volume changes\textsuperscript{88}. We specifically addressed the hypothesis that structural changes across the neuroaxis are associated with the presence and intensity of below-level NP. Thus, we investigated healthy individuals, individuals with SCI with NP, and pain-free individuals with SCI.

6.3 Material and Methods

Data collection

Participants

We enrolled 30 individuals with a chronic traumatic SCI (mean (SD) 46.3 (11.9) years; gender: 3 female, 27 male) including lesions at cervical (n=15), thoracic (n=13), and lumbar levels (n=2). Time since injury spanned from 2 to 27 years (mean 10.5 yrs) and 2 to 26 years (mean 13 yrs) for the individuals with tetraplegia and paraplegia, respectively. We also recruited 31 neurologically healthy individuals (mean (SD) 31.9 (9.9) years; gender: 14 female, 17 male). All participants provided written informed consent and all procedures described below were in accordance with the Declaration of Helsinki and approved by the local Ethics committee (ref. number: EK-04/2006).

Clinical Assessment

The neurological examination was performed according to International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) published by the American Spinal Injury Association.
Briefly, sensory, motor, and neurological levels of injury were identified allowing characterization of sensory/motor functioning as well as determination of the completeness of injury by means of the ISNCSCI Impairment Scale (AIS)\textsuperscript{62}. A ‘Touch-test Sensory Evaluator’ (North Coast Medical Inc, California, USA) was used for the evaluation of the cutaneous sensation level (i.e., Von Frey Filament testing) of the participants’ left and right C6 dermatomes. Beginning with the smallest filament size (2.83), the stimulus was applied three times, whereas a single response indicated a positive response. In cases in which the participant did not respond to the stimulus, the next largest monofilament was chosen and the process repeated. At maximum, a set of five of monofilaments was used (2.83, 3.61, 4.31, 4.56, and 6.65). All participants were interviewed to determine the existence of pain using the ‘European Multicenter Study about SCI’ (EMSCI) pain questionnaire (V4.2, \url{http://www.emsci.org/}). The pain questionnaire examines various aspects of pain (e.g., duration (years), maximal and average pain intensity) as well as pain-associated psychosocial factors (pain interference). The pain intensity was rated using an 11-point numeric rating scale with “0” indicating no pain to “10” indicating worst pain imaginable. Pain interference was measured with a 7-point numeric rating scale with “0” for “not affected me” and “6” for “affected me completely”. Accordingly, pain can be grouped into nociceptive (e.g., musculoskeletal or visceral) or neuropathic pain (e.g., at or below the lesion). To be classified as below-level NP, ongoing pain had to be located three or more segments below the level of lesion. Lastly, warm perception and pain thresholds were recorded using the method of levels starting at a baseline temperature of 32°C\textsuperscript{168}. Briefly, single stimuli with increasing temperature (1°C/stimulus) were sequentially presented by a contact heat stimulator (Pathway, Medoc, RamatYishai, Israel) until the individual reported to perceive a thermal sensation (i.e., perception threshold) or rated the thermal sensation as painful (i.e., pain perception).

**Image acquisition**

Magnetic resonance imaging (MRI) data was collected on a Philips 3 T Ingenia system (Philips Medical Systems, Best, the Netherlands) using a 15-channel Philips Sense head coil. A 3D-GRE T1-weighted (T1w) sequence was used to acquire a structural scan optimized for simultaneous assessment of the brain and spinal cord\textsuperscript{267}. The imaging parameters were: isotropic 1 mm\textsuperscript{3} resolution, field of view 256 x 256 x 180, repetition time=6.88 ms, echo time=3.1 ms, flip angle 8°, fat saturation, scan resolution 256x256 voxels, and a scan time of 6:31min. Prior to analysis the MRI data were screened for movement artefacts.

**Data Analysis**

**Statistics**

All statistical procedures were performed using SPSS (version 19.0, Armonk, New York, U.S.). Mann-Whitney-U tests were applied and P < 0.05 was considered significant after Bonferroni correction.

**MRI Analysis**
Voxel-based morphometry (VBM) was performed in SPM8 in order to perform voxel-wise comparisons of gray (GM) and white matter (WM) volume between the three groups of individuals. The preprocessing included four steps. Firstly, all 3D-GRE T1-weighted images were reoriented to set the image origin (0/0/0) to the anterior commissure. Then, a unified model inversion (unified segmentation) was used for bias correction and segmentation of 3D-GRE T1-weighted (T1w) images into GM, WM, and CSF. In the next step, through iterative nonlinear registration (DARTEL) the GM and WM segments were warped into an optimal (average) space. The resulting GM and WM images were modulated and affine transformed to MNI space. Lastly, the modulated normalized GM and WM segments were smoothed using an isotropic Gaussian kernel with 6 mm full width at half-maximum prior to between-group analyses at the 2nd level.

**Voxel-based cortical thickness**

A voxel-based cortical thickness (VBCT) map was created for each participant using the GM, WM, and CSF segments created in the preprocessing step of the VBM analysis. The input tissue segments were sub-sampled from 1 mm to 0.5 mm using trilinear interpolation to increase resolution for narrow CSF spaces. In order to compute VBCT maps, cortical GM boundaries were extracted and the distance between the inner and outer GM boundaries were estimated for each voxel in the cortex. The VBCT maps comprised of a value for cortical thickness at each voxel in GM and zeros elsewhere. Afterwards, the VBCT maps were warped into the same reference space as the GM probability maps. Subsequently, smoothing was performed by using an isotropic 6mm full width at half-maximum Gaussian kernel including a correction in order to maintain local cortical thickness.

**Region of interest (ROI) Analysis**

Based on previous neuroimaging evidence, we chose an ROI approach incorporating the bilateral primary motor cortex (M1), somatosensory cortices (S1 and S2), premotor cortex (PMC), insulae, thalamus, and ACC using the WFU Pickatlas. Group comparisons were performed between healthy controls and individuals with SCI, as well as between individuals with SCI suffering from NP and pain-free individuals with SCI. All results reported are family-wise error (FWE) corrected for multiple comparisons within ROI. Age, gender, level of lesion, and total intracranial volume (TIV) were included in the model as nuisance variables. Then we extracted the contrast estimates of all ROIs from the individuals with SCI in order to perform Spearman correlations to identify associations between structural changes and clinical characteristics (i.e., spinal cord area (SCA), ISNCSCI motor and sensory scores, disease duration, level of lesion, pinprick and light touch scores, pain intensity and duration).

**Spinal Cord Area (SCA), anterior-posterior width (APW), and left-right width (LRW)**

From 3D-GRE T1 images five contiguous 3 mm axial slices were reformatted using the center of C2/C3 intervertebral disc as a caudal landmark, with the slices perpendicular to the spinal cord. In order to
identify the cross-sectional cord area a well-established semi-automated segmentation method was used\textsuperscript{88}. In addition, the anterior-posterior width (APW) and the left-right width (LRW) of the SCA was calculated\textsuperscript{88}. The Kruskal–Wallis one-way analysis of variance was used to identify differences in cord area between controls and individuals with SCI. Regression analysis was performed to test for an association between impairment and SCA, in individuals with SCI. The behavioral measure was set as the dependent variable, while SCA, age, and lesion level were included as independent variables.

**Table 6.1:** Clinical data for the spinal cord injured individuals

<table>
<thead>
<tr>
<th>ID</th>
<th>Age [yrs]</th>
<th>Gender</th>
<th>Etiology of the injury</th>
<th>Time since Injury [yrs]</th>
<th>Level of Lesion\textsuperscript{‡}</th>
<th>AIS*</th>
<th>Motor score (0-100)</th>
<th>Sensory score (0-224)\textsuperscript{+}</th>
<th>Neuropathic pain</th>
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</thead>
<tbody>
<tr>
<td>P01</td>
<td>50</td>
<td>m</td>
<td>Vehicle accident</td>
<td>7</td>
<td>Th4/Th5</td>
<td>A</td>
<td>50</td>
<td>90</td>
<td>yes</td>
</tr>
<tr>
<td>P02</td>
<td>50</td>
<td>m</td>
<td>Vehicle accident</td>
<td>9</td>
<td>Th4</td>
<td>B</td>
<td>50</td>
<td>200</td>
<td>no</td>
</tr>
<tr>
<td>P03</td>
<td>35</td>
<td>m</td>
<td>Gunshot</td>
<td>12</td>
<td>Th4</td>
<td>B</td>
<td>50</td>
<td>144</td>
<td>yes</td>
</tr>
<tr>
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<td>m</td>
<td>Vehicle accident</td>
<td>16</td>
<td>Th6/Th7</td>
<td>A</td>
<td>50</td>
<td>112</td>
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<td>50</td>
<td>96</td>
<td>no</td>
</tr>
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<td>m</td>
<td>Sports accident</td>
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<td>Th11</td>
<td>A</td>
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<td>128</td>
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<td>Th8</td>
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</tr>
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<td>L3</td>
<td>D</td>
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<td>A</td>
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<td>158</td>
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</tr>
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<td>m</td>
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<td>Th12</td>
<td>D</td>
<td>100</td>
<td>185</td>
<td>no</td>
</tr>
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<td>m</td>
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<td>Th11</td>
<td>A</td>
<td>60</td>
<td>148</td>
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</tr>
<tr>
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<td>m</td>
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<td>C6/ C7</td>
<td>D</td>
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<td>134</td>
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<td>C5</td>
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<td>126</td>
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<td>Vehicle accident</td>
<td>11</td>
<td>C4</td>
<td>D</td>
<td>95</td>
<td>170</td>
<td>no</td>
</tr>
<tr>
<td>T04</td>
<td>53</td>
<td>m</td>
<td>Gun shot</td>
<td>10</td>
<td>C4</td>
<td>B</td>
<td>27</td>
<td>102</td>
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<tr>
<td>T05</td>
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<td>m</td>
<td>Vehicle accident</td>
<td>6</td>
<td>C6/ C7</td>
<td>D</td>
<td>96</td>
<td>202</td>
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<tr>
<td>T06</td>
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<td>m</td>
<td>Vehicle accident</td>
<td>19</td>
<td>C6</td>
<td>B</td>
<td>24</td>
<td>78</td>
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<td>51</td>
<td>m</td>
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<td>27</td>
<td>C6/ C7</td>
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<td>30</td>
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<tr>
<td>T08</td>
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<td>m</td>
<td>Sports accident</td>
<td>5</td>
<td>C6/ C7</td>
<td>D</td>
<td>93</td>
<td>176</td>
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<td>T09</td>
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<td>m</td>
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<td>D</td>
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<td>m</td>
<td>Vehicle accident</td>
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<td>C6/ C7</td>
<td>A</td>
<td>40</td>
<td>185</td>
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<td>T11</td>
<td>55</td>
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<td>C3</td>
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<td>C6</td>
<td>D</td>
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<td>T13</td>
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<td>C4/C5</td>
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<td>m</td>
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<td>C2</td>
<td>D</td>
<td>100</td>
<td>224</td>
<td>yes</td>
</tr>
</tbody>
</table>

\textsuperscript{‡} The level of lesion refers to the neurological level

\textsuperscript{*}ASIA impairment scale: A, no sensory or motor function is preserved; B, sensory function is preserved below the level of the injury, but there is no motor function; C, motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade of < 3; D, motor function is preserved below the neurological level, and at least half of the key muscles below the neurological level have a muscle grade of > 3.

\textsuperscript{+}Sensory Score: Sum of segmental light touch and pinprick classifications
6.4 Results

Clinical measurements
According to the ISNCSCI impairment classification, 12 of 30 SCI individuals had a complete (2 tetraplegic, 10 paraplegic) and 18 incomplete (13 tetraplegic, 5 paraplegic) injury of the spinal cord. Two SCI individuals (1 individual with tetraplegia, 1 with paraplegia) were excluded due to incomplete datasets (i.e., measurement errors). All individuals with SCI had reduced ISNCSCI motor and sensory scores of upper and lower limbs. From the remaining 28 individuals with SCI, 13 (8 with paraplegia, 5 with tetraplegia) suffered from NP (Table 6.1). The average and maximal pain intensities were 4.0 ± 2.1 and 5.5 ± 3.0, respectively, and the duration of ongoing pain ranged from 4 to 33 years (mean 19.8 ± 12.3). All other individuals were pain-free at the time-points of measurements. Individuals with SCI showed elevation of thermal perception (paraplegic SCI: F=13.4, df:56, p=0.010, tetraplegic SCI: F=13.4, df:56, p<0.001) and pain thresholds (paraplegic SCI: F=14.39, df:56, p<0.001; tetraplegic SCI: F=15.1, df: 56 , p=0.003) compared to healthy controls (Table 6.2).

Table 6.2: Main clinical and demographic findings from the cohorts studied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Controls</th>
<th>tSCI</th>
<th>pSCI</th>
<th>Significant pairwise comparisons (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender [m:f]</td>
<td>17:14</td>
<td>14:0</td>
<td>12:2</td>
<td>HC-tSCI (&lt;0.001); HC-pSCI (&lt;0.001)</td>
</tr>
<tr>
<td>Age [yrs]</td>
<td>31.9 ± 9.9</td>
<td>47.6 ± 14.5</td>
<td>45.2 ± 10.3</td>
<td>HC-tSCI (&lt;0.001); HC-pSCI (&lt;0.002)</td>
</tr>
<tr>
<td>Duration of SCI [yrs]</td>
<td>NA</td>
<td>10.5 ± 7.2</td>
<td>14.5 ± 8.9</td>
<td>ns</td>
</tr>
<tr>
<td>SCA [mm²]</td>
<td>76.6 ± 8.4</td>
<td>56.0 ± 10.2</td>
<td>65.1 ± 5.1</td>
<td>HC-tSCI (&lt;0.001); HC-pSCI (&lt;0.001); pSCI+tSCI (&lt;0.015)</td>
</tr>
<tr>
<td>AP-Width [mm]</td>
<td>6.7 ± 0.5</td>
<td>5.4 ± 0.9</td>
<td>6.2 ± 1.5</td>
<td>HC-tSCI (&lt;0.001)</td>
</tr>
<tr>
<td>LR-Width [mm]</td>
<td>10.2 ± 0.8</td>
<td>9.4 ± 1.0</td>
<td>10.2 ± 0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Von Frey Filaments</td>
<td>2.83 ± 0</td>
<td>3.1 ± 0.5</td>
<td>2.83 ± 0</td>
<td>HC-tSCI (&lt;0.001); HC-pSCI (&lt;0.001)</td>
</tr>
<tr>
<td>Perception Threshold [°C]</td>
<td>38.7 ± 1.0</td>
<td>41.6 ± 2.6</td>
<td>39.3 ± 2.5</td>
<td>HC-tSCI (&lt;0.001); HC-pSCI (&lt;0.010)</td>
</tr>
<tr>
<td>Pain Threshold [°C]</td>
<td>0.63 ± 0.3</td>
<td>0.86 ± 0.2</td>
<td>0.3 ± 0.3</td>
<td>HC-tSCI (&lt;0.003); HC-pSCI (&lt;0.001); pSCI+tSCI (&lt;0.001)</td>
</tr>
</tbody>
</table>

Results are displayed as mean ± standard deviation. ‡: Bonferroni corrected
ns= not significant
SCA= Spinal Cord Area; AP= Anterior-posterior width of the spinal cord area; LR= Left-right width of the spinal cord area
HC= healthy controls, tSCI= Individuals with tetraplegia, pSCI= Individuals with paraplegia

Structural changes at spinal cord and brain
Overall, cross-sectional SCA was reduced by 21.7% (mean (SD) 76.6 ± 8.4) in the SCI cohort compared to healthy controls (i.e., 16.5% (65.1 ± 5.1) paraplegic SCI; 28.2% (56.0 ± 10.2) tetraplegic SCI). The degree of cord atrophy in SCI individuals with tetraplegia was greater than in individuals with paraplegia (p=0.015, -14.0%) (Figure 6.1 and Table 6.2). Differences in anterior-posterior width (APW) were only
observed in individuals with tetraplegia (p=0.001, -17.0%) when compared to healthy controls. No differences between groups were detected regarding left-right width (LRW) (p> 0.05). At the brain level, decreased GM and WM cortical volumes were detected in individuals with SCI compared to healthy controls (Table 6.3).

**Figure 6.1: Spinal cord area reduction as a consequence of SCI.** (A) A significant reduction in SCA was found in individuals with paraplegia and tetraplegia. The degree of reduction was more pronounced in individuals with tetraplegia. (B) In individuals with tetraplegia, a significant decrease of the anterior-posterior (AP) width is observed compared to healthy controls, while (C) the left-right (LR) width is indifferent between the groups.

*pSCI: Individuals with paraplegia
*tSCI: Individuals with tetraplegia*
Specifically, lower GM volumes were found in ACC (Z-score=3.68, p=0.049), left insula (Z-score=3.81, p=0.015), left secondary somatosensory cortex (Z-score=4.00, p=0.018 and Z-score=4.00, p=0.021), and bilateral thalamus (Z-score=3.84, p=0.023 (left) and Z-score=3.72, p=0.038 (right)). White matter volumes were decreased in the pyramids (Z-score=4.61, p=0.001, (left) and Z-score=3.99, p=0.007 (right)) and the left internal capsule (Z-score=3.47, p=0.045) in individuals with SCI. Similar to and beyond the area identified by VBM, VBCT analysis revealed decreased cortical thickness in left secondary somatosensory cortex (Z-score=3.58, p=0.041) in individuals with SCI compared with controls. The cluster extended in a rostral–caudal direction from y = -49.5 to -64.5, in the ventral–dorsal direction from z = 9 to 31.5 and left-right direction x = -9 to -21, thus additionally encompassing left M1 and right S1.

### Relationship between neuropathic pain and structural changes across the neuroaxis

Individuals with paraplegia suffering from NP, but not the individuals with tetraplegia (p>0.05), exhibited a more decreased cord area compared to their pain-free counterparts (p=0.002, greater mean reduction of -11.6%) (Figure 6.2). At the spinal level, cord area was associated with level of lesion (r=0.558, p=0.020) and pathologically increased warm perception threshold (r=-0.486, p=0.049) – that is, warm perception thresholds were increased with smaller SCA. Cord area changes were independent of severity of injury (ISNSCI Score) (r=-0.108, p=0.583), time since

![Figure 6.2: The relationship between the presence of below-level neuropathic pain (np) and spinal cord atrophy. Subjects with paraplegia suffering from neuropatic pain (SCI-NP) exhibit lower cord area compared to their pain-free counterparts (SCI). Such a difference was not observed in subjects with tetraplegia.](image)

**SCI-NP**: Individuals suffering from neuropathic pain

**SCI**: Pain-free individuals

<table>
<thead>
<tr>
<th>Table 6.3: Region of reduced gray and white matter as well as thinner cortical thickness in individuals with SCI compared to healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region</strong></td>
</tr>
<tr>
<td>Gray Matter (VBM): Controls&gt;SCI</td>
</tr>
<tr>
<td>ACC</td>
</tr>
<tr>
<td>Insula</td>
</tr>
<tr>
<td>S2</td>
</tr>
<tr>
<td>Thalamus</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>White Matter (VBM): Controls&gt;SCI</td>
</tr>
<tr>
<td>Pyramids</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Capsula Interna</td>
</tr>
<tr>
<td>Cortical Thickness (VBCT): Controls&gt;SCI</td>
</tr>
<tr>
<td>S2</td>
</tr>
<tr>
<td>FWE = Family wise error; ACC = Anterior cingulate cortex; S2 = secondary somatosensory cortex; L = Left; R = Right; MNI = Montreal Neurological Institute</td>
</tr>
</tbody>
</table>
injury ($r=-0.103, p=0.600$), and age ($r=-0.091, p=0.644$). At the brain level, individuals suffering from below-level NP exhibited more or less decrease (i.e., preservation) in GM volumes in the left ACC ($Z$-score=3.85, $p=0.034$) and right M1 ($Z$-score=4.33, $p=0.005$) than NP-free individuals with SCI. On the other hand, NP-free individuals with SCI show greater GM volumes in the right S1 ($Z$-score=3.79, $p=0.046$), and thalamus ($Z$-score=4.16, $p=0.010$) (Table 6.4 as well as Figures 3 and 4). In addition, greater GM volume in M1 is associated with higher on-going pain intensity ($r=0.637; p=0.001$).

Table 6.4: Differences in gray matter volume between individuals with SCI suffering from neuropathic pain (SCI-NP) compared to pain-free individuals with SCI (SCI)

<table>
<thead>
<tr>
<th>Region</th>
<th>Side</th>
<th>P-value (FWE-corrected)</th>
<th>Z-Score</th>
<th>T-Value</th>
<th>Cluster size</th>
<th>Coordinates in MNI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>L</td>
<td>0.034</td>
<td>3.85</td>
<td>4.58</td>
<td>46</td>
<td>-2 36 27</td>
</tr>
<tr>
<td>Gray Matter (VBM): SCI-NP&gt;SCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>R</td>
<td>0.005</td>
<td>4.33</td>
<td>5.40</td>
<td>99</td>
<td>12 -32 59</td>
</tr>
<tr>
<td>Gray Matter (VBM): SCI&gt;SCI-NP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>R</td>
<td>0.046</td>
<td>3.79</td>
<td>4.01</td>
<td>27</td>
<td>24 -33 48</td>
</tr>
<tr>
<td>Thalamus</td>
<td>L/R</td>
<td>0.010</td>
<td>4.16</td>
<td>5.10</td>
<td>152</td>
<td>0  -6 3</td>
</tr>
</tbody>
</table>

FWE = Family wise error; ACC = Anterior cingulate cortex; M1 = primary motor cortex; S1 = primary somatosensory cortex; L= Left; R = Right, MNI=Montreal Neurological Institute

6.5 Discussion

This study shows distinct association of below-level NP with structural changes of the spinal cord and brain after SCI. In individuals with paraplegia, the reduction in SCA was associated with below-level NP independent of the level or completeness of lesion. At the brain level, pain-related changes were of bi-directional nature (i.e., increased and decreased volumes) and located in areas relevant to nociceptive processing (i.e., including S2, insula, ACC, thalamus) as well as brain areas not associated with the sensation of NP (i.e., S1, M1, pyramids, internal capsule). These findings provide insights into the complex pattern of neuro-adaptive processes due to below-level NP following SCI.
Figure 6.3: Trauma-induced and pain-related changes of the cerebral structure. (A) Volumetric changes between healthy subjects (HC) and individuals with SCI. Individuals with SCI exhibit decreased gray matter volume (red) in the left anterior cingulate cortex, left insula, left secondary sensory cortex, as well as bilateral thalamus compared to healthy controls. Further, decreased white matter volumes (green) were detected in bilateral pyramids and left internal capsule. (B) Bidirectional pain-related morphological changes in individuals with SCI (SCI-NP) experiencing neuropathic pain. In comparison to their pain-free counterparts (SCI), spinally injured individuals with neuropathic pain exhibit decreased gray matter volumes (blue) in the right primary sensory cortex (S1) and in the thalamus. In pain-free individuals with SCI, smaller gray matter volumes (yellow) were observed in the right primary motor cortex and the left anterior cingulate cortex compared to the individuals with SCI suffering from neuropathic pain. For display reason $p=0.001$. 
Below-level neuropathic pain and structural changes of the spinal cord

The magnitude of cord atrophy above the level of lesion (all measures performed at C2) was associated with the presence of below-level NP in individuals with paraplegia. That is, individuals with greater cord atrophy were more likely to exhibit below-level NP independent of the level of lesion. Due to the cross-sectional nature of this study, the question of whether below-level NP is causal or consequential cannot be addressed. Based on recent findings, cord atrophy is considered to be due to anterograde and retrograde axonal degeneration of spinal pathways\textsuperscript{192}.

However, pain might arise from both the abolishment of specific fiber tracts reflected by atrophic changes, but also changes through aberrant sprouting of spinal circuits may cause disinhibition or imbalance between sensory and motor pathways\textsuperscript{61}. On the contrary, degeneration of myelinated efferent fibers is sufficient to elicit spontaneous activity in uninjured C fiber afferents and has been associated with hyperalgesia and allodynia\textsuperscript{272}. Another key factor in individuals with SCI might result from reduced neuro-muscular activity evident in individuals with chronic pain\textsuperscript{273}. Typically, individuals suffering from pain tend to be less active due to the ongoing pain and its concomitants (e.g., depression, lack of motivation) or side-effects of pain medication. Increased activity promotes plasticity at multiple levels of the neuroaxis including spinal cord circuitry rostral and caudal to injury\textsuperscript{274}. Thus, pain-related neuro-muscular inactivity leading to lower brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) levels might inhibit these plastic processes resulting in atrophic structures as seen in the present study\textsuperscript{275}.
Up-regulated BDNF levels following intense exercise were associated with neuronal plasticity in skeletal muscles but also in the innervating level of the spinal cord\textsuperscript{276, 277}.

No such difference in the magnitude of cord atrophy related to pain was observed in individuals with tetraplegia. A potential reason might be a ‘flooring effect’ of atrophic progression towards a ‘stable’ SCA\textsuperscript{83, 88}. Cord atrophy rostral to the injury (i.e., C2 vertebrae) is dependent on the level of lesion, as shown in the present study, – with more pronounced changes at higher levels of lesion\textsuperscript{88}. Notably, given the anatomical properties of the spinal cord, a lesion to the cervical cord impacts the structural integrity of a higher number of fibers and neurons than a comparable thoracic lesion. The level-dependent degree of atrophy suggests axonal degeneration inducing cord atrophy as the morphometric changes were predominantly in the anterior-posterior axis of the cord. Changes in the anterior-posterior axis might represent Wallerian degeneration of afferents running in the dorsal columns\textsuperscript{88}.

**Below-level neuropathic pain and structural changes of the brain**

In individuals with SCI, below-level NP was accompanied with reduced GM volume in S1 and the thalamus, but increased GM in the M1 and ACC. Numerous studies demonstrated volumetric bi-directional alterations of GM in areas belonging to the nociceptive matrix (e.g., thalamus, insula, cingulate cortex)\textsuperscript{231}. While, decreased GM volume in pain-regulating areas is often put in context with maladaptive structural plasticity\textsuperscript{231, 232}, increased GM is rather linked with preserved structure as the underlying source of chronic pain\textsuperscript{244}. ‘Maladaptive plasticity’ predicts that following loss of efferent output and afferent input deprived central areas undergo structural alterations of synaptic connections thereby triggering NP. The neuronal substrates underlying injury-induced alterations remain incompletely understood. VBM is sensitive to structural volume changes (i.e., alterations in WM and GM), but it cannot disclose the neuronal substrates underlying the structural differences that are revealed in the present study and thus assumptions regarding the underlying neuronal substrates remain speculative. Alterations in GM could result from varying numbers of neurons, interneurons, or glia cells, but also by differently sized cells. Based on animal studies, proposed mechanisms for the volume alterations include axonal sprouting, neuronal death, synaptic pruning, and activity-dependent plasticity\textsuperscript{278}. Moore and colleagues proposed in their pre-clinical study that a decline in GM, potentially due to persistent noiceptive input to the brain, could result in neural hyperexcitability along the neuroaxis\textsuperscript{279}. Morphometric alteration in the thalamus, as seen in the present study, is a robust and consistent observation across various pain conditions. Anatomical deafferentation is likely the underlying mechanism resulting in a general thalamic hypoactivity (e.g., deficit in thalamic metabolism, decreased thalamic blood flow, functional thalamic depression)\textsuperscript{230, 280}. Furthermore, the decreased GM volumes in primary somatosensory cortex is in line with previous studies associating structural and functional alterations with the presence of chronic pain following deafferentation\textsuperscript{230, 281}. Similar to Makin\textsuperscript{244}, we show not only the relationship of below-level NP and maintained local GM in M1, but also the correlation between pain intensity and preserved GM
volume. Maintained local structure with disturbed long-range connectivity and diminished inhibition by surrounding representational areas may lead to ‘autonomous-acting’ areas responsible for the experience of pain. The positive correlation with the intensity of ongoing below-level NP strengthens the assumption that pain is the consequence of structural preservation.

Finally, in this cohort, structural alterations of the WM and cortical thickness were not related with pain, but with the trauma-induced deafferentation. Based on animal studies, retrograde axonal degeneration is also suspected to induce volumetric changes of WM tissue at multiple levels of the corticospinal tract as in the present study in the pyramids and internal capsule. Lastly, cortical thinning of the secondary somatosensory cortex was detected in the SCI group compared to healthy controls indicating atrophy of neurons, glial cells, and/or extracellular space. Again, no link with the ongoing below-level NP and degree of cortical thinning was found.

**Limitations**
A major limiting factor constitutes that individuals with SCI and healthy volunteers are neither matched with respect to gender nor age. This can confound the group comparison, given possible differences in structure according to age and possibly gender. DARTEL was used to improve the equalization between the groups through normalization processes. Furthermore, the cross-sectional nature of the study restricts conclusions to a single time point only and thus, temporal information of the above-described structural changes remains speculative. Outside the scope of this study, the assessment of depression and anxiety indices would be interesting to show that observed effects cannot be attributed to highly prevalent psychiatric comorbidity in this clinical group.

**Conclusion**
We provide evidence of extensive changes in sensory function (i.e., below-level neuropathic pain) and micro-structural morphology of the CNS rostral to the level of lesion (spinal cord and brain). Besides the trauma-dependent changes also volumetric changes become discerned that are related to below-level NP with both decreases and increases. The CNS shows extensive and complex changes (multi-directional with decrease – increase) in response to an injury. These complex interactions need to be considered in interventional studies that often simultaneously may affect many modalities like sensor-motor function and pain syndromes. Future investigations are required to better understand the role of below-level NP in the process of dynamic structural changes after spinal cord injury. It is conceivable that neuro-imaging biomarkers are sensitive in detecting these complex pain related neuro-adaptive changes.

**Acknowledgement**
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all of the individuals participating in our study, and Jenny Haefeli and Alexandra Schaettin for their support in collecting data. The authors report no conflict of interests in this work.

**Author’s contribution**

Catherine R. Jutzeler contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Furthermore, she drafted the research article. Eveline Huber made substantial contribution to the data acquisition and revising the research article Martina F. Callagan contributed substantially to the study by introducing a new method to assess the axes width of the spinal cord. She further revised the research article critically for important intellectual content. Roger Luechinger contributed substantially to the data acquisition, analysis, and interpretation. Armin Curt made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content. John L. Kramer contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Patrick Freund made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content.
Study 5

Neuropathic pain is not associated with functional reorganization in the primary sensorimotor cortex after spinal cord injury

Catherine R. Jutzeler, Patrick Freund, Eveline Huber, Armin Curt, and John L.K. Kramer

Pain (submitted)

~ We would like to thank all of the individuals participating in our study.~
7.1 Abstract

There is still a limited understanding whether cortical reorganization following spinal cord injury (SCI) is the cause or consequence of the development of neuropathic pain. Addressing the concept of maladaptive plasticity, the aim of the present study was to investigate the relation of cortical reorganization, intensity of neuropathic pain and sensorimotor function after SCI. A total of 28 individuals with sensorimotor complete and incomplete para- and tetraplegia (13 suffering from NP, 15 pain-free) and 30 healthy individuals were examined. Functional magnetic resonance imaging was used to assess cortical activation in response to active and passive wrist extension, and heat and brushing applied at the C6 dermatome. In individuals with SCI, there were no group-level differences in task related activation (i.e., movement or sensory) compared to healthy control individuals. However, compared to healthy controls, peak activity in primary sensory and motor cortices shifted laterally in individuals with intact C6 spinal segments (p<0.05). Among individuals with NP, pain intensity inversely correlated with magnitude of the shift in the primary motor cortex. After SCI, task-related shifts in primary motor cortex topography were significantly reduced with increased intensity of neuropathic pain. Contrary to findings in support of the concept of maladaptive plasticity in the sensory cortex, NP had the effect of preserving cortical topography in the motor system.
7.2 Introduction

Animal studies have consistently demonstrated large-scale reorganization in primary sensory and motor cortices after spinal cord injury (SCI)\(^{286-288}\). Comparatively, evidence in humans is much less robust\(^{30, 84, 246, 288, 289}\). Several factors may contribute to reorganization in humans with SCI, including injury severity and level of lesion and amount of structural damage. Similar to phantom limb pain\(^{230, 252}\), cortical reorganization may also be affected by the presence and intensity of below-level neuropathic pain (NP). Neuropathic pain represents a major secondary complication for people currently living with SCI, refractory to treatment and negatively impacting quality of life and functional independence\(^{38, 146}\). Among difficulties related to the development of more effective interventions, there is currently a limited understanding of neuropathic pain mechanisms after SCI. One proposal put forward is that below-level neuropathic pain is the result of maladaptive changes in supraspinal anatomy and physiology\(^{290}\).

Supporting the concept of ‘maladaptive plasticity’, Wrigley and colleagues recently demonstrated greater reorganization in S1 in response to brushing associated with more severe neuropathic pain\(^{246}\). The primary aim of the present study was to address the relationship between the intensity of reported below-level NP and cortical reorganization in sensory and motor areas after SCI. We hypothesized that SCI would induce sensory and motor reorganization, the degree of which would be associated with the intensity of NP symptoms. Using functional magnetic resonance imaging (fMRI), individuals with SCI were examined during sensory stimulation (i.e., brushing and heat), and movement tasks (i.e., active and passive wrist extension). Based on the presence and intensity of individuals reported NP symptoms, the analysis focused on addressing group-level differences in activity, as well as changes in the location of peak activity (i.e., Euclidean distance) in primary sensory and motor areas.

7.3 Material and Methods

Participants

A total of 30 individuals with a chronic traumatic SCI (mean (SD) 46.3 (11.9) years; gender: 3 female, 27 male) including individuals with tetra- (n=15) and paraplegia (n=15) were recruited. Only individuals that could perceive brushing and heat stimulation applied on the C6 dermatome as well as independently perform active wrist extension were included in the study. Two individuals with SCI (1 individual with tetraplegia and 1 with paraplegia) were excluded due technical measurement errors. Additionally, 31 neurologically healthy individuals (mean (SD) 32.6 (13) years; gender: 14 female, 17 male) were enrolled in the study. Participants’ demographic and clinical details are summarized in Table 7.1. All participants provided written informed consent and all procedures described below were in accordance with the Declaration of Helsinki and approved by the local ethics board (ref. number: EK-04/2006).
Clinical assessments
Prior to the functional magnetic resonance imaging (fMRI), all participants were interviewed to determine
handedness and the existence of pain using the German versions of the Edinburgh inventory (14 item
version\(^{291}\)) and the European Multicenter Study about SCI (EMSCI) pain questionnaire (V4.2,
http://www.emsci.org/), respectively. The pain questionnaire examines various aspects of pain (e.g.,
duration, maximal and average pain intensity) as well as pain associated psychosocial factors.
Accordingly, pain can be grouped into nociceptive (e.g., musculoskeletal or visceral) or neuropathic pain
(e.g., at or below the lesion). To be classified as below-neuropathic pain, symptoms (e.g., burning, cold,
tingling) reported had to be located three or more segments below the neurological level of lesion. In
individuals with SCI, the neurological level of injury was assessed using the International Standards for
Neurological Classification of Spinal Cord Injury published by the American Spinal Injury Association
(ASIA)\(^{62, 63, 167}\).

Image acquisition
MRI data was collected on a Philips 3 T Achieva system (Philips Medical Systems, Best, the Netherlands)
using an eight-channel Philips Sense head coil. Functional time series were acquired with a sensitivity-
encoded (reduction factor 2), single-shot echo-planar sequence (SENSE-sshEPI)\(^{292}\) with a measured
resolution of 2.75 x 2.75 x 4 mm. The 29 axial slices without interslice gaps covered the entire cerebrum.

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Table 7.1: Demographic and clinical details of the sample

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>Significant pairwise comparisons (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>tSCI</td>
</tr>
<tr>
<td>Gender [m : f]</td>
<td>9:12</td>
<td>14 : 0</td>
</tr>
<tr>
<td>Age [yrs]</td>
<td>31.9 ± 9.9</td>
<td>47.6 ± 14.5</td>
</tr>
<tr>
<td>Handedness[r : l]</td>
<td>30:2</td>
<td>13:1</td>
</tr>
<tr>
<td>ASIS motor score</td>
<td>100 ± 0</td>
<td>69.0 ± 29.9</td>
</tr>
<tr>
<td>AIS sensory score</td>
<td>224 ± 0</td>
<td>154.6 ± 50.1</td>
</tr>
<tr>
<td>Duration of SCI [yrs]</td>
<td>7.0 ± 2.8</td>
<td>16.5 ± 9.4</td>
</tr>
<tr>
<td>Injury severity [cSCI: iSCI]</td>
<td>2 : 12</td>
<td>9 : 5</td>
</tr>
<tr>
<td>Neuropathic pain (y:n)*</td>
<td>5 : 9</td>
<td>8 : 6</td>
</tr>
<tr>
<td>Duration of pain [yrs]*</td>
<td>6.9 ± 2.3</td>
<td>16.1 ± 8.2</td>
</tr>
<tr>
<td>Mean pain intensity*</td>
<td>3.6 ± 2.0</td>
<td>4.3 ± 2.3</td>
</tr>
<tr>
<td>Max pain intensity*</td>
<td>4.4 ± 2.9</td>
<td>6.3 ± 3.0</td>
</tr>
</tbody>
</table>

Results are displayed as mean ± standard deviation.
\(^{‡}\): Bonferroni corrected
\(^{†}\): German version of the Edinburgh inventory questionnaire
*: EMSCI pain questionnaire with incorporated visual analogue scale ranging from 0 (no pain) to 10 (worst pain imaginable).
y= yes, n= no, l=left, r=right, cSCI= complete SCI, iSCI= incomplete SCI, EMSCI= European Multicenter Study about SCI

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Slices were aligned to the anterior commissure–posterior commissure line. Other scan parameters were as follows: echo time=35 ms; flip angle=90°; repetition time=3000s; field of view=220 x 135 x 220mm; reconstruction matrix of scan resolution 72x72 voxels with 3 x 3 x 3 mm, and scan time of 16:12min. The first three scans were acquired to reach steady-state magnetization and then discarded. In total, 320 volumes were acquired.

A 3D-GRE T1-weighted (T1w) sequence was used to acquire a whole-brain, structural scan optimized for simultaneous assessment of the brain and spinal cord. The imaging parameters were: isotropic 1 mm$^3$ resolution, field of view 256 x 256 x 180, repetition time=6.88 ms, echo time=3.1 ms, flip angle 8°, fat saturation, scan resolution 256x256 voxels, and a scan time of 6:31min. Prior to analysis the MRI data were screened for movement artefacts.

**Functional MRI paradigm**
The functional task comprised active 20s blocks of uni-lateral (i) active and (ii) passive wrist extension, (iii) heat stimulation, and (iv) brushing. Six repetitions of each task blocks were performed alternating with 20s rest blocks (starting with a rest block). The active blocks were presented in pseudo-randomized order (Figure 7.1). All participants were scanned lying in a supine position and viewed visual stimuli projected screen via a mirror system mounted above the magnetic resonance head-coil. The right hand was fixed to the MR bed only allowing the participant to execute the wrist extension through normal range of motion.

![Figure 7.1: Functional magnetic resonance imaging (fMRI) paradigm.](image-url)Cortical activation was assessed in response to sensory and motor task. The functional paradigm was composed of active 20s blocks of uni-lateral brushing, heat stimulation, active and passive wrist extension. Six repetitions of each task blocks were performed alternating with 20s rest blocks (starting with a rest block). The active blocks were presented in pseudo-randomized order. Heat stimulation and brushing were applied to the C6 dermatome. Active and passive wrist extensions were conducted 10 times/ block. In order to reduce the sensory input, a handle was used to execute the passive wrist extension. In order to correct for the sensory input of the handle, participants were holding it during the entire experiment.
motion (ROM). In order to reduce sensory input, participants hold a handle in the hand during the entire time of scanning session (Figure 7.1). The strap attached to the handle was used to perform the passive wrist extension without touching the participant. During the passive wrist extension, the examiner extended the participants right hand-wrist along the ROM and then brought it back to its original starting position using the strap. For the active wrist extension task, participants were asked to actively extend their right wrist along the full ROM and bring it back to the original starting position. For both motor tasks, a cadence of 10 movements/block (i.e., 2Hz) was chosen. Each participant practiced this cadence prior to entering the scanner. Heat stimulation comprised of placing heat packs (average temp: 52.3°C; Trevolution, Zurich, Switzerland) onto the right C6 dermatome for 20s. A new heat pack was used for each 20s block. Brushing involved consistently brushing the right C6 dermatome with cotton swabs. Brushing was performed at the base of the thumb. Importantly, the area of brushing did not overlap with the area of heating. For both sensory tasks, participants were instructed to lie quietly and minimize the eye-movement.

**MRI Data Analysis**

Functional volumes were preprocessed and analyzed in Matlab 2010b using Statistical Parametric Mapping 8 (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). The images were initially realigned to the first scan and unwarped to control for movement- and susceptibility-induced image distortions. Movement parameter were calculated to be later included in the statistical model. Following coregistration of the anatomical and functional images, spatial normalization of the functional images was executed. Using a unified segmentation approach, individual brains were normalized to the Montreal Neurological Institute standard space (MNI space). Lastly, spatial smoothing was conducted by applying an isotropic 8-mm full-width-at-half-maximum (FWHM) Gaussian kernel to reduce image noise.

A voxel-wise general mixed model was used for the first-level analysis (i.e., within an individual) in order to calculate contrast images for each task separately (i.e., heat, brushing, active and passive wrist extension). Significant increases in BOLD signal were identified using a repeated box car model convolved with a canonical form of the hemodynamic response function. The second-level analysis (i.e., group analysis) was performed to identify task-specific pattern of activation for each group (i.e., healthy controls, individuals with paraplegia, and individuals with tetraplegia). Significant differences between the control and SCI groups, between SCI without pain and SCI with pain groups during each task were also determined. Age, gender, level of lesion, and total intracranial volume (TIV) were included in the model as nuisance variables. All results reported were corrected for multiple-comparisons using the family-wise error (FWE). For group comparisons, a priori regions of interest (ROI) were used for small volume correction. These ROIs incorporated primary (S1) and secondary (S2) somatosensory cortices, primary motor cortex (M1), premotor cortex (PMC), supplementary motor area (SMA), anterior cingulate cortex (ACC), and cerebellum and were generated using the WFU Pickatlas.
Euclidean Distance

The point at which the central sulcus meets the longitudinal fissure at the dorsal aspect of the brain was set as anatomical marker\textsuperscript{246}. The Euclidean distance (ED) between the anatomical marker and the maximally activated voxel in task-specific ROIs (i.e., S1/S2 for heat/brushing, M1 for active/passive wrist extension) was computed for the anterior–posterior, medial–lateral and superior–inferior coordinates. The ED between two points in a plane is calculated with the Pythagorean theorem and provides an absolute value independently of direction\textsuperscript{295}.

Statistics

All statistical procedures were performed using IBM’s Statistical Package for the SocialSciences (SPSS) version 19.0 (Armonk, New York, U.S.). Non-parametric tests (Mann-Whitney-U and Kruskal-Wallis) were applied to determine significant differences in ED, peak activation, and cluster size between healthy controls, individuals with SCI and an intact C6 dermatome, as well as individuals with SCI and an impaired C6 dermatome. Pain-specific changes of ED, peak activation, and cluster size were explored by comparing the control group with the neuropathic pain SCI group as well as the pain-free SCI group. \(P<0.05\) was considered significant. All multiple comparisons were Bonferroni corrected. Spearman correlation was applied to assess the relationship of EDs and pain parameters (e.g., intensity, duration). Level of lesion and injury severity (i.e., AIS score) were included as covariates.

Contrast estimates of all ROIs from the individuals with SCI were extracted in order to perform Spearman correlations to identify associations between task-specific cortical activation and clinical characteristics (i.e., SCA, AIS motor and sensory scores, disease duration, level of lesion, pinprick and light touch scores, as well as pain intensity and duration).
### 7.4 Results

**Injury and pain characteristics, individuals’ demographics**

Of the 28 individuals with SCI, 11 had AIS complete (2 tetraplegic, 9 paraplegic) and 17 incomplete (12 tetraplegic, 5 paraplegic) injuries. In total, 13 patients (8 with paraplegia, 5 with tetraplegia) reported neuropathic pain (NP) (Table 7.2). The mean and maximal pain intensities were 4.0 ± 2.1 and 5.5 ± 3.0, respectively, and the duration of ongoing pain ranged from 4 to 33 years (mean 19.8 +/-12.3 years). In all cases, locations of on-going pain were below the level of injury and away from the C6 examination site.

### Table 7.2: Individual clinical data for the spinal cord injured subject

<table>
<thead>
<tr>
<th>ID</th>
<th>Age [yrs]</th>
<th>Gender</th>
<th>Aetiology of the injury</th>
<th>Time since Injury [yrs]</th>
<th>Level of Lesion</th>
<th>AIS*</th>
<th>Motor score (0-100)</th>
<th>Sensory score (0-224)</th>
<th>Neuropathic pain</th>
<th>Mean pain intensity</th>
<th>Max pain intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01</td>
<td>50</td>
<td>m</td>
<td>Vehicle accident</td>
<td>7</td>
<td>Th4/Th5</td>
<td>A</td>
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**Individuals with paraplegic SCI**

**Individuals with tetraplegic SCI**

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*ASIA impairment scale: A, no sensory or motor function is preserved; B, sensory function is preserved below the level of the injury, but there is no motor function; C, motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade of ≥ 3; D, motor function is preserved below the neurological level, and at least half of the key muscles below the neurological level have a muscle grade of ≥ 3.

**Shifts in S1 and M1 topography: Single-subject level analysis**

Although the pattern of the task-specific brain activation was similar across the group, there were significant differences in the location of peak activation. The analysis yielded in significant differences of the EDs for active movement (Figure 7.2A), as well as for heat and brushing (Figure 7.3), respectively, in individuals with SCI compared to control individuals. Left coordinates (x,y,z) and the mean Euclidean distance (ED) for each task and group are listed in Table 7.3. Pain-related changes in ED were only found for M1 during the active wrist extension task (Figure 7.2B). While pain-free individuals with SCI exhibit an increase in ED compared to healthy controls, the ED of individuals with SCI reporting neuropathic pain...
did not differ from healthy control values. Based on the classification of pain, EDs for brushing, heat

![Figure 7.2](image)

**Figure 7.2:** (A) Significant differences in Euclidean distances (ED) for active movement were found for the SCI group when compared to the control group (B) ED for active movement dependent on the presence of below level neuropathic pain. Pain-free individuals with SCI exhibit significant increased ED compared to healthy controls. The ED of individuals with SCI suffering from below-level neuropathic pain did not differ from the controls. (C) Inverse relationship between the degree of reorganization and the intensity of neuropathic pain. The more pain individuals with SCI experience, the smaller the degree of reorganization of M1 was observed. Stimulation, and passive movement were not significantly different.
Correlation with pain intensity and other variables

The magnitude of cortical reorganization of M1 during active wrist extension negatively correlated with the intensity of ongoing neuropathic pain, independent of level and severity of injury. That is, greater task-related cortical reorganization was associated with lower pain ratings (Figure 7.2C). No significant correlation between the ongoing pain and the task-specific brain activation during passive movement, heat stimulation and brushing.

![Figure 7.3](image-url)

**Figure 7.3:** Task-specific Euclidean distances (ED) of healthy controls and individuals with SCI. Significant differences in EDs for heat, brushing were found in individuals with spinal cord injury (SCI) when compared to the control group. Selected region of interest (ROI) was for both task the primary sensory cortex.
Patterns of brain activation in response to brushing, heat stimulation, active and passive wrist extension: Group-level analysis

Similar locations of task-specific signal intensity increases were observed in all three groups (i.e., healthy, tetra-, and paraplegia) (Figure 7.4). Active and passive wrist extension evoked significant increases in signal intensity in contralateral primary motor cortex, primary and secondary sensory cortex, premotor cortex, and cerebellum. Heat and brushing stimulation resulted in significant signal intensity increases in left (heat and brushing) and right secondary sensory cortex (heat only), and left primary somatosensory cortex (brushing only).

Figure 7.4: Task-specific brain activations in healthy controls, individuals with paraplegic SCI, and individuals with tetraplegic SCI. (A) Active wrist extension evoked BOLD signal increases in M1, S1, and thalamus (unilateral), as well as S2 and cerebellum (bilateral). (B) Similar areas were significantly activated during passive wrist extension with the exception of unilateral signal increase in cerebellum. (C) Heat and (D) brushing stimulation resulted in significant signal intensity increases in left (heat and brushing) and right secondary sensory cortex (heat only), and left primary somatosensory cortex (brushing only).
7.5 Discussion

This study is about cortical reorganization of sensorimotor cortical areas, as well as the relationship between cortical reorganization and neuropathic pain in individuals with SCI. Cortical reorganization was observed in S1 and M1 following sensory stimulation (brushing and heat) and active wrist extension, respectively. Compared to healthy individuals and individuals with neuropathic pain, individuals that were pain-free demonstrated significantly larger shifts in peak M1 activation during active wrist extension. Less reorganization in M1 correlated with the reported intensity of chronic neuropathic pain, such that individuals with more severe neuropathic pain underwent less extensive changes in terms of location of peak activity.

Preserved functional organization associated with neuropathic pain

While pain-free individuals with SCI demonstrate trauma-induced cortical reorganization in the primary motor cortex, the location of peak M1 activation inversely correlated with pain intensity (i.e., more intense pain, less prominent shifts in M1). This observation is in contrast to the maladaptive plasticity model, which states that greater cortical reorganization is associated with more severe pain symptoms. To our knowledge, this is the first study examining motor organization above the level of injury related to neuropathic pain in individuals with SCI.

Makin and colleagues recently highlighted the need to revisit the relationship between pain and brain organization, calling into question the concept of maladaptive plasticity by demonstrating disrupted connectivity but intact functional and structural representations in amputees with phantom limb pain. Pain was postulated to replace lost peripheral afferent input, contributing to “maintained cortical representation” of the missing hand. Similar to amputation, cortical organization of the hand in M1 after SCI may be preserved by painful sensory input arising from areas of the body deafferented by damage in the spinal cord. Such interpretation inherently suggests that preserved functional organization in M1 is a consequence of neuropathic pain. Alternatively, cortical reorganization could also be preventing the development of neuropathic pain after SCI – a form of adaptive plasticity. As such, reorganization in M1 is protective and the failure to reorganize is maladaptive. To address whether neuropathic pain is causing preserved cortical organization or vice versa, longitudinal studies performed during the transition from acute (i.e., before onset of below-level neuropathic pain) to chronic SCI (i.e., after onset) are needed. Interestingly, we did not observe an effect of injury severity on cortical reorganization. On one hand, this finding conflicts with the idea that residual sensation below the level of injury preserves functional activity in the brain. Several factors could explain why pain but not other forms of sensory sparing maintained cortical organization. First, clinical methods to assess residual sensory and motor sparing below the level of lesion (i.e., the typical clinical classification: ISNCSCI) may not be sensitive to detect subtle differences in injury severity. Second, the intensity and persistence of below-level neuropathic pain...
may represent greater afferent input compared to other residual sensation. As such, chronic pain places higher demands on cortical structures, both ascending and descending, and thus has a greater impact on function.

**Spinal cord injury related reorganization of sensory and motor functions**

In preclinical models applying fMRI, large-scale functional reorganization has been reported in primary sensorimotor cortices after experimental lesions in the spinal cord. Characterized by substantial increases in task-related BOLD activity in areas of the brain otherwise not active during the specific task in uninjured animals (e.g., chin stimulation activating the deafferented hand area), these observations largely confirm studies using invasive electrophysiological techniques. Based on findings from fMRI, the impact of deafferentation on cortical reorganization in humans suffering from SCI is less clear. In support, shifts in BOLD activity in S1 towards the deafferented leg area during brushing, as well as increased M1 activity during movement of intact muscles (e.g., lips, hand, biceps) has been reported in individuals with paraplegia above the level of injury. Additionally, Freund and colleagues found increased BOLD activation in the leg area of individuals with tetraplegia during hand grasping, and increased BOLD activation in the face area in response to median nerve stimulation. Interestingly, transcranial magnetic stimulation applied above the cortical representing leg area of the primary motor cortex of individuals with SCI did not translate into TMS induced muscle activity, suggesting that task-related increases in BOLD activation is due to a hyperactive M1 with no functional consequence during voluntary arm movement. Wrigley et al., reported no overall group level differences between individuals with SCI and healthy individuals (i.e., same as reported here), and Curt et al., observed no shifts in peak M1 topography using center of gravity. Overall, our group level findings do not support large-scale changes in cortical organization after SCI in humans – similar patterns of cortical activation in response to sensory stimulation and movement in individuals with SCI and healthy individuals (i.e., no leg area activation during wrist movement or afferent stimulation of the hand). Comparable to Wrigley, single-subject level analysis revealed subtle shifts in peak BOLD activity in S1 during brushing and heat, as well as in M1 during active wrist extension. The less extensive demonstration of reorganization in humans compared to animals may be related to several factors, including the heterogeneity of SCI and the insensitivity of the methods. To assess reorganization in preclinical models, specific spinal cord pathways (e.g., dorsal columns) are often completely transected. Comparatively, even the most severe human injuries are typically accompanied by some afferent and efferent sparing across the lesion site. Additional variability may be introduced by differences in how fMRI data is analyzed and the sensitivity and validity of these methods. For example, Curt et al. examined center of gravity rather than Euclidean Distance, and Freund and colleagues specifically examined the leg area using a directed ROI approach.
Limitations
In contrast to previous studies, we found no evidence for shifts in S1 topography related to the presence and intensity of neuropathic pain during brushing in individuals with SCI compared to healthy control individuals\textsuperscript{246, 301, 302}. A previous study has shown that cortical reorganization was most closely related to neuropathic pain in response to brushing of the little finger, but not the thumb\textsuperscript{246}. The present study examined only the base of the thumb using multiple sensory modalities, and thus did not address reorganization in response to stimulation in other areas (e.g., little finger, lip). Another limitation of our study is the heterogeneity of the SCI sample, which included both individuals with tetra- and paraplegia, as well as all severities of injury. While our analysis took these differences into account, a more powerful study design may be required to examine the interaction between pain and injury characteristics.

Conclusion
Cortical reorganization was observed in both sensory and motor systems after SCI. In agreement with a recent study\textsuperscript{244}, shifts in M1 topography during an executed hand movement negatively correlated with pain intensity. In general, our findings support more limited reorganization in humans after SCI compared to preclinical studies, and that neuropathic pain may preserve cortical function in response to deafferentation.

Acknowledgement
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Author’s contribution
Catherine R. Jutzeler contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Furthermore, she drafted the research article. Eveline Huber made substantial contribution to the data acquisition and revising the research article. Armin Curt made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content. Patrick Freund made substantial contributions to conception and design and participated in revising the research article critically for important intellectual content. John L. Kramer contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. He was further involved in the drafting process of the manuscript.
General Discussion
8 General Discussion

The primary aim of the thesis was to investigate the plasticity of the sensory system along the neuroaxis, providing novel insight in potential mechanisms and processes underlying sensory impairments and discomfort (e.g., pain and paraesthesia). The multi-methodological approach (i.e., behavioral, neurophysiology, and neuroimaging) enabled the detection of alterations of the sensory system’s integrity on multiple levels of the neuroaxis (i.e., brain and spinal cord), as well as the assessment of trauma-induced and/or pain-associated functional and anatomical plasticity. The first study (Chapter 2) yielded marked alterations in modulation of noxious input based on the sensitization state (i.e., presence/absence of capsaicin) of healthy control individuals emphasizing the importance of interactions between different sensory fibers.

A number of the major findings, including altered modulation of noxious afferent input in individuals with spinal cord injury (SCI) suffering from neuropathic pain (NP) (Chapter 3) as well as the pain-related changes of the central nervous system (CNS) anatomy and function (Chapter 6 and 7), highlight the plasticity of the sensory system and how it globally affects the function and recovery following SCI. Physiological mechanisms processing afferent information were found to be divergent in individuals with SCI suffering from pain compared to pain-free individuals (Chapter 3). In fact, lack of habituation and reduced perception to prolonged noxious input (i.e., capsaicin application) indicate crucial deficiencies of the descending control system associated with the presence of pain. Moreover, multifaceted adaptive changes of CNS were identified as a consequence of the insult by employing voxel-based morphometry (VBM) and functional MRI (fMRI) techniques (Chapter 6 and 7). Importantly, the observed alterations in structure and function (i.e., decreases in brain and spinal cord volumes, shifts in topography of areas responsible for encoding sensory stimuli and motor command) were partially associated with the presence and amount of experienced pain. That is, preserved structure and function were accompanied by the presence of neuropathic pain and the magnitude of preservation was correlated with the pain intensity (i.e., the higher the pain, the more preservation of function and structure).

The findings of each individual study have been discussed in the specific discussion sections. In the following, the findings are discussed in the conjunction with each other and briefly summarized in context of respective research questions stated in the general introduction of the thesis.

8.1 The sensori-sensory interactions - a delicate balance

The first study (Chapter 2) addressed the question how noxious stimuli were modulated in the presence of altered nociception (i.e., induced hyperalgesia) and, if modulation persists, what physiological and anatomical substrates could mediate such analgesia (e.g., spinal versus supra-spinal). Under physiological conditions, several lines of evidence suggest that the co-activation of large myelinated sensory fibers has an inhibitory effect on the conductivity of thinly myelinated/unmyelinated sensory nerve fibers (i.e., Aβ-
mediated inhibition on C fibers)\textsuperscript{303-306}. The modulation of afferent input (i.e., noxious and non-noxious) is thought to take place on multiple levels along the neuroaxis (i.e., spinal and supraspinal relays). In response to a painful stimulus, afferent volley travels along the peripheral nociceptor and enters the spinal cord via the dorsal horn\textsuperscript{307}. Within the dorsal horn, the terminal of the afferent nociceptor synapses with a dorsal horn neuron and, depending on the intensity of stimulation, a postsynaptic output is projected to supraspinal networks involved in the pain modulation. In 1965, Melzack and Wall first postulated possible association of A\(\beta\) on C inhibition with spinal inhibitory mechanisms within the scope of their ‘gate control theory of pain’\textsuperscript{101}. Thus, nociception information conveyed by small diameter afferents is prevented from reaching supraspinal centers due to the activation of large diameter fibers. Similarly, A\(\beta\) on A\(\delta\) inhibition in spinocervical cells as well as inhibition of glutamate induced activity was described in the cat also indicating a spinal mode of action\textsuperscript{308}. Concurrently, pharmacologic and physiologic evidence support the role of supraspinal centers to be involved in the pain reduction induced by activation of large diameter fibers\textsuperscript{139, 309, 310}. Release of endogenous opioids could be mediated through the activation of local spinal circuits and/or activation of descending inhibitory pathways. Our observations also potentially highlight differential modulation of sharp pain (i.e., contact heat; linked with A\(\delta\) fiber activation) and dull, spontaneous like pain (i.e., capsaicin; linked with C fiber activation) by TENS-mediated release of opioids\textsuperscript{106}.

**Challenging the balance: Capsaicin and neuromodulation**

Disturbances of these sensori-sensory interactions, for instance induced by deafferentation, are suspected to contribute to altered modulation of afferent input which in turn potentially leads to sensory complications ranging from dysaesthesia to chronic pain symptoms. The underlying mechanisms are largely not understood. Along with the mechanistic understanding of changes to the single primary afferent tracts (i.e., dorsal column and spinothalamic tract), it is also of great interest to elucidate alterations of the interaction of these primary afferents. Amongst others, an imbalance in the sensory interaction between information conveyed by the dorsal column system and spinothalamic system is suspected to be a potential mechanism of sensory impairment and discomfort. Examining both pain thresholds and evoked potentials in response to noxious stimulation (e.g., radiant and contact heat), the modulatory effect of conditioning electrical nerve stimulation has been extensively investigated in healthy individuals\textsuperscript{90-96}. In general, these studies support that electrical stimulation moderately reduces sensitivity to noxious stimuli, findings that have been associated with the reported beneficial effects of TENS in chronic pain patient populations\textsuperscript{311-314}. Compared to studies in humans, experiments in animals have long adopted experimental models of pain to measure changes in the modulation of noxious stimuli following conditioning electrical stimulation. This includes the use of primary and secondary hyperalgesia models\textsuperscript{115, 132, 315, 316}. The use of experimental pain models in animals recognizes that TENS is typically not applied in...
the “healthy” central nervous system, and that the presence of experimental pain may reveal a different modulating capacity of electrical stimulation. Models of induced pain symptoms (i.e., painful sensitization) serve as an intermediate step to gain knowledge regarding mechanism underlying the modulation of noxious input in the presence of altered nociception and further, to test efficacy of therapeutically derived analgesia in patient populations. The advantage of modeling pain is that afferent processing can be altered to resemble chronic pain symptoms, without having to consider many of the confounding variables and heterogeneity (e.g., age, duration of symptoms, comorbidities) associated with chronic pain patients. In terms of inducing hyperalgesia, one such readily applicable and widely studied model is the use of capsaicin, a TRPV-1 agonist known to induce burning-like sensations.107-109, 317. The first study (Chapter 2) revealed marked differences in modulation of noxious input based on the presence/absence of capsaicin in healthy control individuals. Importantly, the large-diameter fiber mediated modulatory effects only became evident following sensitization with capsaicin and were restricted to subjective pain reports (i.e., reflected as changes in peak temperature) while perception thresholds (i.e., thermal and electrical) and evoked potentials (i.e., CHEP and SSEP parameters) remained unaffected. Interestingly, these findings do not confirm the sensitivity of CHEPs to detect analgesia induced by cutaneous electrical stimulation – even in the presence of a marked reduction in evoked pain intensity. The dissociation of CHEP amplitude and pain rating is not entirely unexpected. Indeed, the vertex potential of CHEPs (i.e., N2P2) is closely related to stimulation saliency.184, 318 Therefore, the effect of reduced evoked pain after conditioning electrical stimulation may be overcome by increased saliency detection related to modulated perception – that is, while perceived as less intense, noxious stimuli are, perhaps somewhat surprisingly, perceived as more salient. The net effect of changes in intensity competing with changes in stimulus saliency may be no change in evoked potential outcomes. In terms of clinical applications, this represents a major limitation of CHEPs/LEPs to objectively track the induction of analgesia.

Taken together, the findings from the first study (Chapter 2) highlight the complexity of the sensory system and diversity of interactions between the various types of sensory tracts. Under physiological condition, the sensory system is in ‘homeostatic state’ that is temporarily destabilized when an acute afferent stimulus triggers excitatory pain signals reaching the brain via the spinal cord. As the stimulus is short lived, so is the neuronal response of the central nervous system and eventually, the physiological state is restored. However, prolonged noxious stimulation leads to continued discharge of peripheral nociceptors and changes in excitability of dorsal horn neurons that in turn enables non-nociceptive peripheral nerves (e.g., brush-sensitive Aβ fibers) access to the ascending pain system (i.e., evoke painful sensation). As a consequence, hypersensitivity arises due to the increased sensitivity of nociceptive pathways when they relay nociceptive input. The term “central sensitization” was coined to describe these changes in the CNS.
Out of balance: Disturbed sensori-sensory interaction

The aforementioned resultant plastic changes of the CNS, so-called central sensitization, not only inhibit the reestablishment and maintenance of homeostatic state, but also corrupt the modulation of additional noxious input. Indeed, the second study (Chapter 3) of the thesis indicates that some features (e.g., habituation and perception) of the descending control of pain are globally disturbed in individuals with SCI suffering from chronic below-level neuropathic pain compared to healthy control individuals and pain-free individuals with SCI. In concert with other studies, the overall concept is that damage in the spinal cord and the presence of below-level neuropathic pain results in subtle changes in processing of sensation, such as altered thresholds and perception to noxious input, in dermatomes that are otherwise intact (i.e., remain innervated with the brain). Dysfunction of the descending control (e.g., reduced habituation, deficient pain modulation) is not an exclusive feature of neuropathic pain, but rather hallmarks a variety of chronic pain conditions, including fibromyalgia and migraine, as well as small diameter fiber neuropathies. The majority of the studies focused on examining how an external noxious (i.e., counterirritant) input influences the perception of the ongoing pain or induced experimental pain (i.e., irritant). Conventionally, the modulation mediated by the descending control of pain in humans has been assessed by employing a conditioned pain modulation (CPM) paradigm. Briefly, normal modulation to painful test stimuli can be readily demonstrated in healthy individuals by applying equally or more painful conditioning stimulation (i.e., “pain inhibits pain” or counter-irritation), akin to diffuse-noxious inhibitory control (DNIC) in animals. The general finding derived from available studies delineates a one-directional mechanism of the CPM/DNIC, namely the counterirritant modulates the perception of the irritant. However, the findings of second study (Chapter 3) indicate a bi-directional action of mechanism of the CPM/DNIC as the in presence of neuropathic pain the perception of prolonged capsaicin and heat stimuli is seemingly attenuated and the habituation ability deficient. As such, the irritant (i.e., neuropathic pain) seems to have a modulatory effect on the counterirritant (i.e., thermal stimuli and capsaicin). Thus, our findings give rise to consider that a globally altered processing of afferent input may influence the effectiveness of CPM. Lastly, the hypothesis that modulation of afferent input is impaired was also tested by examining behavioral responses to contact heat stimulation before and after non-noxious ipsilateral and contralateral TENS, respectively. The rationale for applying non-noxious electrical stimulation was to modulate sensation through spinal mechanisms (i.e., “active” modulation) and to investigate if the analgesic effect of TENS may be mediated through interactions with sensitized small diameter afferents. In contrast to the inhibitory modulation by CPM (i.e., ‘pain inhibits pain’), TENS represents an excitatory modulation of afferent input by deliberately stimulating large fibers with a innocuous intensity. Interestingly, pain-free individuals (SCI and healthy controls) retained a normal capacity to modulate noxious stimuli in response to both types of conditioning (i.e., ipsilateral and contralateral), but among those with neuropathic pain, contralateral conditioning had no appreciable effect.
on sensation evoked by capsaicin and contact heat. We interpret this observation to mean that individuals with neuropathic pain have a reduced capacity to modulate sensation. Closing the circle, initial damage to the central nervous system, as seen following spinal cord disruption, profoundly disturbs the integrity of the sensory system reflected as loss of function, sensory impairments, sensory discomfort (e.g., neuropathic pain), but also alterations in the modulation of afferent input. These observations immediately lead to the chicken or egg question: Could this failure to modulate be giving rise to neuropathic pain? Or is failed modulation a consequence of neuropathic pain? If impaired modulation is a source of neuropathic pain, targeting it with treatment could lead to improved outcomes. Considering the burden to the individuals with SCI and in particular those suffering from neuropathic pain, further studies are warranted in order to simply spoken restore the balance of the sensory system.

8.2 Neuropathic pain: Role of maladaptive plasticity?

Employing structural and functional MRI in a cohort of individuals with SCI and healthy control individuals yielded cortical reorganization of sensory and motor cortices due to SCI. Neuro-adaptive processes, such as cortical reorganization, after deafferentation (e.g., amputation and spinal cord injury) have been frequently reported in humans as well in animals\textsuperscript{30, 286, 287, 297}. Generally, the disruption of afferent input is designated by structural and functional degeneration in the deprived sensorimotor cortex, findings which are in agreement with post-mortem histological observations associating loss of sensory input with neurodegeneration\textsuperscript{241, 321, 322}. The application of multiple stimulation modalities (i.e., heat stimulation, brushing, active and passive wrist extension) enabled us to scrutinize the reorganization of various cortical regions at the same time. Primary motor and sensory cortices underwent cortical reorganization, however in other areas involved in sensory (i.e., secondary sensory cortex) or motor (i.e., cerebellum, premotor cortex) information processing reorganization processes were not detected. One possibility, although speculative, might be the impact of deafferentation on the function of the distinct regions and the somatotopic organization of the motor and sensory cortices. Both primary motor and sensory cortices are directly engaged in the processing and encoding of the type and intensity of the motor or sensory input, respectively. The primary motor and sensory cortices are systematically organized – processing and integration of sensory and motor information of each body part takes place in allocated representations within S1 and M1, respectively. Missing inputs due to deafferentation entail plasticity resulting in reallocation of responsiveness within these cortices – neighboring areas invade the area responsive to denervated body parts. The premotor cortex and cerebellum are involved in the planning/initiation of movements and while S2 is engaged in the processing of high-order features of the stimulus (e.g., attention, memory, learning)\textsuperscript{323}. The impact of deafferentation in terms of location of responsiveness seems to be less pronounced, potentially due to absence of or less pronounced somatotopic organization of these areas. Nevertheless, the underlying neuronal substrates of cortical reorganization and its role of in
the process of pain development remain incompletely understood. Altered local brain chemistry and functional reorganization are traits of various pain disorders and are understood as altered functional state caused by central plasticity\textsuperscript{235}. Our study further provides precious evidence that in distinct brain regions (i.e., M1 and S1) the reorganization is more emphasized in pain-free individuals with SCI compared to individuals suffering from neuropathic pain. That is, the presence neuropathic pain is associated with preserved function and structure. These observations potentially indicate that the pain hampers *adaptive* processes like functional reorganization and anatomical reallocation or for unsolved reasons adaption is lacking which in turn causes pain. In this respect, cortical reorganization could be preventing the development of neuropathic pain after SCI – a form of adaptive plasticity. As such, reorganization is protective and the failure to reorganize is maladaptive. Similarly, Makin and colleagues observed that chronic phantom limb pain drives plasticity by maintaining local cortical representations and disrupting inter-regional connectivity\textsuperscript{244}. However, our findings contrast the widely held belief of the maladaptive plasticity (i.e., aberrant cortical reorganization) claiming that pain is the trigger for functional and structural reorganization\textsuperscript{235, 242, 243, 246, 259}. Briefly, cortical areas encoding for deafferented regions (e.g., legs) shrink and allow for invasion of expanding neighboring areas\textsuperscript{195, 289, 301, 302}. The maladaptive plasticity model predicts that sensorimotor cortex representations of deafferented body parts are smaller in individuals who suffer from more intense pain, owing to greater reorganization. Since the majority of the studies, including ours, are of cross-sectional nature the question if pain is casual or consequential to cortical reorganization remains unresolved. Future longitudinal studies are warranted to examine how the development of pain and reorganization of function and structure are allied. Early disclosure of spatially and temporally distinct neuropathological alterations along the neuro-axis relevant to the development of sensory impairments and discomfort would provide essential targets for (non-) pharmacological treatment interventions. Conceivably, elaborate structural and functional MRI protocols may serve as neuroimaging biomarkers for interventional studies to complement clinical outcome parameters during recovery from acute spinal cord injury.

### 8.3 Does pain have a neurological signature?

While the integrity of small diameter afferents conveying temperature and pain sensation to the brain in the spinothalamic tract can be reliably assessed with the objective methods contact heat evoked potentials (CHEPs) and laser evoked potentials (LEPs), pain is not easy to ascertain at the present\textsuperscript{180}. The *clinical gold standard* of pain evaluation by means of self-report (i.e., description and intensity) is an imperfect measure of subjective experience. An objective assessment of pain is desirable which also provides a basis for understanding the neurophysiological processes underlying different types of pain. The quest for more objective appraisal of pain, namely physiological responses that reflect a person’s pain, has gained steam with the employment of imaging methods. As such, structural MRI was used in 30 neurologically healthy
individuals in order to pinpoint areas of the brain with structural variations (gray and white matter) accounting for the variability in processing of contact heat mediated pain (Chapter 4). Pivotal, distinct areas in gray and white matter yielded a significant relationship between the amplitude of CHEPs and pain rating. Revealed findings indicate that the discrepancy between pain ratings and the amplitude of evoked potentials is not solely attributable to measurement artifact (i.e., differences in the interpretation of pain rating scales between individuals), but rather ascribed to anatomical differences between individuals. From a clinical point of view, these results are of high relevance since it contributes to our apprehension of the high between-subject variability in ratings and amplitude of prominent cortical waveforms (e.g., N2P2)\textsuperscript{73, 181}. Crucially, the dissociation between pain rating and amplitude (e.g., low rating allied with large amplitude cortical potentials, and vice versa) is a frequent phenomenon seen in individuals with SCI and typically deafferentation is accounted for. Considering the presented findings, it is plausible that so-called neurological signatures of pain (anatomical traits across brain regions) could influence how pain is perceived and processed. Collaterally, previous studies employing functional MRI identified patterns of activity across brain regions that could predict pain intensity induced by thermal pain\textsuperscript{324}. Collectively, these findings are compelling, but caution should be exercised when trying to translate these findings to patients’ population. Pain-associated functional and structural MRI patterns likely differ according type of pain (visceral vs. cutaneous)\textsuperscript{325} and clinical cause. Furthermore, previous studies considering the “neurological signature of pain” have done so in healthy brains and in response to experimental pain. However, that is rather a simplistic model compared to clinical pain, especially when in fact the brain mechanisms and signature of ongoing chronic pain may be deviant from the physiological brain state. Lastly, the use of brain imaging to disprove that a person is in pain should be avoided complying with the saying ‘the absence of evidence is not the evidence of absence’ or not treating someone because their neurological signature of pain doesn’t say they are “in pain”. Since neuroimaging results are extremely unreliable at a single-subject level, false negative findings are likely and as such very dangerous. In the case of pain, a false negative means someone with pain does not get treated because the employed diagnostic tool was incorrect.

8.4 General Strengths and Limitations

The specific strengths and limitations of each study were highlighted in the research chapters and will not be repeated here. Generally, all studies were of prospective nature allowing controlled preselection of participants to be enrolled and permit multiple outcomes to be assessed in the same study. All studies involved proper control groups (i.e., healthy control individuals and SCI cohorts) strengthening our findings and conclusions. Future studies can use the knowledge gained from these studies to further elucidate segmental afferent pathology in affected dermatomes in SCI.
A limitation of this thesis is that all studies conducted were of cross-sectional nature which does not allow any conclusion regarding temporal progression (i.e., dynamics) of observed structural and functional alterations following SCI. The understanding of dynamics is essential in order to further elucidate mechanisms potentially driving the observed results. Future longitudinal studies are warranted which include individuals with SCI in the acute stage of injury and follow them over the course of recovery (i.e., sub-acute and chronic stage of injury). Although the strict inclusion of individuals with chronic SCI provides a ‘neurologically stable’ environment and observed alterations are likely robust, it limits the explanatory power regarding appearance and development of these changes and how they are related to primary and secondary injury mechanisms. Given the heterogeneity of the thesis’ study samples (e.g., lesion level, completeness of injury, presence/absence of spasms, absence/presence of pain, etc.), single-subject fMRI data would probably have revealed individual-specific findings relevant for understanding phenotypes. However, while CHEPs and SSEPs emerged to valid and reliable assessment tools in the clinical setting, functional MRI is not yet established in the clinic for prognosis and diagnosis of SCI, mainly due to susceptible single-subject test–retest reliability. Amongst others, individual’s motion has been reported to non-negligibly influence the reliability. Accordingly, a considerable proportion of individuals with SCI suffer from spasticity which likely leads to an increased head motion and impinges the reliability.

8.5 Future Directions

This thesis contributed to the apprehension concerning the plasticity of the sensory system after a spinal cord injury, but a number of questions remain unanswered. Further research is required to improve our understanding of how the descending control of afferent stimuli is altered in response to deafferenation (i.e., changes in the modulation of innocuous and nociceptive thermal inputs), the impact of central versus peripheral deafferenation, and the evolution of changes throughout the course of disease (i.e., acute versus chronic stages of deafferenation). Future projects should evaluate the relationship between changes in descending control following deafferenation and the development of clinically relevant sensory impairments (e.g., hypoalgesia) and complications (e.g., hyperalgesia, allodynia, and neuropathic pain). Central to the future studies will be the direct comparison of differences in sensory modulation related to central (i.e., SCI) and peripheral deafferenation (i.e., amputation). Consolidated mechanistic understanding of the underlying sensory plasticity (i.e., sensory interactions at spinal and supraspinal levels) is highly relevant in the process of evaluation and design of novel therapeutic interventions in human SCI. Lastly, a priority should also be to evaluate how clinical deficits relate to findings from advanced neuroimaging and as such, longitudinal studies combining neurophysiological and advanced neuroimaging techniques are inevitable. Assessing the magnitude and dynamics of alteration in spinal
Sensory Plasticity in Cervical Spinal Cord Injury

General Discussion

cord and brain microstructure will provide essential information regarding mechanisms initiating and maintaining sensory deficits and complication, in particular neuropathic pain.

Segmental recovery following SCI reflects clinically relevant functional changes in sensorimotor behavior. Novel approaches to further assess segmental neurophysiology are likely required to dissociate the extent of repair of damaged segmental pathways (i.e., afferent conductivity), and plasticity of segmental spinal cord organization to form new functional projections either due to unmasking of pre-established pathways or new projections (i.e., sensori-sensory interactions). The longstanding debate as to whether partial damage in the spinothalamic tract is a requisite for the onset sensory complications (i.e., neuropathic pain) would benefit from such investigations. As such, the application of non-noxious cool stimuli allows selectively stimulating defined subpopulations of thinly myelinated and unmyelinated sensory fibers that are suspected to be involved in the pathology of cold allodynia, a frequently reported symptom in individuals with SCI. Using cold-evoked potentials (CEPs) might be an objective and quantifiable method to assess the function/dysfunction of the underlying spinal pathways. CEPs provide an additional electrophysiological outcome parameter for the function of the ventral cord, and might add further insights into spinothalamic function beyond contact heat evoked potentials and clinical pinprick testing. 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. The changes in spinal cord neurophysiology specific to neuroplasticity are expected to serve as important outcome measure for detecting the efficacy and mechanism of therapeutic intervention.

There is an emerging interest in the development and adoption of quantitative approaches to assess how noxious afferent input is modulated in chronic pain patients – so-called dynamic tests of sensation. To date, this area of investigation has been intensely focused on measuring diffuse noxious inhibitory control using a conditioned pain modulation (CPM) paradigm. Briefly, perception to noxious stimuli is examined during painful conditioning stimulation applied heterotopically (i.e., pain inhibits pain). As a measure of descending inhibitory control, the utility of CPM has been demonstrated in a number of chronic pain conditions, relieving impaired endogenous modulation. Suggesting different underlying mechanisms, CPM and TENS show distinct effects on perception to noxious afferent stimuli. In patient populations, changes in perception to noxious afferent input following TENS could be used to assess impaired modulation at the spinal level. To determine specific types of impairments in endogenous pain modulation, future studies should address the feasibility and specificity/sensitivity of CPM and TENS in patient populations. These studies could involve the application of capsaicin, but could also focus on the modulating effects on pressure pain, potentially negating the need for the induction of sensitization.
8.6 Concluding Remarks

The present thesis extensively investigated the plasticity of the sensory system after deafferentation due to spinal cord injury. Of particular interest were pain-associated alterations of the sensory system (i.e., anatomical and functional) as well as how neuropathic pain alters the modulation of noxious afferent input. The following conclusion can be drawn from the presented results. Firstly, the presence of neuropathic pain was associated altered modulation of noxious afferent input mirrored as reduced habituation and reduced perception, important features of the descending control of pain. Secondly, preservation of structure and function was found in individuals with SCI suffering from neuropathic pain indicating a potential lack of neuronal adaption in response of deafferentation. Lastly, both studies in healthy control individuals contribute to our understanding of the modulation of afferent input by disclosing traits of cortical structures responsible for the variability in pain processing as well as highlighting the differential modulation of A\(\delta\) fiber (i.e., sharp pain) and C fiber activation (dull spontaneous pain) mediated by large fiber excitation. Conclusively, the present findings deepen our understanding of the relationship between pain and associated alterations of brain structure and function.
References

37. Siddall PJ, Taylor DA, McClelland JM, Rutkowski SB, Cousins MJ. Pain report and the relationship of pain to physical factors in the first 6 months following spinal cord injury. Pain 1999;81
38. Siddall PJ, McClelland JM, Rutkowski SB, Cousins MJ. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain 2003;103
46. Finnerup NB, Sorensen L, Biering-Sorensen F, Johannesen IL, Jensen TS. Segmental hypersensitivity and spinothalamic function in spinal cord injury pain. Exp Neurol 2007;207
47. Hari AR, Wyndkeller C, Dokladal P, Halder P. Enhanced recovery of human spinothalamic function is associated with central neuropathic pain after SCI. Exp Neurol 2009;216
54. Dostrovsky JO. Role of thalamus in pain. Prog Brain Res 2000;129
60. Farrar JT, Young JP, Jr., LaMoreaux L, Wether JL, Poole RM. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain 2001;94
69. Dawson GD. Cerebral Responses to Electrical Stimulation of Peripheral Nerve in Man. J Neurol Neurosurg Psychiatry 1947;10

Sensory Plasticity in Cervical Spinal Cord Injury
95. Kakigi R, Watanabe S. Pain relief by various kinds of interference stimulation applied to the peripheral skin in humans: pain-related brain potentials following CO2 laser stimulation. J Peripher Nerv Syst 1996;1
97. Chesterton LS, Foster NE, Wright CC, Baxter GD, Barlas P. Effects of TENS frequency, intensity and stimulation site parameter manipulation on pressure pain thresholds in healthy human subjects. Pain 2003;106
102. Wall PD. The gate control theory of pain mechanisms. A re-examination and re-statement. Brain 1978;101

References
References

104. Leonard G, Goffaux P, Marchand S. Deciphering the role of endogenous opioids in high-frequency TENS using low and high doses of naloxone. Pain 2010;151
105. Lu Y, Pierre V, Yeomans DC. Differential antinociceptive effects of spinal opioids on foot withdrawal responses evoked by C fibre or A delta nociceptor activation. Br J Pharmacol 1997;121
111. Fitzgerald M. Alterations in the ipsi- and contralateral afferent inputs of dorsal horn cells produced by capsaicin treatment of one sciatic nerve in the rat. Brain Res 1982;248
112. Jin YH, Nishikawa H, Wakabayashi K, Fujita T, Yonehara N. Effect of morphine on the release of excitatory amino acids in the rat hind instep: Pain is modulated by the interaction between the peripheral opioid and glutamate systems. Neuroscience 2006;138
115. LaMotte RH, Lundberg LE, Torebjork HE. Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. J Physiol 1992;448
136. Babbar S, Marier JF, Mouksassi MS, et al. Pharmacokinetic analysis of capsaicin after topical administration of a high-concentration capsaisin patch to patients with peripheral neuropathic pain. Ther Drug Monit 2009;31
139. DeSantana JM, Da Silva LF, De Resende MA, Sluka KA. Transcutaneous electrical nerve stimulation at both high and low frequencies activates ventrolateral periaqueductal grey to decrease mechanical hyperalgesia in arthritic rats. Neuroscience 2009;163
141. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. Curr Opin Anaesthesiol 2010;23
152. Finnerup NB, Johannesen IL, Bach FW, Jensen TS. Sensory function above lesion level in spinal cord injury patients with and without pain. Somatosens Mot Res 2003;20
156. Sluka KA, Deacon M, Sibal A, Strissel S, Terpstra A. Spinal blockade of opioid receptors prevents the analgesia produced by TENS in arthritic rats. J Pharmacol Exp Ther 1999;289
157. Radhakrishnan R, Sluka KA. Spinal muscarinic receptors are activated during low or high frequency TENS-induced antihyperalgesia in rats. Neuropearmacology 2003;45
158. Sluka KA. Systemic morphine in combination with TENS produces an increased antihyperalgesia in rats with acute inflammation. J Pain 2000;1
159. Somers DL, Clemente FR. Contralateral high or a combination of high- and low-frequency transcutaneous electrical nerve stimulation reduces mechanical allodynia and alters dorsal horn neurotransmitter content in neuropathic rats. J Pain 2009;10
205. Ashburner J, Friston KJ. Voxel-based morphometry—the methods. Neuroimage 2000;11
209. Tseng MT, Tseng WY, Chao CC, Lin HE, Hsieh ST. Distinct and shared cerebral activations in processing innocuous versus noxious contact heat revealed by functional magnetic resonance imaging. Hum Brain Mapp 2010;31
211. Maldjian JA, Laurieniti PI, Burdette JH. Precentral gyrus discrepancy in electronic versions of the Talairach atlas. Neuroimage 2004;21
231. May A. Chronic pain may change the structure of the brain. Pain 2008;137

Sensory Plasticity in Cervical Spinal Cord Injury

References
257. Chen A, Yao J, Kuiken T, Dewald JP. Cortical motor activity and reorganization following upper-limb amputation and 2012;135
References

282. Lemon RN. Descending pathways in motor control. Annu Rev Neurosci 2008;31
284. Kim BG, Dai HN, McAtee M, Vicini S, Bregman BS. Remodeling of synaptic structures in the motor cortex following spinal cord injury. Exp Neurol 2006;198
303. Mendell LM. Physiological properties of unmyelinated fiber projection to the spinal cord. Exp Neurol 1966;16

Sensory Plasticity in Cervical Spinal Cord Injury


308. Hongo T, Jankowska E, Lundberg A. Post-synaptic excitation and inhibition from primary afferents in neurones of the spino-cervical tract. J Physiol 1968;199


310. Sluka KA, Walsh D. Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness. J Pain 2003;4


316. Mohammadian P, Andersson OK, Arendt-Nielsen L. Correlation between local vascular and sensory changes following tissue inflammation induced by repetitive application of topical capsaicin. Brain Res 1998;792


319. Staud R. Abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions. Expert Rev Neurother 2012;12


321. Nava E, Roder B. Adaptation and maladaptation insights from brain plasticity. Prog Brain Res 2011;191


333. Garrison DW, Foreman RD. Decreased activity of spontaneous and noxiously evoked dorsal horn cells during transcutaneous electrical nerve stimulation (TENS). Pain 1994;58
## 9 List of Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>anterior cingulate gyrus</td>
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<tr>
<td>ADL</td>
<td>activities of daily living</td>
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<td>AIS</td>
<td>American Spinal Injury Association International Standards</td>
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<tr>
<td>APW</td>
<td>anterior-posterior width</td>
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<tr>
<td>ASIA</td>
<td>American Spinal Injury Association</td>
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<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
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<td>BOLD</td>
<td>blood oxygen level detection</td>
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<td>CDT</td>
<td>cold detection threshold</td>
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<td>CHEP</td>
<td>contact heat evoked potential</td>
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<td>CMAP</td>
<td>compound muscle action potential</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
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<td>CPM</td>
<td>conditioned pain modulation</td>
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<td>CPT</td>
<td>cold pain threshold</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>DARTEL</td>
<td>diffeomorphic anatomical registration through exponentiated lie algebra</td>
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<tr>
<td>dSSEP</td>
<td>dermatomal somatosensory evoked potential</td>
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<td>DNIC</td>
<td>diffuse noxious inhibitory control</td>
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<td>DTI</td>
<td>diffusion tensor imaging</td>
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<td>ED</td>
<td>Euclidean distance</td>
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<td>EDT</td>
<td>electrical detection threshold</td>
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<td>EEG</td>
<td>electroencephalography</td>
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<td>EMG</td>
<td>electromyography</td>
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<td>EPT</td>
<td>electrical perception threshold</td>
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<td>EM-SCI</td>
<td>European Multi-Center Study about Spinal Cord Injury</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>FLS</td>
<td>FMRIB software library</td>
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<td>FWE</td>
<td>family-wise error</td>
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<td>FWHM</td>
<td>full-width-at-half-maximum</td>
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<td>GM</td>
<td>gray matter</td>
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<td>IFOF</td>
<td>inferior frontal-occipital fasciculus</td>
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<td>ISNCSCI</td>
<td>International Standards for the Neurological Classification of Spinal Cord Injury</td>
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<td>LEP</td>
<td>laser evoked potential</td>
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<td>LRW</td>
<td>left-right width</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MCID</td>
<td>minimally clinically important difference</td>
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<td>M1</td>
<td>primary motor cortex</td>
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<td>MEG</td>
<td>magnetoencephalography</td>
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<td>MEP</td>
<td>motor evoked potential</td>
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<td>MeSH</td>
<td>Medical Subject Headings</td>
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<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance image</td>
</tr>
<tr>
<td>MVA</td>
<td>motor vehicle accident</td>
</tr>
<tr>
<td>NP</td>
<td>neuropathic pain</td>
</tr>
<tr>
<td>NRS</td>
<td>numerical rating scale</td>
</tr>
<tr>
<td>QST</td>
<td>quantitative sensory testing</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>RVM</td>
<td>rostroventral medulla</td>
</tr>
<tr>
<td>S1</td>
<td>primary somatosensory cortex</td>
</tr>
<tr>
<td>S2</td>
<td>secondary somatosensory cortex</td>
</tr>
<tr>
<td>SCA</td>
<td>spinal cord area</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>SNF</td>
<td>Swiss National Foundation</td>
</tr>
<tr>
<td>SPM</td>
<td>statistical parametric mapping</td>
</tr>
<tr>
<td>SSEP</td>
<td>somatosensory evoked potential</td>
</tr>
<tr>
<td>TENS</td>
<td>transcutaneous electrical nerve stimulation</td>
</tr>
<tr>
<td>TIV</td>
<td>total intracranial volume</td>
</tr>
<tr>
<td>TBM</td>
<td>tensor-based morphometry</td>
</tr>
<tr>
<td>TrpV1</td>
<td>transient receptor potential cation channel subfamily V member 1</td>
</tr>
<tr>
<td>UEMS</td>
<td>upper extremity motor score</td>
</tr>
<tr>
<td>VBM</td>
<td>voxel-based morphometry</td>
</tr>
<tr>
<td>VBCT</td>
<td>voxel-based cortical thickness</td>
</tr>
<tr>
<td>WDT</td>
<td>warm detection threshold</td>
</tr>
<tr>
<td>WM</td>
<td>white matter</td>
</tr>
<tr>
<td>WPT</td>
<td>warm pain threshold</td>
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</tbody>
</table>
Curriculum Vitae

Personal Data

Working address: University Hospital Balgrist
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Cell phone: +41 79 543 54 46

E-mail: cjutzeler@paralab.balgrist.ch

Date of birth: October 9th, 1985

Citizenship: Swiss

Educational Background

2012-2015 PhD Candidate
Focus on sensory systems after spinal cord injury
Spinal Cord Center Balgrist, Zürich
Supervisor: Prof. Dr. med. Armin Curt

2011 Research Assistant
Internship with focus on electrophysiology in rodents, Dr. Siegel’s Laboratory, Psychiatry Department, Translational Research Laboratory, Philadelphia, PA

February 2011 Master Degree in Pharmaceutical Sciences and Pharmacology, University of Basel

2010 Master’s Thesis, Dr. Siegel’s Laboratory, Psychiatry Department, Translational Research Laboratory, Philadelphia, PA

2006-2009 Bachelor Degree in Pharmaceutical Sciences and Pharmacology, University in Basel

Professional Experience

2012-2015 Proxorso - Centre for Spine Medicine, Zurich
Medical Technical Assistant (10%)

2012-2015 University Hospital Balgrist, Zurich, Neurophysiology Lab
Medical Technical Assistant (10%)

January-February 2009 Bosnaliek, Sarajevo (BIH)
Internship in the pharmaceutical technology and supply department

September 2006 Fisher Clinical Services, Basel
Internship in drug supply development and production
June - August 2006  Hospital Bruderholz Basel  
Internship, Neurology Department

Teaching experience

2012 – 2015  Supervision of Master's Theses in  
- Human Movement Science, ETH Zurich (2)  
- Neurobiology, University of Zurich (1)

2012 – 2015  Supervision of Internships in  
- Human Movement Science, ETH Zurich (1)  
- Biomedicine and Medical Technology, University Mannheim (1)  
- Biomechanics & Neurobiology, University Graz (1)

2012 – 2014  "Functional Assessment of Human Spinal Cord Injury": Conduct of the block course attended by undergraduate students in biology of the University of Zurich and ETH Zurich

Technical Skills

Neuro-electrophysiology  
- Somatosensory evoked potentials, contact heat evoked potentials, nerve conduction velocity, motor evoked potentials

Imaging  
- Functional MRI, voxel-based morphometry, voxel-based cortical thickness

Personal Skills

Languages  
- German  Mother tongue  
- French  Fluent  
- English  Fluent  
- Spanish  Beginner

Computer skills  
- Microsoft software, MatLab, SPM, SPSS

Society Memberships

Society for Neuroscience  
International Association for the Study of Pain (IASP)
Conference Presentation

Posters
1. **C.R. Jutzeler; M. Freitag; A. Curt; J.K. Kramer;** SCI patients with neuropathic pain show reduced capacity to modulate noxious afferent input. International Symposium KFSP, Ittingen, Switzerland, 2015

2. J. Rosner; D. Pfau; **C.R. Jutzeler;** A. Curt; R.D. Treede; W. Greffrath; Using cold evoked potentials (cCEPs) to assess (residual) spinothalamic function in healthy volunteers and patients with spinal cord injuries: a pilot study. DGKN Kongress, Tuebingen, Germany, 2015


4. **C.R. Jutzeler;** A. Curt; J.K. Kramer; Preserved Interaction between Aβ- and Aδ-fibers in Capsaicin-induced Pain. 16th annual ZNZ Symposium, Zurich 2013


6. **C.R. Jutzeler;** J. Haefeli; J.K. Kramer; P. Freund; A. Curt. Cortical reorganization of dorsal columns and spinothalamic tract input after spinal cord injury. 15th annual ZNZ Symposium, Zurich 2012


8. V. M.Tatard-Leitman; M. J. Gandal; **C. R. Jutzeler;** S. J. Siegel, NMDA Receptor-1 hypomorphic affect on GABAergic interneurons subpopulations. Annual Convention for the Society of Cognitive Neuroscience, San Diego 2010

9. R. Lin; Y. Liang; J. Suh; **C. R. Jutzeler;** S. J. Siegel, Cognitive impairment and catalepsy in the absence of positive symptoms with chronic amphetamine administration in mice. Annual Convention for the Society of Cognitive Neuroscience, San Diego 2010

Talks/ Workshops
1. Talk: Neuropathic Pain Is Not Associated With Functional Reorganization In S1 After Spinal Cord Injury, ISCoS and ASIA Joint Meeting, Montreal, 2015


**Awards**

1. ICORD scholarship for International Trainees (VISIT program), ICORD, Vancouver, Canada 2014
2. Poster prize, 3rd Place, International Symposium KFSP, Ittingen, Switzerland, 2015
Publications


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