MicroRNA and Neuroimaging Biomarkers of Neuropathic Pain Severity After Spinal Cord Injury: Results from a Robotic-Assisted Gait Training Study

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA
BY

Jesse Lin Kowalski

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Leslie R. Morse, DO, Advisor

July 2022

Acknowledgements

This work would not have been completed without my heart and soul, my family. Thank you to my parents, **Karl and Jodi Kowalski**, and my sister, **Sadie Boley**, for the unwavering support, love, and encouragement every step of my journey since my very first ones, even though they carried me far away from home. All I have become and achieved is because of you. To my nieces, **Blake and Harlyn Boley**, may this help you remember that you can grow up to be and do anything you wish to – I cannot wait to see who you become. I love you all to the moon and back always and forever.

Dr. Leslie Morse: Thank you for your phenomenal mentorship, guidance, support, and for the precious gift of time you dedicate to selflessly fostering my growth and development. You have taught me so much I am grateful for, but most importantly, you have shown me the kind of mentor I aspire to be.

Dr. Dawn Lowe: I would not be where I am today without your belief in me when I did not believe in myself. Thank you for seeing the best in me, and helping me become the researcher and woman that I am. I can never thank you enough. Without your compassion, constancy, guidance, support, and championship, none of this would be possible.

Dr. Clas Linnman: Thank you for graciously and patiently sharing your immense knowledge and expertise with me. Your mentorship knows no limits, across miles, software systems, analysis approaches, brain systems and functions… you inspire me to chase the unattainable goal of becoming as brilliant, giving, and kind.

To **Drs. Ricardo Battaglino and Laura Stone**: Thank you for serving on my committee, and for the guidance and expertise you graciously share with me, in order to help me become a better scientist.

To co-authors **Nguyen Nguyen** and **Drs. Karen Troy, Scott Falci**, and **Susan Charlifue**: Thank you for your contributions to this work.

To **Rich Adamczak**, thank you for helping me make it through, always saving the day, making me laugh, and for climbing under the stairs.

To dear friends near and far, and especially to my PhD program cohort, **Dr. Rebekah Summers, Dr. Leah Johnson**, and soon to be Drs. **Gaura Saini** and **Aileen Lee**: thank you for your cherished friendship, support, and all the wonderful memories and laughter we have shared along the way.

Last but not least, to **Bunny:** Thank you for being by my side through every late night, early morning, all-nighter, exam, paper, trial, and tribulation. You are my unofficial co-author always. I love you just as much as you love me. Unconditionally.

Doctoral training support was provided the National Center for Advancing Translational Sciences of the National Institutes of Health [TL1R002493 and UL1TR002494], the University of Minnesota's MnDRIVE (Minnesota's Discovery, Research and Innovation Economy) Initiative, Florence P. Kendall and Promotional of Doctoral Studies I Scholarships from the Foundation for Physical Therapy Research, and the University of Minnesota Divisions of Rehabilitation Science and Physical Therapy. This work was additionally supported by the National Institute on Disability, Independent Living, and Rehabilitation Research (NIDILRR) [90SI5015-01-00] and the Department of Defense [W81XWH-10-1-1043].

Dedication

“Do all the good you can,
by all the means you can,
in all the ways you can,
in all the places you can,
at all the times you can,
to all the people you can,
as long as ever you can.”

- John Wesley

To all those struggling with the excruciating and debilitating condition of neuropathic pain, may this work offer some much needed good.

Table of Contents

List of Abbreviations

3D: three-dimensional

Aβ: amyloid-β

ACC: anterior cingulate cortex

AD: Alzheimer's disease

AG: angular gyrus

AIS: American Spinal Injury Association Impairment Scale

AL: at-level spinal cord injury

AMPK/mTOR: adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin signaling pathway

aPaHC: anterior parahippocampal gyrus

APP: amyloid precursor protein

Arl2: ADP ribosylation factor-like 2 protein

ATP: adenosine triphosphate

B: bilateral

$BACE_1$: β-site amyloid precursor protein-cleaving enzyme 1

BCL-2: B-cell lymphoma 2

BCL2L11: Bcl-2-like 11

BDNF: brain derived neurotrophic factor

BL: below-level spinal cord injury

BOLD: blood-oxygen-level-dependent

CIR: cerebral ischemia/reperfusion

CONSORT: Consolidated Standards of Reporting Trials

CSF: cerebral spinal fluid

CTGF: connective tissue growth factor

CRPS: complex regional pain syndrome

CXC4: chemokine (C-X-C motif) receptor-4

DARTEL: Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra

dmMPFC: medial prefrontal cortex division of the default mode network

dmPCC: posterior cingulate cortex division of the default mode network

DRG: dorsal root ganglia

EPI: echo planar imaging

FOV: field of view

fMRI: functional magnetic resonance imaging

FP: frontal pole

FWE: family-wise error

GM: gray matter

GMV: gray matter volume

HC: hippocampus

ICC: intracalcarine cortex

IL-1β: interleukin-1β

IL-6: interleukin-6

IκB: inhibitor of NF-κB

IKK-α: inhibitor of NF-κB kinase α

ISCIPBDS: International Spinal Cord Pain Basic Dataset

ITG: inferior temporal gyrus

L: left

LP: lateral parietal

LSmean: least squares mean

M: midline

M1: primary motor cortex

M1 macrophages: proinflammatory macrophages

M2 macrophages: wound resolving macrophages

MAS: Modified Ashworth Scale

MFG: middle frontal gyrus

miRNA: microRNA

MNI: Montreal Neurological Institute

mPFC: medial prefrontal cortex

MR: magnetic resonance

MRI: magnetic resonance imaging

mRNA: messenger RNA

MS: multiple sclerosis

NC: nociceptive

nDSC: normalized deep sequencing count

NF-κB: nuclear factor κB

NP: neuropathic pain

NSAIDS: nonsteroidal anti-inflammatory drugs

PAG: periaqueductal gray matter

PCC: posterior cingulate cortex

PFC: prefrontal cortex

PHQ-9: Patient Health Questionnaire-9

PI: pain interference

Pmax: maximal power

pPaHC: posterior parahippocampal gyrus

R: right

RAGT: robotic-assisted gait training

RF: radiofrequency

RNA: ribonucleic acid

ROI: region of interest

rsFC: resting state functional connectivity

rs-fMRI: resting state functional magnetic resonance imaging

S1: primary somatosensory cortex

SBC: seed-based correlation

SC: spinal cord

SCI: spinal cord injury

SMA: supplementary motor area

SPL: superior parietal lobule

SPM: Statistical Parametric Mapping

TE: echo time

TFC: temporal fusiform cortex

TR: repetition time

TNF-α: tumor necrosis factor-α

VO^2 max: maximal oxygen uptake

VO^2 peak: peak oxygen uptake

WM: white matter

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 1: Literature Review

1.1 Introduction

Spinal cord injury (SCI) results in chronic neuroinflammation which contributes to altered neural function and the development of neuropathic pain.[1] Differential expression of microRNA regulators of neuroinflammatory pathways[2-5] and alterations in brain structure[6,7] and functional connectivity[8-10] may contribute to the development or severity of neuropathic pain. Exercise has been shown to reduce neuroinflammation[11] and chronic pain[11-13] and alter brain structure[14,15] in human and animal models, yet little is known about how exercise interventions influence pain processing in human populations with SCI.

This doctoral dissertation aimed to identify 1) novel microRNA biomarkers of neuropathic pain, 2) neuropathic pain-related alterations in brain functional connectivity, and 3) the efficacy of an exercise intervention of robotic-assisted gait training to reduce neuropathic pain and alter brain volume in individuals with SCI. Successful identification of underlying mechanisms of neuropathic pain and potential exercise induced mitigation of these factors will guide the development of targeted interventions and provide useful biomarkers to predict and optimize prognosis, and subsequent care management for individuals with SCI.

1.2 Spinal Cord Injury and Neuropathic Pain

Prevalence and health impacts

Spinal cord injury (SCI) affects approximately 935,000 individuals worldwide each year, with an estimated prevalence of 27 million global cases.[16] The United States alone demonstrates an estimated incidence and prevalence of 17,810 and 294,000 SCI cases respectively.[17,18] SCI profoundly impacts function, independence, and quality of life and can lead to development of secondary conditions including chronic pain, depression, infections, fracture, and skin pressure injuries.[19-21] Total direct costs for all causes of SCI in the United States are estimated to be $7.736 billion, with a total lifetime cost of medical care for an individual with SCI of up to $969,659.[22]

Chronic pain is a particularly salient secondary condition of SCI as it is reported in as high as 81% of this population.[23] SCI-related pain is categorized as nociceptive (musculoskeletal, visceral, or other pain arising from non-neural tissue) or neuropathic (stemming from somatosensory nervous system injury or disease).[23-25] Neuropathic pain is typically more severe and persistent than nociceptive pain[24,26], with an estimated 56% of pain SCI-related pain complaints classified as neuropathic.[27] In individuals with SCI, pain intensity ratings are higher for neuropathic pain compared to nociceptive pain (Figure 1.1)[26], and a greater proportion report neuropathic pain as being severe or excruciating compared to nociceptive pain.[23]

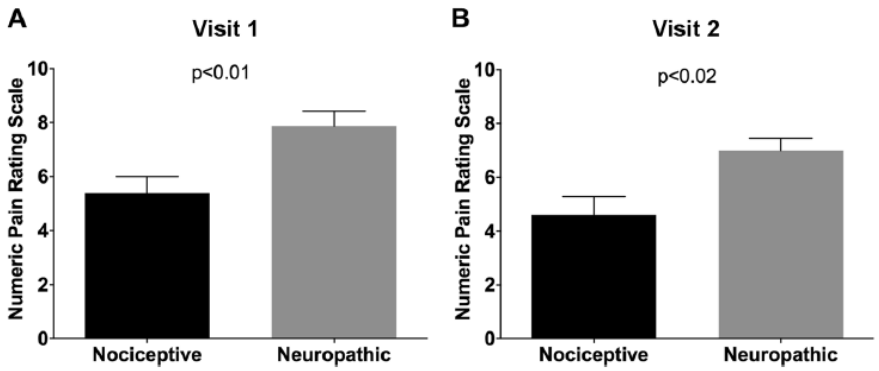


Figure 1.1. Pain intensity for the worst reported pain problem (either nociceptive or neuropathic) by type in individuals with chronic spinal cord injury for Visit 1 (A) and Visit 2 (B). Observational study visits 1 and 2 were held 6 months apart to collect data on pain symptoms and quality of life in adults with chronic spinal cord injury.[26] Unmodified figure utilized from Gibbs et al 2019 (https://creativecommons.org/licenses/by/4.0).

In addition to greater severity of pain, a high proportion of individuals with neuropathic pain also report pain interference with daily activities (61%), mood (57%), and ability to get a good night of sleep (76%).[26] Pain intensity has been demonstrated to correlate with interference in these domains, as well as domains of mobility, interpersonal relationships, and self-care.[28] Those with higher pain intensities utilize greater healthcare services, and those with neuropathic pain are more likely to be unemployed.[29] Overall, individuals with SCI with pain rate lower global health than those without pain.[23]

Neuropathic pain poses an additional challenge due to its resistance to pharmacologic intervention.[24] First line pharmacologic interventions include gabapentinoids, tricyclic antidepressants, and serotonin norepinephrine reuptake inhibitors, which offer limited efficacy.[30,31] These drugs commonly produce negative side effects of somnolence, fatigue, dizziness, orthostatic hypotension,

edema, and urinary retention.[30,31] When first line strategies fail, opioids are commonly prescribed.[32] These drugs also offer limited efficacy, and hold additional risk of unwanted side effects and complications such as drowsiness, nausea, constipation, respiratory depression, and potential dependency.[32] As respiratory, autonomic, bladder, and bowel function are commonly impaired in individuals with SCI, pharmacologic management is not a feasible treatment option for all individuals due to further limitation of these functions.[19-21] Approximately 20% of individuals with SCI-related pain will discontinue pharmacologic treatment due to unfavorable side effects.[26] Greater elucidation of the underlying pathophysiologic mechanisms that lead to the development of neuropathic pain, and interventions to target these mechanisms, is required to develop optimal treatment strategies and improve quality life for individuals with SCI.

Pathophysiology of Neuropathic Pain After Spinal Cord Injury

Trauma to the spinal cord initiates immediate inflammatory and immune responses similar to peripheral injuries such as damage to skin or muscle. However, unlike peripheral injuries which progress through a sequence of inflammatory, proliferative, and remodeling phases resolving in wound healing, the inflammatory response persists after SCI, resulting in a chronic state of neuroinflammation.[33,34]

One contributing mechanism to chronic inflammation after SCI is the observed difference in macrophage phenotype expression.[33] Macrophage

phenotypes are broadly classified as proinflammatory (M1), or wound resolving (M2).[34] In peripheral injuries, a cascade of signaling events elicits the transition from M1 to M2 macrophages. However, in SCI, M1 macrophage expression remains elevated compared to M2 macrophages, resulting in continued phagocytosis and production of proinflammatory cytokines (Figure 1.2).[33,34]

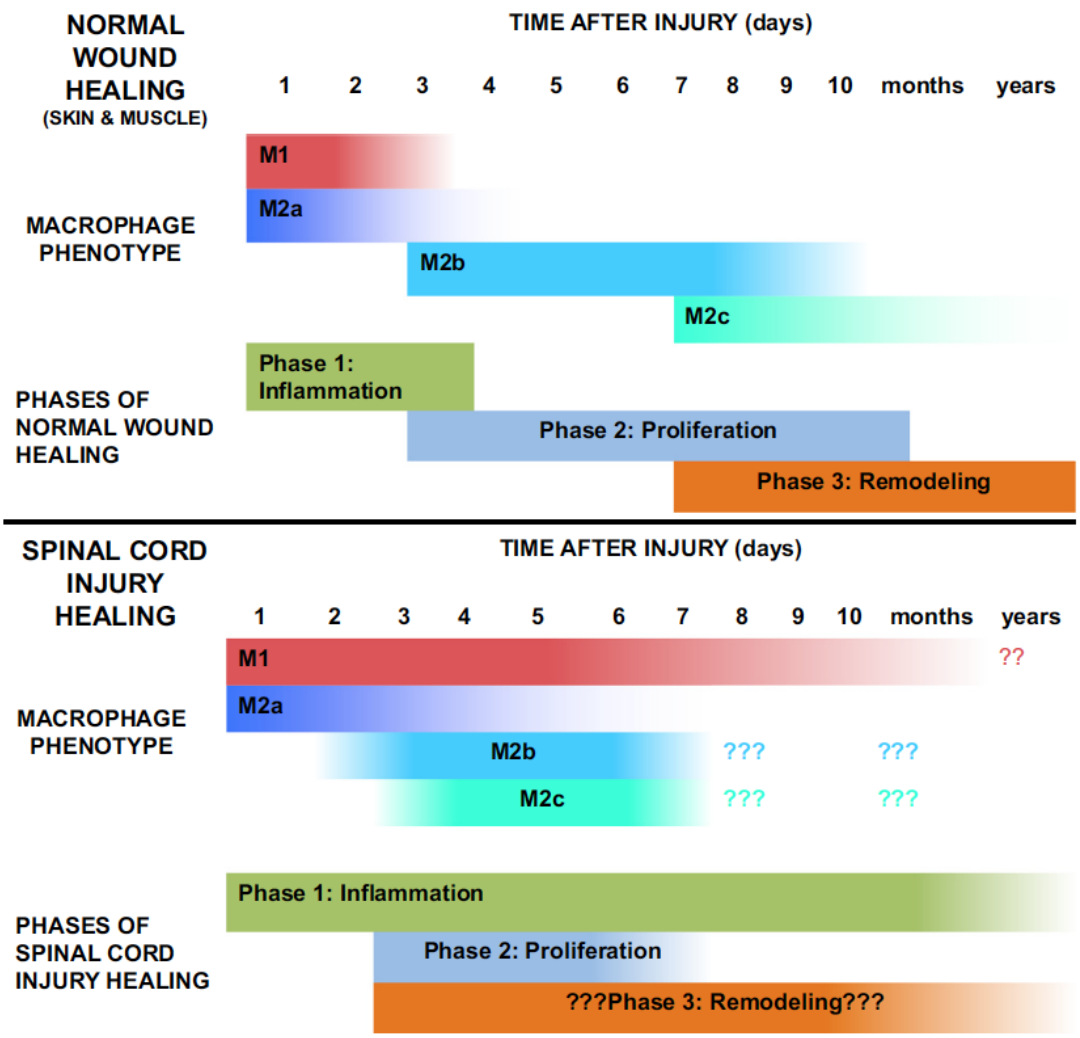


Figure 1.2. Comparison of inflammatory responses and trajectories in normal wound healing and spinal cord injury.[33] Unmodified figure utilized from Gensel and Zhang 2015 (https://creativecommons.org/licenses/by-nc-nd/4.0/).

Proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) contribute to enhanced pain states in part through the activation of nuclear factor κB (NF-κB).[35] Proinflammatory cytokines activate NF-κB, which subsequently promotes the genetic expression of additional proinflammatory cytokines, chemokines, and adhesion molecules, amplifying the neuroinflammatory response after SCI (Figure 1.3).[34-36]

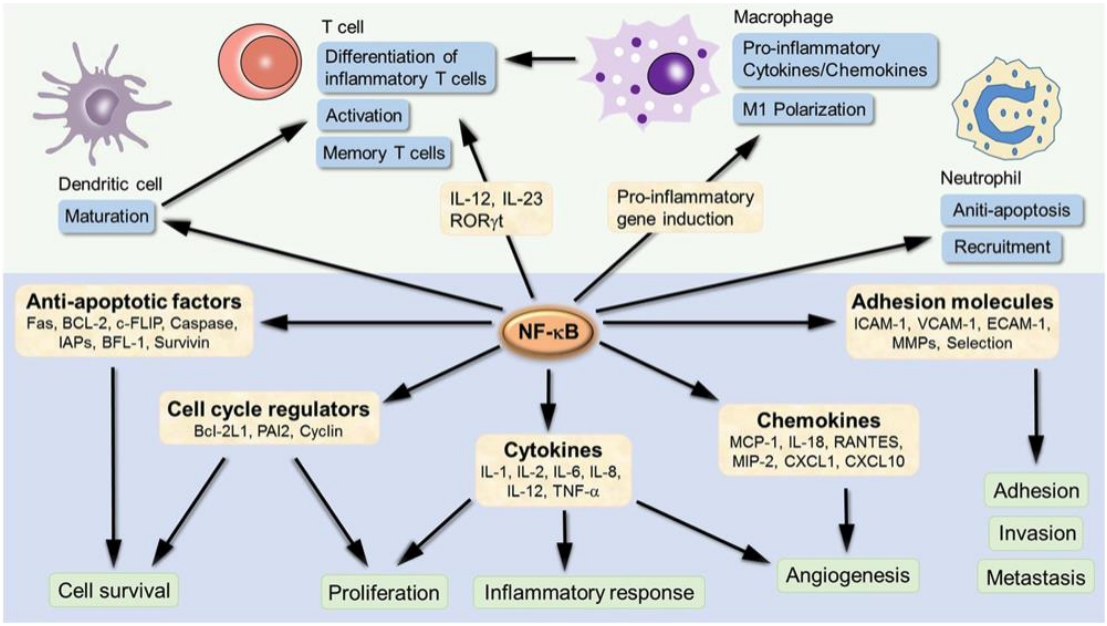


Figure 1.3. NF-κB target genes involved in inflammation development and progression.[36] Unmodified figure utilized From Liu et al 2017 (https://creativecommons.org/licenses/by/4.0/)

The NF-κB signaling pathway is considered a "master regulator of the inflammatory response" and critically regulates hundreds of immune relevant genes.[36,37] NF-κB has been robustly demonstrated to contribute to chronic inflammation, atrophy, and neuropathic pain after SCI and other inflammatory conditions.[35,37-40] NF-κB amplification and prolongation of neuroinflammatory

signaling contributes to neuropathic pain after SCI by promoting persistent glial production of proinflammatory cytokines and other downstream factors, which produce sensitization of dorsal horn neurons resulting in hyperexcitability and perception of pain elicited by non-noxious stimuli.[1,41] Increased neuroinflammatory mediators including the expression of proinflammatory cytokines[41-43], microglia[41,43,44], and macrophages[44] can alter synaptic function and are associated with the development of neuropathic pain after SCI.[1]

1.3 Mechanisms of Neuropathic Pain

Neuroinflammation Induced Alterations of the Sensory System

Sensation is a signal that is encoded at every level of biology from the molecular to systemic level. Chronic inflammation can alter the way signals regarding sensory stimuli are transduced in both ascending and descending pathways.[45] Exposure to noxious and non-noxious stimuli produces a cascade of events beginning with the release of ions that trigger depolarization of sensory neurons. Those neurons encode and transmit the signal, which passes through the dorsal root ganglion to the dorsal spinal cord. The spinal cord transmits the signal to the brain where it arrives at the thalamus, a relay center for a variety of stimuli and processes.[46] The thalamus projects the signal to the somatosensory cortex, where it is decoded within a somatotopic representation of the body allowing interpretation of intensity and location of the stimulus.[45,47]

Once the signal reaches the thalamus, a cascade of activity and interaction between ascending and descending tracts, cortical and subcortical regions, and neural networks occur. These complex interactions facilitate the coordination of behavioral responses, such as programming of muscle activity to initiate a withdrawal of affected region from noxious stimuli, and regulate affective and emotional responses to pain.[47] The ascending lateral pain pathways are primarily responsible for somatosensory aspects of pain processing[45] while the descending pain pathway modulates the suppression of pain signaling.[45,48,49] Of particular note, the periaqueductal gray matter has also been associated with regulating affective responses to pain, such as anxiety and depression, associated with chronic pain.[45] Other regions involved in affective and emotional aspects of pain processing include structures of the limbic system[50-52] and ascending medial pain pathway.[53] The hypothalamus and amygdala, for example, contribute to autonomic function, fear, and anxiety, and also project information to midbrain and brainstem areas that modulate the activity of nociceptive neurons, creating a feedback loop.[45,47]

Heightened and prolonged inflammatory signaling after neural insult results in alteration of synaptic function and activity of sensory neurons.[54] Inflammatory signaling promotes neuronal circuitry changes referred to as central sensitization, or increased responsiveness to normal or subthreshold sensory input.[45] This exaggerated sensory response is caused by the amplification of nociceptor activity through long term potentiation, or the disinhibition of nociceptors due to reduced activity of inhibitory interneurons.[47] This imbalance of

neuronal activity can result in hyperalgesia (hypersensitivity to noxious stimuli) or allodynia (the interpretation of non-noxious stimuli as noxious).[47] These neuroplastic changes at the neuronal and synaptic level lead to subsequent changes throughout ascending and descending nerve tracts and can ultimately alter the structure and function of somatosensory systems and networks in the brain.[45]

Changes in Brain Structure and Function in Neuropathic Pain

Alterations at the cellular level that contribute to the development of neuropathic pain, such as changes in proinflammatory cytokines, can be detected and quantified by biochemical techniques. Structural and functional changes in sensory systems associated with neuropathic pain can also be detected within the central nervous system by neuroimaging. Magnetic resonance imaging (MRI) is a non-invasive neuroimaging method commonly utilized to assess brain structure and function in human populations.

The magnetic resonance (MR) signal consists of resonant frequencies of electromagnetic waves emitted by hydrogen protons, after excitation by radiofrequency (RF) pulses produced by strong magnetic fields.[55] A vast array of MR sequences and analysis techniques can be applied to generate a variety of information about brain structure, biochemistry, and function.[55] MR signal is measured at the voxel-level. Voxels are three-dimensional (3D) parcellations and individual datapoints in the acquired 3D image of the brain. Like the number of pixels in a two-dimensional image, the size and number of voxels that comprise

the 3D image determine the image resolution. The size of the 3D image is determined by the spatial parameters of the sequence - the matrix size, field of view, and slice thickness.[55] Temporal parameters of scan time, repetition time (time between RF pulses), and echo time (time between RF pulse and signal acquisition), as well as the number of RF pulses applied, influence the amount of data collected and subsequent signal-to-noise ratio.[55] Repetition time, echo time, and flip angle also influence the contrast-weighting of MR sequences through production of varying sequences of RF stimuli subsequently eliciting differential responses of hydrogen protons.[55] Contrast-weighting (e.g. T1 and T2) reflects differences in signal intensity, or brightness, in various tissues.[55]

The structure of the brain is analyzed by using voxel-based morphometry to define the volume of tissues, such as the density of gray matter and the thickness of the cortex.[7] Tissue probabilities denote the likelihood that the signal intensity in each voxel represents a specific tissue type. Differing degrees of hydrogen motion in each tissue type result in the emission of distinctive signal intensities, allowing the segmentation of MR data into gray matter, white matter, and cerebral spinal fluid.[55] For example, in T1-weighted sequences, differences in tissue signal intensities are depicted by the white matter appearing brighter, gray matter appearing darker, and cerebral spinal fluid appearing black (Figure 1.4). Differences in brain volume can be determined by comparing the number of voxels classified as a specific tissue type between groups, time points, or conditions. These comparisons can assess structural alterations across the

whole brain or in isolated regions of interest, with voxel-based morphometry most commonly applied to assess differences in gray matter volume.[55]

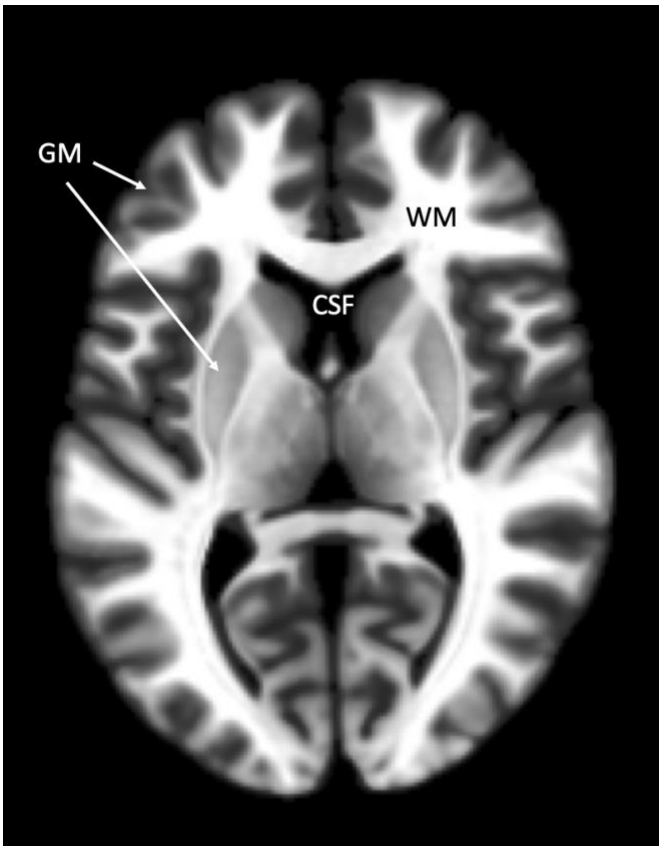


Figure 1.4. T1-weighted magnetic resonance template image demonstrating differing signal intensities between white matter (WM), gray matter (GM), and cerebral spinal fluid (CSF).

Functional activity and connectivity of the brain is measured with functional MRI (fMRI), which also compares voxel signals. fMRI analyzes differences in the blood-oxygen-level-dependent (BOLD) signal, a measure of neuronal activity.[7] Measurement of the BOLD signal is based on the hemodynamic response: an increase in regional blood flow to active neurons

which require additional glucose and oxygen supplied by blood.[56] fMRI can distinguish changes in blood flow in the brain due to alterations in oxyhemoglobin and deoxyhemoglobin concentrations, which are detectable in the MR signal.[56]

The BOLD signal can be measured with an individual at rest, or in conjunction with performance of a task in the scanner. fMRI enables the identification of regions of greater or lesser activity, and regions with fluctuations in synchronous activity, based on alterations in the BOLD signal across voxels.[56] Synchrony or coupling of the BOLD signal between regions is indicative of related activity, and is a measure of functional connectivity between regions.[56] For example, synchronous BOLD signal fluctuations in voxels composing the posterior cingulate cortex, medial prefrontal cortex, and lateral parietal cortices at rest is a hallmark of the default mode network, which is active when the body and mind are not focused on a task (Figure 1.5).[57] The strength of correlation or anticorrelation of the BOLD signal between regions provides a measure of their functional connectivity. fMRI analyses can look for relationships in the BOLD signal across the whole brain and in specified regions of interest. One highly utilized analytic approach is seed-based correlation, in which a seed region of interest is selected and BOLD signal in all other voxels is compared with the signal in the seed region to identify connectivity relationships.[55]

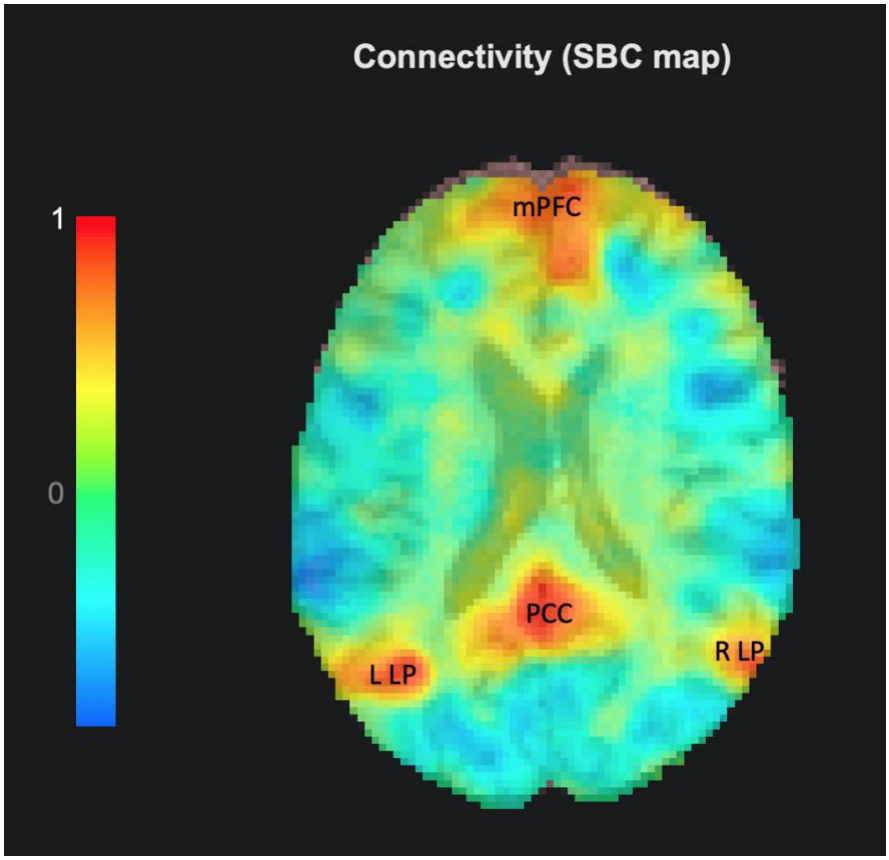


Figure 1.2. Functional connectivity seed-based correlation (SBC) map depicts regions demonstrating greater spontaneous synchronous neural activity (red) with the right lateral parietal (R LP) cortex seed region. R LP displays greater resting state functional connectivity to the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), and left lateral parietal (L LP) cortex. Together, these regions compose the default mode network.

Brain regions that have been previously associated through MRI studies with neuropathic pain after SCI include areas involved in sensorimotor function[6,58-60], emotional regulation[6], executive function and cognition[6,61], and reward motivation and goal directed action.[59,60] Most MRI studies in individuals with SCI and neuropathic pain have been structural, demonstrating decreased gray matter volume in areas with key roles in pain processing (Table 1.1).[46,62]

Regions demonstrating volumetric differences encompass components of the ascending pain pathways and limbic system. Findings of alterations in MRI structure in these areas in individuals with SCI have been validated by work in other neuropathic pain models.[63,64]

Table 1.1. Volumetric differences in individuals with SCI-related neuropathic pain

Region	Role/Function	Volume	Correlation
Primary somatosensory cortex	Sensation[62]	↓[6,58]	negative[49]
Primary motor cortex	Movement[65]	↑[6]	positive[6]
Thalamus	Sensation and movement[46]	↓[6]	negative[8]
Anterior cingulate cortex	Attention[66], reward anticipation and decision making[67], and emotion[68]	↑[6]	negative[8]
Inferior temporal gyrus	Emotional, auditory, and visual processing[69]	--	negative[8]
Middle frontal gyrus	Cognitive processing and motor tasks[70]	--	negative[8]
Temporal pole	Memory, emotional, semantic, and visual processing[71]	--	negative[8]

Summary of literature from structural magnetic resonance imaging analysis in individuals with spinal cord injury (SCI) with neuropathic pain (NP) compared to those with SCI without pain. Correlations with pain intensity are reported in subsamples of individuals with SCI and NP.

Though few studies have explored resting state functional connectivity (rsFC) alterations associated with neuropathic pain after SCI, existing reports have demonstrated connectivity alterations of somatosensory and limbic system structures (Table 1.2).[8,9] In individuals with SCI, stronger rsFC has been identified between limbic, executive, and sensory processing regions in those with neuropathic pain compared to those without pain.[8,9] Additionally, positive

correlations between rsFC and neuropathic pain severity have been reported within the limbic system[8], as well as between limbic and sensory processing regions.[9]

Table 1.2. Functional connectivity differences associated with SCI-related neuropathic pain

Seed Region	Role/Function	Connectivity	Role/Function
Insula	Sensory processing and emotional regulation[72]	↑ to cerebellum[9]+; temporal lobe[9]+; occipital lobe[9]+; amygdala[8]+	Balance, coordination, and movement[73]; memory, auditory and semantic processing[74]; vision[75]; fear, arousal, and emotional regulation[76]
Prefrontal cortex	Executive function, cognition, and working memory[77]	↑ to angular gyrus[8]	Multimodal processing, attention, and spatial cognition[78]
Superior parietal lobule	Visuospatial processing, long-term and working memory[79]	↑ to angular gyrus[8]+	Multimodal processing, attention, and spatial cognition[78]

Summary of literature from resting state functional magnetic resonance imaging seed-based correlation analyses in individuals with spinal cord injury (SCI) with neuropathic pain compared to those with SCI without pain. + signifies a positive correlation between connectivity and pain intensity.

Altered functional activity has also been reported in individuals with multiple sclerosis (MS) and neuropathic pain with decreased coactivation in the bilateral nucleus accumbens and caudate.[80] These areas are involved in reward and behavior, suggesting a possible relationship between neuropathic pain and the dopaminergic system.[80] Additionally, individuals with chronic neuropathic orofacial pain demonstrated increased functional connectivity between the rostral ventromedial medulla, periaqueductal gray matter (PAG), and locus ceruleus, regions involved in descending pain pathways.[64] The PAG and locus ceruleus

also demonstrated increased functional connectivity with the reward center of the nucleus accumbens and with the anterior cingulate cortex, a region known to be associated with neuropathic pain in SCI.[64] As such, these regions could be promising therapeutic targets for interventions to reduce accentuated pain signaling.

The Role of microRNA in Neuroinflammation and Neuropathic Pain

Pain can be also modulated at the molecular level by small non-coding ribonucleic acids (RNA), called microRNA (miRNA), which bind to target messenger RNAs (mRNA) and cause post-transcriptional inhibition or degradation of mRNA resulting in gene silencing.[81,82] Each of the 78 known families of miRNAs conserved in vertebrates has approximately 300-400 targets[83], with a single miRNA regulating as many as 200 mRNAs.[84] Approximately 3,000 connections between 350 miRNAs and 160 diseases have been identified, demonstrating complex interactions of multiple miRNAs contributing to disease states.[85] Preclinical animal model studies have identified differential expression of a number of miRNAs contributing to pathological processes after SCI including apoptosis[86-89], inflammation[90-92], and astrogliosis.[86-88]

miRNAs are emerging potential biomarkers of interest as they can be measured in serum or plasma and may offer the ability to elucidate pathological pathways and processes at the molecular level in clinical populations. Additional factors which make miRNAs promising biomarkers are their intra- and

extracellular stability. Compared to other RNAs with intracellular half-lives of several minutes, miRNAs are more resistant to degradation, with half-lives ranging from 8 hours to 3 weeks.[93] This stability is due to the binding of miRNAs with Argonaute proteins, which also makes them more resistant to extracellular degradation. They are highly stable in plasma and serum up to 24 hours at room temperature or 8 freeze/thaw cycles.[93]

The tissue specificity of miRNAs further supports their potential as biomarkers in disease states. Many miRNAs are more highly expressed in specific tissues, such as neural, cardiac, kidney or liver, enabling the identification of miRNAs contributing to conditions affecting specific systems.[94] As such, miRNAs hold promise as regulatory biomarkers of inflammatory processes within neural tissues which contribute to maladaptive neuroplastic mechanisms after SCI. Identification of miRNAs that regulate genetic transcription of neuroinflammatory cascades after SCI could provide quantitative measures of neuropathology and provide potential targets for downregulation of chronic proinflammatory signaling pathways. Two miRNAs of interest, miR-15b and miR-338, have been found to influence the NF-κB signaling pathway and regulate both neuroinflammation (Table 1.3) and neuropathic pain (Table 1.4).

Table 1.3. Validated microRNA and downstream targets in neuroinflammatory models					
miRNA	**Model**	**microRNA effect**	**Expression compared to controls**	**Tissue**	**Validated targets**
miR-15b	SCI in rat	Neuro-degenerative	↑*	Spinal cord	Bcl-2[12]
	CIR in rat	Neuro-degenerative	↑	Cortex	Arl2, ATP[95]
	In vitro studies of AD	--	--	SH-SY5Y cell line	BACE1/ NF-κB pathway, Aβ, IKK-α[96]
miR-338	CIR in mouse	Neuro-protective	↓	Cortex	CTGF, AMPK/ mTOR pathway[97]
	AD in Human and mouse	Neuro-protective	↓	Hippo-campus	BACE1/ NF-κB pathway[98]

Summary of literature validating microRNA and downstream targets in animal, human, and human derived cell line neuroinflammatory models of spinal cord injury (SCI_, cerebral ischemia/reperfusion (CIR) injury, and Alzheimer's disease (AD). Validated targets include B-cell lymphoma 2 (BCL-2), ADP ribosylation factor-like 2 (Arl2) protein, adenosine triphosphate (ATP), β-site amyloid precursor protein-cleaving enzyme 1 ($BACE_1$), nuclear transcription factor κB (NF-κB) signaling pathway, amyloid- β (Aβ), inhibitor of NF-κB kinase α (IKK-α), connective tissue growth factor (CTGF), and adenosine 5'-monophosphate-activated protein kinase/mammalian target of rapamycin signaling pathway (AMPK/mTOR). *Liu *et al* 2010 identified an increase in expression of miR-15b which did not significantly differ from controls.

Table 1.4. Validated microRNA targets in neuropathic pain models				
miRNA	**NP Model**	**Expression with NP**	**Tissue**	**Validated targets**
miR-15b	Oxaliplatin-induced in rat	↑[3]	DRG	$BACE_1$[3]
miR-338	CRPS in human	↓ [99]	Exosomes	IL-6[99]

Summary of literature validating microRNA and downstream targets in oxaliplatin-induced and complex regional pain syndrome (CRPS) neuropathic pain (NP) models. Tissues studied included dorsal root ganglion (DRG) and exosomes from plasma. β-site amyloid precursor protein-cleaving enzyme 1 ($BACE_1$) and proinflammatory cytokine interleukin 6 (IL-6) were identified as targets.

Though limited information exists regarding the role of miR-15b and miR-338 in SCI-related neuropathic pain, existing literature in both neuroinflammatory and neuropathic pain models suggest they target the NF-κB signaling pathway. In Alzheimer's disease (AD) models of neuroinflammation, both miRNAs have known overlapping targets of the β-site amyloid precursor protein-cleaving enzyme 1 ($BACE_1$)/NF-κB signaling pathway.[96,98] $BACE_1$ cleaves amyloid precursor protein (APP) and generates amyloid-β (Aβ).[96] Aβ activates NF-κB and the inflammatory cascade including production of proinflammatory cytokines.[96] miR-15b and miR-338 inhibit $BACE_1$ and the subsequent downstream activation of the inflammatory effects of the NF-κB signaling pathway. Inhibition of the $BACE_1$/NF-κB signaling pathway could thus reduce the expression of mediators producing central sensitization and mitigate neuropathic pain.

miR-15b can directly inhibit $BACE_1$ and NF-κB, and subsequent production of APP, Aβ, and proinflammatory cytokines in a human-derived neuronal cell line.[96] Interestingly, miR-15b was also found to directly target inhibitor of NF-κB (IκB) kinase-α (IKK-α).[96] Thus miR-15b could produce both direct inhibition of NF-κB, and indirect activation of NF-κB through inhibition of its inhibitor, IKK-α.[96] There is lack of clarity in the literature regarding the role of miR-15b in the development of neuropathic pain. Consistent with previous studies in models of neuroinflammation, miR-15b was found to be upregulated in rats with neuropathic pain, and to produce $BACE_1$ inhibition.[3] However, contradictory to the expected reduction of neuropathic pain due to inflammation, inhibition of $BACE_1$ was found to be associated with the development of

neuropathic pain.[3] One potential explanation for this incongruous finding is that IKK-α or other downstream targets of miR-15b were not assessed. It is possible that differential expression of other mediators of the NF-κB signaling pathway contributed to the induction of neuropathic pain in their model.

The role of miR-388 in a rodent Alzheimer's Disease model mirrors previous findings, identifying inhibition of $BACE_1$ by miR-338, and downregulation of expression of miR-388 in the hippocampus.[98] The downregulation of miR-388 in Alzheimer's disease was found to be associated with elevated $BACE_1$ expression, and over-expression of miR-388 demonstrated neuroprotective effects through inhibition of $BACE_1$ and its downstream target NF-κB.[98] Additionally, IL-6, a proinflammatory cytokine and downstream target of the NF-κB signaling pathway, was identified as a validated target of miR-338 in a neuropathic pain model.[99] Given these findings, miR-15b and miR-338 are promising potential biomarkers of neuroinflammation and neuropathic pain in individuals with SCI, through potential alteration of NF-κB pathway signaling.

1.4 Exercise as a Modulator of Neuropathic Pain

Exercise Reduces Neuroinflammation and Pain

The effects of neuroinflammation on sensory neurons is well-established, but exercise also produces known effects on synaptic activity. Exercise also stimulates a host of molecular and cellular changes that have been shown to produce cellular changes in the dorsal root ganglia (DRG).[11] Exercise promotes expression of neurotrophic factors in the DRG, including brain derived

neurotrophic factor (BDNF), nerve growth factor, growth associated protein-43 and neurotrophin-3 (Figure 1.6).[11]

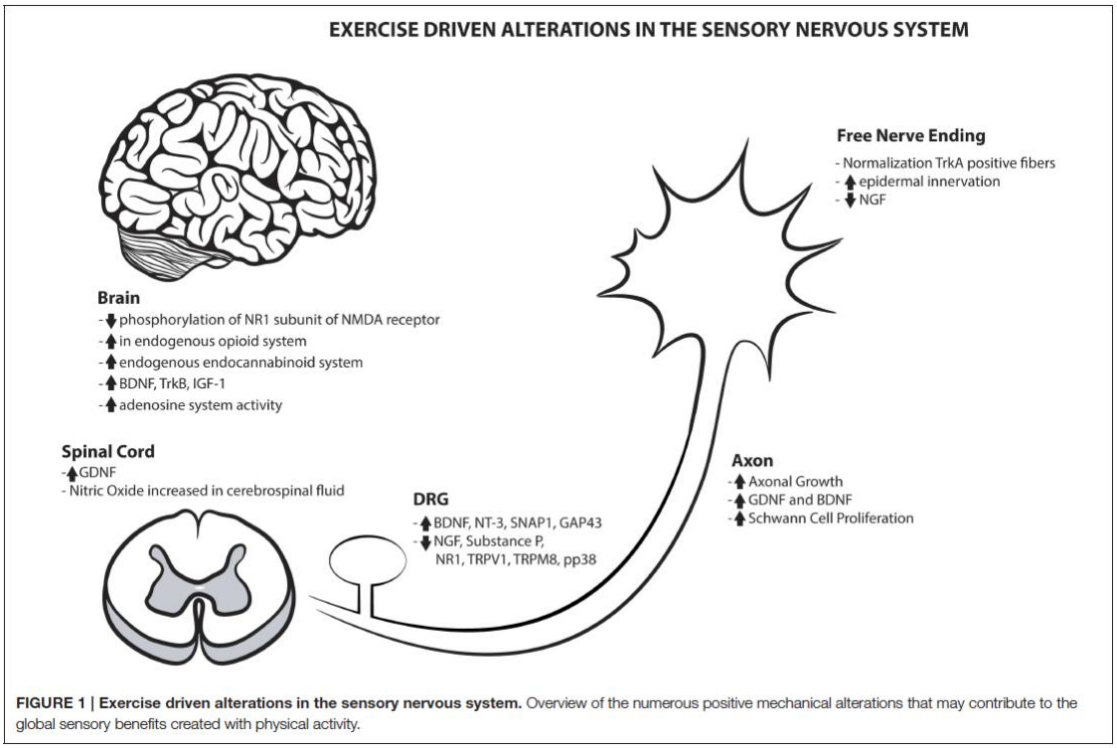


Figure 1.3. Exercise drives alterations in the sensory nervous system through modulation of many factors and signaling pathways, including those involved in neuroinflammation.[11] Unmodified figure utilized from Cooper *et al* 2016 under Creative Commons Attribution License (CC BY).

Increased expression of neurotrophic signaling may explain why exercise interventions can improve neurogenesis and recovery after neurotrauma. In rodent SCI models, exercise stimulates gene expression changes that promote neuroprotective pathways including neurogenesis and remodeling.[100] Exercise also generates a robust anti-inflammatory signaling cascade and secretion of anti-inflammatory cytokines, which can subsequently decrease neuroinflammation based pain (Figure 1.7).[11]

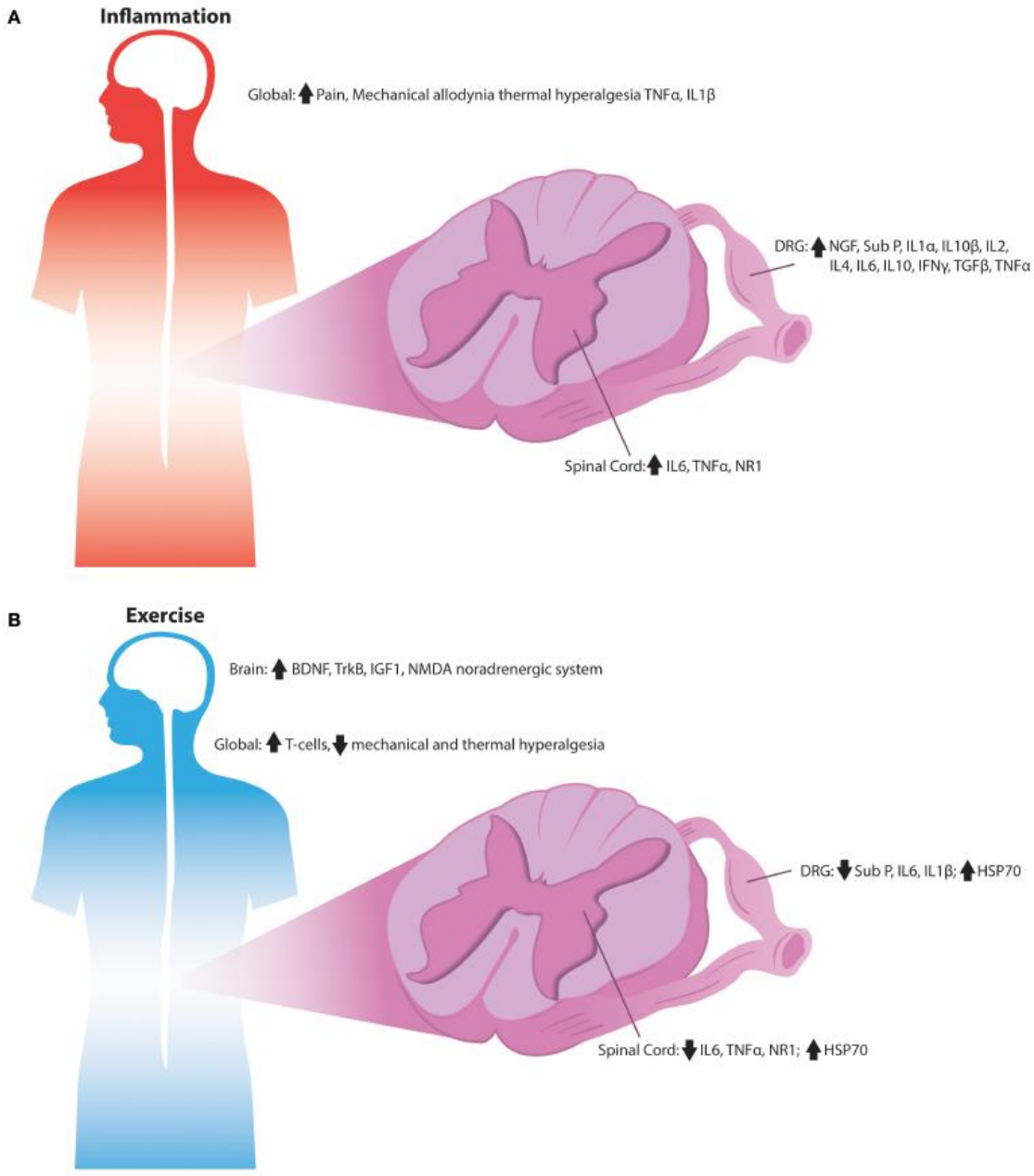


Figure 1.4. Exercise promotes anti-inflammatory signaling and expression, which can counter expression of proinflammatory signaling and expression and resultant neuropathies of sensation. Unmodified figure utilized from Cooper et al 2016 under Creative Commons Attribution License (CC BY).

Exercise has been shown to modify the NF-κB signaling pathway in neuroinflammatory disorders.[101-103] In a rodent traumatic brain injury model, 3 weeks of treadmill training produced neuroprotective effects by inhibiting expression of proinflammatory cytokines and genes, and stimulating production of anti-inflammatory factors.[101] Similarly, in a rodent Parkinson's disease model, 8 weeks of treadmill exercise produced a neuroprotective effect by down-regulating expression of toll-like receptor 2 and subsequently reduced NF-κB signaling.[102] NF-κB regulation was also elicited by 8 weeks of swimming training in a rodent model of Alzheimer's disease, which was found to mitigate neuroinflammatory responses and memory impairments.[103]

The link between neuroinflammation, neuropathic pain, and exercise has also been explored in rodent SCI models. Neuropathic pain after SCI was shown to be associated with increased microglial activation and macrophage presence in the dorsal root ganglion (DRG)[44], and increased density and abnormal distribution of afferent nerve fibers.[104] Early wheel-walking exercise after SCI in rats reduced the number of macrophages[44], aberrant c-fiber sprouting in the DRG, and prevented the onset of pain development.[104] Similar beneficial effects of exercise have been identified in humans with SCI and chronic pain. A 10 week double-poling ergometer exercise program improved both neuropathic and nociceptive pain in individuals with SCI.[13] Post-intervention, median pain intensity rating reduced by 2 points on the numeric rating scale for those with neuropathic pain, and by 4 points for those with nociceptive pain.[13] A virtual walking intervention also reduced neuropathic pain symptoms after SCI.[105]

Exercise Modulates microRNA Expression

Exercise has been shown to modulate the expression of a wide variety of miRNAs.[106] Two miRNAs of particular interest are miR-15b and miR-338. As these miRNAs are implicated in the regulation of the NF-κB signaling pathway, they are promising targets for exercise-induced reduction of neuroinflammation and neuropathic pain in individuals with SCI.[96,98] A summary of literature reporting exercise-induced expression alterations in miR-15b and miR-338 can be found in Table 1.5.

Table 1.5. Validated effects of exercise on microRNA expression

microRNA	Model	Injury model	Expression after exercise	Exercise type	Exercise dose
miR-15b	Rat	SCI	↓ in spinal cord	Passive cycling	30 min, 2x/day, 5 days/week for 10 or 20 days[12]
miR-338	Human	Healthy	↑ in: 1. plasma[107] 2. peripheral blood mono-nuclear cells[108] 3. natural killer cells[109] 4. monocytes[110]	Cycle ergometer	Single session of: 1. 60 min at 65% Pmax[107] 2. 10x 2 min bouts at 76% VO^2 peak[108] 3. 10x 2 min bouts at 77% VO^2 max[109] 4. 10x 2 min bouts at 82% VO^2 max[110]

Summary of the literature identifying effects of exercise on miR-15b and miR-388 expression in rodent SCI and healthy human populations. Abbreviations: maximal power (Pmax), peak oxygen uptake (VO^2 peak), maximal oxygen uptake (VO^2 max).

The inverse relationship between the expression of miR-15b and miR-338 in neuropathic pain and exercise models suggests exercise could normalize their expression and subsequently reduce neuropathic pain (Table 1.6). As such, miR-15b and miR-338 could be promising biomarkers used to assess the influence of neurorehabilitation interventions on biological mechanisms of neuropathic pain.

Table 1.6. microRNA expression in pain and exercise

microRNA	Expression in neuropathic pain models	Expression in exercise models
miR-15b	↑[3]	↓[12]
miR-338	↓[99]	↑[107-110]

Exercise Impacts Brain Structure and Function

Exercise has neuroprotective effects on brain structure and function across various injury and disease models (Table 1.7). Most exercise intervention studies focus on the hippocampus, with well-established neuroprotective effects on hippocampal volume with exercise and BDNF expression.[111,112] In neurological disorders, gait may modulate regions previously identified in neuropathic pain processing. In individuals with SCI, body weight supported treadmill gait training was associated with greater post-intervention activation of primary and secondary somatosensory and cerebellar regions on fMRI.[113] Similarly, robotic-assisted gait training (RAGT) was associated with greater post-intervention activation of the sensorimotor and supplementary motor cortices, as well as enhanced functional connectivity within the motor network in individuals with traumatic brain injury.[114] Individuals with stroke demonstrated increased

activity of the bilateral primary sensorimotor cortices, caudate, and the thalamus of the affected hemisphere after partial body weight supported treadmill training.[115] Treadmill training was also associated with functional connectivity of the thalamus and the right superior frontal and left medial frontal gyri.[116] Additionally, mental imagery of imagined stance and locomotion activated the thalamus and basal ganglia in several studies, suggesting gait may be a successful intervention to target these regions, which are also implicated in neuropathic pain.[117,118]

Aerobic exercise interventions have also demonstrated neuroprotective effects in regions involved in neuropathic pain processing. Adults with Alzheimer's disease who participated in moderate intensity aerobic exercise at least 1 hour a day, 5 days a week, demonstrated greater volumes of the thalamus, caudate, and amygdala.[119] In healthy older adults, aerobic exercise was associated with increased connectivity between the sensorimotor and thalamic networks.[120] Physical activity and energy expenditure were positively associated with gray matter volume in the hippocampus, thalamus, and basal ganglia in older adults.[112] Adults with MS demonstrated an association between cardiorespiratory fitness (VO_2 max) and volumes of the basal ganglia (caudate, putamen, pallidum), and hippocampus.[121] Low impact activity may also produce changes in brain activity and reduce neuroinflammation. Individuals with knee osteoarthritis who completed either Tai Chi, Baduanjin, or stationary cycling demonstrated decreased functional connectivity of the prefrontal cortex (PFC) and supplementary motor area (SMA) and increased functional connectivity

between the PFC and anterior cingulate cortex compared to controls who did not complete an exercise intervention.[122] PFC and SMA connectivity was found to significantly negatively correlate with baseline pain outcome scores, and be associated with serum levels of programmed cell death protein-1.[122]

Table 1.7. Summary of MRI findings across models		
Brain region	**MRI metrics in pain models**	**MRI metrics in exercise models**
S1	↓ volume[6,49]	↑ activity[113-115]
M1	↑ volume[6]	↑ activity[114,115]
Thalamus	↓ volume[6]	↑ volume[112], ↑ activity[115], ↑ connectivity to frontal lobe[116,120]
ACC	↑ volume[6]	↑ connectivity to PFC[123]
PFC	↑ to connectivity to angular gyrus[8]	↓ connectivity to SMA, ↑ connectivity to ACC[123]
Caudate	↓ volume[63]	↑ volume[112,115,121], ↑ activity[101]

Summary of findings of magnetic resonance imaging (MRI) studies assessing the effects of neuropathic pain and exercise on brain structure and functional activity and connectivity. Primary somatosensory (S1) and motor (M1) cortices, anterior cingulate cortex (ACC), prefrontal cortex (PFC), and supplementary motor area (SMA).

1.5 Summary

Prolonged pathologic neuroinflammation after SCI may contribute to the development of neuropathic pain at the molecular, cellular, and brain structural and functional level.[45] One neuroinflammatory pathway that could be a promising target for modulation of neuropathic pain is the $BACE_1$ / NF-κB signaling pathway. Evidence supports the altered expression of miRNA mediators of this pathway, miR-15b and miR-338, in both neuroinflammatory and neuropathic pain models.[3,96,98] These miRNA are of particular interest due to the ability of exercise to induce changes in their expression in an inverse direction of their differential

expression demonstrated in neuropathic pain models (Table 1.8). These findings suggest that exercise could promote normalization of expression levels of miR-15b and miR-338, subsequently reducing BACE1 / NF-κB signaling, and reducing neuroinflammation and neuropathic pain. Given the potential of miR-15b and miR-338 to predict biological changes associated with both neuropathic pain and rehabilitative exercise interventions, validation of these preclinical biomarkers in human populations with SCI is warranted.

Table 1.8. Summary of microRNA expression across models

microRNA	Expression in neuroinflammation models	Expression in neuropathic pain models	Expression in exercise models
miR-15b	↑[12,95]	↑[3]	↓[12]
miR-338	↓[97,98]	↓[99]	↑[107-110]

Existing evidence also suggests exercise modulates neuropathic pain signaling within the brain in regions with roles in both movement production and pain processing (Table 1.9).[6,63,112101,102] As the neuroplastic effects in these regions demonstrate opposite relationships between neuropathic pain and exercise intervention models, it is possible that exercise reduces pain by impacting the structure and activity of these regions. In particular, the demonstrated trophic effects of exercise on thalamus[112] and caudate volume[112,115,121], which is decreased in neuropathic pain models[6,63], could be especially promising. Gait training may be a particularly salient and effective exercise intervention to explore for individuals with SCI-related neuropathic pain as the thalamus and caudate hold known roles in gait function.[124-127]

Table 1.9. Summary of MRI findings across models		
Brain region	**MRI outcomes in neuropathic pain models**	**MRI outcomes in exercise models**
S1	↓ volume[649]	↑ activity[113-115]
Thalamus	↓ volume[6]	↑ volume[112], ↑ activity[115], ↑ connectivity to frontal lobe[116,120]
Caudate	↓ volume[63]	↑ volume[112,115,121], ↑ activity[101]

Magnetic resonance imaging (MRI), primary somatosensory cortex (S1).

1.6 Conclusions

The negative and widespread impacts of neuropathic pain on health and well-being, and the limited efficacy of current interventions establish the need for elucidation of novel biomarkers and potential targets for treatment. Identification of miRNA and neuroimaging biomarkers of neuropathic pain in individuals with SCI could promote more targeted and efficacious interventions. Promising potential biomarkers include miR-338 and miR-15b due to their role in neuroinflammation and differential expression in neuropathic pain models. Brain regions of interest additionally include the primary somatosensory cortex, thalamus, and caudate due to established involvement in neuropathic pain processing. As exercise has been shown to modulate and mitigate the altered states of these miRNAs and brain regions in neuropathic pain models, they hold potential as biomarkers which could guide the optimization of neurorehabilitation interventions for neuropathic pain after SCI.

1.7 Aims and Hypotheses

This dissertation will build upon the existing knowledge in neuroinflammatory and maladaptive neuroplastic associations with neuropathic pain, and the neuroprotective effects of exercise, to address the following limitations in translational science : 1) validation of preclinical miRNA biomarkers of neuropathic pain in a clinical population with SCI, 2) identification of brain regions demonstrating resting state functional connectivity alterations associated with neuropathic pain after SCI, and 3) determine the effects of a robotic-assisted gait training intervention on neuropathic pain and brain structure in individuals with SCI.

Aim 1 (Chapter 2): Determine the association between miRNA levels involved in neuroinflammatory pathways and neuropathic pain severity in individuals with SCI.

> **Hypothesis 1:** Lower expression of miR-338 and higher expression of miR-15b will be associated with neuropathic pain in individuals with SCI, consistent with findings from preclinical studies.

Aim 2 (Chapter 3): Identify resting state functional connectivity alterations associated with neuropathic and nociceptive pain phenotypes in individuals with chronic SCI**.**

Hypothesis: Neuropathic and nociceptive pain will be associated with unique patterns of functional connectivity alterations in pain processing regions.

Aim 3 (Chapter 4): Assess the effects of a robotic-assisted gait training intervention on pain presence and intensity and gray matter volume in individuals with SCI.

Hypothesis 1: Participation in robotic-assisted gait training will be associated with a reduction in neuropathic pain intensity.

Hypothesis 2: Gray matter volume in pain processing regions will be increased after participation in a robotic-assisted gait training intervention.

Chapter 2: MiR-338-5p Levels and Cigarette Smoking are Associated with Neuropathic Pain Severity in Individuals with Spinal Cord Injury: Preliminary Findings from a Genome-Wide microRNA Expression Profiling Screen

This published paper in *The Journal of Physical Medicine and Rehabilitation* has been reformatted but otherwise unmodified for inclusion with author retained copyright permissions.

2.1 Introduction

Approximately 53% of individuals with spinal cord injury (SCI) develop neuropathic pain, a difficult to treat condition that negatively impacts function via interference with mood, sleep, and the ability to complete daily activities.[26,128] Neuropathic pain poses a complex challenge due to its resistance to treatment and patient intolerance of pharmacologic interventions.[30,31] Current first line strategies prescribed to treat neuropathic pain include gabapentinoids, tricyclic antidepressants, and serotonin norepinephrine reuptake inhibitors.[30,31] These primary interventions offer limited efficacy and often produce unwanted side effects and complications including somnolence, fatigue, dizziness, orthostatic hypotension, edema, and urinary retention.[30,31] Novel pharmacological interventions for neuropathic pain, which offer improved efficacy and tolerability, are greatly needed. Therefore, elucidation of the underlying pathophysiologic mechanisms that lead to the development of neuropathic pain may facilitate the

development of innovative treatment strategies and improve function and quality life for individuals with SCI.

SCI causes a state of chronic inflammation that contributes to neuronal apoptosis and necrosis of the injured tissue.[1,33,34] Neuronal apoptosis contributes to neuropathic pain development after neural injury, and mitigation of pro-apoptotic signaling molecules has been reported to alleviate allodynia in preclinical models.[129-132] This is mediated in part by microRNAs (miRNAs), small non-coding ribonucleic acids (RNAs), which bind to target messenger RNAs (mRNAs) and cause post-transcriptional inhibition or degradation of mRNAs resulting in gene silencing.[81,82] Preclinical animal model studies have identified differential expression of a number of miRNAs which mediate neuronal apoptosis signaling pathways[2,12,90,133-144] and contribute to neuropathic pain after central and peripheral nerve injury[2-5], but translation to clinical studies remains lacking. The ability to measure circulating miRNA levels in blood provides an opportunity to validate preclinical work in human populations. In addition to miRNAs, factors of sex[145-147], age[146,147], and cigarette smoke exposure[146-150] have been associated with neuropathic pain in human populations, as well as neuronal apoptosis in preclinical animal studies[151-155], and may contribute to neuropathic pain outcomes after SCI. Successful identification of the biological mechanisms and clinical factors contributing to the development of neuropathic pain after SCI could facilitate better prediction of those at risk of neuropathic pain development and identify new targets for optimized treatment interventions. Therefore, this study sought to determine whether validated miRNA mediators of neuronal

apoptosis signaling pathways in preclinical models and biologically relevant clinical variables are associated with neuropathic pain severity in individuals with SCI.

2.2 Materials and Methods

Selection and Description of Participants

We conducted a secondary analysis of data from a convenience sample of participants with SCI enrolled in one of two ongoing clinical trials assessing bone health (ClinicalTrials.gov Identifiers: NCT02533713 and NCT02946424) or in an observational study assessing surgical treatment for severe neuropathic pain. Criteria for enrollment for each study can be found in Table 2.1. For all participants, data were derived from baseline testing which occurred between 05/15/2017 and 11/05/2019. Two samples were utilized for analyses: a screening sample and a larger validation sample. Data from the screening analyses were utilized to identify differentially expressed miRNA of interest for validation in the larger sample. All analyses were conducted on de-identified data by a member of the research team not involved in data collection. The study protocol was approved by our Institutional Review Board.

Table 2.1. Study criteria	
NCT02946424	**NCT02533713**
Inclusion Criteria • 18-60 years of age • Motor complete SCI (AIS A-C) • Medically stable • Able to follow directions • Within 3 months of injury **Exclusion Criteria** • Enrollment in another clinical trial • Simvastatin contraindications • Moderate or heavy alcohol intake • Renal dysfunction • Concurrent use of drugs that cause or increase risk of myopathy with simvastatin therapy • Uncontrolled or poorly controlled diabetes • Unstable anti-coagulation treatment • History of statin treatment within the preceding year • Metabolic bone disease • Untreated thyroid disorder • Bilateral oophorectomy • Current use of medications potentially affecting bone health • Current pregnancy or lactation • Postmenopausal women with pre-existing osteoporosis • Vitamin D deficiency	**Inclusion Criteria** • ≥ 18 years of age • ≥ 3 years post-injury • Motor complete SCI (AIS A and B) or AIS C and D who use a wheelchair > 50% of the time • SCI C7-T12 • Height of 155-191 cm • Weight < 113 kg • MAS rating < 3 in bilateral lower extremities • Sufficient upper body strength to complete sit to sit transfers **Exclusion Criteria** • Enrollment in another clinical trial • Pregnancy • Symptomatic orthostatic hypotension • Active grade 2 or > pressure ulcer that could be worsened by exoskeleton device • Lower extremity contractures that interfere with device fit • Unhealed limb or pelvic bone fracture • Other neurological disease • Active treatment for epilepsy or thyroid disorders • Current use of medications potentially affecting bone health • Women with osteoporosis
Surgical Intervention Study	
Inclusion Criteria • ≥ 18 years of age • SCI-related neuropathic pain	**Exclusion Criteria:** • MRI contraindications

American Spinal Injury Association Impairment Scale (AIS), spinal cord injury (SCI), Modified Ashworth Scale (MAS), magnetic resonance imaging (MRI).

Screening Sample

An untargeted genome wide miRNA expression profile screening on banked baseline serum samples was conducted in a cohort of individuals with chronic paraplegia based on the presence of severe neuropathic pain (pain score range 8-10) versus no neuropathic pain. Participants with neuropathic pain (n = 5) were enrolled in a surgical intervention cohort study planning to undergo dorsal root entry zone lesioning surgery for alleviation of neuropathic pain. Participants without neuropathic pain (n = 4) were enrolled in a randomized, controlled clinical trial (NCT02533713).

Validation Sample

Baseline miRNA and clinical data collected from two ongoing randomized, controlled trials (NCT02533713 and NCT02946424) and the surgical intervention cohort study were utilized for validation of findings from the screening sample. A total of 89 participants with SCI were enrolled across all three studies. All participants with miRNA and clinical datasets (n = 43) were included in the validation sample. Participant data from the screening sample were included in the validation sample.

Clinical Outcome Measures

Pain presence, type (neuropathic or nociceptive), intensity, and interference with mood, sleep, and activity were assessed with the International Spinal Cord Pain Basic Dataset (ISCIPBDS). If neuropathic and/or nociceptive

pain were reported on the ISCIPBDS, the highest rated average weekly intensity of each pain type across the three worst pain problems was defined as the participants' worst neuropathic or nociceptive pain intensity. Depressive symptoms were assessed with the Patient Health Questionnaire-9 (PHQ-9). Participant intake questionnaires included demographic factors (age, sex), injury characteristics (injury duration, level of injury), medication use, and smoking history. Smoking history was classified dichotomously (ever smoker or never smoker) by a health questionnaire based on the American Thoracic Society adult respiratory disease questionnaire.[156] Ever smokers were defined as smoking one or more cigarettes a day for at least one year or 20 or more packs of cigarettes in a lifetime.[157,158] Spinal Injury Association Impairment Scale (AIS) classification was confirmed by physical exam.

Serum Collection and Processing

Blood samples were collected in EDTA tubes by venipuncture and immediately delivered to the core blood research laboratory at our facility. The samples were centrifuged for 15 min at 2600 rpm (1459 x g) at 40C and stored at -80C until batch analysis.

miRNA Bioinformatics Pipeline

Serum miRNA Next Generation sequencing was completed by LC Sciences (Houston, TX). Briefly, comprehensive miRNA/small RNA sequencing service included sample QC, library preparation, and sequencing (50

base pair sequencing, on average a minimum of 7-10 million reads per sample). Raw reads were subjected to an in-house program, ACGT101-miR (LC Sciences, Houston, Texas, USA) to remove adapter dimers, junk, low complexity, common RNA families and repeats. Subsequently, unique sequences with length in 18~26 nucleotide were mapped to specific species precursors in miRBase 21.0 by BLAST search to identify known miRNAs and novel 3p- and 5p- derived miRNAs. Length variation at both 3' and 5' ends and one mismatch inside of the sequence were allowed in the alignment. The unique sequences mapping to specific species mature miRNAs in hairpin arms were identified as known miRNAs. The unique sequences mapping to the other arm of known specific species precursor hairpin opposite to the annotated mature miRNA-containing arm were considered novel 5p- or 3p derived miRNA candidates. The remaining sequences were mapped to other selected species precursors (with the exclusion of specific species) in miRBase 21.0 by BLAST search, and the mapped pre-miRNAs were further BLASTed against the specific species genomes to determine their genomic locations. The unmapped sequences were BLASTed against the specific genomes, and the hairpin RNA structures containing sequences were predicated from the flank 80 nucleotide sequences using RNAfold software (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). The criteria for secondary structure prediction were: (1) number of nucleotides in one bulge in stem (≤12), (2) number of base pairs in the stem region of the predicted hairpin (≥16), (3) cutoff of free energy (kCal/mol ≤-15), (4) length of hairpin (up and down stems + terminal loop ≥50),

(5) length of hairpin loop (≤20), (6) number of nucleotides in one bulge in mature region (≤8), (7) number of biased errors in one bulge in mature region (≤4), (8) number of biased bulges in mature region (≤2), (9) number of errors in mature region (≤7), (10) number of base pairs in the mature region of the predicted hairpin (≥12), and (11) percent of mature in stem (≥80).

Normalization of sequence counts in each sample (or data set) was achieved by dividing the counts by a library size parameter of the corresponding sample. The library size parameter is a median value of the ratio between the counts a specific sample and a pseudo-reference sample. A count number in the pseudo-reference sample is the count geometric mean across all samples.

$$s_j = \underset{i}{median}\left(\frac{c_{ij}}{\left(\prod_{k=1}^{m} c_{ik}\right)^{1/m}} \right)$$

, where Sj is the library size parameter; cij is the count number of sequence i of sample j; m is the total number of samples involved.

Target Prediction Analysis

Target genes potentially regulated by hsa-miR-338-5p were predicted using the publicly available miRNA target database TargetScan (Release 7.2, Whitehead Institute for Biomedical Research). The predicted target false-positive rate was reduced by applying a cut-off of –0.3 in the context scores for TargetScan results. Targets with strong experimental evidence for hsa-miR-338-

5p were retrieved, following which, an extended literature search of the PubMed database was performed to further confirm neuronal apoptosis-associated validated targets.

Statistical Analyses

Normalized deep-sequencing counts from the untargeted genome wide miRNA expression profile screening were analyzed by independent t-test. Because False Discovery Rate adjusted *p*-values may be too conservative for screening studies, we determined differential expression based on log twofold concentration change and raw *p*-value < 0.05. This study is adequately powered to detect twofold differences in expression between groups.[159] Descriptive statistics include frequency counts and percentages for nominal variables, and log twofold changes, means, standard deviations, and ranges for continuous variables. Least squares regression was utilized to analyze univariate associations with neuropathic pain severity and multivariable associations between miR-338-5p expression level and neuropathic pain severity. Factors with a *p*-value of ≤ 0.10 in the univariate models or considered biologically relevant (age, sex, level of injury, AIS classification, injury duration and chronicity) were included in the multivariable models of neuropathic pain severity. Models and factors with a *p*-value of < 0.05 were considered statistically significant. Participants with missing data (n = 6 with no medication use or smoking data and 2 additional participants missing smoking data) were included in the study and all available data were used in the analyses. All analyses were

conducted using JMP version 15 (SAS Institute Inc., Cary, NC) assuming a 5% level of significance.

2.3 Results

Cohort Characteristics

Demographic data for the genome wide miRNA expression profile screening cohort (n = 9) are presented in Table 2.2. Data from a total of 43 participants with SCI (n = 28 with neuropathic pain, n = 15 without neuropathic pain) were included in analyses for subsequent validation studies (Table 2.3). Ever smokers consumed a mean of 10.71 ± 7.57 cigarettes a day (ranging 2 – 20 per day) over a span of an average of 14.88 ± 8.34 years (ranging 1 – 33 years).

Table 2.2. Characteristics of microRNA screening cohort

Variable	Neuropathic Pain (n = 5)	No Pain (n = 4)	Total (n = 9)
Demographics			
Age (years)	50.16 ± 16.95	30.14 ± 6.73	41.26 ± 16.49
Male	5 (100.0)	3 (75.0)	8 (88.9)
Injury Characteristics			
Injury duration (years)	16.63 ± 14.01	8.03 ± 6.13	12.81 ± 11.52
AIS classification • A • B	 5 (100.0) 0 (0.0)	 3 (75.0) 1 (25.0)	 8 (88.9) 1 (11.1)

Continuous variables are presented as mean ± SD and categorical variables as N (%). American Spinal Injury Association Impairment Scale (AIS).

Table 2.3. Characteristics of validation cohort			
Variable	**Neuropathic Pain (n=28)**	**No Neuropathic Pain (n=15)**	**Total (n=43)**
Demographics			
Age (years)	40.12 ± 12.77	35.91 ± 10.67	38.65 ± 12.12
Male	24 (85.7)	11 (73.3)	35 (81.4)
Nociceptive pain	18 (64.3)	10 (66.7)	28 (65.1)
Injury Characteristics			
Injury duration (years)	8.82 ± 9.07	6.67 ± 8.10	8.07 ± 8.71
Acute (≤3 months post-injury)	7 (25.0)	6 (40.0)	13 (30.2)
Tetraplegia	6 (21.4)	4 (26.7)	10 (23.3)
AIS classification • A • B • C	 20 (71.4) 4 (14.3) 4 (14.3)	 10 (66.7) 4 (26.7) 1 (6.7)	 30 (69.8) 8 (18.6) 5 (11.6)
Active Medication Use			
Opioid	8/22 (36.4)	4/15 (26.7)	12/37 (32.4)
Gabapentin	10/22 (45.5)	2/15 (13.3)	12/37 (32.4)
Spasmolytic	15/22 (68.2)	11/15 (73.3)	26/37 (70.3)
Smoking History			
Cigarettes (ever)	11/21 (52.4)	6/14 (42.9)	17/35 (48.6)
Clinical Outcome Measures (ISCIPBDS and PHQ-9)			
Worst neuropathic	5.82 ± 2.29	-	-
Worst nociceptive	3.43 ± 2.86	3.33 ± 3.06	3.40 ± 2.90
PI with activity	4.96 ± 2.90	3.90 ± 2.85	4.68 ± 2.89
PI with mood	4.00 ± 2.76	2.90 ± 2.73	3.71 ± 2.76
PI with sleep	4.89 ± 2.59	3.90 ± 2.69	4.63 ± 2.61
PHQ-9 total	7.93 ± 6.88	4.33 ± 4.17	6.67 ± 6.26

Continuous variables are presented as mean ± SD and categorical variables as N (%). Data provided includes the full sample of participants (n = 43) except for active medication use (n = 37) and smoking history (n = 35). American Spinal Injury Association Impairment Scale (AIS), International Spinal Cord Injury Basic Pain Dataset (ISCIPBDS), Pain interference (PI), Patient Health Questionnaire-9 (PHQ-9).

Differential Expression of miR-338-5p Levels

A total of 2255 unique miRNA sequences were identified from serum samples and 71 miRNAs were significantly ($p < 0.05$) up- or down-regulated in

participants with chronic paraplegia reporting severe neuropathic pain versus no pain. We selected miR-338-5p for validation based on the down-regulated expression in this subsample of participants with neuropathic pain (n = 5, mean miR-338-5p expression = 88) versus without neuropathic pain (n = 4, mean miR-338-5p expression = 187) (log twofold change = –1.09, *p* = 0.004, Figure 2.1) and its reported role in neuronal apoptosis.

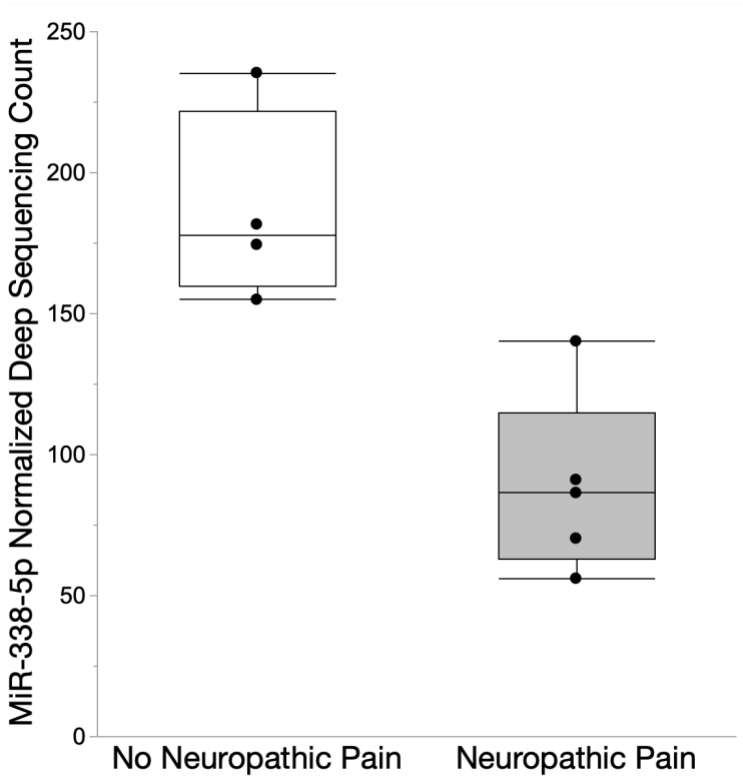


Figure 2.1. Downregulated expression of miR-338-5p in a preliminary screening subset of participants with chronic paraplegia with severe neuropathic pain (n = 5) and without neuropathic pain (n = 4).

Validated targets of miR-338-5p in preclinical models

Validated miRNA targets from preclinical studies meeting all methodological criteria for inclusion are provided in Table 2.4. Predicted targets

inhibited by miR-338-5p include proinflammatory cytokine interleukin-6 (IL-6)[99], chemokine (C-X-C motif) receptor-4 (CXC4)[160], and apoptosis facilitators Bcl-2-like 11 (BCL2L11)[161] and connective tissue growth factor (CTGF).[97]

Table 2.4. Validated targets of miR-338-5p in vitro

Target	Condition	Tissue	microRNA Effect	References
Interleukin-6 (IL-6)	Complex regional pain syndrome	Human embryonic kidney 293 cells and human monocytic leukemia cells	Anti-inflammatory	Ramanathan *et al* 2019
Bcl-2-like protein 11 (BCL2L11)	Alzheimer's disease	Mouse hippocampal neurons	Anti-apoptotic, neuroprotective	Li *et al* 2020
Connective tissue growth factor (CTGF)	Cerebral ischemia/ reperfusion injury	Mouse Neuro-2a cells	Anti-apoptotic, neuroprotective, enhanced neural proliferation	Yi *et al* 2010
Chemokine (C-X-C motif) receptor-4 (CXCR4)	Bone cancer pain	Rat bone cancer cells	Neuroprotective, inhibition of morphine tolerance	Mei *et al* 27

Univariate associations with severity of neuropathic pain

Neuropathic pain severity was negatively associated with miR-338-5p expression level (R^2 = 0.19, $F_{1,26}$ = 6.09, β = –0.03, p = 0.02). Those with paraplegia also had more severe neuropathic pain than those with tetraplegia (R^2 = 0.21, $F_{1,26}$ = 7.01, *mean difference* = 2.53, p = 0.01). Though not statistically significant in univariate analyses, additional univariate factors with a p-value of ≤ 0.10 which were subsequently included in multivariable analyses included AIS

level (R^2 = 0.19, $F_{2,25}$ = 3.02, p = 0.07) and history of cigarette smoking (R^2 = 0.13, $F_{1,19}$ = 2.89, p = 0.10). Results of all univariate analyses can be found in Table 2.5.

Table 2.5. Univariate associations with neuropathic pain severity		
Continuous Variable	**β ± SE**	***p***
miR-338-5p (unit pain score / nDSC)	-0.03 ± 0.01	0.02
Age (unit pain score / year)	0.03 ± 0.03	0.35
Injury duration (unit pain score / year)	0.06 ± 0.05	0.22
PHQ-9 total (unit pain score / unit PHQ-9 score)	0.07 ± 0.06	0.27
Categorical Variable	**LSMean Pain Score ± SE**	***p***
Sex • Male • Female	 5.63 ± 0.47 7.00 ± 1.14	0.28
Injury chronicity • Acute (within 3 months of injury) • Chronic (≥ 3 years post injury)	 4.86 ± 0.86 6.14 ± 0.49	0.20
Injury level • Paraplegia • Tetraplegia	 6.36 ± 0.44 3.83 ± 0.84	0.01
AIS classification • A • B • C	 5.45 ± 0.48 5.25 ± 1.07 8.25 ± 1.07	0.07
Active opioid use • Yes • No	 6.38 ± 0.90 5.36 ± 0.68	0.38
Active gabapentin use • Yes • No	 5.70 ± 0.82 5.75 ± 0.75	0.96
Active spasmolytic use • Yes • No	 5.33 ± 0.65 6.57 ± 0.95	0.30
Cigarette use • Ever • Never	 6.64 ± 0.75 4.80 ± 0.78	0.10

All univariate analyses utilized the sample of participants with neuropathic pain (n = 28) except for active opioid, gabapentin, and spasmolytic use (n = 22) and cigarette use (n = 21). Normalized deep sequencing count (nDSC), Patient Health Questionnaire-9 (PHQ-9), American Spinal Injury Association Impairment Scale (AIS), least squares mean (LSmean).

Multivariable predictors of neuropathic pain severity

In multivariable models, neuropathic pain severity was not associated with biologically relevant factors of injury level, AIS classification, age, sex, or injury duration when considered continuously or categorically (acute vs chronic). Neuropathic pain severity remained significantly associated with both smoking status and miR-338-5p expression levels (Table 2.5, Figure 2.2). Ever cigarette smokers had significantly greater neuropathic pain severity than never smokers (*mean difference* = 1.84, $p = 0.04$). For a 1-point expression in miR-338-5p, pain severity decreased by 0.04 ($p = 0.02$). This model explained 37% of the variation in pain severity (model $p = 0.02$). This association was restricted to neuropathic pain severity as we found no association between miR-338-5p levels and most severe nociceptive pain rating on the ISCIPBDS ($R^2 = 0.002$, $F_{1,26} = 0.06$, $\beta = -0.003$, $p = 0.81$).

Table 2.6. Multivariable associations with neuropathic pain severity		
Model $p = 0.02$, $F_{2,18} = 5.31$		
Continuous Variable	**β ± SE**	***p***
miR-338-5p (unit pain score / nDSC)	-0.04 ± 0.01	0.02
Categorical Variable	**LSMean Pain Score ± SE**	***p***
Cigarette use • Ever • Never	 6.77 ± 0.65 4.65 ± 0.69	0.04

Analyses included all participants with neuropathic pain and smoking history data (n = 21). Normalized deep sequencing count (nDSC), least squares mean (LSmean).

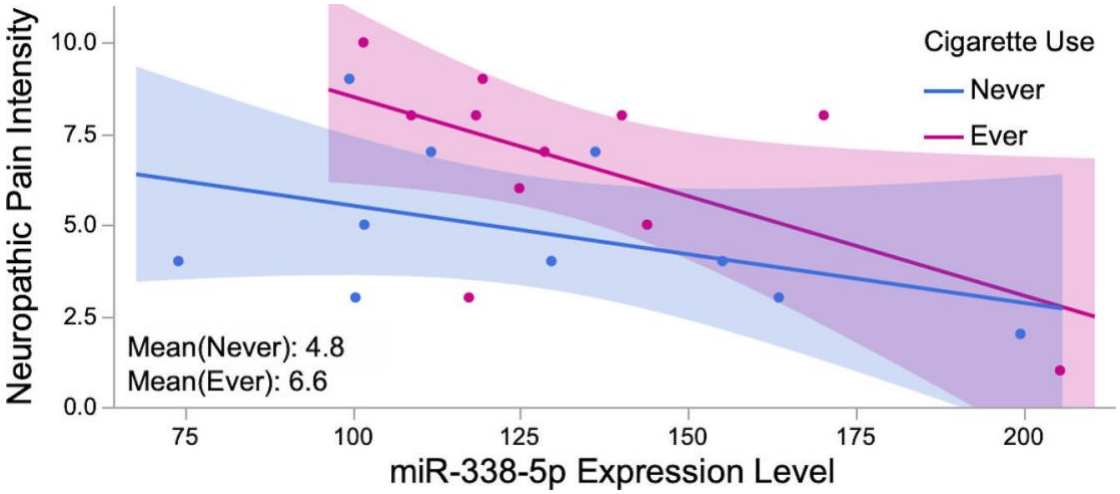


Figure 2.2. Association of neuropathic pain intensity with miR-338-5p expression level and history of cigarette use (ever/never) among individuals with spinal cord injury related neuropathic pain (n = 21).

2.4 Discussion

We aimed to investigate the association between miRNA mediators of neuronal apoptosis signaling pathways, biologically relevant clinical variables, and neuropathic pain severity in a clinical population of individuals with SCI. We identified a multivariable association between lower expression of miR-338-5p, history of ever smoking cigarettes, and greater severity of neuropathic pain in individuals with SCI. This association was restricted to neuropathic pain severity as we found no association between nociceptive pain severity and miR-338-5p levels. Our results are consistent with prior studies which identified reduced expression of miR-338-5p[99,160] or history of cigarette smoking[148-150] as influential factors in clinical and preclinical neuropathic pain models.

The link between miR-338-5p expression, cigarette smoking, and neuropathic pain may stem from their role in neuroinflammatory processes

involved in sensitization and neuronal apoptosis. MiR-338-5p is well-established as a neuroprotective, anti-apoptotic, and anti-inflammatory mediator in neurological injury and disease models.[97-99,144,161] Validated targets of miR-338-5p, IL-6 and CXC4, support the neuroprotective role of this miRNA in suppression of mediators of neuroinflammation and sensory receptor sensitization. Elevated expression of the proinflammatory cytokine IL-6 is involved in the pathogenesis of neuropathic pain after central and peripheral nerve injury through an influential role in multiple inflammatory signaling pathways.[162-165] CXC4 is a member of the G-protein coupled receptor family present in nociceptive pre- and post-synaptic sensory nerve terminals.[166,167] Its increased expression is associated with hyperalgesia and sensitization after nerve injury.[166,168] Lower expression of miR-338-5p may insufficiently suppress these pro-inflammatory mediators, subsequently increasing neuroinflammatory signaling contributing to the sensitization of sensory synapses. Cigarette smoking may additionally drive increased expression of these pro-inflammatory mediators. Current or former smoking has been positively associated with the expression of CXC4.[169] Cigarette smoking also promotes inflammation through the production of proinflammatory cytokines IL-6, interleukin-1, interleukin-8, and tumor necrosis factor-α (TNF-α).[170] Elevated levels of TNF-α have been reported in ever smokers compared to never smokers[171], which may be an underlying factor contributing to our findings of greater neuropathic pain severity in ever smokers.

Neuronal apoptosis additionally contributes to mechanisms of pain after spinal cord injury, with heightened neuronal cell death associated with

development and longer lasting symptoms of allodynia,[129,130] and mitigation of neuronal apoptosis associated with reduction of allodynia.[131,132] Prior preclinical work established miR-338-5p expression and exposure to cigarette smoke as contributors in mechanisms of neuronal apoptosis. Pro-apoptotic drivers of neuronal death, BCL2L11[172-174] and CTGF[175,176] are validated targets inhibited by miR-338-5p.[97,161] Reduction of the expression of these mediators of neuroinflammation and neuronal apoptosis by miR-338-5p may contribute to lower severity of neuropathic pain in individuals with SCI with higher expression of this neuroprotective miRNA. Studies in preclinical animal models have corroborated these findings and demonstrated that exposure to cigarette smoke, as well as the carcinogen acrolein, generated neuronal apoptosis[154,155,177,178] and heightened sensitization and neuropathic pain behaviors[179] supporting the role of smoking in neuropathic pain.

Collectively, these findings suggest that lower miR-338-5p expression and history of smoking cigarettes may worsen neuropathic pain severity after SCI. Circulating expression of miR-338-5p is a promising biomarker of SCI-related neuropathic pain. Identification and implementation of circulating biomarkers of neuropathic pain after SCI, such as miR-338-5p, could facilitate prediction of those at risk and early intervention to mitigate the severity of neuropathic pain after SCI. Circulating biomarkers of SCI-related neuropathic pain may also serve as new targets for treatment and their measurement could aid in the optimization of rehabilitation interventions. History of ever smoking cigarettes may also serve as a clinically relevant risk factor for heightened severity of neuropathic pain after

SCI. Our findings that history of smoking cigarettes is associated with higher neuropathic pain severity are consistent with a report in individuals with herpes zoster.[148] Though the number of currently active smokers in our sample was limited (n = 5), the majority of active smokers (80%) reported neuropathic pain suggesting smoking cessation programs for currently active smokers may aid in pain alleviation. Smoking cessation has been reported to reduce pain in various clinical populations and may benefit individuals with SCI as well.[180-183] Further exploration of the role of miR-338-5p expression and cigarette smoking in neuropathic pain is warranted to improve pain and quality of life outcomes for individuals with SCI.

Study Limitations

This is a preliminary study with a small sample of individuals with SCI. As this is a cross-sectional analysis, no conclusions can be made regarding causality. Additionally, several participants were missing data on smoking and medication history, and it is possible that missing data influenced our findings. Therefore, larger longitudinal studies are needed to confirm and expand our findings regarding the relationship between miR-338-5p expression, smoking, and neuropathic pain. Longitudinal studies with larger sample sizes could elucidate whether smoking cessation, smoking duration, and pack years influence miR-338-5p expression levels and neuropathic pain. Additional studies are needed to directly evaluate the relationship between neuropathic pain, miR-338-5p levels and IL-6, CXC4, BCL2L11, and CGTF expression. Despite these

limitations, this study provides important insight into potential factors associated with neuropathic pain following SCI and supports future research promoting targeted interventions.

Conclusions

Reduced expression of neuroprotective miR-388-5p and history of cigarette smoking are associated with greater severity of neuropathic pain after SCI. The specificity of the association between miR-388-5p differential expression and neuropathic pain offers a potential circulating biomarker of neuropathic pain and potential target for intervention in individuals with SCI. Our findings also suggest that smoking cessation programs should be explored as potential strategies to prevent or reduce neuropathic pain after SCI.

2.5 Addendum: Assessment of the Association Between miR-15b-3p Expression and Neuropathic Pain Severity

Preliminary analyses of miR-15b were simultaneously conducted utilizing the same samples and methodology of the above publication assessing miR-338 expression. Significantly up-regulated expression of miR-15b-3p was identified in our screening sample (n = 9) of individuals with chronic SCI with severe neuropathic pain (n = 5, mean miR-15b-3p expression = 1279) versus those without pain (mean miR-15b-3p expression = 953) (log twofold change = 0.42, $p = 0.01$). However, preliminary analyses did not identify a significant association between neuropathic pain severity and expression of miR-15b-3p ($F_{1,26} = 0.69$, $R^2 = 0.03$, $p = 0.42$) in the subset of individuals with SCI and neuropathic pain in the validation sample (n = 28). As such, the role of miR-15b-3p was not explored further in this dissertation work. However, given the upregulated expression of miR-15b-3p in the screening sample, consistent with a preclinical neuropathic pain model[3], future analyses of smaller homogeneous subsamples (e.g. only individuals with acute or chronic SCI, complete or incomplete SCI) or larger datasets should be considered to overcome potential issues of heterogeneity in the present sample. Exploration of the miR-15b-3p association with other aspects of chronic pain, such as pain interference and pain catastrophizing, or comorbidities of depression and anxiety may additionally be warranted.

Chapter 3: Resting State Functional Connectivity Differentiation of Neuropathic and Nociceptive Pain in Individuals with Chronic Spinal Cord Injury

3.1 Introduction

Chronic pain affects as many as four out of five individuals with chronic spinal cord injury (SCI) and negatively impacts participation in daily tasks, life satisfaction, and overall health and wellbeing.[27] The primary chronic pain complaints reported by individuals with chronic SCI are nociceptive (affects 49%) and neuropathic (affects 56%) in nature.[27] Nociceptive pain arises from non-neural tissues and often develops due to postural impairments and positioning, compensatory movement patterns, and musculoskeletal overuse injuries during mobility tasks such transfers and wheelchair propulsion in individuals with chronic SCI.[25,184] Nociceptive pain is typically managed with nonsteroidal anti-inflammatory drugs (NSAIDS) and modifications to reduce musculoskeletal stresses.[184] Neuropathic pain stems from neural injury and tends to be more severe in intensity and refractory to treatment in most individuals with chronic SCI.[185] Current pharmacologic treatment strategies for neuropathic pain consist of gabapentinoids, tricyclic antidepressants, and serotonin norepinephrine reuptake inhibitors, which offer limited efficacy.[30,31] Opioids are not recommended as a first-line therapy for pain after SCI, but are still commonly prescribed and often at high dosages as a last resort. Concurrent use of

benzodiazepines, sedatives, and hypnotics further increase the risk of adverse outcomes.[186]

Limitations in pain management support the need for a better understanding of brain mechanisms contributing to nociceptive and neuropathic pain processing for the development of more efficacious treatments. Prior volumetric and functional connectivity differences have been demonstrated between individuals with chronic neuropathic pain after SCI and those without pain or healthy controls, with some overlap of regions of interest, but no replication of findings across studies.[7-10] These studies did not account for the possible co-occurrence of both neuropathic and nociceptive pain or medication use as confounding factors in their analyses, which may contribute to the discrepancy in results. Similar to experimental activation of a-delta and c-fiber pain, the experience of chronic neuropathic and nociceptive pain may engage both overlapping and segregated neural systems.[187-189] Opioids and gabapentin, which are commonly used in this population for neuropathic pain management, may also limit replicability of findings as these drugs can alter resting state functional connectivity.[190-192] Considering the high prevalence of both pain types as well as pain medication use in individuals with chronic SCI, an analysis of potential segregated and combinatorial effects of pain phenotypes controlling for potential confounds of medication may reduce the variability in findings reported across neuroimaging studies.

Identification of resting state functional connectivity (rsFC) alterations associated with specific pain phenotypes could begin to isolate regions that

uniquely contribute to nociceptive and neuropathic pain experiences and guide the development of more specific and efficacious therapeutic interventions. This exploratory study aimed to identify rsFC alterations associated with neuropathic and nociceptive pain severity, the combinatorial effects of both pain types, and differences based on pain type in individuals with chronic SCI.

3.2 Methods

Subjects

This cross-sectional analysis included participants with chronic SCI who were at least three years post injury. Participants were enrolled in a clinical trial assessing changes in bone health (primary outcome) and brain connectivity (secondary outcome) in response to robotic-assisted gait training (ClinicalTrials.gov Identifier: NCT02533713) or in an observational study assessing surgical treatment for severe neuropathic pain (IRB ID 1235452-13). Criteria for enrollment for each study can be found in Table 3.1. For all participants, data were derived from baseline testing which occurred between 08/16/2017 and 04/02/2021. All analyses were conducted on de-identified data by a member of the research team not involved in data collection. The study protocols were approved by our Institutional Review Boards and all participants gave their written informed consent to participate. A total of 71 individuals with SCI were enrolled across both studies. Complete magnetic resonance imaging (MRI) and pain datasets were required for inclusion in analyses. MRI data were unobtainable in 32 participants due to incompatible implanted devices or other

magnetic resonance safety concerns, inability to self-transfer onto the scanning bed, or discontinued study participation. MRI data from 2 participants were excluded due to high levels of motion artifact. In total, 33 participants were excluded due to unobtainable (n=31) or poor-quality (n=2) MRI data resulting in a total sample of 37 included in this analysis.

Clinical Outcome Measures

Neuropathic and nociceptive pain presence and intensity were assessed with the International Spinal Cord Injury Pain Basic Dataset (ISCIPBDS).[193] The ISCIPBDS follows the International Spinal Cord Injury Pain Classification definitions to classify pain as nociceptive or neuropathic, and further classifies neuropathic pain as at-level or below-level SCI pain.[194] Participants' worst neuropathic and nociceptive pain intensities were defined as the highest rated average weekly intensity of each pain type across the three worst pain problems on the ISCIPBDS. Pain intensity was rated on a 0-10 scale, with 0 being no pain, and 10 being worst imaginable pain. Individuals who denied neuropathic and/or nociceptive pain were included in analyses with pain intensities of 0. Demographic factors (age, sex), injury characteristics (injury duration, level of injury), and pain medication use were attained from study intake questionnaires. Pain medication use was defined as current use of opioids or gabapentin. American Spinal Injury Association Impairment Scale (AIS) classification was confirmed by physical exam.

Table 3.1. Study criteria
Robotic-assisted gait training trial (NCT02533713)
Inclusion criteria • ≥ 18 years of age • ≥ 3 years post-injury • Motor complete SCI (AIS A and B) or AIS C and D who use a wheelchair > 50% of the time • SCI C7-T12 • Height of 155-191 cm • Weight < 113 kg • MAS rating < 3 in bilateral lower extremities • Sufficient upper body strength to complete sit to sit transfers **Exclusion criteria** • Enrollment in another clinical trial • Pregnancy • Symptomatic orthostatic hypotension • Active grade 2 or > pressure ulcer that could be worsened by exoskeleton device • Lower extremity contractures that interfere with device fit • Unhealed limb or pelvic bone fracture • Other neurological disease • Active treatment for epilepsy or thyroid disorders • Current use of medications potentially affecting bone health • Women with osteoporosis
Surgical intervention study (IRB ID 1235452-13)
Inclusion criteria • ≥ 18 years of age • SCI-related neuropathic pain rated at least 9/10 • Scheduled to undergo Dorsal Root Entry Zone Lesioning **Exclusion criteria:** • MRI contraindications including MRI incompatible implants, pumps, or neurostimulators

American Spinal Injury Association Impairment Scale (AIS), spinal cord injury (SCI), Modified Ashworth Scale (MAS), magnetic resonance imaging (MRI).

Neuroimaging

Data were obtained on a 3 tesla Siemens Trio using a 12-channel head coil. The scanning protocol sequences utilized for resting state functional

connectivity analysis consisted of a high resolution structural T1 image (1x1x1 mm) and two sets of 6-minute, 18 sec resting state echo planar imaging (EPI) datasets.

T1 weighted structural images were acquired with a gradient echo sequence with GRAPPA parallel imaging with an acceleration factor of 2, 256 mm field of view (FOV), 1x1x1 mm voxel size, 1 mm slice thickness, sagittal acquisition (interleaved), 20 ms echo time (TE), 4.92 ms repetition time (TR), flip angle of 25 degrees, and 5:17 scan time. Resting state functional images were acquired with an EPI sequence with a 216 mm FOV, 3x3x3 mm voxel size, 3 mm slice thickness, interleaved acquisition, 30 ms TE, 3,000 ms TR, flip angle of 85 degrees, 6:18 scan time (2 repeated scans collected in each subject).

Resting State Connectivity Analysis

All functional data was preprocessed and statistically analyzed using the CONN Toolbox (version 20b)[195], a cross-platform software operating under Statistical Parametric Mapping (SPM12)[196] and MATLAB (version R2020b, The Mathworks Inc., Natick, MA). Preprocessing was completed using default parameters, including slice timing, motion correction, spatial normalization to the Montreal Neurological Institute (MNI) template, spatial smoothing with an 8-mm Gaussian kernel, and high-pass temporal filtering (cutoff 128 s). The Artifact Detection Toolbox (ART, https://www.nitrc.org/projects/artifact_detect) was used to detect and remove frames with excessive motion (global signal value $z > 5$, interscan motion > 0.9 mm). Seed-based correlations were utilized to identify

brain regions correlated with seed regions of interest. Four linear regressions were conducted to identify alterations in rsFC associated with: 1) worst neuropathic pain intensity controlling for worst nociceptive pain severity, 2) worst nociceptive pain intensity controlling for worst neuropathic pain severity, 3) the sum of each participant's worst neuropathic and nociceptive pain intensities, and 4) the contrast of worst neuropathic and nociceptive pain intensities. All analyses controlled for current pain medication use. 25 seed regions were selected based on prior literature supporting their role in pain processing and consisted of the lower body representation of the primary motor and somatosensory cortices, anterior and posterior cingulate, insula, hippocampus, parahippocampal gyri, thalamus, amygdala, caudate, putamen, periaqueductal gray matter, and the default mode network. Right and left seeds were included for all bilateral structures. A 10-voxel threshold and significance level of $p<0.05$ after family-wise error (FWE) correction were utilized to define resulting regions where seed connectivity was significantly associated with pain ratings.

Descriptive Statistical Analyses

Descriptive statistics of demographic variables included percentages, mean, range, and standard deviation. Pearson's correlation was utilized to identify the relationship between neuropathic and nociceptive pain intensities. All analyses were conducted using JMP version 16 (SAS Institute Inc., Cary, NC) assuming a 5% level of significance. All available data for demographic variables not included as variables of interest in analyses were reported.

3.3 Results

Cohort Characteristics

Of the 37 participants with chronic SCI in our sample, 70.3% (n=26) reported neuropathic pain, 45.9% (n=17) reported nociceptive pain, and 16.2% (n=6) reported no pain. Specific pain presentations included only neuropathic pain 37.8% (n=14), only nociceptive pain 13.5% (n=5), and both neuropathic and nociceptive pain 32.4% (n=12). In those with each pain type, average neuropathic pain intensity (5.92 ± 2.35, range 2-10) was higher than average nociceptive pain intensity (4.71 ± 1.61, range 2-8). Of the 26 participants with neuropathic pain, 8 were classified as at-level and 18 as below-level SCI pain. 14 participants (37.8%) utilized opioids or gabapentin for pain management at the time of assessment. Additional demographic data (Table 3.2) and data regarding pain type, severity and location for each participant can be found in Table 3.3. For the analysis attempting to segregate neuropathic and nociceptive pain, no significant correlation between neuropathic and nociceptive pain ratings was identified in the total sample (r = 0.03, p = 0.84) (Figure 3.1). However, the subgroup of participants with both pain types demonstrated a significant positive correlation between neuropathic and nociceptive pain ratings (r = 0.63, p 0.03).

Table 3.2. Participant characteristics	
Variable	**Total (n=37)**
Demographics	
Age (years)	41.24 ± 13.48
Male	30 (81.1)
No pain	6 (16.2)
Neuropathic pain	26 (70.3)
Only neuropathic pain	14 (37.8)
Nociceptive pain	17 (45.9)
Only nociceptive pain	5 (13.5)
Neuropathic + Nociceptive Pain	12 (32.4)
Injury Characteristics	
Injury duration (years)	10.17 ± 8.05
Tetraplegia	5 (13.5)
AIS classification* • A • B • C	 26/36 (72.2) 6/36 (16.7) 4/36 (11.1)
Active Medication Use	
Opioid	9 (24.3)
Gabapentin	5 (13.5)
ISCIPBDS Pain Ratings	
Worst neuropathic	5.92 ± 2.35
Worst nociceptive	4.71 ± 1.61

Continuous variables are presented as mean ± SD and categorical variables as N (%). *Data provided includes the full sample of participants (n = 37) except for AIS classification (n = 36). American Spinal Injury Association Impairment Scale (AIS), International Spinal Cord Injury Pain Basic Dataset (ISCIPBDS).

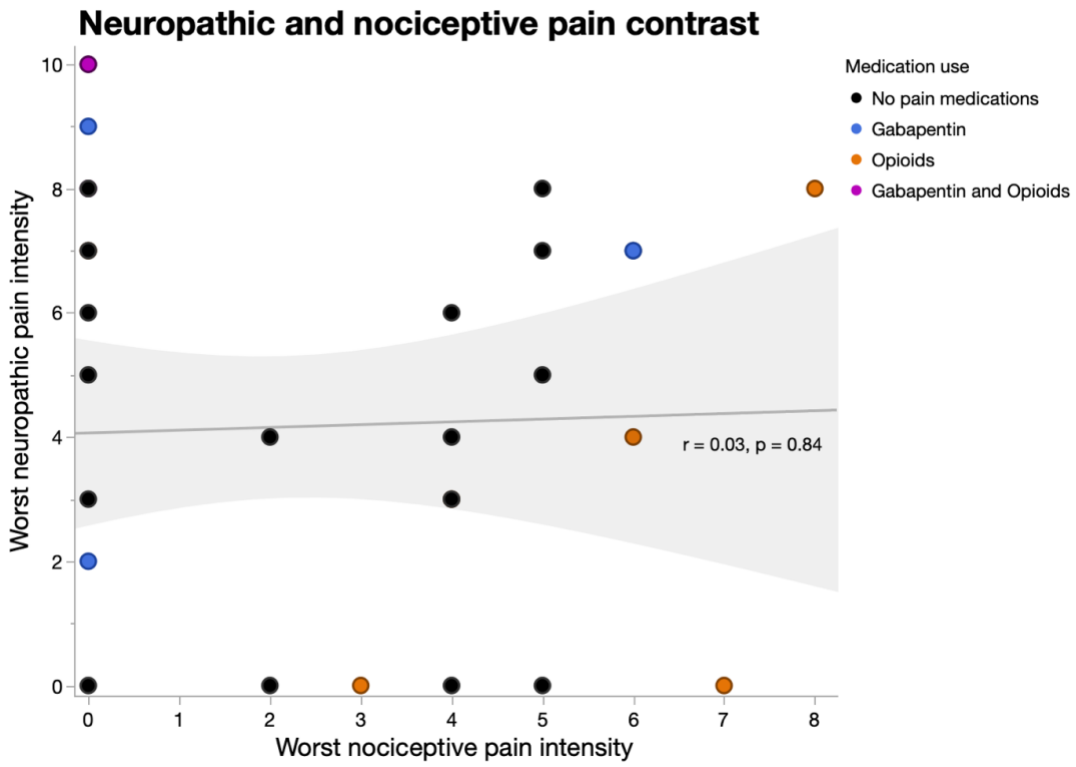


Figure 3.1. No significant correlation of neuropathic and nociceptive pain severities was identified (r = 0.03, p = 0.84) in participants with chronic spinal cord injury (n = 37). Data points reflect participants' neuropathic pain ratings plotted against their nociceptive pain ratings. Participants are defined as currently taking gabapentin (blue), opioids (orange), both medications (purple), or no medications (black).

Table 3.3. Participant pain characteristics						
ID	**NP type**	**NP rating**	**NP pain location**	**NC rating**	**NC pain location**	**Pain Sum**
1	AL	5	Chest (R, M)	0	-	5
2	BL	8	B buttocks, hips, upper legs/thighs, knees, shins, calves, ankles, feet/toes	0	-	8
3	BL	6	B feet/toes	4	B shoulders	10
4	BL	6	R abdomen	0	-	6
5	AL	7	B lower back and hips, R upper leg/thigh	0	-	7
6	BL	7	R buttocks and hip, anus (M)	0	-	7
7	BL	10	Pelvis/genitalia (B, M), upper and lower back (R, M), R buttocks and hip, anus	0	-	10
8	BL	8	L abdomen, pelvis/genitalia, upper and lower back, buttocks and hip, anus, L upper leg/thigh, knee, shin, calf, ankle, foot/toes	0	-	8
9	BL	7	Pelvis/genitalia (B, M), B hips and upper legs/thighs	0	-	7
10	-	0	-	0	-	0
11	AL	8	M upper back	8	M neck	16
12	-	0	-	0	-	0
13	AL	3	B chest	4	Upper and lower back (B, M)	7
14	AL	5	Lower back (B, M)	5	L hip	10
15	AL	3	B chest	0	-	3
16	-	0	-	0	-	0
17	-	0	-	0	-	0
18	-	0	-	4	R neck	4
19	BL	2	B upper legs/thighs, knees, shins, calves, ankles, and feet/toes	0	-	2

20	-	0	-	0	-	0
21	BL	4	R elbow	4	L chest	8
22	BL	7	B upper legs/thighs, knees, shins, calves, ankles, and feet/toes	6	M lower back	13
23	BL	7	B buttocks and hips, anus, B upper thighs, knees, shins, calves, ankles, and feet/toes	6	Abdomen (B, M)	13
24	BL	7	L buttocks and hip, anus	5	R elbow and forearm	12
25	AL	4	Lower back (R, M)	6	L upper back	10
26	BL	3	L buttocks and hip, anus	4	R neck	7
27	BL	8	R upper arm, elbow, forearm, wrist, and hand/fingers	5	L shoulder	13
28	-	0	-	2	L shoulder	2
29	-	0	-	7	B shoulders	7
30	-	0	-	5	Throat (B, M)	5
31	BL	10	B knees, shins, and calves	0	-	10
32	BL	9	B knees, shins, calves, ankles, and feet/toes	0	-	9
33	-	0	-	0	-	0
34	-	0	-	3	Neck (B, M)	3
35	BL	4	Neck (B, M), B shoulders and upper arms, chest (B, M), upper and lower back (B, M)	2	Neck (B, M), B shoulders and upper arms, upper back (B, M), lower back (M)	6
36	AL	3	Lower back (B, M)	0	-	3
37	BL	3	B buttocks	0	-	3

Pain is defined as neuropathic (NP), nociceptive (NC), at-level (AL) or below-level (BL) of spinal cord injury NP, bilateral (B), midline (M), right (R), and left (L)

Effect of Neuropathic Pain Severity

In the linear regression assessing the effect of worst neuropathic pain intensity on rsFC controlling for worst nociceptive pain severity and medication use, we identified altered rsFC between 3 seed regions and 5 clusters (Table 3.4, Figure 3.2). Greater neuropathic pain severity was significantly associated with lower connectivity between the 1) right posterior parahippocampal gyrus (pPaHC) to the right putamen and amygdala, 2) posterior cingulate cortex component of the default mode network (dmPCC) to right occipital regions, and 3) the periaqueductal gray matter (PAG) to the left angular and supramarginal gyri. Greater neuropathic pain severity was significantly associated with greater connectivity between the dmPCC and regions in the bilateral frontal lobes.

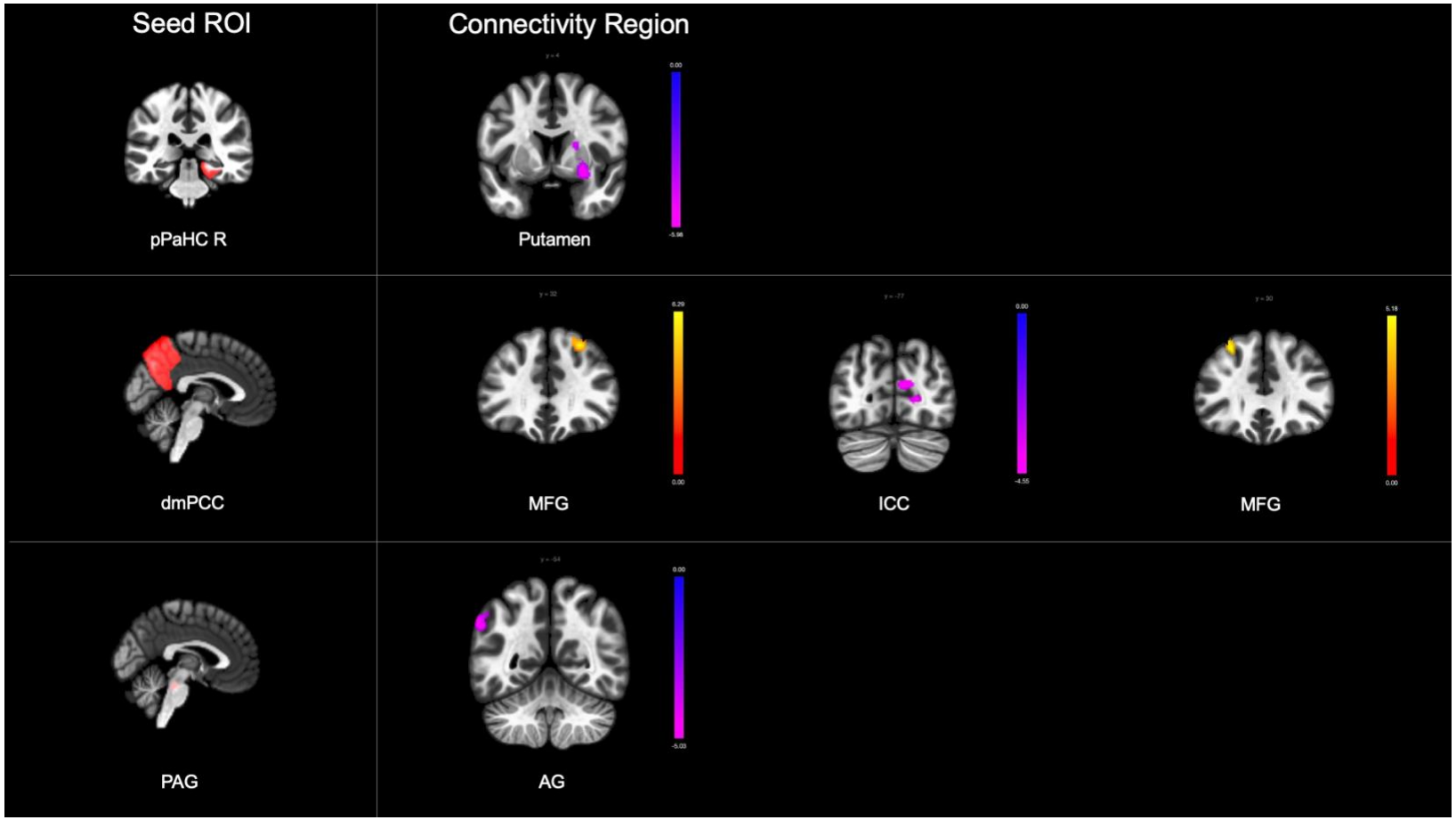


Figure 3.2. Connectivity alterations associated with neuropathic pain severity. Color bars indicate connectivity strength with warmer colors indicating greater connectivity and colder colors indicating lower connectivity. Posterior parahippocampal gyrus (pPaHC), default mode posterior cingulate cortex division (dmPCC), middle frontal gyrus (MFG), intracalcarine cortex (ICC), periaqueductal gray matter (PAG), angular gyrus (AG). Laterality indicated by right (R) or left (L).

Table 3.4. Neuropathic pain severity connectivity alterations							
Seed	**Resulting regions**	**MNI X**	**MNI Y**	**MNI Z**	**Cluster size**	**T**	**p-FWE**
Parahippocampal gyrus (posterior division, R)	R putamen and amygdala	26	4	-10	366	-6.41	0.0002
Default mode (posterior cingulate cortex division)	R frontal pole, R middle and superior frontal gyri	26	32	48	242	6.78	0.005
	R intracalcarine, cuneal, and supracalcarine cortices	22	-78	6	201	-5.30	0.01
	L superior and middle frontal gyri, L frontal pole	-28	30	48	163	6.41	0.03
Periaqueductal gray matter	L angular and supramarginal (posterior division) gyri	-58	-54	36	196	-5.18	0.008

Positive and negative T-values reflect positive and negative correlation between neuropathic pain severity and connectivity respectively. Laterality is defined as right (R) and left (L). Montreal Neurological Institute (MNI) system coordinates for resulting regions are provided.

Effect of Nociceptive Pain Severity

In the linear regression assessing the effect of worst nociceptive pain severity on rsFC controlling for worst neuropathic pain severity and medication use, we identified altered rsFC between 6 seed regions and 8 clusters (Table 3.5, Figure 3.3). Greater nociceptive pain severity was significantly associated with lower connectivity of the 1) right pPaHC to the right angular and supramarginal gyri and lateral occipital cortex, 2) right thalamus to the right hippocampus and amygdala, and temporal regions in both hemispheres, 3) left thalamus to right hippocampus, amygdala, anterior parahippocampal gyrus (aPaHC), and temporal regions, and 4) the medial prefrontal cortex component of the default mode network (dmMPFC) to the right cerebellum. Greater nociceptive pain severity was significantly associated with greater connectivity between the 1) left aPaHC to the right inferior temporal gyrus and 2) the left amygdala to the left superior parietal lobule and supramarginal gyrus.

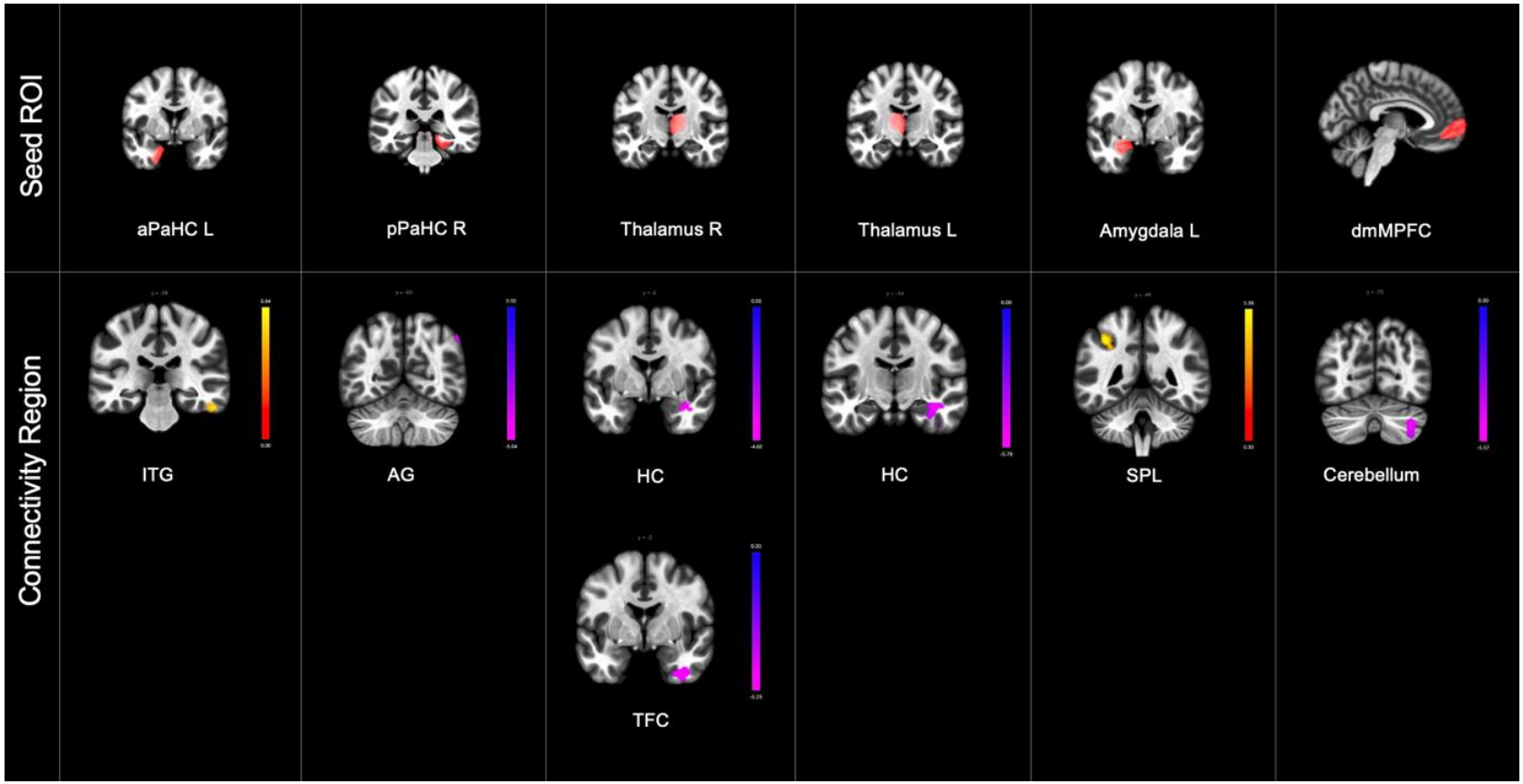


Figure 3.3. Connectivity alterations associated with nociceptive pain severity. Color bars indicate connectivity strength with warmer colors indicating greater connectivity and colder colors indicating lower connectivity. Anterior (aPaHC) and posterior parahippocampal gyrus (pPaHC), default mode medial prefrontal cortex division (dmMPFC), inferior temporal gyrus (ITG), angular gyrus (AG), hippocampus (HC), temporal fusiform cortex (TFC), superior parietal lobule (SPL). Laterality indicated by right (R) or left (L).

Table 3.5. Nociceptive pain severity connectivity alterations							
Seed	**Resulting regions**	**MNI X**	**MNI Y**	**MNI Z**	**Cluster size**	**T**	**p-FWE**
Parahippocampal gyrus (anterior division L)	Inferior temporal gyrus (posterior division R)	54	-28	-24	148	5.51	0.04
Parahippocampal gyrus (posterior division R)	R angular gyrus, lateral occipital cortex (superior division R), supramarginal gyrus (posterior division R)	54	-60	48	203	-4.73	0.009
Thalamus R	R hippocampus and amygdala	32	-2	-24	171	-5.95	0.03
	Temporal fusiform cortex (anterior and posterior divisions R), inferior temporal gyrus (anterior division R)	36	-2	-38	219	-6.15	0.01
	Temporal pole L	-20	10	-40	149	-6.04	0.046
Thalamus L	R hippocampus, temporal fusiform cortex (anterior and posterior divisions R), inferior temporal gyrus (anterior division R), amygdala R, parahippocampal gyrus (anterior division R)	38	-14	-18	379	-7.29	0.0005
Amygdala L	Superior parietal lobule L, supramarginal gyrus (anterior and posterior divisions L)	-38	-46	44	221	5.93	0.006
Default mode (medial prefrontal cortex division)	Cerebellum crus 1 and 2 R, cerebellum 7b and 8 R	36	-70	-38	180	-5.68	0.03

Positive and negative T-values reflect positive and negative correlation between nociceptive pain severity and connectivity respectively. Laterality is defined as right (R) and left (L). Montreal Neurological Institute (MNI) coordinates for resulting regions are provided

Effect of Additive Pain Severities

In the linear regression assessing the effect of the sum of each participant's neuropathic and nociceptive pain severities on rsFC controlling for medication use, we identified significantly altered rsFC between 5 seed regions and 7 clusters (Table 3.6, Figure 3.4). Higher additive pain intensities were significantly associated with lower connectivity between 1) the left aPaHC to the left middle and superior temporal gyri, 2) the right pPaHC to the right putamen, amygdala, angular and supramarginal gyri, and lateral occipital cortex, and 3) the PAG to the left angular and supramarginal gyri. Higher additive pain intensities were significantly associated with greater connectivity between the 1) anterior cingulate to the left lateral occipital cortex, 2) left amygdala to the left lateral occipital cortex, inferior and middle temporal gyri, superior parietal lobule, and supramarginal gyrus.

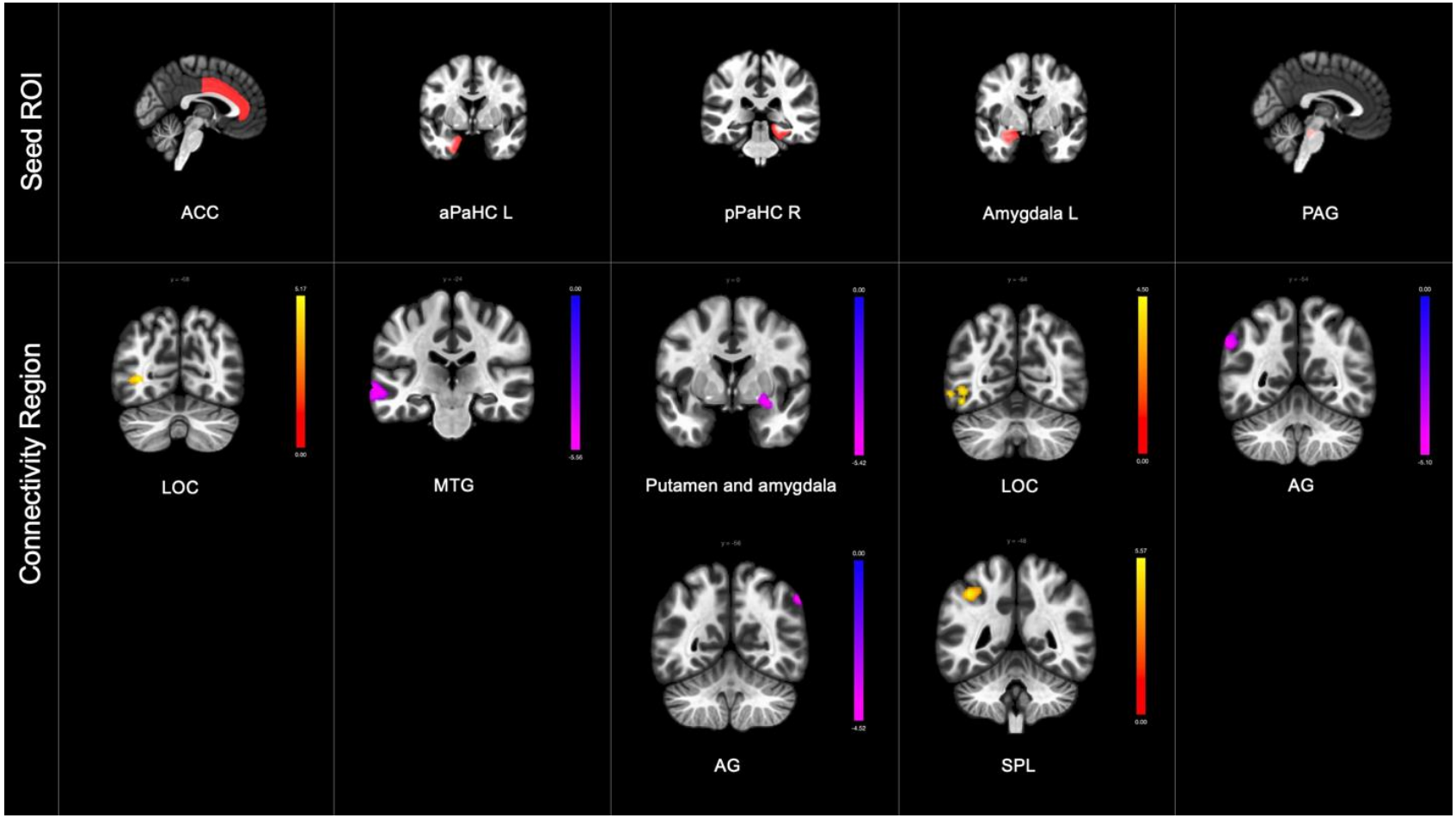


Figure 3.4. Connectivity alterations associated with additive neuropathic and nociceptive pain severities. Color bars indicate connectivity strength with warmer colors indicating greater connectivity and colder colors indicating lower connectivity. Anterior cingulate cortex (ACC), anterior (aPaHC) and posterior parahippocampal gyrus (pPaHC), periaqueductal gray matter (PAG), lateral occipital cortex (LOC), middle temporal gyrus (MTG), angular gyrus (AG), superior parietal lobule (SPL). Laterality indicated by right (R) or left (L).

Table 3.6. Additive pain severity connectivity alterations							
Seed	**Resulting regions**	**MNI X**	**MNI Y**	**MNI Z**	**Cluster size**	**T**	**p-FWE**
Cingulate gyrus (anterior division)	Lateral occipital cortex (inferior division L)	-36	-68	4	189	5.13	0.02
Parahippocampal gyrus (anterior division L)	Middle temporal gyrus (posterior division L), superior temporal gyrus (posterior division L)	-64	-24	-4	228	-6.00	0.006
Parahippocampal gyrus (posterior division R)	R putamen and amygdala	30	0	-14	363	-6.19	0.0003
	Angular gyrus R, lateral occipital cortex (superior division R), supramarginal gyrus (posterior division R)	56	-56	44	145	-4.42	0.04
Amygdala L	Lateral occipital cortex (inferior division L), Inferior and middle temporal (temporoocipital part L) gyri	-46	-64	-12	270	5.59	0.002
	Superior parietal lobule L, supramarginal gyrus (posterior and anterior divisions L)	-40	-48	44	175	5.38	0.02
Periaqueductal gray matter	L angular and supramarginal (posterior division L) gyri	-58	-54	36	162	-4.89	0.02

Positive and negative T-values reflect positive and negative correlation between overall pain severity and connectivity respectively. Laterality is defined as right (R) and left (L). Montreal Neurological Institute (MNI) coordinates for resulting regions are provided.

Contrast of Neuropathic and Nociceptive Pain Severities

In the linear regression assessing the difference between neuropathic and nociceptive pain severities on rsFC controlling for medication use, we identified opposing rsFC relationships between 3 seed regions and 5 clusters (Table 3.7, Figure 3.5). Connectivity was negatively correlated with neuropathic pain severity and positively correlated with nociceptive pain severity between the 1) posterior cingulate to bilateral occipital regions, right lingual gyrus, and left cerebellum, and 2) dmPCC to bilateral occipital regions (Figure 3.6 A and B). Connectivity was positively correlated with worst neuropathic pain severity and negatively correlated with worst nociceptive pain severity between the 1) posterior cingulate to the right frontal pole and superior and middle frontal gyri, 2) right thalamus to right temporal regions, and 3) dmPCC to the right frontal pole and superior and middle frontal gyri (Figure 3.6 C-E).

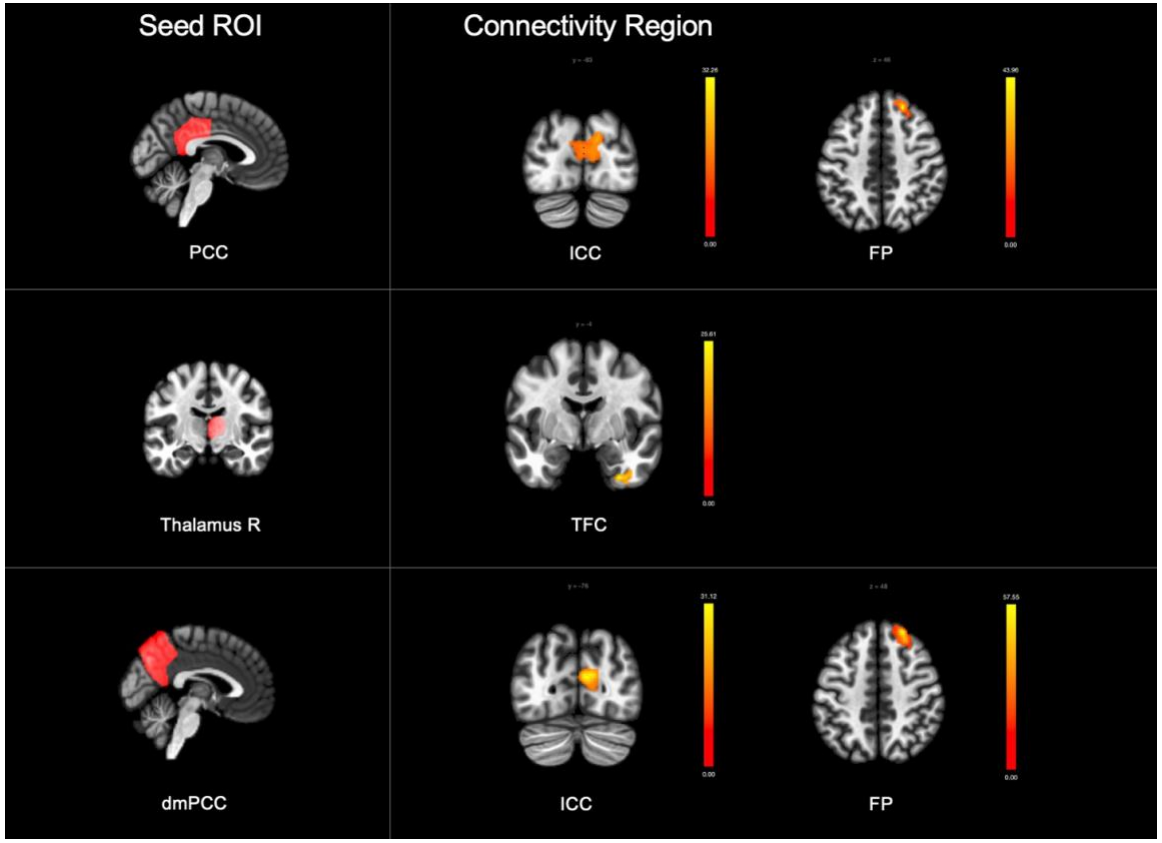


Figure 3.5. Connectivity alterations associated with contrast of neuropathic and nociceptive pain severities. Color bars indicate connectivity strength with warmer colors indicating greater connectivity associated with higher neuropathic pain and lower nociceptive pain intensities and colder colors indicating lower connectivity associated with higher neuropathic and lower nociceptive pain intensities. Posterior cingulate cortex (PCC), intracalcarine cortex (ICC), frontal pole (FP), temporal fusiform cortex (TFC), default mode network posterior cingulate cortex division (dmPCC). Laterality indicated by right (R) or left (L).

Table 3.7. Contrast of pain severities connectivity alterations							
Seed	**Resulting regions**	**MNI X**	**MNI Y**	**MNI Z**	**Cluster size**	**T**	**p-FWE**
Cingulate gyrus (posterior division)	Intracalcarine cortex R and L, lingual gyrus R and L, cuneal cortex R and L, supracalcarine cortex R and L, cerebellum 6 R, occipital pole L	14	-74	18	1422	-5.39	<0.000001
	Frontal pole R, superior and middle frontal gyri	20	36	46	194	6.37	0.02
Thalamus R	Temporal fusiform cortex (anterior and posterior division R), inferior temporal gyrus (anterior division R), R temporal pole	30	-4	-40	182	5.80	0.02
Default mode (posterior cingulate cortex division)	Intracalcarine cortex R, cuneal cortex R and L, supracalcarine cortex R	6	-76	16	509	-5.37	0.00002
	R frontal pole, superior and middle frontal gyri	22	40	48	348	7.60	0.0004

Positive T-values reflect positive and negative correlation between neuropathic and nociceptive pain severity and connectivity respectively. Negative T-values reflect negative and positive correlation between neuropathic and nociceptive pain severity and connectivity respectively. Laterality is defined as right (R) and left (L). Montreal Neurological Institute (MNI) coordinates for resulting regions are provided.

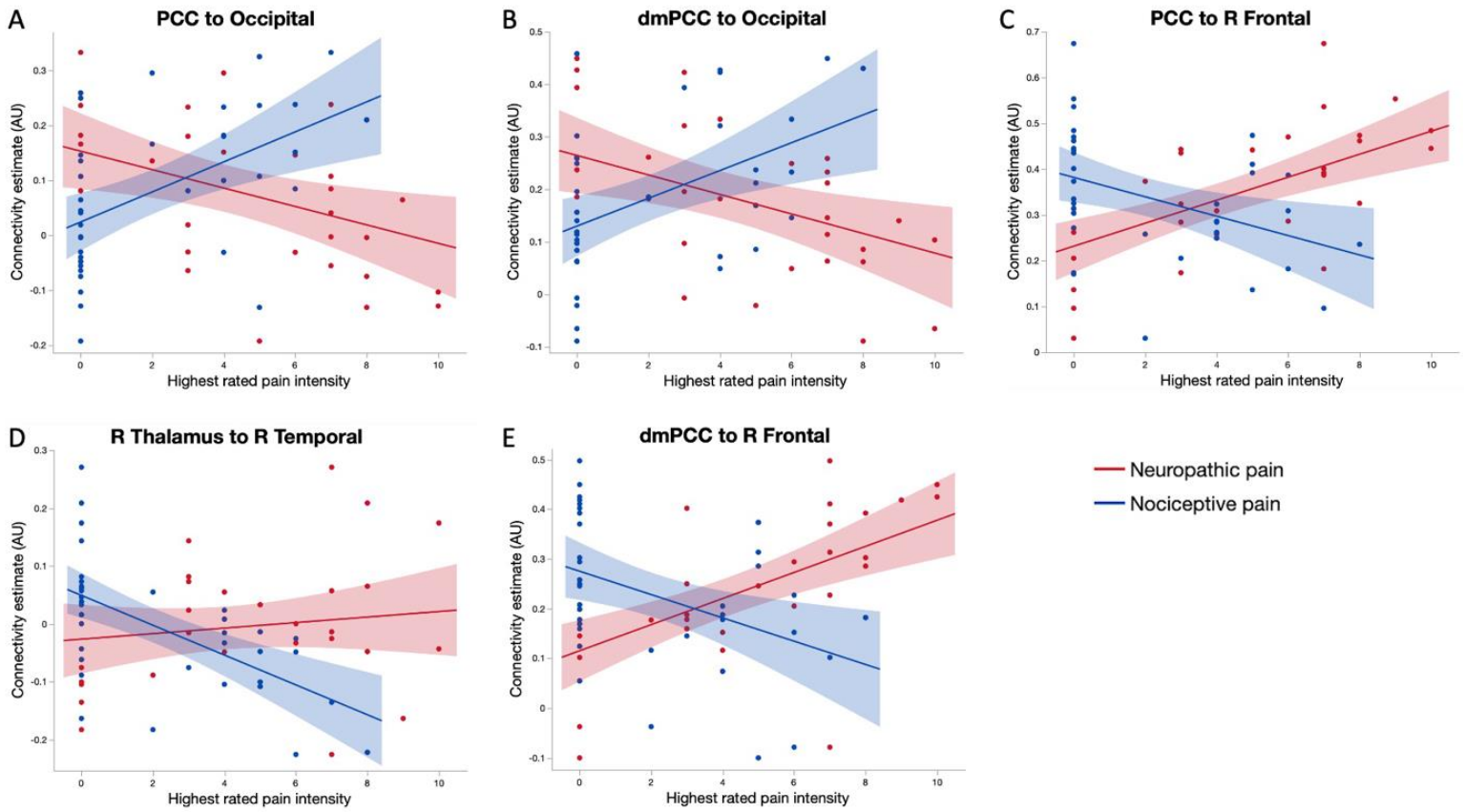


Figure 3.6. Differences in connectivity alterations associated with neuropathic (red) and nociceptive (blue) pain intensities identified between A) the posterior cingulate (PCC) to the bilateral occipital lobes, B) default mode posterior cingulate cortex division (dmPCC) to the bilateral occipital lobes, C) PCC to right frontal lobe, D) right thalamus to right temporal lobe, E) dmPCC to right frontal lobe.

3.4 Discussion

The present study represents a first attempt at segregating the experience of neuropathic and nociceptive pain in terms of alterations in resting state networks. To our knowledge, this is the first report of rsFC alterations associated with neuropathic and nociceptive pain phenotypes in individuals with chronic SCI, or any other clinical population. Our findings support the existence of rsFC alterations uniquely associated with pain type and severity involving regions in the limbic system, striatum, medial and lateral pain pathways, and default mode network.

Limbic System Connectivity Alterations

Across all analyses, most functional connectivity alterations involved limbic system structures. Lower intralimbic connectivity alterations between the right pPaHC and amygdala were restricted to the neuropathic and additive pain analyses. This suggests altered intralimbic connectivity may be unique to neuropathic pain, which likely drives the replicated findings in additive analyses. Prior studies suggest the parahippocampal gyrus and amygdala contribute to psychological and emotional regulation of pain processing[50-52] and sensitivity[51,52,197], and have been implicated in other populations with neuralgia.[198-200] A previously identified negative correlation between neuropathic pain severity in individuals with SCI and mean diffusivity of the amygdala suggest microstructural changes could contribute to our findings of altered amygdala connectivity.[59]

Limbic to striatal functional connectivity alterations may also be unique to neuropathic pain. As a negative association between neuropathic pain severity and connectivity of the right pPaHC to putamen was identified only in neuropathic and additive pain analyses, neuropathic pain may drive the overlapping findings in additive pain analyses. The striatum is involved with production of movement, reward, and multisensory integration of noxious and non-noxious stimuli.[201,202] Alterations in R2* signal, reflective of iron content, in the parahippocampal gyrus and basal ganglia were previously identified in individuals with SCI with neuropathic pain [203], further supporting the role of these regions in neuropathic pain processing.

Altered connectivity from the limbic system was also identified in regions encompassing the parietal, temporal, occipital, and frontal lobes, and cerebellum. Limbic to parietal connectivity alterations were restricted to and consistent across nociceptive and additive analyses, suggesting nociceptive pain likely drives the replicated connectivity alterations in additive analyses. Pain intensity and functional connectivity were negatively correlated in the right pPaHC gyrus to the right angular and supramarginal gyri, and positively correlated in the left amygdala to the left supramarginal gyrus and superior parietal lobule. The angular and supramarginal gyri hold roles in multisensory integration[78] and regulating emotional and empathetic responses to pain.[51,204] Prior studies also implicated the parietal lobe in pain experience after SCI, with greater pain intensity positively associated with mean diffusivity of the parietal cortex[59] and rsFC of the superior parietal lobule to the angular gyrus.[8]

Limbic to temporal and occipital lobe connectivity alterations were identified across multiple analyses. Temporal and occipital lobe involvement in pain, though poorly understood, has previously been identified in SCI populations. Volumetric differences of the temporal and occipital lobes [58,203], as well temporal functional connectivity[9] and metabolic[205] alterations have been reported in individuals with neuropathic pain after SCI, supporting their involvement in pain experience in this population.

Limbic to frontal lobe and cerebellar connectivity was differentially associated with pain type, and specifically involved the posterior cingulate. Posterior cingulate to frontal lobe connectivity demonstrated positive and negative associations with neuropathic and nociceptive pain intensities respectively. The posterior cingulate is a component of both the limbic system and the default mode network and holds a well-established role in pain experience.[206] The frontal lobe is involved in planning and execution of movement, executive function, and pain processing.[207] Neuropathic pain in individuals with SCI has been previously associated with differences in mean diffusivity[59], gray matter volume (GMV)[203] and altered rsFC[8] of the frontal lobe. Posterior cingulate to cerebellar connectivity demonstrated negative and positive correlations with neuropathic and nociceptive pain severity respectively. The cerebellum also holds a known but poorly understood role in pain processing[189] and has been previously implicated in neuropathic pain after SCI through reduced GMV[203] and altered rsFC of the cerebellum[9] in those with neuropathic pain after SCI. The identification of differential posterior cingulate to frontal and

cerebellar connectivity in our sample suggests these regions may uniquely contribute to the modulation of both pain phenotypes.

Ascending and Descending Pain Pathway Alterations

The ascending medial and lateral pain pathways contribute to the affective and sensory aspects of pain processing, while the descending pain pathway modulates pain inhibition. Both ascending and descending pathways demonstrated functional connectivity alterations uniquely associated with nociceptive and neuropathic pain respectively.

The thalamus is a sensory relay center that serves as a primary component of ascending medial and lateral pain pathways.[48,49] Lower thalamic to limbic system connectivity alterations were uniquely negatively associated with nociceptive pain, with lower connectivity between the bilateral thalamus to the right hippocampus and amygdala, as well as the left thalamus to right pPaHC. Thalamic to temporal connectivity was also lower in individuals with higher levels of nociceptive pain in both nociceptive and pain type contrast analyses. Previously identified greater thalamic mean diffusivity[59] and lower GMV of the thalamus[6,8] and temporal lobe[8] in those with neuropathic pain after SCI suggest a potential role of microstructural changes and neuronal atrophy in diminished functional connectivity of these regions.

The PAG component of the descending pain pathway modulates the suppression of pain signaling.[48,49] The PAG was uniquely associated with neuropathic pain and demonstrated lower connectivity with the angular and

supramarginal gyri, consistent with the majority of limbic to angular and supramarginal gyri seed based correlations. Lower connectivity of the PAG to these regions may reflect reduced descending inhibitory pain modulation associated with higher neuropathic pain severities. The PAG has been previously implicated in neuropathic pain after SCI, with a negative correlation of pain intensity and R1 signal, reflective of lower myelination, in the PAG identified in those with greater pain.[203] Lower myelination could suggest a reduction in the size or number of neural tracts extending from the PAG limiting inhibitory pain modulation, which may overlap with our findings of reduced connectivity from this region.

Default Mode Network Connectivity Alterations

The default mode network is comprised of the posterior cingulate cortex, medial prefrontal cortex, and inferior parietal lobule components and is spontaneously active when the mind and body are not actively engaged in a task.[57] The default mode network is associated with internal mental processes and demonstrates altered functional connectivity in chronic pain conditions.[208,209] Default mode network connectivity was increased between dmPCC and frontal lobe regions and decreased in occipital regions in those with higher levels of neuropathic pain in both neuropathic and pain contrast analyses. Default mode connectivity between the dmMPFC and cerebellum was lower in those with higher levels of nociceptive pain. Individuals with SCI related neuropathic pain have demonstrated differences in GMV of frontal[8,210] and occipital regions[58], as

well as metabolic[210] and microstructural[59] alterations of the prefrontal cortex further supporting the role of these regions in pain experience after SCI.

Summary

Our findings suggest that intralimbic, limbic to striatal, and descending pain pathway connectivity alterations may be uniquely associated with neuropathic pain severity, whereas thalamic to limbic and limbic to parietal connectivity alterations may be associated specifically with nociceptive pain severity. Opposing connectivity relationships dependent upon pain type suggest the posterior cingulate and thalamus differentially modulate the experience of both neuropathic and nociceptive pain. Additional connectivity alterations associated with pain phenotypes were identified involving the limbic system and default mode network. In line with our findings, the default mode network and ascending and descending pain pathways displayed distinct neural signatures of neuropathic and non-neuropathic pain phenotypes measured by magnetoencephalography in individuals with multiple sclerosis.[211] Taken together, these findings suggest altered neural activity in these regions may uniquely contribute to neuropathic and nociceptive pain experiences.

Limitations and Future Considerations

Our sample size was modest and as such, we were limited in the number of variables of interest included in our statistical models. Future studies with larger sample sizes should consider including additional factors such as age and

duration of injury[212] and injury classification (tetraplegia vs paraplegia)[23,213], which may contribute to pain outcomes after SCI to further elucidate underlying pain mechanisms in this heterogeneous population. As NSAIDs were not previously found to alter rsFC[190] and only 2 individuals in our sample currently utilized them, we did not control for NSAID use in our sample. However, as only the short-term effects of NSAID use on connectivity have been assessed after administration in an experimental pain condition[190], future studies investigating the effects of long-term use of NSAIDs as well as other medications typically utilized in individuals with SCI and chronic pain may be warranted.

Conclusions

Alterations in rsFC between regions involved in sensory and emotional aspects of pain processing were found to be associated with both pain severity and phenotype in individuals with chronic pain after SCI. Unique connectivity patterns dependent upon pain phenotype suggest differential relationships between brain regions may exist for neuropathic and nociceptive pain and could guide further study and future interventions.

Chapter 4: Robotic-Assisted Gait Training Increases Subcortical Gray Matter Volume in Individuals with Chronic Spinal Cord Injury

4.1 Introduction

Prior research estimates the prevalence of neuropathic pain among individuals who have experienced spinal cord injury (SCI) is 56%.[27,128] Individuals with neuropathic pain after SCI report poorer physical and psychological health, which can impair life satisfaction and quality.[214] The high prevalence of neuropathic pain and negative impacts on overall well-being are further complicated by lack of effective pharmacologic therapies, and the refractory nature of neuropathic pain.[30,31] These challenges establish a need for the investigation of alternative interventions that may alleviate neuropathic pain among populations with SCI. Though its underlying mechanisms are still poorly understood, neuropathic pain is associated with alterations in gray matter volume (GMV) in sensory and pain processing regions after SCI.[6]

Epidemiological studies indicate that both muscle strengthening and aerobic exercise have dose dependent benefits on mood and depression.[215] Likewise, muscle strengthening and aerobic exercise can both be beneficial for reducing chronic pain [216], although the evidence is moderate. Among individuals with SCI, there is a close correlation between pain and the frequency of engaging in heavy intensity physical activity. [217] This may be explained in part by pain being a barrier to engage in physical activity, thereby creating a downward spiral

of pain, depression and inactivity.[218] This cycle may be reversible, as exercise interventions can reduce musculoskeletal and neuropathic pain.[13,219-225]

Robotic-assisted gait training (RAGT) in particular is associated with improved markers of mobility, spasticity, and psychosocial well-being in individuals with SCI, and could be an especially salient intervention in this population.[226-229] Some evidence suggests RAGT may reduce pain in individuals with SCI, but its efficacy as an intervention for pain management is largely undetermined.[227,230-232] In individuals with multiple sclerosis, as well as in healthy elderly adults, low-intensity walking exercise led to increased GMV and greater function.[233-235] Likewise, in individuals with stroke and multiple sclerosis, ambulatory interventions including RAGT, treadmill training with and without body weight support, and overground training have also been associated with neuroplastic changes in the brain measured by magnetic resonance imaging (MRI).[115,116,236] These findings suggest RAGT could facilitate structural neuroplastic changes of regions which may contribute to pain experience.

Further support of the potential benefit of ambulatory exercise for the reduction of neuropathic pain and facilitation of neuroplastic changes has been reported in preclinical studies. Ambulatory exercise interventions in models of SCI induced neuropathic pain in rodents have identified reduced pain behaviors associated with increased expression of neuroprotective proteins involved in the modulation of neuroplasticity and synaptic remodeling.[104,237,238] Additionally, ambulatory exercise in rodent SCI-induced neuropathic pain models produced analgesic effects associated with structural changes in the amygdala.[239]

Though evidence supports the benefit of RAGT in individuals with SCI, its potential impact on neuropathic pain and neuroplasticity has yet to be established. This study aimed to identify changes in pain intensity and neuroplastic alterations in brain volume associated with the effects of a RAGT intervention in individuals with chronic SCI.

4.2 Methods

Study Design

Effects of a RAGT intervention were assessed with a cross-over design randomized, controlled clinical trial (Effects of exoskeleton-assisted gait training on Bone Health and Quality of Life: A Randomized Clinical Trial; NCT02533713). Participants were randomized to Initial Treatment Arm 1) Up to 78 sessions of RAGT or Delayed Treatment Arm 2) 6 months of standard treatment (i.e., no restrictions on routine medical care that an individual with chronic SCI may receive during this period) (Figure 4.1). After the initial period of either RAGT or standard treatment, the groups then switched to the opposite intervention group (i.e., the RAGT group underwent 6 months of standard treatment, and the standard treatment group completed up to 78 sessions of RAGT).

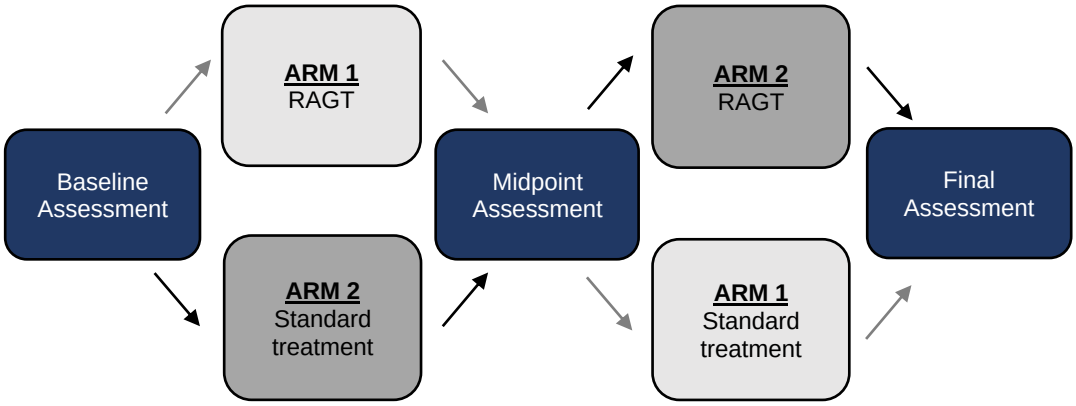


Figure 4.1. Study design depicting assessment time points and timing of group assignment to either robotic-assisted gait training (RAGT) or standard treatment. Both groups received the RAGT intervention, with Arm 1 as the initial treatment arm, and Arm 2 as the delayed treatment arm.

For data analysis, study assessments for all treatment arms were defined as 4 distinct time points: 1) standard care baseline (Delayed Treatment Arm 2 only), 2) pre-gait assessment (both treatment arms), 3) post-gait assessment (both treatment arms), and 4) longitudinal follow up (Initial Treatment Arm 1 only). The study protocol was approved by our Institutional Review Board and all participants gave their written informed consent to participate. All analyses were conducted on de-identified data, collected between 08/16/2017 and 04/02/2021, by a member of the research team not involved in data collection.

Subjects

A total of 61 participants with chronic SCI, who were at least three years post-injury and met study criteria, were enrolled (Table 4.1, Figure 4.2). After enrollment, one participant was considered unsafe to use the device based on physical assessment and was removed from the study. The 60 individuals

deemed eligible to initiate the RAGT intervention were randomized to Initial Treatment Arm 1 (n=33) or Delayed Treatment Arm 2 (n=27).

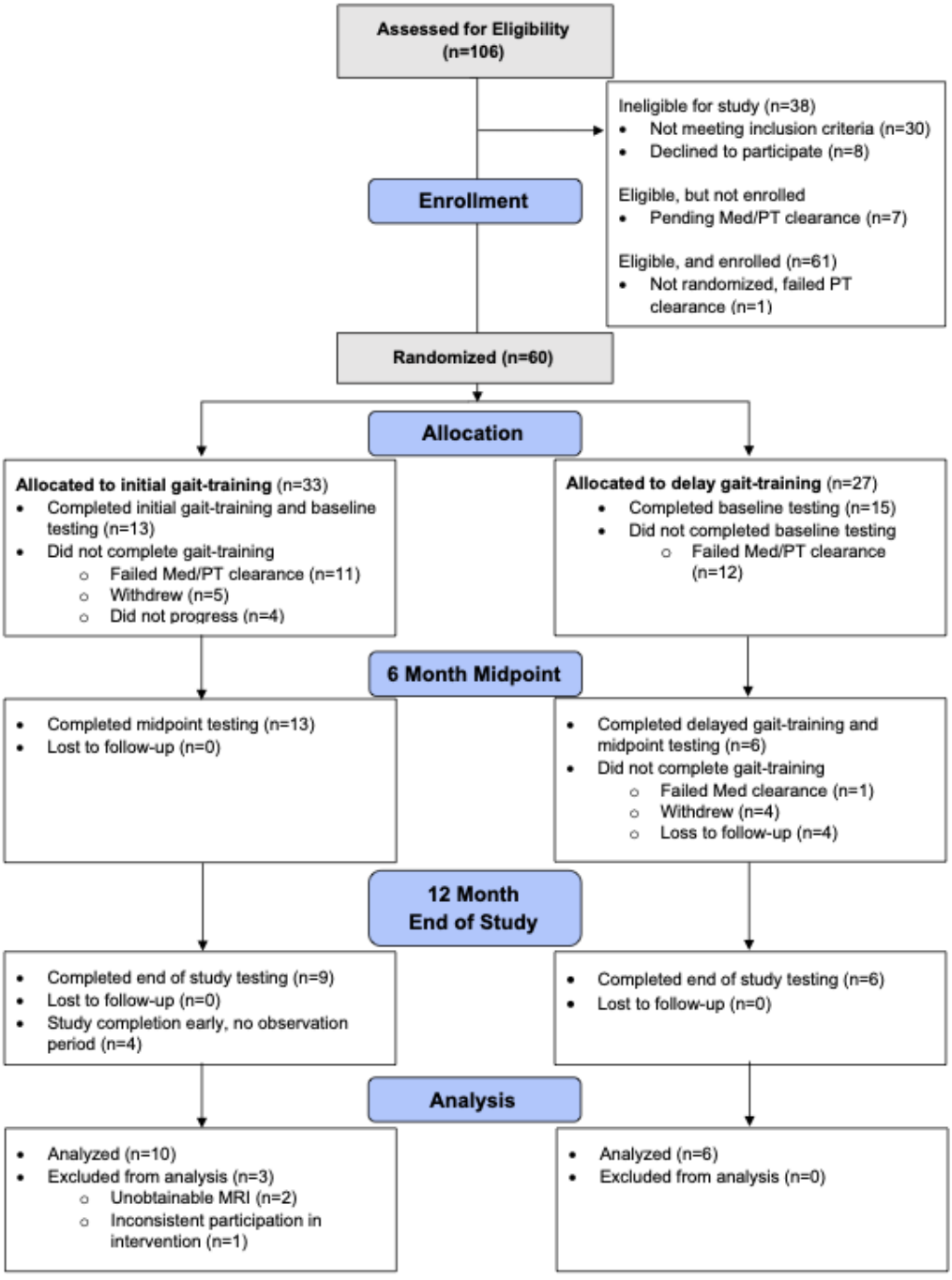


Figure 4.2. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Table 4.1. Study criteria
Effects of exoskeleton-assisted gait training on bone health and quality of life: A randomized clinical trial (NCT02533713)
Inclusion criteria • ≥ 18 years of age • ≥ 3 years post-injury • Motor complete SCI (AIS A and B) or AIS C and D who use a wheelchair > 50% of the time • SCI C7-T12 • Height of 155-191 cm • Weight < 113 kg • Modified Ashworth Scale rating < 3 in bilateral lower extremities • Sufficient upper body strength to complete sit to sit transfers **Exclusion criteria** • Enrollment in another clinical trial • Pregnancy • Symptomatic orthostatic hypotension • Active grade 2 or > pressure ulcer that could be worsened by exoskeleton device • Lower extremity contractures that interfere with device fit • Unhealed limb or pelvic bone fracture • Other neurological disease • Active treatment for epilepsy or thyroid disorders • Current use of medications potentially affecting bone health • Women with osteoporosis

American Spinal Injury Association Impairment Scale (AIS), spinal cord injury (SCI)

Participants who completed immediate pre- and post-gait assessments (time points 2 and 3) and consistently participated in at least 39 of the 78 RAGT sessions, with MRI data, were included in analyses. Of the 19 participants who completed immediate pre- and post-gait assessments, 3 were excluded due to unobtainable MRI data (n=2) or lack of consistent RAGT participation due to medical issues (n=1), resulting in a total sample of 16 included in this analysis. Of the 16 included in the analysis, 3 participants in Initial Treatment Arm 1 did not

complete the longitudinal follow up assessment after 6 months of standard treatment (time point 4). All other participants had complete datasets across 3 time points: time points 2, 3, and 4 for Initial Treatment Arm 1 and time points 1, 2, and 3 for Delayed Treatment Arm 2.

Robotic-Assisted Gait Training Intervention

Participants completed up to 78 sessions of a clinically supervised RAGT intervention. Each subject received one hour of individual walking therapy, up to three days per week, over a period of 5-8 months, supervised by an exoskeleton-trained clinical physical therapist. During the first training session the fitting of the RAGT device (Indego, Parker Hannifin Corporation, Human Motion & Control, Macedonia, OH, USA; EksoNR, Ekso Bionics, Richmond, CA, USA) was maximized.

Participants were trained in donning and doffing the device. Gait training occurred in bouts of 10 minutes, with rest periods as needed, and progressed up to 60 minutes of continuous walking as tolerated. The total number of steps taken, and minutes spent walking, were recorded for each subject for each session and over the course of the intervention. Total number of sessions completed were tracked through attendance records.

Clinical Outcome Measures

Demographic and SCI characteristic data were attained from study intake questionnaires. Pain type and intensity were rated on a 0-10 scale with the

International Spinal Cord Pain Basic Dataset (ISCIPBDS). Participants' highest rated average weekly intensity of neuropathic and/or nociceptive pain on the ISCIPBDS were attained at each assessment time point. Overall pain burden was defined as the sum of each participant's highest rated neuropathic and nociceptive pain complaints. Participants without pain were included in analyses with a rating of 0. A clinically meaningful change in pain severity was defined as a 30% difference in pre- and post-intervention pain intensity ratings.[240] American Spinal Injury Association Impairment Scale (AIS) classification was confirmed by physical exam.

Neuroimaging

Data were obtained on a 3-Tesla Siemens Trio using a 12-channel head coil. High resolution T1 weighted structural images were acquired for volumetric analyses. The T1 scanning protocol consisted of a gradient echo sequence with GRAPPA parallel imaging with an acceleration factor of 2, 256 mm field of view (FOV), 1x1x1 mm voxel size, and 1 mm slice thickness, sagittal acquisition (interleaved), 20 ms echo time (TE), 4.92 ms repetition time (TR), flip angle of 25 degrees, and 5:17 scan time.

Volumetric Analyses

All structural data were preprocessed and statistically analyzed with SPM12[196], a MATLAB (version R2019b, The Mathworks Inc., Natick, MA) operated MRI software. Longitudinal whole-brain volumetric analyses were

performed using voxel-based morphometry to identify brain regions demonstrating altered GMV after the RAGT intervention. T1 images from each assessment time point were serially co-registered for each participant using the SPM longitudinal registration package[241], and the resulting mean structural image (from up to 3 time points) were segmented. The gray matter segmentation was multiplied with the Jacobian determinants from each time point, and then spatially normalized to the Montreal Neurological Institute (MNI) template and smoothed with an 8 mm Gaussian kernel using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL).[242] Normalized gray matter masked Jacobians were entered in a flexible factorial including the main effects of subject and time (four possible study time points) and covariates of age and sex. Control covariates of age and sex were included in the model due to prior reported correlations between these variables and GMV.[243] Planned comparisons contrasted T changes in GMV between the immediate pre- and post-RAGT intervention assessment time points. Significance level was set at $p<0.05$ after family-wise error (FWE) correction with a 10-voxel cluster size threshold.

Statistical Analysis of Descriptive Factors

Descriptive statistics of demographic factors included percentages, mean, range, and standard deviation. Normality of continuous data distributions was assessed with Shapiro Wilk Goodness-of-Fit Tests. Proportional differences in neuropathic and nociceptive pain presence between study assessment time

points were assessed with Chi-square tests. Wilcoxon signed-rank tests assessed differences in pain ratings pre- and post-RAGT intervention. Bivariate linear regression assessed the relationship between continuous variables. All analyses were conducted using JMP version 16 (SAS Institute Inc., Cary, NC).

4.3 Results

Cohort Characteristics

Our sample consisted of 15 males (94%) and 1 female (6%), with a mean age of 37 years and an average of 9 years post-injury. Most participants reported neuropathic (75.0%) and/or nociceptive (56.3%) pain prior to initiating the RAGT intervention. Participants took an average of 145,460 steps and spent an average of 53 hours walking in the exoskeleton devices. 14 participants (87.5%) completed the full 78 sessions. Additional demographic data are presented in Table 4.2.

Pain Changes Associated with RAGT

After the RAGT intervention, there was a 28.3% reduction in the proportion of individuals who reported neuropathic pain and a clinically meaningful 1.06-point (32.0%) reduction in mean neuropathic pain intensity (S = -25.00, p = 0.21).[240] A 0.19-point (6.4%) reduction in nociceptive pain intensity (S = -6.00, p = 0.89) was identified with no change in proportion of those reporting nociceptive pain. Overall pain burden was reduced by 1.25 points (20.4%, S = -21.50, p = 0.36). Total steps and hours spent walking in the RAGT

device did not significantly predict change in intensity of neuropathic, nociceptive, or overall pain burden (Table 4.3).

Table 4.2. Participant characteristics		
Variable	**Total (n=16)**	**Range**
Demographics		
Age (years)	37.18 ± 10.55	23-60
Male	15 (93.8)	-
Pre-RAGT neuropathic pain	12 (75.0)	-
Pre-RAGT nociceptive pain	9 (56.3)	-
Post-RAGT neuropathic pain	7 (46.7)	-
Post-RAGT nociceptive pain	9 (56.3)	-
Injury Characteristics		
Injury duration (years)	8.98 ± 6.33	3.10-19.84
Tetraplegia	4 (25.0)	-
AIS classification* • A • B • C	 10/15 (66.7) 4/15 (26.7) 1/15 (6.7)	 - - -
Pain Ratings		
Pre-RAGT neuropathic pain	3.31 ± 2.68	0-8
Pre-RAGT nociceptive pain	2.81 ± 2.88	0-8
Pre-RAGT overall pain burden	6.13 ± 4.98	0-16
Post-RAGT neuropathic pain	2.25 ± 3.02	0-8
Post-RAGT nociceptive pain	2.63 ± 2.83	0-8
Post-RAGT overall pain burden	4.88 ± 4.30	0-16
Change in neuropathic pain	-1.06 ± 3.09	-8 to 4
Change in nociceptive pain	-0.19 ± 1.87	-5 to 3
Change in overall pain burden	-1.25± 4.20	-13 to 5
Gait data		
Total steps taken	145,460 ± 23,883	102,090-187,484
Total walking time (hours)	52.98 ± 5.47	42.30-60.48
Number of sessions	75.81 ± 7.52	48-78

Continuous variables are presented as mean ± SD and range, and categorical variables as N (%). Negative change in pain values reflect decrease in pain intensity after robotic-assisted gait training (RAGT). *Data provided include the full sample of participants (n=16) except for American Spinal Injury Association Impairment (AIS) classification (n=15).

Table 4.3. Bivariate associations of robotic-assisted gait training dosage			
Change in neuropathic pain	$F_{1,14}$	R^2	*p*
Total minutes spent walking	<0.001	<0.001	0.98
Total steps taken	1.22	0.08	0.29
Change in nociceptive pain	$F_{1,14}$	R^2	*p*
Total minutes spent walking	0.16	0.01	0.69
Total steps taken	0.26	0.02	0.62
Change in overall pain burden	$F_{1,14}$	R^2	*p*
Total minutes spent walking	0.03	0.50	0.87
Total steps taken	1.09	0.07	0.31
Change in right caudate volume	$F_{1,14}$	R^2	*p*
Total minutes spent walking	8.95	0.39	0.01*
Total steps taken	3.54	0.20	0.08
Change in right thalamus volume	$F_{1,14}$	R^2	*p*
Total minutes spent walking	0.73	0.05	0.41
Total steps taken	0.47	0.03	0.51

*Statistically significant association ($p < 0.05$).

Volumetric Changes Associated with RAGT

Post-RAGT intervention, increased GMV was identified in the right caudate ($t = 6.03$, $p_{FWE} = 0.02$) and thalamus ($t = 6.03$, $p_{FWE} = 0.02$) (Table 4.4, Figure 4.3). No significant decreases in GMV were identified post-intervention. Total number of steps taken in the exoskeleton device were predictive of change in GMV volume in the right caudate ($F_{1,14} = 8.95$, $p = 0.01$), but not thalamus ($F_{1,14} = 3.54$, $p = 0.08$) (Figure 4.4). No relationship was identified between total minutes spent walking in the device and change in GMV for either region.

Table 4.4. Volumetric changes post-intervention						
Brain region	**MNI X**	**MNI Y**	**MNI Z**	**Cluster size**	**T**	**p-FWE**
Right caudate	18	21	0	33	6.03	0.02
Right thalamus	10	-33	3	18	6.03	0.02

Montreal Neurological Institute (MNI) system coordinates for resulting regions are provided.

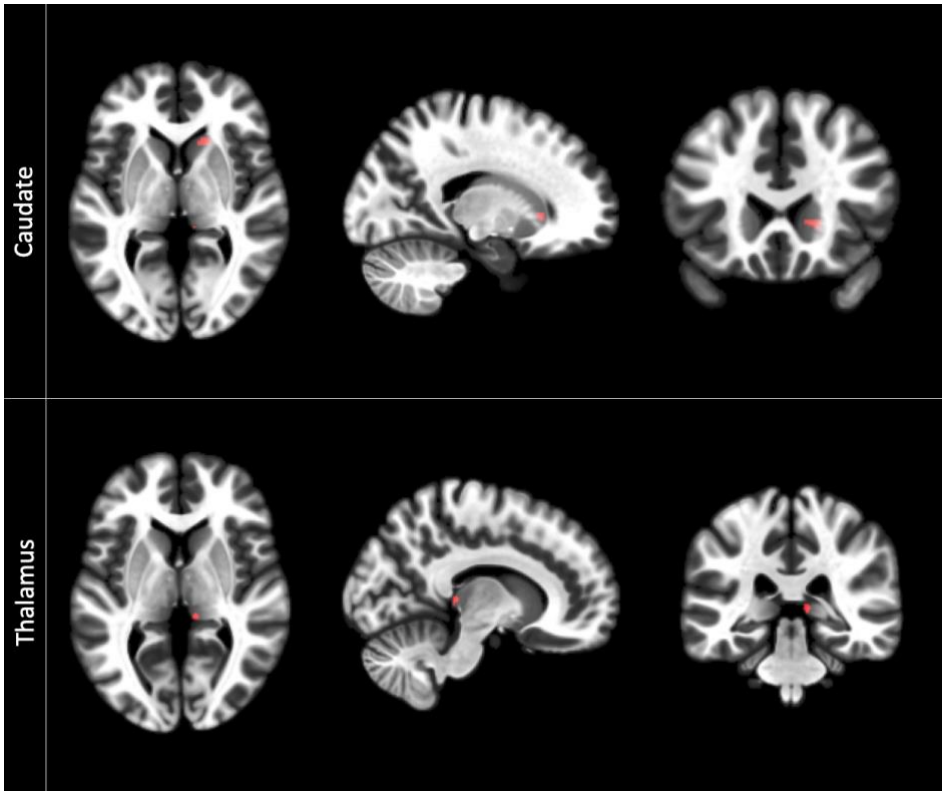


Figure 4.3. Clusters demonstrating increased gray matter volume after the robotic-assisted gait training intervention (axial, sagittal, and coronal views).

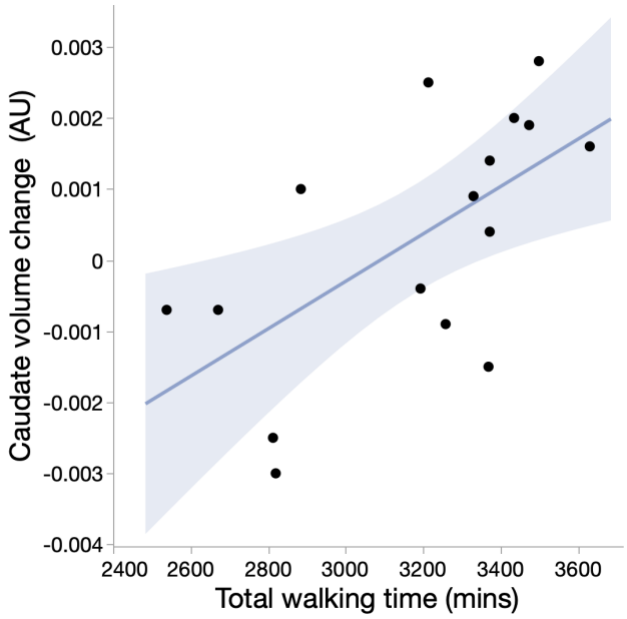


Figure 4.4. Bivariate linear regression demonstrated a positive predictive association between total minutes spent walking in the robotic-assisted gait training device and caudate volume change ($\beta = 3.34e^{-6}$, $p = 0.01$).

4.4 Discussion

This study aimed to identify the effects of a RAGT intervention on pain intensity and brain volume in individuals with chronic SCI. A clinically meaningful reduction in neuropathic pain intensity, as well as significantly increased GMV of the right caudate and thalamus were identified after RAGT intervention. Additionally, the number of steps taken in the RAGT device were predictive of GMV change in the right caudate.

RAGT Associated Reduction of Neuropathic Pain

Average neuropathic pain intensity was reduced by 32%, constituting a clinically meaningful decrease in pain post-RAGT intervention.[240] Additionally, the overall proportion of individuals reporting neuropathic pain decreased after the RAGT intervention, with 28.3% achieving complete relief of neuropathic pain. Our results support the findings of a prior case study which reported reduced neuropathic pain severity in two individuals with SCI after RAGT.[230] Additional studies have demonstrated reductions in pain post-RAGT in SCI populations, though the effects on neuropathic or nociceptive pain were not specifically assessed.[230,231] Smaller post-intervention reductions in overall pain burden (21%) and nociceptive pain (6%), suggest RAGT may more meaningfully influence neuropathic pain intensities. The lack of reduction in proportion and intensity of nociceptive pain post-intervention is likely due to RAGT engagement and challenge of the musculoskeletal system in positions and movements not typically performed by previously non-ambulatory individuals with SCI.

The clinically meaningful reduction in neuropathic pain post-RAGT may be due to the neuroprotective effects of ambulatory exercise previously demonstrated in clinical and preclinical studies. Ambulatory exercise in spinal cord injured rodent models has been shown to reduce neuropathic pain symptoms and onset and alter gene expression.[44,104,237,244-247] Reduction in neuropathic pain after ambulatory exercise was associated with increased expression of neurotrophic factors in the spinal cord dorsal horn and dorsal root ganglia (DRG) sensory neurons.[104] Reduction of neuropathic pain after ambulatory exercise was also associated with reduced expression of pro-inflammatory factors known to contribute to sensitization and neuropathic pain in the DRG and dorsal horn.[33,248] These preclinical studies establish the potential benefit of ambulatory exercise in reducing neuropathic pain as well as regulation of underlying cellular level changes in the spinal cord and DRG after SCI. Exercise induced modulation of these neuroprotective and neurodegenerative factors could contribute to mechanisms underlying reduced neuropathic pain severity demonstrated after RAGT-intervention. However, as these preclinical studies consisted primarily of wheel running that was likely at higher aerobic stress levels than walking in an RAGT device, the cellular level changes produced by RAGT may be entirely unique and require further study.

RAGT Associated Increases in Brain Volume

Brain volume significantly increased in the right caudate and thalamus after the RAGT intervention. These deep gray matter structures are both key

components of the sensorimotor system and contribute to the production of volitional movement[249,250] as well as pain processing.[201,251] Both structures lie in proximity to one another at the center of the brain and are components of the cortico-basal ganglia-thalamo-cortical feedback loop involved in regulation of motor, limbic, and associative function.[249,252] The caudate nucleus and putamen form the striatum, the major input nuclei of the basal ganglia, which provides efferent signaling to the thalamus.[249] The thalamus serves as a sensory relay center receiving afferent signaling from the ascending sensory tracts and basal ganglia, and transmits efferent signals to the sensorimotor cortex, which subsequently efferently synapses with the caudate.[249,252,253] Alterations in this loop of transmission and integration of neural signaling in the deep gray matter have been associated with disorders of movement and sensation.[249,253] Lower GMV of the caudate and thalamus has been previously reported in populations with neuropathic pain, suggesting the post-intervention reduction in neuropathic pain in our sample could be associated with simultaneous GMV increases in these regions.[63,254]

Greater ambulatory function is also associated with larger GMV of the caudate and thalamus. Prior studies identified positive correlations between gait speed and caudate volume in individuals with multiple sclerosis (MS)[124], stroke[255], mild cognitive impairment[256-258], type 1 diabetes[259], and community dwelling older adults.[260] Greater thalamic volume was also associated with faster gait speed in individuals with MS[125-127] and community dwelling older adults[256], as well as in individuals with cerebral small vessel disease.[261] In contrast, older

adults with dual decline in cognition and gait speed demonstrated reduced thalamic volume.[262] Gait impairments associated with lower caudate volume include shuffling[263], freezing[264], and falls[265] in individuals with Parkinson's disease. This well-established association between caudate and thalamic volume and gait supports their role in the regulation of ambulatory movement. Our findings of increased right caudate and thalamic volume after gait training in individuals with SCI, who were non-ambulatory for 3 years or more, provide further support that these regions are key contributors to ambulatory function.

This is the first known study to establish significant changes in GMV of the caudate and thalamus after gait training, however, higher volume in these regions has been previously associated with exercise and aerobic health. In older adults, a 12 month coordination training intervention consisting of exercises to improve balance, reaction time, and upper and lower extremity coordination was associated with increased caudate volume.[14] Likewise, a moderate intensity aerobic cycling intervention was found to increase thalamic volume in individuals with traumatic brain injury.[15] Participation in moderate intensity aerobic exercise has been associated with increased caudate and thalamic volume in individuals with Alzheimer's disease[119], as well as greater thalamic volume in individuals with MS. [266] In older adults, greater thalamic volume was associated with long-term Tai Chi participation[267] and higher levels of energy expenditure. [112] Caudate and thalamic volumes were also found to positively correlate with VO_2 peak and VO_2 max respectively in individuals with MS, suggesting higher levels of cardiorespiratory fitness are associated with greater volume of these

regions.[121,268] Higher glucose uptake in the caudate of individuals who engaged in high intensity interval training compared to sedentary individuals support the existence of greater brain metabolism in the caudate associated with exercise.[269] Our findings of increased right caudate and thalamic volume after RAGT provide support for the potential use of RAGT as an adaptive exercise intervention for individuals with SCI, capable of replicating previously reported exercise-associated neuroplastic changes.

The effects of exercise on brain plasticity are best described for the hippocampus. It is well established that exercise in the form of voluntary wheel-running robustly enhances cell proliferation and the number of newly generated neurons in the dentate subgranular zone of the hippocampus.[270-272] In humans, the impact of exercise on brain function has primarily been studied in the elderly, where higher levels of aerobic fitness are associated with increased hippocampal volume[273], and aerobic exercise can halt aging-related hippocampal volume reductions, and even increase hippocampal volumes by 2% to 4%. [111,274] In addition to exercise induced effects on the hippocampus, various forms of exercise interventions have been correlated with a reduction in neuropathic pain and sensory dysfunction[275], as well as volumetric alteration of the basal ganglia.[14,15]

We did not observe changes in hippocampal gray matter volume, as has previously been reported in multiple animal and human aerobic exercise studies. [111,270-274]. This may indicate that aerobic exercise uniquely contributes to hippocampal change, while the RAGT intervention puts more emphasis on

muscle strength and motor coordination training. This appears to be in line with studies on elite athletes, where endurance athletes, but not martial arts athletes had larger hippocampi[276], and elite sprinters had larger basal ganglia volumes that long distance runners.[277]It is also possible that this study was not adequately powered to detect changes in hippocampal gray matter volume or that a higher dose of gait training is required to induce these changes in paralyzed individuals.

Dosing of exercise intervention may also contribute to volumetric increases in gray matter structures. Increased caudate and thalamic volume was identified in ultramarathoners but not marathoners immediately after a race, suggesting the amount exercise, such as time spent running, may influence neuroplasticity of these regions.[278] These prior findings of higher volumes associated with greater exercise duration could support the positive association we identified between right caudate volume and total number of minutes spent walking in the RAGT device.[278] However, as this report assessed the effects of acute exertion during a single session of endurance exercise, as opposed to long-term low intensity exercise participation, the similarity in findings between the two studies may be due to other mechanisms. Further exploration of RAGT dosing parameters is warranted to determine optimal duration and frequency of use to promote neuroplasticity after SCI.

Limitations and Future Directions

Though a clinically meaningful reduction in neuropathic pain was identified, this difference did not reach statistical significance. Our sample may

have been too small and heterogeneous to detect statistically significant changes in pain severity associated with the intervention. Additional studies are planned to investigate the effects of RAGT on pain modulation with a larger sample size not restricted by those with complete MRI data, as well as additional pain outcomes such as pain interference and pain catastrophizing. Some individuals in our sample reported no improvement or increased pain after RAGT. Additional study is needed to identify characteristics of candidates likely to attain optimal outcomes from RAGT interventions. As our sample consisted exclusively of individuals with SCI, our findings may not be generalizable to other clinical populations in which RAGT devices are utilized and will require further investigation. The underlying mechanisms which contribute to the neuroplastic effects of RAGT in individuals with SCI were not assessed.

Summary and Conclusions

RAGT produced clinically meaningful reductions in neuropathic pain and increased volume of the right caudate and thalamus in individuals with SCI. These findings support the potential benefit of RAGT as an adaptive exercise intervention for pain management and facilitation of neuroplastic changes in the sensorimotor system in SCI populations. Consistent participation in at least 6 months of RAGT resulted in a 32% reduction in mean neuropathic pain intensity and facilitation of neuroplastic change in regions previously associated with exercise-related volumetric increases in other populations. The positive association between time spent walking in the RAGT device and amount of GMV

change in the caudate support the influence of RAGT dosage on striatal neuroplasticity, and the need for further exploration of optimal dosing parameters of RAGT interventions.

Chapter 5: Summary, Future Directions, and Conclusions

5.1 Summary

This dissertation work established miRNA and neuroimaging biomarkers of neuropathic pain, as well as the ability of RAGT to promote neuroplastic changes in brain structure in individuals with spinal cord injury. Results of the completed studies identified novel targets for treatment at the molecular, cellular and brain organ level, and support RAGT as an intervention capable of enacting change in sensorimotor system structures involved in pain processing.

miR-338-5p was identified as a biomarker of neuropathic pain severity in individuals with SCI. Lower expression of miR-338-5p, as well as history of cigarette smoking, were associated with higher levels of neuropathic pain severity. These findings are consistent with the reduced expression of miR-338 in preclinical models of neuropathic pain[97,98], elucidating its role as a neuroprotective inhibitor of pro-inflammatory mediators (IL-6[99] and CXC4[160]) and facilitators of neuronal apoptosis (BCL2L11[161] and CTGF[97]). Reduced expression of miR-338-5p was uniquely associated with the severity of neuropathic but not nociceptive pain. These findings suggest miR-338-5p could serve as a biomarker and potential target for treatment of neuropathic pain in individuals with SCI.

Neuroimaging biomarkers of neuropathic pain were also identified with resting-state functional connectivity analyses. Significant functional connectivity alterations associated with the type and severity of pain in the limbic system, striatum, medial and lateral pain pathways, and default mode network were

identified. Functional connectivity alterations associated uniquely with neuropathic pain severity suggest altered intralimbic, limbic to striatal, and descending pain pathway connectivity uniquely contribute to the experience of neuropathic pain. Likewise, thalamic to limbic and limbic to parietal connectivity alterations may be associated specifically with nociceptive pain. The identification of functional connectivity alterations exclusively associated with neuropathic pain could guide further study and future targeted pain management interventions to improve neuropathic pain outcomes after SCI.

RAGT was established as capable of promoting increased volume of the caudate and thalamus, regions which demonstrate reduced volume in populations with neuropathic pain.[6,63] Additionally, there was a 32% reduction in neuropathic pain severity and 28% decrease in neuropathic pain presence after RAGT intervention. Volumetric increases in the caudate and thalamus, in addition to neuropathic pain reduction, suggest RAGT may promote neuroprotective plasticity and normalization of neuronal activity in regions contributing to the experience of neuropathic pain.

In summary, neuropathic pain severity was associated with reduced miR-338-5p and altered functional connectivity in limbic, striatal, and descending pain pathways. RAGT facilitated volumetric increases of subcortical gray matter structures involved in sensorimotor function, and may be an effective intervention for neuropathic pain management in individuals with SCI.

5.2 Future Directions

Expanded study is warranted to assess the relationship between exercise, such as RAGT or other adaptive exercise interventions, and miRNA expression and structural and functional neuroplastic changes within the brain. Exploration of the effects of exercise type and dosing could promote optimization of rehabilitation interventions and clinical outcomes for individuals with SCI.

Specifically, assessment of the effects of exercise on miR-338-5p could determine the ability of exercise to upregulate and normalize its expression, subsequently reducing neuropathic pain. Additionally, future work to assess the association between the expression of miR-338-5p and its validated downstream targets in human populations would further elucidate the potential role of miR-338 in modulating neuroinflammatory mechanisms of neuropathic pain after SCI. One promising validated target is IL-6[99], a proinflammatory cytokine and circulating inflammatory marker which can be measured in the blood in clinical populations.[279] Assessment of the effects of exercise on miR-338-5p and its downstream target IL-6 could provide objective assessment of the efficacy of rehabilitative interventions to modulate neuroinflammatory signaling mechanisms contributing to neuropathic pain.

Assessment of the effects of exercise on rsFC, particularly involving limbic, striatal, and descending pain pathway structures identified to be uniquely associated with neuropathic pain severity, could assess the efficacy of exercise to normalize regional neuronal activity and altered functional connectivity associated with neuropathic pain severity. Assessment of the effects of RAGT on

limbic to striatal activity is of particular interest as connectivity alterations of these regions were specifically associated with neuropathic pain severity, and the striatum demonstrates known association with ambulatory function.[124,255-260]

Modest reductions in neuropathic pain intensity and presence were identified after the RAGT intervention, suggesting gait training can provide clinically meaningful pain relief. Additional assessments of pain data in a larger sample of participants who completed the RAGT intervention with unobtainable MRI data could increase the power to detect significant changes in pain. These expanded analyses could additionally assess how RAGT affects other clinically relevant domains of pain-related psychoemotional function, such as pain interference with mood, sleep, and activity as well as anxiety and depression.

5.3 Conclusions

This dissertation work established miR-338-5p expression and altered functional connectivity of intralimbic, limbic to striatal, and descending pain pathway structures as biomarkers of neuropathic pain after SCI. Participation in a RAGT intervention resulted in volumetric increases of the caudate and thalamus in individuals with SCI. Results of the completed studies identified novel targets for treatment at the cellular and systemic level, and support RAGT as an intervention capable of eliciting change in sensorimotor system structures involved in pain processing. Next steps to advance current findings include investigation of the effects of additional exercise types and dosing, miR-338 downstream target expression, the effects of RAGT on functional connectivity,

and expanded analyses of the effects of RAGT on pain experience and psychoemotional health. Continued work in these domains could further elucidate the potential of these biomarkers and interventions to facilitate optimized neurorehabilitation treatment strategies and improved outcomes for individuals with SCI.

Bibliography

1. Hulsebosch CE. Gliopathy ensures persistent inflammation and chronic pain after spinal cord injury. *Exp Neurol*. Nov 2008;214(1):6-9. doi:10.1016/j.expneurol.2008.07.016

2. Li T, Wan Y, Sun L, et al. Inhibition of MicroRNA-15a/16 Expression Alleviates Neuropathic Pain Development through Upregulation of G Protein-Coupled Receptor Kinase 2. *Biomol Ther (Seoul)*. Jul 1 2019;27(4):414-422. doi:10.4062/biomolther.2018.073

3. Ito N, Sakai A, Miyake N, et al. miR-15b mediates oxaliplatin-induced chronic neuropathic pain through BACE1 down-regulation. *Br J Pharmacol*. Mar 2017;174(5):386-395. doi:10.1111/bph.13698

4. Ma Y, Deng Q, Li S, Chen M, Jin B, Wang M. TRPV1, Targeted by miR-338-3p, Induces Neuropathic Pain by Interacting with NECAB2. *J Mol Neurosci*. Jun 19 2020;doi:10.1007/s12031-020-01626-4

5. Sakai A, Suzuki H. Nerve injury-induced upregulation of miR-21 in the primary sensory neurons contributes to neuropathic pain in rats. *Biochem Biophys Res Commun*. May 31 2013;435(2):176-81. doi:10.1016/j.bbrc.2013.04.089

6. Jutzeler CR, Huber E, Callaghan MF, et al. Association of pain and CNS structural changes after spinal cord injury. *Sci Rep*. Jan 6 2016;6:18534. doi:10.1038/srep18534

7. Solstrand Dahlberg L, Becerra L, Borsook D, Linnman C. Brain changes after spinal cord injury, a quantitative meta-analysis and review. *Neurosci Biobehav Rev*. Jul 2018;90:272-293. doi:10.1016/j.neubiorev.2018.04.018

8. Huynh V, Lütolf R, Rosner J, et al. Supraspinal nociceptive networks in neuropathic pain after spinal cord injury. *Human brain mapping*. Aug 15 2021;42(12):3733-3749. doi:10.1002/hbm.25401

9. Li X, Wang L, Chen Q, et al. The Reorganization of Insular Subregions in Individuals with Below-Level Neuropathic Pain following Incomplete Spinal Cord Injury. *Neural Plast*. 2020;2020:2796571. doi:10.1155/2020/2796571

10. Huynh V, Rosner J, Curt A, Kollias S, Hubli M, Michels L. Disentangling the Effects of Spinal Cord Injury and Related Neuropathic Pain on Supraspinal Neuroplasticity: A Systematic Review on Neuroimaging. *Frontiers in neurology*. 2019;10:1413. doi:10.3389/fneur.2019.01413

11. Cooper MA, Kluding PM, Wright DE. Emerging Relationships between Exercise, Sensory Nerves, and Neuropathic Pain. *Front Neurosci*. 2016;10:372. doi:10.3389/fnins.2016.00372

12. Liu G, Keeler BE, Zhukareva V, Houle JD. Cycling exercise affects the expression of apoptosis-associated microRNAs after spinal cord injury in rats. *Exp Neurol*. Nov 2010;226(1):200-6. doi:10.1016/j.expneurol.2010.08.032

13. Norrbrink C, Lindberg T, Wahman K, Bjerkefors A. Effects of an exercise programme on musculoskeletal and neuropathic pain after spinal cord injury--results from a seated double-poling ergometer study. *Spinal Cord*. Jun 2012;50(6):457-61. doi:10.1038/sc.2011.160

14. Niemann C, Godde B, Staudinger UM, Voelcker-Rehage C. Exercise-induced changes in basal ganglia volume and cognition in older adults. *Neuroscience*. Dec 5 2014;281:147-63. doi:10.1016/j.neuroscience.2014.09.033

15. Wender CLA, Sandroff BM, Krch D, et al. The preliminary effects of moderate aerobic training on cognitive function in people with TBI and significant memory impairment: a proof-of-concept randomized controlled trial. *Neurocase*. Oct 2021;27(5):430-435. doi:10.1080/13554794.2021.1990964

16. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. Jan 2019;18(1):56-87. doi:10.1016/s1474-4422(18)30415-0

17. Jain NB, Ayers GD, Peterson EN, et al. Traumatic spinal cord injury in the United States, 1993-2012. *Jama*. Jun 9 2015;313(22):2236-43. doi:10.1001/jama.2015.6250

18. Lasfargues JE, Custis D, Morrone F, Carswell J, Nguyen T. A model for estimating spinal cord injury prevalence in the United States. *Paraplegia*. Feb 1995;33(2):62-8. doi:10.1038/sc.1995.16

19. Haisma JA, van der Woude LH, Stam HJ, et al. Complications following spinal cord injury: occurrence and risk factors in a longitudinal study during and after inpatient rehabilitation. *J Rehabil Med*. May 2007;39(5):393-8. doi:10.2340/16501977-0067

20. Noreau L, Proulx P, Gagnon L, Drolet M, Laramée MT. Secondary impairments after spinal cord injury: a population-based study. *Am J Phys Med Rehabil*. Nov-Dec 2000;79(6):526-35. doi:10.1097/00002060-200011000-00009

21. Craven C, Hitzig SL, Mittmann N. Impact of impairment and secondary health conditions on health preference among Canadians with chronic spinal cord injury. *J Spinal Cord Med*. Sep 2012;35(5):361-70. doi:10.1179/2045772312y.0000000046

22. DeVivo MJ. Causes and costs of spinal cord injury in the United States. *Spinal Cord*. Dec 1997;35(12):809-13. doi:10.1038/sj.sc.3100501

23. 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*. Jun 2003;103(3):249-57. doi:10.1016/s0304-3959(02)00452-9

24. Widerström-Noga E. Neuropathic Pain and Spinal Cord Injury: Phenotypes and Pharmacological Management. *Drugs*. Jun 2017;77(9):967-984. doi:10.1007/s40265-017-0747-8

25. Scholz J, Finnerup NB, Attal N, et al. The IASP classification of chronic pain for ICD-11: chronic neuropathic pain. *Pain*. Jan 2019;160(1):53-59. doi:10.1097/j.pain.0000000000001365

26. Gibbs K, Beaufort A, Stein A, Leung TM, Sison C, Bloom O. Assessment of pain symptoms and quality of life using the International Spinal Cord Injury Data Sets in persons with chronic spinal cord injury. *Spinal Cord Ser Cases*. 2019;5:32. doi:10.1038/s41394-019-0178-8

27. Felix ER, Cardenas DD, Bryce TN, et al. Prevalence and Impact of Neuropathic and Nonneuropathic Pain in Chronic Spinal Cord Injury. *Arch Phys Med Rehabil*. Jul 31 2021;doi:10.1016/j.apmr.2021.06.022

28. Jensen MP, Hoffman AJ, Cardenas DD. Chronic pain in individuals with spinal cord injury: a survey and longitudinal study. *Spinal Cord*. Dec 2005;43(12):704-12. doi:10.1038/sj.sc.3101777

29. Burke D, Fullen BM, Lennon O. Pain profiles in a community dwelling population following spinal cord injury: a national survey. *J Spinal Cord Med*. Mar 2019;42(2):201-211. doi:10.1080/10790268.2017.1351051

30. Bates D, Schultheis BC, Hanes MC, et al. A Comprehensive Algorithm for Management of Neuropathic Pain. *Pain Med*. Jun 1 2019;20(Suppl 1):S2-s12. doi:10.1093/pm/pnz075

31. Guy SD, Mehta S, Casalino A, et al. The CanPain SCI Clinical Practice Guidelines for Rehabilitation Management of Neuropathic Pain after Spinal Cord: Recommendations for treatment. *Spinal Cord*. Aug 2016;54 Suppl 1:S14-23. doi:10.1038/sc.2016.90

32. Benyamin R, Trescot AM, Datta S, et al. Opioid complications and side effects. *Pain Physician*. Mar 2008;11(2 Suppl):S105-20.

33. Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Res*. Sep 4 2015;1619:1-11. doi:10.1016/j.brainres.2014.12.045

34. Brennan FH, Popovich PG. Emerging targets for reprograming the immune response to promote repair and recovery of function after spinal cord injury. *Curr Opin Neurol*. Jun 2018;31(3):334-344. doi:10.1097/wco.0000000000000550

35. Ledeboer A, Gamanos M, Lai W, et al. Involvement of spinal cord nuclear factor kappaB activation in rat models of proinflammatory cytokine-mediated pain facilitation. *Eur J Neurosci*. Oct 2005;22(8):1977-86. doi:10.1111/j.1460-9568.2005.04379.x

36. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2:17023-. doi:10.1038/sigtrans.2017.23

37. Mitchell JP, Carmody RJ. NF-κB and the Transcriptional Control of Inflammation. *Int Rev Cell Mol Biol*. 2018;335:41-84. doi:10.1016/bs.ircmb.2017.07.007

38. Mitchell S, Vargas J, Hoffmann A. Signaling via the NFκB system. *Wiley Interdiscip Rev Syst Biol Med*. May 2016;8(3):227-41. doi:10.1002/wsbm.1331

39. Yarar-Fisher C, Bickel CS, Kelly NA, et al. Heightened TWEAK-NF-κB signaling and inflammation-associated fibrosis in paralyzed muscles of men with chronic spinal cord injury. *Am J Physiol Endocrinol Metab*. May 1 2016;310(9):E754-61. doi:10.1152/ajpendo.00240.2015

40. Brandolini L, d'Angelo M, Antonosante A, Allegretti M, Cimini A. Chemokine Signaling in Chemotherapy-Induced Neuropathic Pain. *Int J Mol Sci*. Jun 14 2019;20(12)doi:10.3390/ijms20122904

41. Detloff MR, Fisher LC, McGaughy V, Longbrake EE, Popovich PG, Basso DM. Remote activation of microglia and pro-inflammatory cytokines predict the onset and severity of below-level neuropathic pain after spinal cord injury in rats. *Exp Neurol*. Aug 2008;212(2):337-47. doi:10.1016/j.expneurol.2008.04.009

42. Peng XM, Zhou ZG, Glorioso JC, Fink DJ, Mata M. Tumor necrosis factor-alpha contributes to below-level neuropathic pain after spinal cord injury. *Ann Neurol*. May 2006;59(5):843-51. doi:10.1002/ana.20855

43. Lee JY, Choi HY, Ju BG, Yune TY. Estrogen alleviates neuropathic pain induced after spinal cord injury by inhibiting microglia and astrocyte activation.

Biochim Biophys Acta Mol Basis Dis. Jul 2018;1864(7):2472-2480. doi:10.1016/j.bbadis.2018.04.006

44. Chhaya SJ, Quiros-Molina D, Tamashiro-Orrego AD, Houlé JD, Detloff MR. Exercise-Induced Changes to the Macrophage Response in the Dorsal Root Ganglia Prevent Neuropathic Pain after Spinal Cord Injury. *J Neurotrauma*. Mar 19 2019;36(6):877-890. doi:10.1089/neu.2018.5819

45. Colloca L, Ludman T, Bouhassira D, et al. Neuropathic pain. *Nat Rev Dis Primers*. Feb 16 2017;3:17002. doi:10.1038/nrdp.2017.2

46. Kandel ER, Schwartz JH, Jesse TM, Sigelbaum SA, Hudspeth AJ. *Principles of Neural Science*. 5th ed. The McGraw-Hill Companies, Inc.; 2013.

47. Basbaum AI, Jessell TM. Pain. In: Kandel ER, Schwartz JH, Jessell TM, Sigelbaum SA, Hudspeth AJ, eds. *Principles of Neural Science*. 5th ed. The McGraw-Hill Companies, Inc.; 2013:530-555:chap 24.

48. De Ridder D, Vanneste S, Smith M, Adhia D. Pain and the Triple Network Model. *Frontiers in neurology*. 2022;13:757241. doi:10.3389/fneur.2022.757241

49. Willis WD, Westlund KN. Neuroanatomy of the pain system and of the pathways that modulate pain. *J Clin Neurophysiol*. Jan 1997;14(1):2-31. doi:10.1097/00004691-199701000-00002

50. Allen HN, Bobnar HJ, Kolber BJ. Left and right hemispheric lateralization of the amygdala in pain. *Prog Neurobiol*. Jan 2021;196:101891. doi:10.1016/j.pneurobio.2020.101891

51. Naor N, Rohr C, Schaare LH, Limbachia C, Shamay-Tsoory S, Okon-Singer H. The neural networks underlying reappraisal of empathy for pain. *Social*

cognitive and affective neuroscience. Sep 24 2020;15(7):733-744. doi:10.1093/scan/nsaa094

52. Meerwijk EL, Ford JM, Weiss SJ. Brain regions associated with psychological pain: implications for a neural network and its relationship to physical pain. *Brain Imaging Behav*. Mar 2013;7(1):1-14. doi:10.1007/s11682-012-9179-y

53. De Ridder D, Adhia D, Vanneste S. The anatomy of pain and suffering in the brain and its clinical implications. *Neurosci Biobehav Rev*. Nov 2021;130:125-146. doi:10.1016/j.neubiorev.2021.08.013

54. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci*. Nov 2007;10(11):1361-8. doi:10.1038/nn1992

55. Yousaf T, Dervenoulas G, Politis M. Advances in MRI Methodology. *Int Rev Neurobiol*. 2018;141:31-76. doi:10.1016/bs.irn.2018.08.008

56. Lv H, Wang Z, Tong E, et al. Resting-State Functional MRI: Everything That Nonexperts Have Always Wanted to Know. *AJNR Am J Neuroradiol*. Aug 2018;39(8):1390-1399. doi:10.3174/ajnr.A5527

57. Buckner RL. The brain's default network: origins and implications for the study of psychosis. *Dialogues Clin Neurosci*. Sep 2013;15(3):351-8. doi:10.31887/DCNS.2013.15.3/rbuckner

58. Mole TB, MacIver K, Sluming V, Ridgway GR, Nurmikko TJ. Specific brain morphometric changes in spinal cord injury with and without neuropathic pain. *Neuroimage Clin*. 2014;5:28-35. doi:10.1016/j.nicl.2014.05.014

59. Gustin SM, Wrigley PJ, Youssef AM, et al. Thalamic activity and biochemical changes in individuals with neuropathic pain after spinal cord injury. *Pain*. May 2014;155(5):1027-36. doi:10.1016/j.pain.2014.02.008

60. Micalos PS, Korgaonkar MS, Drinkwater EJ, Cannon J, Marino FE. Cerebral responses to innocuous somatic pressure stimulation following aerobic exercise rehabilitation in chronic pain patients: a functional magnetic resonance imaging study. *Int J Gen Med*. 2014;7:425-32. doi:10.2147/ijgm.s55169

61. Pascoal-Faria P, Yalcin N, Fregni F. Neural markers of neuropathic pain associated with maladaptive plasticity in spinal cord injury. *Pain Pract*. Apr 2015;15(4):371-7. doi:10.1111/papr.12237

62. Kim W, Kim SK, Nabekura J. Functional and structural plasticity in the primary somatosensory cortex associated with chronic pain. *J Neurochem*. May 2017;141(4):499-506. doi:10.1111/jnc.14012

63. Li M, Yan J, Li S, et al. Reduced volume of gray matter in patients with trigeminal neuralgia. *Brain Imaging Behav*. Apr 2017;11(2):486-492. doi:10.1007/s11682-016-9529-2

64. Mills EP, Di Pietro F, Alshelh Z, et al. Brainstem Pain-Control Circuitry Connectivity in Chronic Neuropathic Pain. *J Neurosci*. Jan 10 2018;38(2):465-473. doi:10.1523/jneurosci.1647-17.2017

65. Kadmon Harpaz N, Ungarish D, Hatsopoulos NG, Flash T. Movement Decomposition in the Primary Motor Cortex. *Cereb Cortex*. Apr 1 2019;29(4):1619-1633. doi:10.1093/cercor/bhy060

66. Pardo JV, Pardo PJ, Janer KW, Raichle ME. The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. *Proc Natl Acad Sci U S A*. Jan 1990;87(1):256-9. doi:10.1073/pnas.87.1.256

67. Bush G, Vogt BA, Holmes J, et al. Dorsal anterior cingulate cortex: a role in reward-based decision making. *Proc Natl Acad Sci U S A*. Jan 8 2002;99(1):523-8. doi:10.1073/pnas.012470999

68. Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci*. Jun 2000;4(6):215-222. doi:10.1016/s1364-6613(00)01483-2

69. Lin YH, Young IM, Conner AK, et al. Anatomy and White Matter Connections of the Inferior Temporal Gyrus. *World Neurosurg*. Nov 2020;143:e656-e666. doi:10.1016/j.wneu.2020.08.058

70. Li W, Qin W, Liu H, et al. Subregions of the human superior frontal gyrus and their connections. *Neuroimage*. Sep 2013;78:46-58. doi:10.1016/j.neuroimage.2013.04.011

71. Herlin B, Navarro V, Dupont S. The temporal pole: From anatomy to function-A literature appraisal. *J Chem Neuroanat*. Apr 2021;113:101925. doi:10.1016/j.jchemneu.2021.101925

72. Uddin LQ, Nomi JS, Hébert-Seropian B, Ghaziri J, Boucher O. Structure and Function of the Human Insula. *J Clin Neurophysiol*. Jul 2017;34(4):300-306. doi:10.1097/wnp.0000000000000377

73. Koziol LF, Budding D, Andreasen N, et al. Consensus paper: the cerebellum's role in movement and cognition. *Cerebellum*. Feb 2014;13(1):151-77. doi:10.1007/s12311-013-0511-x

74. Jackson RL, Bajada CJ, Rice GE, Cloutman LL, Lambon Ralph MA. An emergent functional parcellation of the temporal cortex. *Neuroimage*. Apr 15 2018;170:385-399. doi:10.1016/j.neuroimage.2017.04.024

75. Bender MB, Postel DM, Krieger HP. Disorders of oculomotor function in lesions of the occipital lobe. *J Neurol Neurosurg Psychiatry*. May 1957;20(2):139-43. doi:10.1136/jnnp.20.2.139

76. Gallagher M, Chiba AA. The amygdala and emotion. *Curr Opin Neurobiol*. Apr 1996;6(2):221-7. doi:10.1016/s0959-4388(96)80076-6

77. Barbey AK, Koenigs M, Grafman J. Dorsolateral prefrontal contributions to human working memory. *Cortex*. May 2013;49(5):1195-205. doi:10.1016/j.cortex.2012.05.022

78. Seghier ML. The angular gyrus: multiple functions and multiple subdivisions. *Neuroscientist*. Feb 2013;19(1):43-61. doi:10.1177/1073858412440596

79. Koenigs M, Barbey AK, Postle BR, Grafman J. Superior parietal cortex is critical for the manipulation of information in working memory. *J Neurosci*. Nov 25 2009;29(47):14980-6. doi:10.1523/jneurosci.3706-09.2009

80. Seixas D, Palace J, Tracey I. Chronic pain disrupts the reward circuitry in multiple sclerosis. *Eur J Neurosci*. Aug 2016;44(3):1928-34. doi:10.1111/ejn.13272

81. Yun SJ, Byun K, Bhin J, et al. Transcriptional regulatory networks associated with self-renewal and differentiation of neural stem cells. *J Cell Physiol*. Nov 2010;225(2):337-47. doi:10.1002/jcp.22294

82. Shi Z, Zhou H, Lu L, et al. The roles of microRNAs in spinal cord injury. *Int J Neurosci*. Dec 2017;127(12):1104-1115. doi:10.1080/00207454.2017.1323208

83. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. Jan 23 2009;136(2):215-33. doi:10.1016/j.cell.2009.01.002

84. Chen X, Xie D, Zhao Q, You ZH. MicroRNAs and complex diseases: from experimental results to computational models. *Brief Bioinform*. Mar 22 2019;20(2):515-539. doi:10.1093/bib/bbx130

85. Oulas A, Karathanasis N, Louloupi A, et al. Prediction of miRNA targets. *Methods Mol Biol*. 2015;1269:207-29. doi:10.1007/978-1-4939-2291-8_13

86. Liu NK, Wang XF, Lu QB, Xu XM. Altered microRNA expression following traumatic spinal cord injury. *Exp Neurol*. Oct 2009;219(2):424-9. doi:10.1016/j.expneurol.2009.06.015

87. Strickland ER, Hook MA, Balaraman S, Huie JR, Grau JW, Miranda RC. MicroRNA dysregulation following spinal cord contusion: implications for neural plasticity and repair. *Neuroscience*. Jul 14 2011;186:146-60. doi:10.1016/j.neuroscience.2011.03.063

88. Yunta M, Nieto-Diaz M, Esteban FJ, et al. MicroRNA dysregulation in the spinal cord following traumatic injury. *PLoS One*. 2012;7(4):e34534. doi:10.1371/journal.pone.0034534

89. Izumi B, Nakasa T, Tanaka N, et al. MicroRNA-223 expression in neutrophils in the early phase of secondary damage after spinal cord injury. *Neurosci Lett*. Apr 1 2011;492(2):114-8. doi:10.1016/j.neulet.2011.01.068

90. Hu JZ, Huang JH, Zeng L, Wang G, Cao M, Lu HB. Anti-apoptotic effect of microRNA-21 after contusion spinal cord injury in rats. *J Neurotrauma*. Aug 1 2013;30(15):1349-60. doi:10.1089/neu.2012.2748

91. Hu J, Zeng L, Huang J, Wang G, Lu H. miR-126 promotes angiogenesis and attenuates inflammation after contusion spinal cord injury in rats. *Brain Res*. May 22 2015;1608:191-202. doi:10.1016/j.brainres.2015.02.036

92. Johnnidis JB, Harris MH, Wheeler RT, et al. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature*. Feb 28 2008;451(7182):1125-9. doi:10.1038/nature06607

93. Muth DC, Powell BH, Zhao Z, Witwer KW. miRNAs in platelet-poor blood plasma and purified RNA are highly stable: a confirmatory study. *BMC Res Notes*. May 4 2018;11(1):273. doi:10.1186/s13104-018-3378-6

94. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. *Curr Biol*. Apr 30 2002;12(9):735-9. doi:10.1016/s0960-9822(02)00809-6

95. Liu X, Wu D, Wen S, et al. Mild therapeutic hypothermia protects against cerebral ischemia/reperfusion injury by inhibiting miR-15b expression in rats. *Brain Circ*. Oct-Dec 2017;3(4):219-226. doi:10.4103/bc.bc_15_17

96. Li J, Wang H. miR-15b reduces amyloid-β accumulation in SH-SY5Y cell line through targetting NF-κB signaling and BACE1. *Biosci Rep*. Dec 21 2018;38(6)doi:10.1042/bsr20180051

97. Yi X, Fang Q, Li L. MicroRNA-338-5p alleviates cerebral ischemia/reperfusion injury by targeting connective tissue growth factor through the adenosine 5'-monophosphate-activated protein kinase/mammalian target of

rapamycin signaling pathway. *Neuroreport*. Feb 5 2020;31(3):256-264. doi:10.1097/wnr.0000000000001404

98. Qian Q, Zhang J, He FP, et al. Down-regulated expression of microRNA-338-5p contributes to neuropathology in Alzheimer's disease. *Faseb j*. Mar 2019;33(3):4404-4417. doi:10.1096/fj.201801846R

99. Ramanathan S, Douglas SR, Alexander GM, et al. Exosome microRNA signatures in patients with complex regional pain syndrome undergoing plasma exchange. *J Transl Med*. Mar 14 2019;17(1):81. doi:10.1186/s12967-019-1833-3

100. Perreau VM, Adlard PA, Anderson AJ, Cotman CW. Exercise-induced gene expression changes in the rat spinal cord. *Gene Expr*. 2005;12(2):107-21. doi:10.3727/000000005783992115

101. Chio CC, Lin HJ, Tian YF, et al. Exercise attenuates neurological deficits by stimulating a critical HSP70/NF-κB/IL-6/synapsin I axis in traumatic brain injury rats. *J Neuroinflammation*. Apr 24 2017;14(1):90. doi:10.1186/s12974-017-0867-9

102. Koo JH, Jang YC, Hwang DJ, et al. Treadmill exercise produces neuroprotective effects in a murine model of Parkinson's disease by regulating the TLR2/MyD88/NF-κB signaling pathway. *Neuroscience*. Jul 25 2017;356:102-113. doi:10.1016/j.neuroscience.2017.05.016

103. Souza LC, Jesse CR, Del Fabbro L, et al. Swimming exercise prevents behavioural disturbances induced by an intracerebroventricular injection of amyloid-β(1-42) peptide through modulation of cytokine/NF-kappaB pathway and indoleamine-2,3-dioxygenase in mouse brain. *Behav Brain Res*. Jul 28 2017;331:1-13. doi:10.1016/j.bbr.2017.05.024

104. Detloff MR, Smith EJ, Quiros Molina D, Ganzer PD, Houlé JD. Acute exercise prevents the development of neuropathic pain and the sprouting of non-peptidergic (GDNF- and artemin-responsive) c-fibers after spinal cord injury. *Exp Neurol*. May 2014;255:38-48. doi:10.1016/j.expneurol.2014.02.013

105. Richardson EJ, McKinley EC, Rahman A, Klebine P, Redden DT, Richards JS. Effects of virtual walking on spinal cord injury-related neuropathic pain: A randomized, controlled trial. *Rehabil Psychol*. Feb 2019;64(1):13-24. doi:10.1037/rep0000246

106. Silva GJJ, Bye A, El Azzouzi H, Wisløff U. MicroRNAs as Important Regulators of Exercise Adaptation. *Prog Cardiovasc Dis*. Jun-Jul 2017;60(1):130-151. doi:10.1016/j.pcad.2017.06.003

107. Nielsen S, Åkerström T, Rinnov A, et al. The miRNA plasma signature in response to acute aerobic exercise and endurance training. *PLoS One*. 2014;9(2):e87308. doi:10.1371/journal.pone.0087308

108. Radom-Aizik S, Zaldivar F, Jr., Leu SY, Adams GR, Oliver S, Cooper DM. Effects of exercise on microRNA expression in young males peripheral blood mononuclear cells. *Clin Transl Sci*. Feb 2012;5(1):32-8. doi:10.1111/j.1752-8062.2011.00384.x

109. Radom-Aizik S, Zaldivar F, Haddad F, Cooper DM. Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults. *J Appl Physiol (1985)*. Mar 1 2013;114(5):628-36. doi:10.1152/japplphysiol.01341.2012

110. Radom-Aizik S, Zaldivar FP, Jr., Haddad F, Cooper DM. Impact of brief exercise on circulating monocyte gene and microRNA expression: implications

for atherosclerotic vascular disease. *Brain Behav Immun*. Jul 2014;39:121-9. doi:10.1016/j.bbi.2014.01.003

111. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A*. Feb 15 2011;108(7):3017-22. doi:10.1073/pnas.1015950108

112. Raji CA, Merrill DA, Eyre H, et al. Longitudinal Relationships between Caloric Expenditure and Gray Matter in the Cardiovascular Health Study. *J Alzheimers Dis*. 2016;52(2):719-29. doi:10.3233/jad-160057

113. Winchester P, McColl R, Querry R, et al. Changes in supraspinal activation patterns following robotic locomotor therapy in motor-incomplete spinal cord injury. *Neurorehabil Neural Repair*. Dec 2005;19(4):313-24. doi:10.1177/1545968305281515

114. Sacco K, Cauda F, D'Agata F, et al. A combined robotic and cognitive training for locomotor rehabilitation: evidences of cerebral functional reorganization in two chronic traumatic brain injured patients. *Front Hum Neurosci*. 2011;5:146. doi:10.3389/fnhum.2011.00146

115. Enzinger C, Dawes H, Johansen-Berg H, et al. Brain activity changes associated with treadmill training after stroke. *Stroke*. Jul 2009;40(7):2460-7. doi:10.1161/strokeaha.109.550053

116. Sandroff BM, Wylie GR, Sutton BP, Johnson CL, DeLuca J, Motl RW. Treadmill walking exercise training and brain function in multiple sclerosis: Preliminary evidence setting the stage for a network-based approach to rehabilitation. *Mult Scler J Exp Transl Clin*. Jan-Mar 2018;4(1):2055217318760641. doi:10.1177/2055217318760641

117. Ionta S, Ferretti A, Merla A, Tartaro A, Romani GL. Step-by-step: the effects of physical practice on the neural correlates of locomotion imagery revealed by fMRI. *Hum Brain Mapp*. May 2010;31(5):694-702. doi:10.1002/hbm.20898

118. Wang C, Wai Y, Kuo B, Yeh YY, Wang J. Cortical control of gait in healthy humans: an fMRI study. *J Neural Transm (Vienna)*. Aug 2008;115(8):1149-58. doi:10.1007/s00702-008-0058-z

119. Um YH, Wang SM, Kim NY, et al. Effects of Moderate Intensity Exercise on the Cortical Thickness and Subcortical Volumes of Preclinical Alzheimer's Disease Patients: A Pilot Study. *Psychiatry Investig*. Jun 2020;17(6):613-619. doi:10.30773/pi.2020.0214

120. Flodin P, Jonasson LS, Riklund K, Nyberg L, Boraxbekk CJ. Does Aerobic Exercise Influence Intrinsic Brain Activity? An Aerobic Exercise Intervention among Healthy Old Adults. *Front Aging Neurosci*. 2017;9:267. doi:10.3389/fnagi.2017.00267

121. Motl RW, Pilutti LA, Hubbard EA, Wetter NC, Sosnoff JJ, Sutton BP. Cardiorespiratory fitness and its association with thalamic, hippocampal, and basal ganglia volumes in multiple sclerosis. *Neuroimage Clin*. 2015;7:661-6. doi:10.1016/j.nicl.2015.02.017

122. Liu J, Chen L, Tu Y, et al. Different exercise modalities relieve pain syndrome in patients with knee osteoarthritis and modulate the dorsolateral prefrontal cortex: A multiple mode MRI study. *Brain Behav Immun*. Nov 2019;82:253-263. doi:10.1016/j.bbi.2019.08.193

123. Dai X, Cai Z, Liu J. Up-regulation of miR-338-5p after spinal cord injury enhances the neuronal repair via inhibition of inflammation aggravation and

oxidative stress. *Minerva Med*. Dec 13 2019;doi:10.23736/s0026-4806.19.06280-3

124. Motl RW, Hubbard EA, Sreekumar N, et al. Pallidal and caudate volumes correlate with walking function in multiple sclerosis. *J Neurol Sci*. Jul 15 2015;354(1-2):33-6. doi:10.1016/j.jns.2015.04.041

125. Baird JF, Cutter GR, Motl RW. Do physical activity, cardiorespiratory fitness, and subcortical brain structures explain reduced walking performance in older adults with multiple sclerosis? *Mult Scler Relat Disord*. Apr 2022;60:103702. doi:10.1016/j.msard.2022.103702

126. Jakimovski D, Weinstock-Guttman B, Hagemeier J, et al. Walking disability measures in multiple sclerosis patients: Correlations with MRI-derived global and microstructural damage. *J Neurol Sci*. Oct 15 2018;393:128-134. doi:10.1016/j.jns.2018.08.020

127. Motl RW, Zivadinov R, Bergsland N, Benedict RH. Thalamus volume and ambulation in multiple sclerosis: a cross-sectional study. *Neurodegener Dis Manag*. 2016;6(1):23-9. doi:10.2217/nmt.15.71

128. Burke D, Fullen BM, Stokes D, Lennon O. Neuropathic pain prevalence following spinal cord injury: A systematic review and meta-analysis. *European journal of pain*. Jan 2017;21(1):29-44. doi:10.1002/ejp.905

129. Sekiguchi M, Sekiguchi Y, Konno S, Kobayashi H, Homma Y, Kikuchi S. Comparison of neuropathic pain and neuronal apoptosis following nerve root or spinal nerve compression. *Eur Spine J*. Dec 2009;18(12):1978-85. doi:10.1007/s00586-009-1064-z

130. Meng C, Liang X, Li Q, Chen G, Liu H, Li K. Changes of GTP cyclohydrolase I and neuronal apoptosis in rat spinal dorsal cord induced by sciatic nerve injury. *Neurol Sci*. Dec 2013;34(12):2145-50. doi:10.1007/s10072-013-1353-5

131. Fu H, Li F, Thomas S, Yang Z. Hyperbaric oxygenation alleviates chronic constriction injury (CCI)-induced neuropathic pain and inhibits GABAergic neuron apoptosis in the spinal cord. *Scand J Pain*. Oct 2017;17:330-338. doi:10.1016/j.sjpain.2017.08.014

132. Li J, Chen X, Li X, et al. Upregulation of Cdh1 in the trigeminal spinal subnucleus caudalis attenuates trigeminal neuropathic pain via inhibiting GABAergic neuronal apoptosis. *Neurochem Int*. Feb 2020;133:104613. doi:10.1016/j.neuint.2019.104613

133. Kang J, Li Z, Zhi Z, Wang S, Xu G. MiR-21 derived from the exosomes of MSCs regulates the death and differentiation of neurons in patients with spinal cord injury. *Gene Ther*. Dec 2019;26(12):491-503. doi:10.1038/s41434-019-0101-8

134. Zhang T, Ni S, Luo Z, Lang Y, Hu J, Lu H. The protective effect of microRNA-21 in neurons after spinal cord injury. *Spinal Cord*. Feb 2019;57(2):141-149. doi:10.1038/s41393-018-0180-1

135. Xu G, Ao R, Zhi Z, Jia J, Yu B. miR-21 and miR-19b delivered by hMSC-derived EVs regulate the apoptosis and differentiation of neurons in patients with spinal cord injury. *J Cell Physiol*. Jul 2019;234(7):10205-10217. doi:10.1002/jcp.27690

136. Wang Z, Song Y, Han X, Qu P, Wang W. Long noncoding RNA PTENP1 affects the recovery of spinal cord injury by regulating the expression of miR-19b and miR-21. *J Cell Physiol*. Apr 2020;235(4):3634-3645. doi:10.1002/jcp.29253

137. Li M, Jiang WT, Li J, Ji WC. Exercise protects against spinal cord injury through miR-21-mediated suppression of PDCD4. *Am J Transl Res*. 2020;12(9):5708-5718.

138. Liu R, Wang W, Wang S, Xie W, Li H, Ning B. microRNA-21 regulates astrocytic reaction post-acute phase of spinal cord injury through modulating TGF-β signaling. *Aging (Albany NY)*. Jun 23 2018;10(6):1474-1488. doi:10.18632/aging.101484

139. Chung HJ, Chung WH, Do SH, Lee JH, Kim HY. Up-regulation of MicroRNAs-21 and -223 in a Sprague-Dawley Rat Model of Traumatic Spinal Cord Injury. *Brain Sci*. Mar 2 2020;10(3)doi:10.3390/brainsci10030141

140. Bai G, Jiang L, Meng P, et al. LncRNA Neat1 Promotes Regeneration after Spinal Cord Injury by Targeting miR-29b. *J Mol Neurosci*. Nov 11 2020;doi:10.1007/s12031-020-01740-3

141. Liu XJ, Zheng XP, Zhang R, Guo YL, Wang JH. Combinatorial effects of miR-20a and miR-29b on neuronal apoptosis induced by spinal cord injury. *Int J Clin Exp Pathol*. 2015;8(4):3811-8.

142. Kang J, Zhang C, Zhi Z, et al. Stem-like cells of various origins showed therapeutic effect to improve the recovery of spinal cord injury. *Artif Cells Nanomed Biotechnol*. Dec 2020;48(1):627-638. doi:10.1080/21691401.2020.1725031

143. Nguyen LH, Ong W, Wang K, Wang M, Nizetic D, Chew SY. Effects of miR-219/miR-338 on microglia and astrocyte behaviors and astrocyte-oligodendrocyte precursor cell interactions. *Neural Regen Res*. Apr 2020;15(4):739-747. doi:10.4103/1673-5374.266922

144. Liu C, Cao Y, Ko TC, Chen M, Zhou X, Wang R. The Changes of MicroRNA Expression in the Corpus Cavernosum of a Rat Model With Cavernous Nerve Injury. *J Sex Med*. Jul 2018;15(7):958-965. doi:10.1016/j.jsxm.2018.05.006

145. Abraham A, Barnett C, Katzberg HD, Lovblom LE, Perkins BA, Bril V. Sex differences in neuropathic pain intensity in diabetes. *J Neurol Sci*. May 15 2018;388:103-106. doi:10.1016/j.jns.2018.03.008

146. Boogaard S, Heymans MW, de Vet HC, et al. Predictors of Persistent Neuropathic Pain--A Systematic Review. *Pain Physician*. Sep-Oct 2015;18(5):433-57.

147. Momi SK, Fabiane SM, Lachance G, Livshits G, Williams FMK. Neuropathic pain as part of chronic widespread pain: environmental and genetic influences. *Pain*. Oct 2015;156(10):2100-2106. doi:10.1097/j.pain.0000000000000277

148. Parruti G, Tontodonati M, Rebuzzi C, et al. Predictors of pain intensity and persistence in a prospective Italian cohort of patients with herpes zoster: relevance of smoking, trauma and antiviral therapy. *BMC Med*. Oct 11 2010;8:58. doi:10.1186/1741-7015-8-58

149. Moulin DE, Clark AJ, Gordon A, et al. Long-Term Outcome of the Management of Chronic Neuropathic Pain: A Prospective Observational Study. *J Pain*. Sep 2015;16(9):852-61. doi:10.1016/j.jpain.2015.05.011

150. Çelik SB, Can H, Sözmen MK, et al. Evaluation of the neuropathic pain in the smokers. *Agri*. Jul 2017;29(3):122-126. doi:10.5505/agri.2017.68815

151. Zhang L, Li PP, Feng X, Barker JL, Smith SV, Rubinow DR. Sex-related differences in neuronal cell survival and signaling in rats. *Neurosci Lett*. Feb 6 2003;337(2):65-8. doi:10.1016/s0304-3940(02)01179-5

152. Andreasson KI, Savonenko A, Vidensky S, et al. Age-dependent cognitive deficits and neuronal apoptosis in cyclooxygenase-2 transgenic mice. *J Neurosci*. Oct 15 2001;21(20):8198-209. doi:10.1523/jneurosci.21-20-08198.2001

153. Fan ZK, Cao Y, Lv G, Wang YS, Guo ZP. The effect of cigarette smoke exposure on spinal cord injury in rats. *J Neurotrauma*. Mar 15 2013;30(6):473-9. doi:10.1089/neu.2012.2574

154. Anbarasi K, Kathirvel G, Vani G, Jayaraman G, Shyamala Devi CS. Cigarette smoking induces heat shock protein 70 kDa expression and apoptosis in rat brain: Modulation by bacoside A. *Neuroscience*. 2006;138(4):1127-35. doi:10.1016/j.neuroscience.2005.11.029

155. Wallauer MM, Huf F, Tortorelli LS, et al. Morphological changes in the cerebellum as a result of ethanol treatment and cigarette smoke exposure: A study on astrogliosis, apoptosis and Purkinje cells. *Neurosci Lett*. Apr 13 2018;672:70-77. doi:10.1016/j.neulet.2018.02.047

156. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis*. Dec 1978;118(6 Pt 2):1-120.

157. Morse LR, Battaglino RA, Stolzmann KL, et al. Osteoporotic fractures and hospitalization risk in chronic spinal cord injury. *Osteoporos Int*. Mar 2009;20(3):385-92. doi:10.1007/s00198-008-0671-6

158. Battaglino RA, Nguyen N, Summers M, Morse LR. B Cell-Activating Factor Is Associated with Testosterone and Smoking Status in Non-Ambulatory Men with Chronic Spinal Cord Injury. *J Neurotrauma*. Dec 15 2019;36(24):3332-3337. doi:10.1089/neu.2018.6221

159. Kok MGM, de Ronde MWJ, Moerland PD, Ruijter JM, Creemers EE, Pinto-Sietsma SJ. Small sample sizes in high-throughput miRNA screens: A common pitfall for the identification of miRNA biomarkers. *Biomol Detect Quantif*. May 2018;15:1-5. doi:10.1016/j.bdq.2017.11.002

160. Mei HX, Zhou MH, Zhang XW, et al. Effects of miR-338 on morphine tolerance by targeting CXCR4 in a rat model of bone cancer pain. *Biosci Rep*. Apr 30 2017;37(2)doi:10.1042/bsr20160517

161. Li J, Li D, Zhou H, et al. MicroRNA-338-5p alleviates neuronal apoptosis via directly targeting BCL2L11 in APP/PS1 mice. *Aging (Albany NY)*. Oct 21 2020;12(20):20728-20742. doi:10.18632/aging.104005

162. Zhou YQ, Liu Z, Liu ZH, et al. Interleukin-6: an emerging regulator of pathological pain. *J Neuroinflammation*. Jun 7 2016;13(1):141. doi:10.1186/s12974-016-0607-6

163. Murakami T, Kanchiku T, Suzuki H, et al. Anti-interleukin-6 receptor antibody reduces neuropathic pain following spinal cord injury in mice. *Exp Ther Med*. Nov 2013;6(5):1194-1198. doi:10.3892/etm.2013.1296

164. Chen SX, Wang SK, Yao PW, et al. Early CALP2 expression and microglial activation are potential inducers of spinal IL-6 up-regulation and bilateral pain following motor nerve injury. *J Neurochem*. Apr 2018;145(2):154-169. doi:10.1111/jnc.14317

165. Lee KM, Jeon SM, Cho HJ. Interleukin-6 induces microglial CX3CR1 expression in the spinal cord after peripheral nerve injury through the activation of p38 MAPK. *Eur J Pain*. Aug 2010;14(7):682.e1-12. doi:10.1016/j.ejpain.2009.10.017

166. Luo X, Wang X, Xia Z, Chung SK, Cheung CW. CXCL12/CXCR4 axis: an emerging neuromodulator in pathological pain. *Rev Neurosci*. Jan 2016;27(1):83-92. doi:10.1515/revneuro-2015-0016

167. Reaux-Le Goazigo A, Rivat C, Kitabgi P, Pohl M, Melik Parsadaniantz S. Cellular and subcellular localization of CXCL12 and CXCR4 in rat nociceptive structures: physiological relevance. *Eur J Neurosci*. Sep 2012;36(5):2619-31. doi:10.1111/j.1460-9568.2012.08179.x

168. Bhangoo S, Ren D, Miller RJ, et al. Delayed functional expression of neuronal chemokine receptors following focal nerve demyelination in the rat: a mechanism for the development of chronic sensitization of peripheral nociceptors. *Mol Pain*. Dec 12 2007;3:38. doi:10.1186/1744-8069-3-38

169. Samman Tahhan A, Hammadah M, Raad M, et al. Progenitor Cells and Clinical Outcomes in Patients With Acute Coronary Syndromes. *Circ Res*. May 25 2018;122(11):1565-1575. doi:10.1161/circresaha.118.312821

170. Strzelak A, Ratajczak A, Adamiec A, Feleszko W. Tobacco Smoke Induces and Alters Immune Responses in the Lung Triggering Inflammation, Allergy, Asthma and Other Lung Diseases: A Mechanistic Review. *Int J Environ Res Public Health*. May 21 2018;15(5)doi:10.3390/ijerph15051033

171. Olsson P, Skogstrand K, Nilsson A, et al. Smoking, disease characteristics and serum cytokine levels in patients with primary Sjögren's syndrome. *Rheumatol Int*. Aug 2018;38(8):1503-1510. doi:10.1007/s00296-018-4063-8

172. Zhao Y, Zhu L, Yu S, Zhu J, Wang C. CaMKII inhibition promotes neuronal apoptosis by transcriptionally upregulating Bim expression. *Neuroreport*. Sep 28 2016;27(14):1018-23. doi:10.1097/wnr.0000000000000648

173. Wu Y, Ma S, Xia Y, et al. Loss of GCN5 leads to increased neuronal apoptosis by upregulating E2F1- and Egr-1-dependent BH3-only protein Bim. *Cell Death Dis*. Jan 26 2017;8(1):e2570. doi:10.1038/cddis.2016.465

174. Zimmermann AK, Loucks FA, Le SS, et al. Distinct mechanisms of neuronal apoptosis are triggered by antagonism of Bcl-2/Bcl-x(L) versus induction of the BH3-only protein Bim. *J Neurochem*. Jul 2005;94(1):22-36. doi:10.1111/j.1471-4159.2005.03156.x

175. Zhao B, Qian M, Zhang Y, Yin F. Stem cells from human exfoliated deciduous teeth transmit microRNA-26a to protect rats with experimental intracerebral hemorrhage from cerebral injury via suppressing CTGF. *Brain Res Bull*. Dec 14 2020;doi:10.1016/j.brainresbull.2020.12.006

176. Pei G, Xu L, Huang W, Yin J. The protective role of microRNA-133b in restricting hippocampal neurons apoptosis and inflammatory injury in rats with depression by suppressing CTGF. *Int Immunopharmacol*. Jan 2020;78:106076. doi:10.1016/j.intimp.2019.106076

177. Eser O, Cosar M, Sahin O, et al. The neuroprotective effects of caffeic acid phenethyl ester (CAPE) in the hippocampal formation of cigarette smoke exposed rabbits. *Pathology*. Aug 2007;39(4):433-7. doi:10.1080/00313020701444481

178. Liu-Snyder P, McNally H, Shi R, Borgens RB. Acrolein-mediated mechanisms of neuronal death. *J Neurosci Res*. Jul 2006;84(1):209-18. doi:10.1002/jnr.20863

179. Butler B, Acosta G, Shi R. Exogenous Acrolein intensifies sensory hypersensitivity after spinal cord injury in rat. *J Neurol Sci*. Aug 15 2017;379:29-35. doi:10.1016/j.jns.2017.05.039

180. Pollock A, Baer G, Campbell P, et al. Physical rehabilitation approaches for the recovery of function and mobility following stroke. *Cochrane Database Syst Rev*. Apr 22 2014;(4):Cd001920. doi:10.1002/14651858.CD001920.pub3

181. Zhao S, Chen F, Wang D, Wang H, Han W, Zhang Y. Effect of preoperative smoking cessation on postoperative pain outcomes in elderly patients with high nicotine dependence. *Medicine (Baltimore)*. Jan 2019;98(3):e14209. doi:10.1097/md.0000000000014209

182. Behrend C, Prasarn M, Coyne E, Horodyski M, Wright J, Rechtine GR. Smoking Cessation Related to Improved Patient-Reported Pain Scores Following Spinal Care. *J Bone Joint Surg Am*. Dec 5 2012;94(23):2161-6. doi:10.2106/jbjs.k.01598

183. Bastian LA, Fish LJ, Gierisch JM, Stechuchak KM, Grambow SC, Keefe FJ. Impact of Smoking Cessation on Subsequent Pain Intensity Among Chronically Ill Veterans Enrolled in a Smoking Cessation Trial. *J Pain Symptom Manage*. Dec 2015;50(6):822-9. doi:10.1016/j.jpainsymman.2015.06.012

184. Cardenas DD, Felix ER. Pain after spinal cord injury: a review of classification, treatment approaches, and treatment assessment. *Pm r*. Dec 2009;1(12):1077-90. doi:10.1016/j.pmrj.2009.07.002

185. Kosek E, Cohen M, Baron R, et al. Do we need a third mechanistic descriptor for chronic pain states? *Pain*. Jul 2016;157(7):1382-1386. doi:10.1097/j.pain.0000000000000507

186. DiPiro ND, Murday D, Corley EH, DiPiro TV, Krause JS. Opioid Use Among Individuals With Spinal Cord Injury: Prevalence Estimates Based on State Prescription Drug Monitoring Program Data. *Arch Phys Med Rehabil*. May 2021;102(5):828-834. doi:10.1016/j.apmr.2020.10.128

187. Bishop GH, Landau WM, Jones MH. Evidence for a double peripheral pathway for pain. *Science*. Sep 26 1958;128(3326):712-4. doi:10.1126/science.128.3326.712

188. Matre DA, Hernandez-Garcia L, Tran TD, Casey KL. "First pain" in humans: convergent and specific forebrain responses. *Mol Pain*. Nov 17 2010;6:81. doi:10.1186/1744-8069-6-81

189. Veldhuijzen DS, Nemenov MI, Keaser M, Zhuo J, Gullapalli RP, Greenspan JD. Differential brain activation associated with laser-evoked burning and pricking pain: An event-related fMRI study. *Pain*. Jan 2009;141(1-2):104-13. doi:10.1016/j.pain.2008.10.027

190. Wanigasekera V, Mezue M, Andersson J, Kong Y, Tracey I. Disambiguating Pharmacodynamic Efficacy from Behavior with Neuroimaging: Implications for Analgesic Drug Development. *Anesthesiology*. Jan 2016;124(1):159-68. doi:10.1097/aln.0000000000000924

191. Gorka SM, Fitzgerald DA, de Wit H, Angstadt M, Phan KL. Opioid modulation of resting-state anterior cingulate cortex functional connectivity. *J Psychopharmacol*. Dec 2014;28(12):1115-24. doi:10.1177/0269881114548436

192. Croosu SS, Frøkjaer JB, Drewes AM, Hansen TM. Tapentadol and oxycodone affect resting-state functional brain connectivity: A randomized, placebo-controlled trial. *J Neuroimaging*. Sep 2021;31(5):956-961. doi:10.1111/jon.12902

193. Widerström-Noga E, Biering-Sørensen F, Bryce TN, et al. The International Spinal Cord Injury Pain Basic Data Set (version 2.0). *Spinal Cord*. Apr 2014;52(4):282-6. doi:10.1038/sc.2014.4

194. Bryce TN, Biering-Sørensen F, Finnerup NB, et al. International spinal cord injury pain classification: part I. Background and description. March 6-7, 2009. *Spinal Cord*. Jun 2012;50(6):413-7. doi:10.1038/sc.2011.156

195. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain connectivity*. 2012;2(3):125-41. doi:10.1089/brain.2012.0073

196. Friston KJ, Holmes AP, Worsley KJ, Poline J-P, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. *Human brain mapping*. 1994;2(4):189-210. doi:https://doi.org/10.1002/hbm.460020402

197. Grant JA, Courtemanche J, Duerden EG, Duncan GH, Rainville P. Cortical thickness and pain sensitivity in zen meditators. *Emotion*. Feb 2010;10(1):43-53. doi:10.1037/a0018334

198. Tang Y, Ren C, Wang M, et al. Altered gray matter volume and functional connectivity in patients with herpes zoster and postherpetic neuralgia. *Brain Res*. Oct 15 2021;1769:147608. doi:10.1016/j.brainres.2021.147608

199. Zhang Y, Mao Z, Pan L, et al. Dysregulation of Pain- and Emotion-Related Networks in Trigeminal Neuralgia. *Front Hum Neurosci*. 2018;12:107. doi:10.3389/fnhum.2018.00107

200. Geha PY, Baliki MN, Chialvo DR, Harden RN, Paice JA, Apkarian AV. Brain activity for spontaneous pain of postherpetic neuralgia and its modulation

by lidocaine patch therapy. *Pain*. Mar 2007;128(1-2):88-100. doi:10.1016/j.pain.2006.09.014

201. Borsook D, Upadhyay J, Chudler EH, Becerra L. A key role of the basal ganglia in pain and analgesia--insights gained through human functional imaging. *Mol Pain*. May 13 2010;6:27. doi:10.1186/1744-8069-6-27

202. Chudler EH, Dong WK. The role of the basal ganglia in nociception and pain. *Pain*. Jan 1995;60(1):3-38. doi:10.1016/0304-3959(94)00172-b

203. Kyathanahally SP, Azzarito M, Rosner J, et al. Microstructural plasticity in nociceptive pathways after spinal cord injury. *J Neurol Neurosurg Psychiatry*. May 26 2021;92(8):863-71. doi:10.1136/jnnp-2020-325580

204. Zhao Y, Zhang L, Rütgen M, Sladky R, Lamm C. Neural dynamics between anterior insular cortex and right supramarginal gyrus dissociate genuine affect sharing from perceptual saliency of pretended pain. *Elife*. Aug 19 2021;10doi:10.7554/eLife.69994

205. Widerström-Noga E, Cruz-Almeida Y, Felix ER, Pattany PM. Somatosensory phenotype is associated with thalamic metabolites and pain intensity after spinal cord injury. *Pain*. Jan 2015;156(1):166-174. doi:10.1016/j.pain.0000000000000019

206. Benarroch EE. What is the role of the cingulate cortex in pain? *Neurology*. Oct 20 2020;95(16):729-732. doi:10.1212/wnl.0000000000010712

207. Ong WY, Stohler CS, Herr DR. Role of the Prefrontal Cortex in Pain Processing. *Mol Neurobiol*. Feb 2019;56(2):1137-1166. doi:10.1007/s12035-018-1130-9

208. Baliki MN, Mansour AR, Baria AT, Apkarian AV. Functional reorganization of the default mode network across chronic pain conditions. *PloS one*. 2014;9(9):e106133. doi:10.1371/journal.pone.0106133

209. Wu Y, Wang C, Qian W, et al. Disrupted default mode network dynamics in recuperative patients of herpes zoster pain. *CNS Neurosci Ther*. Dec 2020;26(12):1278-1287. doi:10.1111/cns.13433

210. Yoon EJ, Kim YK, Shin HI, Lee Y, Kim SE. Cortical and white matter alterations in patients with neuropathic pain after spinal cord injury. *Brain Res*. Dec 2 2013;1540:64-73. doi:10.1016/j.brainres.2013.10.007

211. Kim JA, Bosma RL, Hemington KS, et al. Cross-network coupling of neural oscillations in the dynamic pain connectome reflects chronic neuropathic pain in multiple sclerosis. *Neuroimage Clin*. 2020;26:102230. doi:10.1016/j.nicl.2020.102230

212. Finnerup NB, Norrbrink C, Trok K, et al. Phenotypes and predictors of pain following traumatic spinal cord injury: a prospective study. *J Pain*. Jan 2014;15(1):40-8. doi:10.1016/j.jpain.2013.09.008

213. Kennedy P, Frankel H, Gardner B, Nuseibeh I. Factors associated with acute and chronic pain following traumatic spinal cord injuries. *Spinal Cord*. Dec 1997;35(12):814-7. doi:10.1038/sj.sc.3100569

214. Burke D, Lennon O, Fullen BM. Quality of life after spinal cord injury: The impact of pain. *Eur J Pain*. Oct 2018;22(9):1662-1672. doi:10.1002/ejp.1248

215. Bennie JA, De Cocker K, Biddle SJH, Teychenne MJ. Joint and dose-dependent associations between aerobic and muscle-strengthening activity with

depression: A cross-sectional study of 1.48 million adults between 2011 and 2017. *Depression and anxiety*. Feb 2020;37(2):166-178. doi:10.1002/da.22986

216. Geneen LJ, Moore RA, Clarke C, Martin D, Colvin LA, Smith BH. Physical activity and exercise for chronic pain in adults: an overview of Cochrane Reviews. *The Cochrane database of systematic reviews*. Apr 24 2017;4:CD011279. doi:10.1002/14651858.CD011279.pub3

217. Tawashy AE, Eng JJ, Lin KH, Tang PF, Hung C. Physical activity is related to lower levels of pain, fatigue and depression in individuals with spinal-cord injury: a correlational study. *Spinal Cord*. Apr 2009;47(4):301-6. doi:10.1038/sc.2008.120

218. Mulroy SJ, Hatchett PE, Eberly VJ, et al. Objective and Self-Reported Physical Activity Measures and Their Association With Depression and Satisfaction With Life in Persons With Spinal Cord Injury. *Archives of physical medicine and rehabilitation*. Oct 2016;97(10):1714-20. doi:10.1016/j.apmr.2016.03.018

219. Ditor DS, Latimer AE, Ginis KA, Arbour KP, McCartney N, Hicks AL. Maintenance of exercise participation in individuals with spinal cord injury: effects on quality of life, stress and pain. *Spinal Cord*. Aug 2003;41(8):446-50. doi:10.1038/sj.sc.3101487

220. Hicks AL, Martin KA, Ditor DS, et al. Long-term exercise training in persons with spinal cord injury: effects on strength, arm ergometry performance and psychological well-being. *Spinal Cord*. Jan 2003;41(1):34-43. doi:10.1038/sj.sc.3101389

221. Curtis KA, Tyner TM, Zachary L, et al. Effect of a standard exercise protocol on shoulder pain in long-term wheelchair users. *Spinal Cord*. Jun 1999;37(6):421-9.

222. Mulroy SJ, Thompson L, Kemp B, et al. Strengthening and optimal movements for painful shoulders (STOMPS) in chronic spinal cord injury: a randomized controlled trial. *Physical therapy*. Mar 2011;91(3):305-24. doi:10.2522/ptj.20100182

223. Serra-Ano P, Pellicer-Chenoll M, Garcia-Masso X, Morales J, Giner-Pascual M, Gonzalez LM. Effects of resistance training on strength, pain and shoulder functionality in paraplegics. *Spinal Cord*. Nov 2012;50(11):827-31. doi:10.1038/sc.2012.32

224. Kressler J, Thomas CK, Field-Fote EC, et al. Understanding therapeutic benefits of overground bionic ambulation: exploratory case series in persons with chronic, complete spinal cord injury. *Archives of physical medicine and rehabilitation*. Oct 2014;95(10):1878-1887 e4. doi:10.1016/j.apmr.2014.04.026

225. Sato G, Osumi M, Morioka S. Effects of wheelchair propulsion on neuropathic pain and resting electroencephalography after spinal cord injury. *J Rehabil Med*. Jan 31 2017;49(2):136-143. doi:10.2340/16501977-2185

226. Nam KY, Kim HJ, Kwon BS, Park JW, Lee HJ, Yoo A. Robot-assisted gait training (Lokomat) improves walking function and activity in people with spinal cord injury: a systematic review. *J Neuroeng Rehabil*. Mar 23 2017;14(1):24. doi:10.1186/s12984-017-0232-3

227. Fang CY, Tsai JL, Li GS, Lien AS, Chang YJ. Effects of Robot-Assisted Gait Training in Individuals with Spinal Cord Injury: A Meta-analysis. *Biomed Res Int*. 2020;2020:2102785. doi:10.1155/2020/2102785

228. Fundarò C, Giardini A, Maestri R, Traversoni S, Bartolo M, Casale R. Motor and psychosocial impact of robot-assisted gait training in a real-world rehabilitation setting: A pilot study. *PLoS One*. 2018;13(2):e0191894. doi:10.1371/journal.pone.0191894

229. Mazzoleni S, Battini E, Rustici A, Stampacchia G. An integrated gait rehabilitation training based on Functional Electrical Stimulation cycling and overground robotic exoskeleton in complete spinal cord injury patients: Preliminary results. *IEEE Int Conf Rehabil Robot*. Jul 2017;2017:289-293. doi:10.1109/icorr.2017.8009261

230. Cruciger O, Schildhauer TA, Meindl RC, et al. Impact of locomotion training with a neurologic controlled hybrid assistive limb (HAL) exoskeleton on neuropathic pain and health related quality of life (HRQoL) in chronic SCI: a case study (.). *Disabil Rehabil Assist Technol*. Aug 2016;11(6):529-34. doi:10.3109/17483107.2014.981875

231. Stampacchia G, Rustici A, Bigazzi S, Gerini A, Tombini T, Mazzoleni S. Walking with a powered robotic exoskeleton: Subjective experience, spasticity and pain in spinal cord injured persons. *NeuroRehabilitation*. Jun 27 2016;39(2):277-83. doi:10.3233/nre-161358

232. Labruyère R, van Hedel HJ. Strength training versus robot-assisted gait training after incomplete spinal cord injury: a randomized pilot study in patients depending on walking assistance. *J Neuroeng Rehabil*. Jan 9 2014;11:4. doi:10.1186/1743-0003-11-4

233. Sandroff BM, Wylie GR, Baird JF, et al. Effects of walking exercise training on learning and memory and hippocampal neuroimaging outcomes in MS: A targeted, pilot randomized controlled trial. *Contemp Clin Trials*. Nov 2021;110:106563. doi:10.1016/j.cct.2021.106563

234. Varma VR, Chuang YF, Harris GC, Tan EJ, Carlson MC. Low-intensity daily walking activity is associated with hippocampal volume in older adults. *Hippocampus*. May 2015;25(5):605-15. doi:10.1002/hipo.22397

235. Best JR, Rosano C, Aizenstein HJ, et al. Long-term changes in time spent walking and subsequent cognitive and structural brain changes in older adults. *Neurobiol Aging*. Sep 2017;57:153-161. doi:10.1016/j.neurobiolaging.2017.05.023

236. Ando D, Yokota C, Koshino K, et al. Microstructural white matter changes following gait training with Hybrid Assistive Limb initiated within 1 week of stroke onset. *J Neurol Sci*. Aug 15 2020;415:116939. doi:10.1016/j.jns.2020.116939

237. Palandi J, Bobinski F, de Oliveira GM, Ilha J. Neuropathic pain after spinal cord injury and physical exercise in animal models: A systematic review and meta-analysis. *Neurosci Biobehav Rev*. Jan 2020;108:781-795. doi:10.1016/j.neubiorev.2019.12.016

238. Ilha J, Meireles A, de Freitas GR, et al. Overground gait training promotes functional recovery and cortical neuroplasticity in an incomplete spinal cord injury model. *Life Sci*. Sep 1 2019;232:116627. doi:10.1016/j.lfs.2019.116627

239. Kami K, Tajima F, Senba E. Plastic changes in amygdala subregions by voluntary running contribute to exercise-induced hypoalgesia in neuropathic pain model mice. *Mol Pain*. Jan-Dec 2020;16:1744806920971377. doi:10.1177/1744806920971377

240. Rowbotham MC. What is a "clinically meaningful" reduction in pain? *Pain*. 2001:131-132. vol. 2.

241. Ashburner J, Ridgway GR. Symmetric diffeomorphic modeling of longitudinal structural MRI. *Front Neurosci*. 2012;6:197. doi:10.3389/fnins.2012.00197

242. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage*. Oct 15 2007;38(1):95-113. doi:10.1016/j.neuroimage.2007.07.007

243. Taki Y, Thyreau B, Kinomura S, et al. Correlations among brain gray matter volumes, age, gender, and hemisphere in healthy individuals. *PLoS One*. 2011;6(7):e22734. doi:10.1371/journal.pone.0022734

244. Hutchinson KJ, Gómez-Pinilla F, Crowe MJ, Ying Z, Basso DM. Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. *Brain*. Jun 2004;127(Pt 6):1403-14. doi:10.1093/brain/awh160

245. Tashiro S, Shinozaki M, Mukaino M, et al. BDNF Induced by Treadmill Training Contributes to the Suppression of Spasticity and Allodynia After Spinal Cord Injury via Upregulation of KCC2. *Neurorehabil Neural Repair*. Aug 2015;29(7):677-89. doi:10.1177/1545968314562110

246. Sliwinski C, Nees TA, Puttagunta R, Weidner N, Blesch A. Sensorimotor Activity Partially Ameliorates Pain and Reduces Nociceptive Fiber Density in the Chronically Injured Spinal Cord. *J Neurotrauma*. Sep 15 2018;35(18):2222-2238. doi:10.1089/neu.2017.5431

247. Nees TA, Tappe-Theodor A, Sliwinski C, et al. Early-onset treadmill training reduces mechanical allodynia and modulates calcitonin gene-related peptide fiber density in lamina III/IV in a mouse model of spinal cord contusion injury. *Pain*. Mar 2016;157(3):687-697. doi:10.1097/j.pain.0000000000000422

248. Iyengar S, Ossipov MH, Johnson KW. The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. *Pain*. Apr 2017;158(4):543-559. doi:10.1097/j.pain.0000000000000831

249. Lanciego JL, Luquin N, Obeso JA. Functional neuroanatomy of the basal ganglia. *Cold Spring Harb Perspect Med*. Dec 1 2012;2(12):a009621. doi:10.1101/cshperspect.a009621

250. Sommer MA. The role of the thalamus in motor control. *Curr Opin Neurobiol*. Dec 2003;13(6):663-70. doi:10.1016/j.conb.2003.10.014

251. Ab Aziz CB, Ahmad AH. The role of the thalamus in modulating pain. *Malays J Med Sci*. Jul 2006;13(2):11-8.

252. Foster NN, Barry J, Korobkova L, et al. The mouse cortico-basal ganglia-thalamic network. *Nature*. Oct 2021;598(7879):188-194. doi:10.1038/s41586-021-03993-3

253. Torrico TJ, Munakomi S. Neuroanatomy, Thalamus. *StatPearls*. StatPearls Publishing
Copyright © 2022, StatPearls Publishing LLC.; 2022.

254. Shen S, Zheng H, Wang J, et al. Gray matter volume reduction with different disease duration in trigeminal neuralgia. *Neuroradiology*. Feb 2022;64(2):301-311. doi:10.1007/s00234-021-02783-y

255. Chen IH, Novak V, Manor B. Infarct hemisphere and noninfarcted brain volumes affect locomotor performance following stroke. *Neurology*. Mar 11 2014;82(10):828-34. doi:10.1212/wnl.0000000000000186

256. Allali G, Montembeault M, Brambati SM, et al. Brain Structure Covariance Associated With Gait Control in Aging. *J Gerontol A Biol Sci Med Sci*. Apr 23 2019;74(5):705-713. doi:10.1093/gerona/gly123

257. Allali G, Montembeault M, Saj A, et al. Structural Brain Volume Covariance Associated with Gait Speed in Patients with Amnestic and Non-Amnestic Mild Cognitive Impairment: A Double Dissociation. *J Alzheimers Dis*. 2019;71(s1):S29-s39. doi:10.3233/jad-190038

258. Beauchet O, Montembeault M, Barden JM, et al. Brain gray matter volume associations with gait speed and related structural covariance networks in cognitively healthy individuals and in patients with mild cognitive impairment: A cross-sectional study. *Exp Gerontol*. Jul 15 2019;122:116-122. doi:10.1016/j.exger.2019.05.001

259. Royse SK, Costacou T, Nunley KA, Orchard TJ, Rosano C. Neural correlates of slower gait in middle-aged persons with childhood-onset type 1 diabetes mellitus: The impact of accelerated brain aging. *J Diabetes Complications*. Feb 2022;36(2):108084. doi:10.1016/j.jdiacomp.2021.108084

260. Dumurgier J, Crivello F, Mazoyer B, et al. MRI atrophy of the caudate nucleus and slower walking speed in the elderly. *Neuroimage*. Apr 2 2012;60(2):871-8. doi:10.1016/j.neuroimage.2012.01.102

261. Su N, Liang X, Zhai FF, et al. The consequence of cerebral small vessel disease: Linking brain atrophy to motor impairment in the elderly. *Hum Brain Mapp*. Nov 2018;39(11):4452-4461. doi:10.1002/hbm.24284

262. Tian Q, Studenski SA, Montero-Odasso M, Davatzikos C, Resnick SM, Ferrucci L. Cognitive and neuroimaging profiles of older adults with dual decline

in memory and gait speed. *Neurobiol Aging*. Jan 2021;97:49-55. doi:10.1016/j.neurobiolaging.2020.10.002

263. Lipat AL, Clark DJ, Hass CJ, Cruz-Almeida Y. Gait subgroups among older adults with chronic pain differ in cerebellum and basal ganglia gray matter volumes. *Exp Gerontol*. Jun 15 2022;163:111773. doi:10.1016/j.exger.2022.111773

264. Herman T, Rosenberg-Katz K, Jacob Y, Giladi N, Hausdorff JM. Gray matter atrophy and freezing of gait in Parkinson's disease: Is the evidence black-on-white? *Mov Disord*. Jan 2014;29(1):134-9. doi:10.1002/mds.25697

265. Rosenberg-Katz K, Herman T, Jacob Y, et al. Fall risk is associated with amplified functional connectivity of the central executive network in patients with Parkinson's disease. *J Neurol*. Nov 2015;262(11):2448-56. doi:10.1007/s00415-015-7865-6

266. Negaresh R, Gharakhanlou R, Sahraian MA, Abolhasani M, Motl RW, Zimmer P. Physical activity may contribute to brain health in multiple sclerosis: An MR volumetric and spectroscopy study. *J Neuroimaging*. Jul 2021;31(4):714-723. doi:10.1111/jon.12869

267. Liu S, Li L, Liu Z, Guo X. Long-Term Tai Chi Experience Promotes Emotional Stability and Slows Gray Matter Atrophy for Elders. *Front Psychol*. 2019;10:91. doi:10.3389/fpsyg.2019.00091

268. Petkus AJ, Jarrahi B, Holschneider DP, et al. Thalamic volume mediates associations between cardiorespiratory fitness (VO(2)max) and cognition in Parkinson's disease. *Parkinsonism Relat Disord*. May 2021;86:19-26. doi:10.1016/j.parkreldis.2021.03.019

269. Robinson MM, Lowe VJ, Nair KS. Increased Brain Glucose Uptake After 12 Weeks of Aerobic High-Intensity Interval Training in Young and Older Adults. *J Clin Endocrinol Metab*. Jan 1 2018;103(1):221-227. doi:10.1210/jc.2017-01571

270. van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature neuroscience*. Mar 1999;2(3):266-70. doi:10.1038/6368

271. Olson AK, Eadie BD, Ernst C, Christie BR. Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus*. 2006;16(3):250-60. doi:10.1002/hipo.20157

272. Brown J, Cooper-Kuhn CM, Kempermann G, et al. Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *The European journal of neuroscience*. May 2003;17(10):2042-6.

273. Erickson KI, Prakash RS, Voss MW, et al. Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus*. Oct 2009;19(10):1030-9. doi:10.1002/hipo.20547

274. ten Brinke LF, Bolandzadeh N, Nagamatsu LS, et al. Aerobic exercise increases hippocampal volume in older women with probable mild cognitive impairment: a 6-month randomised controlled trial. *British journal of sports medicine*. Feb 2015;49(4):248-54. doi:10.1136/bjsports-2013-093184

275. Dobson JL, McMillan J, Li L. Benefits of exercise intervention in reducing neuropathic pain. *Front Cell Neurosci*. 2014;8:102. doi:10.3389/fncel.2014.00102

276. Schlaffke L, Lissek S, Lenz M, et al. Sports and brain morphology - a voxel-based morphometry study with endurance athletes and martial artists. *Neuroscience*. Feb 14 2014;259:35-42. doi:10.1016/j.neuroscience.2013.11.046

277. Zhang K, Jan YK, Liu Y, et al. Exercise Intensity and Brain Plasticity: What's the Difference of Brain Structural and Functional Plasticity Characteristics Between Elite Aerobic and Anaerobic Athletes? *Front Hum Neurosci*. 2022;16:757522. doi:10.3389/fnhum.2022.757522

278. Ozdurak Singin RH, Duz S, Kiraz M. Cortical and Subcortical Brain Volume Alterations Following Endurance Running at 38.6 km and 119.2 km in Male Athletes. *Med Sci Monit*. Jun 22 2021;27:e926060. doi:10.12659/msm.926060

279. Hänsel A, Hong S, Cámara RJ, von Känel R. Inflammation as a psychophysiological biomarker in chronic psychosocial stress. *Neurosci Biobehav Rev*. Sep 2010;35(1):115-21. doi:10.1016/j.neubiorev.2009.12.012

Appendix A: INTERNATIONAL SPINAL CORD INJURY PAIN

BASIC DATA SET Version 2.0

The working-group consists of:
Eva Widerström-Noga, DDS PhD (Chair) represents the International Spinal Cord Society
(ISCoS) and the American Spinal Injury Association (ASIA). She is also a member of the International Association for the Study of Pain (IASP), the American Pain Society (APS) and the Academy of Spinal Cord Injury Professionals.
Fin Biering-Sørensen, MD, PhD represents The Executive Committee of the International Spinal Cord Injury Standards and Data Sets (ASIA/ISCoS).
Thomas N. Bryce, MD represents the ASIA. He is also a member of the IASP and the ISCoS. Diana D Cardenas, MD represents the ASIA and is a member of the Academy of Spinal Cord Injury Professionals.
Nanna Brix Finnerup, MD, PhD, represents the IASP.
Mark P Jensen, PhD, represents the APS. He is also a member of the IASP.
J Scott Richards, PhD, represents the ASIA. He is also a member of the Academy of Spinal Cord Injury Professionals and the IASP.
Philip Siddall, MD, PhD, represents the IASP. He is also a member of the ISCoS.

This interdisciplinary working group was assembled based on published research expertise in the area of spinal cord injury (SCI) related pain. Individuals with expertise in SCI with regard to the clinical condition of pain, pain taxonomy, psychophysics of pain, psychology, epidemiology and assessment of pain were recommended by the presidents of some of the major organizations with an interest in SCI-related pain (i.e., the ISCoS, ASIA, APS and IASP). Most of the committee members have memberships in several of these organizations.

Chronic pain is one of the most frequently reported reasons for reduced quality of life following
SCI (Stensman 1994; Westgren & Levi, 1998). Pain taxonomies for SCI (Siddall et al., 2000; Bryce & Ragnarsson, 2001; Bryce et al., 2012a,b) classify pain as neuropathic or nociceptive, and according to level of injury. The neuropathic pains are usually associated with evoked pain, such as allodynia or hyperalgesia (Eide et al., 1996; Finnerup et al., 2001). The clinical presentation of pain associated with SCI is highly complex in that different pain types are often present simultaneously. Furthermore, the refractory nature of pain following SCI and the associated psychosocial distress emphasize the

need for a greater understanding of not only pathophysiological but also psychosocial mechanisms in the generation and maintenance of SCI-related pain and pain-related suffering. Ideally an effective treatment strategy should be tailored to specific pain-generating mechanisms in each individual. However, because of insufficient knowledge about the precise clinical symptoms and signs associated with a specific mechanism, this is not currently possible (Hansson, 2002).

In the clinical setting, information is collected that is important for the treatment decisions concerning the pain condition. Although physicians who treat individuals with SCI routinely collect clinical information, a standardized way to collect data concerning pain in persons with SCI is lacking. In order to expedite the development of beneficial treatments, it is important to evaluate the outcomes of treatments in a consistent manner. This would facilitate research collaboration between clinical centers and therefore result in larger well designed clinical pain trials in this population. The use of comparable sets of outcome measures in clinical practice and in trials would increase efficiency and greatly facilitate the translation, interpretation, and application of results to enhance the successful management of SCI related pain.

The purpose of the International Spinal Cord Injury Pain Data Set (ISCIPDS) is to standardize the collection and reporting of pain in the SCI population. The ISCIPDS contains a ***basic*** (ISCIPDS:B) and an ***extended*** (ISCIPDS:E) part. The ISCIPDS:B contains a minimal amount of clinically relevant information concerning pain that can be collected in the daily practice of healthcare professionals with expertise in SCI. In addition, the evaluation should be logistically feasible in various settings and countries. Although the intent of the ISCIPDS:B is to evaluate each separate pain problem, it may also be used to only evaluate the most significant or “worst” pain problem if there are time constraints. The ISCIPDS:E is primarily intended to be used for research purposes. The overall purpose of the ISCIPDS concurs with the purpose and vision of the International Spinal Cord Injury Data Sets (Biering-Sørensen et al., 2006) and should be used in conjunction with data in the International SCI Core Data Set (DeVivo et al., 2006). The International SCI Core Data Set includes information on date of birth and injury, gender, the cause of spinal cord lesion, and neurological status. In addition, the Core Data Set contains information on whether a vertebral injury was present, whether spinal surgery was performed, whether associated injuries were present, whether the patient with spinal cord lesion was ventilator-dependent at the time of discharge from initial inpatient care, and the place of discharge from initial inpatient care.

Background

The Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) has recommended that clinical pain trials designed to

evaluate the effectiveness of a therapy, should ***consider*** including a core set of outcomes (Dworkin et al., 2005). It was suggested that the assessment of pain severity, physical and emotional functioning would best capture the multidimensional nature of pain. However, it was also emphasized that complementary measures should be added when appropriate for specific pain populations. After SCI, a decrease in physical function may be more related to the physical impairments of SCI rather than to pain; therefore, a decrease in function ***due to*** pain, i.e., pain interference should be assessed (Widerström & Turk, 2004). These outcome domains are relevant both for clinical trials and clinical practice.

The questions in the ISCIPDS:B are based upon these three domains but adapted to consider the special issues related to SCI (i.e., several simultaneous different pain problems, physical impairments, etc.). The aspects regarding the specific nature of SCI-related pain include a pain intensity rating and a classification for each specific pain. Pain interference is addressed using three questions specifically addressing pain interference with activities, mood and sleep.

Version changes of the International SCI Pain Basic Data Set.

Version 1.0 to Version 1.1:

The only change made was related to the variable **Type of pain**, where the option *"At- and below-level (Neuropathic)"* was removed and merged with the "Below-level (Neuropathic)" pain. The revised "B*elow-level (Neuropathic)"* pain category now includes pain that may be experienced below the level of injury and extends to the level of injury. This modification was made since no current evidence suggests the underlying mechanisms differ between the two categories.

The International SCI Pain Basic Data Set (ISCIPBDS) published in 2008 was Version 1.1:

Widerström-Noga E, Biering-Sørensen F, Bryce T, Cardenas DD, Finnerup NB, Jensen MP, Richards S, Siddall PJ. The International Spinal Cord Injury Pain Basic Data Set. Spinal Cord 2008;46:818-23.

Version 1.1 to Version 2.0 (2.0 version finalized 21 May, 2013):

Several changes have been made due to both updates to the pain classification scheme and desires from the field to shorten the International SCI Pain Basic Data Set to facilitate its clinical usefulness:

1. Related to the variable **Type of pain**, an extra option *"Other"* is inserted, in accordance with the changes made in the International Spinal Cord Injury Pain (ISCIP) Classification (Bryce et al. 2012a). Also only one choice of pain type should now be chosen. This manual has been updated with more detail to facilitate the pain classification according to the ISCIP Classification.

2. The variable **Number of days with pain in the last 7 days including today** has been deleted to shorten the International SCI Pain Basic Data Set.
3. The variable **How long does your pain usually last?** has been deleted to shorten the International SCI Pain Basic Data Set.
4. The variable **When is the pain most intense?** has been deleted to shorten the International SCI Pain Basic Data Set.
5. The variable **How much do you limit your activities in order to keep your pain from getting worse?** has been deleted to shorten the International SCI Pain Basic Data Set.
6. The variable **How much has your pain changed your ability to take part in recreational and other social activities?** has been deleted to shorten the International SCI Pain Basic Data Set.
7. The variable **How much has your pain changed the amount of satisfaction or enjoyment you get from family-related activities?** has been deleted to shorten the International SCI Pain Basic Data Set.
8. The 3 remaining **Pain Interference questions** shall be applied for overall pain rather than differentiated for up to 3 pain types and be scored on a 0 to 10 scale instead of 0 to 6 for consistency with the pain intensity item. Please note that the psychometric properties were evaluated with these items scored between 0 and 6. We expect minimal to no effects on these properties with the revision.

Acknowledgement:

Pfizer Corp supported the initial work involved in developing this Data Set with an unconditional grant.
The authors also want to thank ISCoS, ASIA, and the APS Boards and the IASP Neuropathic Pain Special Interest Group for helpful suggestions. We also want to thank the following individual reviewers for their thoughtful suggestions: Sergio Aito, Susan Charlifue, Michael DeVivo, Petra Dokladal, Robert Dworkin, William Donovan, Pascal Halder, Jennifer Haythornthwaite, Steven Kirshblum, Vanessa Noonan, Lawrence Vogel and Gale Whiteneck.

Endorsement:
The International SCI Pain Basic Data Set Version 1.1 has been officially endorsed by the ISCoS, ASIA, IASP and the APS.

References:
American Spinal Injury Association: International Standards for Neurological Classification of Spinal Cord Injury, revised 2002; Chicago, IL, American Spinal Injury Association; 2002.

Biering-Sørensen F, Charlifue S, DeVivo M, Noonan V, Post M, Stripling T, Wing P. International spinal cord injury data sets. Spinal Cord. 2006;44:530-4.

Bryce TN, Ragnarsson KT. Epidemiology and classification of pain after spinal cord injury. Top Spinal Cord Inj Rehabil. 2001;7:1-17.

Bryce TN, Budh CN, Cardenas DD, Dijkers M, Felix ER, Finnerup NB, Kennedy P, Lundeberg T, Richards JS, Rintala DH, Siddall P, Widerstrom-Noga E. Pain after spinal cord injury: an evidence-based review for clinical practice and research. Report of the National Institute on Disability and Rehabilitation Research Spinal Cord Injury Measures meeting. J Spinal Cord Med. 2007;30:421-40.

Bryce TN, Biering-Sorensen F, Finnerup NB, Cardenas DD, Defrin R, Lundeberg T, Norrbrink C, Richards JS, Siddall PJ, Stripling T, Treede RD, Waxman SG, Widerström-Noga E, Yezierski RP, Dijkers M. International spinal cord injury pain (ISCIP) classification: part I. background and description. Spinal Cord. 2012a: 50: 413-417

Bryce TN, Biering-Sorensen F, Finnerup NB, Cardenas DD, Defrin R, Ivan E, Lundeberg T, Norrbrink C, Richards JS, Siddall PJ, Stripling T, Treede RD, Waxman SG, Widerström-Noga E, Yezierski RP, Dijkers M. International spinal cord injury pain (ISCIP) classification: part 2. initial validation using vignettes. Spinal Cord. 2012b: 50: 404-412

Cardenas DA, Turner JA, Warms CA, Marshall HM. Classification of chronic pain associated with spinal cord injuries. Arch Phys Med Rehabil. 2002;83:1708-14.

DeVivo M, Biering-Sørensen F, Charlifue S, Noonan V, Post M, Stripling T, Wing P. International Spinal Cord Injury Core Data Set. Spinal Cord. 2006;44:535-40.

Donovan WH, Dimitrijevic MR, Dahm L, Dimitrijevic M. Neurophysiological approaches to chronic pain following spinal cord injury. Paraplegia. 1982;20:135-46.

Dworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP, Kerns RD, Stucki G, Allan RR, Bellamy N, Carr DB, Chandler J, Cowan P, Dionne R, Galer BS, Hertz S, Jadad AR, Kramer LD, Manning DC, Martin S, McCormick CG, McDermott, MP, McGrath P, Quessy S, Rappaport BA, Robbins W, Robinson JP, Rothman M, Royal MA, Simon L, Stauffer JW, Stein

W, Tollett J, Wernicke J, Witter J. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain. 2005;113:9-19.

Eide PK, Jorum E, Stenehjelm AE. Somatosensory findings in patients with spinal cord injury and central dyesthesia pain. J Neurol Neurosurg Psychiatry 1996;60:411-5.

Finnerup NB, Johannesen IL, Sindrup SH, Bach FW, Jensen TS. Pain and dysesthesia in patients with spinal cord injury: A postal survey. Spinal Cord. 2001;39:256-62.

Hansson P. Neuropathic pain: clinical characteristics and diagnostic workup. Eur J Pain. 2002;6 Suppl A:47-50.

Hirsh AT, Bockow TB, Jensen MP. Catastrophizing, pain, and pain interference in individuals with disabilities.Am J Phys Med Rehabil. 2011 Sep;90(9):713-22.

Jensen MP, Karoly P. (2001). Self-report scales and procedures for assessing pain in adults. In DC Turk & R Melzack (Eds.), Handbook of pain assessment, 2nd edition. New York: Guilford Publications, pp. 15-34.

Jensen MP, Widerström-Noga E, Richards JS, Finnerup NB, Biering-Sørensen F, Cardenas DD. Reliability and Validity of the International Spinal Cord Injury Basic Pain Dataset Items as SelfReport Measures. Spinal Cord, 2010;48:230-8.

Kirshblum SC, Burns SP, Biering-Sorensen F, Donovan W, Graves DE, Jha A, Johansen M, Jones L, Krassioukov A, Mulcahey MJ, Schmidt-Read M, Waring W. International standards for neurological classification of spinal cord injury (Revised 2011). J Spinal Cord Med. 2011 November; 34(6): 535–546.

Margolis RB, Chibnall JT, Tait RC. Test retest reliability of the pain drawing instrument. Pain. 1988;33:49-51.

Merskey H, Bogduk N. (Eds.). (1994). Classification of chronic pain: Description of chronic pain syndromes and definition of pain terms. Seattle: IASP Press.

Raichle KR, Osborne TL, Jensen MP, Cardenas D. The reliability and validity of pain interference measures in persons with spinal cord injury. J of Pain. 2006;7:179-86.

Rintala DH, Loubser PG, Castro J, Hart KA, Fuhrer MJ. Chronic pain in a community-based sample of men with spinal cord injury: Prevalence, severity, and relationships with impairment, disability, handicap, and subjective well-being. Arch Phys Med Rehabil. 1998;79:604-14.

Siddall PJ, Yezierski RP, Loeser JD. Pain following spinal cord injury: clinical features, prevalence, and taxonomy. International Association for the Study of Pain Newsletter. 2000;3:3-7 (http://www.iasphttp://www.iasp-pain.org/AM/Template.cfm?Section=Technical_Corner&Template=/CM/ContentDisplay.cfm&ContentID=2179pain.org/AM/Template.cfm?Section=Technical_Corner&Template=/CM/ContentDisplay.cfm&Cont entID=2179).

Stensman R. Adjustment to traumatic spinal cord injury. A longitudinal study of self-reported quality of life. Paraplegia. 1994;32:416-22.

Tait RC, Chibnall JT, Krause S. The Pain Disability Index: factor structure and normative data. Arch Phys Med Rehabil. 1994;75:1082-6.

Westgren N, Levi R. Quality of life and traumatic spinal cord injury. Arch Phys Med Rehabil. 1998;79:1433-9.

Widerström-Noga EG, Felipe-Cuervo E and Yezierski RP. Relationships among clinical characteristics of chronic pain following spinal cord injury. Arch Phys Med Rehabil 2001;82:1191-7.

Widerström-Noga EG, Duncan R, Felipe-Cuervo E and Turk DC, Assessment of the impact of pain and impairments associated with spinal cord injuries. Arch Phys Med Rehabil 2002;83:395-404.

Widerström-Noga EG and Turk DC. Outcome measures in chronic pain trials involving people with spinal cord injury. SCI Psychosocial Process 2004; 17:258-267.

Widerström-Noga EG, Cruz-Almeida Y, Martinez-Arizala A, Turk DC. Internal consistency, stability, and validity of the spinal cord injury version of the multidimensional pain inventory. Arch Phys Med Rehabil 2006;87:516-23.

SYLLABUS (instructions) – Version 2.0

Each variable and each response category within each variable have been specifically defined in a way that is designed to facilitate the collection of a uniform basic data set.

VARIABLE NAME: **Date of data collection**

DESCRIPTION: This variable documents the date of data collection

CODES: YYYY/MM/DD

COMMENTS: The collection of data on Pain may be carried out at any time after the spinal cord injury. The *Date of data collection* variable is necessary in order to identify when the data were collected. This variable provides a way to relate the collected data to other data collected on the same individual at various time points.

VARIABLE NAME: **Have you had any pain during the last 7 days including today?**

DESCRIPTION: This variable documents the presence of any type of pain during the last 7 days.

CODES: No
Yes

COMMENTS: To be able to evaluate any present, chronic, and intermittent pain related and unrelated to the spinal cord injury. Pain is defined by the International Association for the Study of Pain (IASP) as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey & Bogduk, 1994).
The seven day interval was chosen in order to be able to capture current pain and both constant and intermittent chronic pain that may be clinically relevant and to have the same time frame in all data sets.

This question can also be used as Basic Pain Question in other questionnaires, i.e. gate question to the Pain Basic Data Set.

Pain Interference

The three interference items were written for and included in the data set given the need for (1) the availability of a single item that could be used to assess the domain of pain interference on physical activity; and (2) the need to ensure assessment of pain interference on mood and sleep, two key interference domains. Widerstrom-Noga et al, 2002; Hirsch et al., 2011. Based on the results from a study testing the

psychometric properties of a self-reported version of the International SCI Pain Basic Data Set (Jensen et al., 2010) the 6 interference items exhibited excellent reliability (0.94). However, a reliability coefficient in this range suggests that some items may provide similar information and could therefore be dropped. Thus, all items were examined regarding internal reliability and validity. Three items asking about interference with day-to-day activities, mood and sleep (AMS) were selected based upon excellent reliability (0.89) and on strong association with the validity criteria (psychological functioning -0.60 and Sleep problems 0.68). Each item has been revised and is now scored on a numerical rating scale from 0 to 10. Please note that the psychometric properties were evaluated with these items scored between 0 and 6. We expect minimal to no effects on these properties with the revision.

In this section pain interference ***during the last week*** apply to all questions and apply to overall pain.

Pain Interference specifically related to General Activity, Mood and Sleep.

VARIABLE NAME: **In general, how much has pain interfered with your day-to-day activities in the last week?**

DESCRIPTION: A 0 – 10 Numerical Rating Scale (ranging from 0 = "No interference" to a maximum of 10 = "Extreme interference") of pain interference with general activity.

CODES:
0
1
2
3
4
5
6
7
8
9
10

COMMENTS: ***This question concerns how a person's specific pain problem interfered with general activity during the last seven days including today.*** Pain interference (the extent to which pain interferes with functioning and mood) is a key pain domain. An interference item that assesses general activity interference was designed specifically for this data-set in order to provide a global summary interference rating.

VARIABLE NAME: In general, how much has pain interfered with your overall mood in the past week?

DESCRIPTION: A 0 – 10 Numerical Rating Scale (ranging from 0 = "No interference" to a maximum of 10 = "Extreme interference") of pain interference of mood.

CODES:
0
1
2
3
4
5
6
7
8
9
10

COMMENTS: ***This question concerns how a person's specific pain problem interfered with mood during the last seven days including today.*** An interference item that assesses mood interference was developed for this data set because pain is known to have a significant negative impact on mood for many patients, and pain's effect on mood is somewhat distinct from its effect on other functioning domains.

VARIABLE NAME: **In general, how much has pain interfered with your ability to get a good night's sleep?**

DESCRIPTION: A 0 – 10 Numerical Rating Scale (ranging from 0 = "No interference" to a maximum of 10 = "Extreme interference") of pain interference of mood.

CODES:
0
1
2
3
4
5
6
7
8
9

COMMENTS: ***This question concerns how a person's specific pain problem interfered with his/her ability to get a good night's sleep during the last seven days including today.*** An interference item that assesses sleep interference was developed for this data set because pain is known to have a significant negative impact on sleep for many patients, and pain's effect on sleep is somewhat distinct from its effect on other functioning domains.

VARIABLE NAME**:** **How many different pain problems do you have?**

DESCRIPTION: This variable determines how many different pain problems an individual perceives that he or she has experienced during the last seven days including today. A "pain problem" is defined by the person himself as a pain that has a specific character. Please note that one pain problem can be located in one or several areas.

CODES:
1 - One pain problem
2 - Two pain problems
3 - Three pain problems
4 - Four pain problems
5 - Five or more pain problems

COMMENTS: Data from previous studies suggest that persons with SCI rarely have more than 5 different pain problems. Persons who experience SCI related chronic pain can usually differentiate between different pain problems. Although unusual, it is possible to have two different types of pain in overlapping areas. An example would be musculoskeletal shoulder pain in a person with cervical injury and neuropathic pain at the level of injury.

Description of the three worst pain problems

COMMENTS: Each person is only required to describe the three worst pain problems he or she is currently experiencing (within the last 7 days). The reasons for this are twofold. First, most people with SCI experience three or fewer pain problems. Second, describing the details of more than three different simultaneous pain problems may induce errors in the data collection. Please note that the forms should be completed in a columnar fashion for each pain problem and not be read across.

VARIABLE NAME: **Location(s) of pain** (check all that apply including right side, midline and/or left side)

DESCRIPTION: This variable contains information concerning the location of pain.

CODES:

Head		right side, midline and/or left side
Neck/shoulders		
	throat	right side, midline and/or left side
	neck	right side, midline and/or left side
	shoulder	right and/or left side
Arms/hands		
	upper arm	right and/or left side
	elbow	right and/or left side
	forearm	right and/or left side
	wrist	right and/or left side
	hand/fingers	right and/or left side
Frontal torso/genitals		
	chest	right side, midline and/or left side
	abdomen	right side, midline and/or left side
	pelvis/genitalia	right side, midline and/or left side
Back		
	upper back	right side, midline and/or left side
	lower back	right side, midline and/or left side
Buttocks/hips		
	buttocks	right and/or left side
	hip	right and/or left side
	anus	midline
Upper legs/thighs		right and/or left side
Lower legs/feet		
	knee	right and/or left side
	shin	right and/or left side
	calf	right and/or left side
	ankle	right and/or left side
	foot/toes	right and/or left side

COMMENTS: This division into pain areas is based on a pain drawing originally described by Margolis et al.,1988 but which was since recalculated into 8 principal areas (Widerström-Noga et al., 2001): (1) head; (2) neck/shoulders; (3) arms/hands; (4) frontal torso/genitals; (5) back; (6) buttocks/hips; (7) Upper legs/thighs; and (8) Lower legs/feet. Within each of these 8 pain

locations, further divisions into more precise locations can be made. For example, in the “arms/hand” category specification of wrist, elbow pain etc. can be made if needed. Each individual is asked to describe the location of all present pain. Please indicate right (R), midline (M) and/or left (L) side.

The descriptions of the pain locations in the Basic Pain Data Set are meant to be based on each individual’s perception of the location of pain, and can be used to follow pain at subsequent visits. Therefore, the delineations of these areas are not defined with precise anatomical landmarks. Several locations may be given for each pain problem, e.g., neck and either shoulders, or pain in the abdomen extending into the buttocks and thighs areas and further down to the feet.

VARIABLE NAME: **Type of pain**
DESCRIPTION: This variable documents the type of pain present.

CODES:
Musculoskeletal (Nociceptive)
Visceral (Nociceptive)
Other (Nociceptive)
At-level SCI (Neuropathic)
Below-level SCI (Neuropathic)
Other (Neuropathic)
Other
Unknown

COMMENTS: Seven broad types of pain are specified based on pain types identified in previous SCI pain taxonomies (Donovan et al., 1982; Siddall et al., 2000; Bryce & Ragnarsson, 2001; Cardenas et al., 2002; Bryce et al., 2012a,b) and based on prevalence in the SCI population. ***Please note that the ASIA Impairment scale (AIS) and the associated dermatomal map (Kirshblum et al., 2011) are to be used as integral parts of the SCI pain classification.*** Nociceptive pains that are less prevalent or not directly related to SCI and not categorized as musculoskeletal or visceral can be classified as “*Other (Nociceptive)*”. Pains that are not associated with a lesion or disease affecting the spinal cord or nerve roots yet are nevertheless neuropathic can be classified as “*Other (Neuropathic)”*. *”Unknown”* should be used when it is not possible to classify the pain into one of the categories listed above. *”Unknown”* pain refers only to pain of unknown etiology and not to pains with both nociceptive and neuropathic qualities, nor to defined pain syndromes of unknown etiology,

like fibromyalgia. For pains that seem to have both nociceptive and neuropathic qualities the two components should be classified separately. Defined pain syndromes of unknown etiology (for example, fibromyalgia) should be coded as *"Other"*.

The type of pain should be coded using the following criteria:
Musculoskeletal (Nociceptive) pain refers to pain occurring in a region where there is preserved sensation above, at or below the neurological level of injury and which is believed to be arising from musculoskeletal structures. The presence of this type of pain is suggested by pain descriptors such as dull or aching, pain related to movement, tenderness of musculoskeletal structures on palpation, response to anti-inflammatory or opioid medications and evidence of skeletal pathology on imaging consistent with the pain presentation. Examples include: mechanical pain, spinal fractures, muscular injury, shoulder overuse syndromes and muscle spasm (Donovan et al., 1982; Siddall et al., 2000; Bryce & Ragnarsson, 2001; Cardenas et al., 2002).
Visceral (Nociceptive) pain refers to pain usually located in the thorax, abdomen, or pelvis and believed to be generated in visceral structures. The presence of this type of pain is suggested by characteristics such as dull, tender, or cramping and a relationship to visceral pathology or dysfunction, e.g., infection or obstruction (Donovan et al., 1982; Siddall et al., 2000; Bryce & Ragnarsson, 2001; Cardenas et al., 2002; Bryce et al., 2012a). Examples include urinary tract infection, ureteric calculus and bowel impaction. Note: Failure to find evidence of visceral pathology or failure to respond to treatment directed at visceral pathology may indicate the presence of neuropathic pain (see below).
Other (Nociceptive) pain refers to nociceptive pains that may be present but do not fall into the musculoskeletal or visceral categories (Bryce & Ragnarsson, 2001). Examples include pain associated with ulceration of the skin and headache. These pains may be directly related to SCI (e.g., pressure areas and dysreflexic headache) or unrelated to SCI (e.g., migraine).
At-level SCI (Neuropathic) pain refers to neuropathic pain presenting in a segmental pattern. A necessary condition for this to occur is that there is a lesion or disease affecting the spinal cord or nerve roots. At-level neuropathic pain is perceived anywhere within the dermatome of the level of neurological injury and three dermatomes below this level. Pain which occurs in this distribution which cannot be attributed to a lesion or disease affecting the spinal cord or nerve roots should be classified as "*Other" (Neuropathic)*. This pain is often

characterized as hot-burning, tingling, pricking, pins and needles, squeezing, cold, electric, or shooting. Sensory changes such as allodynia, hypoalgesia, or hyperalgesia within the pain distribution are often found. The pain may be unilateral or bilateral (Siddall et al., 2000; Bryce & Ragnarsson, 2001; Bryce et al., 2012a). Note: Neuropathic pain associated with cauda equina damage is radicular in nature and therefore defined as at level (neuropathic) pain regardless of distribution.

Below-level SCI (Neuropathic) pain refers to neuropathic pain that is present more than three dermatomes below the dermatome of the neurological level of injury; it may in addition be perceived up to the dermatome representing the neurological level of injury and the three dermatomes just below this. A necessary condition for this to occur is that there is a lesion or disease affecting the spinal cord and that the pain is believed to arise as a result of this damage. Pain which occurs in this distribution which cannot be attributed to a lesion or disease affecting the spinal cord should be classified as "*Other" (Neuropathic)*.

This pain is often characterized as hot-burning, tingling, pricking, pins and needles, squeezing, cold, electric, or shooting; it usually has a regional distribution. Sensory changes such as allodynia, hypoalgesia, or hyperalgesia may be present. If two distinct pains are distinguishable in the same region, the two pain types must be classified and documented as separate pains.

Other (Neuropathic) pain refers to neuropathic pains that are present above, at or below the neurological level of injury but are not directly related to the SCI. Examples include postherpetic neuralgia, pain associated with diabetic neuropathy, central post stroke pain, and compressive mononeuropathies (Siddall et al., 2000; Bryce & Ragnarsson, 2001). ***Other pain*** refers to pain that occurs when there is no identifiable noxious stimulus nor any detectable inflammation or damage to the nervous system responsible for the pain and the pain is thought to be unrelated to the underlying SCI, both temporally and mechanistically. It is unclear what causes the pain to develop or persist. Examples include: Complex Regional Pain Syndrome type I, interstitial cystitis pain, irritable bowel syndrome pain and fibromyalgia.

VARIABLE NAME: **Average pain intensity in the last week**

DESCRIPTION: A 0 – 10 Numerical Rating Scale (ranging from 0 = "No pain" to a maximum of 10 = "Pain as bad as you can imagine") of average pain intensity for (up to) three pain problems (the three worst pain

problems respondents experience). Please note that “last week” specifically refers to ***the last seven days including today.***

CODES:
0
1
2
3
4
5
6
7
8
9
10

COMMENTS: Pain intensity is the most common pain domain assessed in research and clinical settings. Although different rating scales have proven to be valid for assessing pain intensity, including the Numerical Rating Scale (NRS), the Verbal Rating Scale (VRS), and the Visual Analogue Scale (VAS), the 0 – 10 NRS has the most strengths and fewest weaknesses of available measures (Jensen & Karoly, 2001). Moreover the 0 – 10 NRS, and specifically the 0 – 10 with the endpoints listed, has been recommended by the IMMPACT consensus group for use in pain clinical trials (Dworkin et al., 2005) and by the 2006 NIDRR SCI Pain outcome measures consensus group (Bryce et al., 2007), so using this measure will help ensure consistency in the assessment of average pain intensity across studies.

The seven day time frame was selected to balance the need to assess pain over a long enough epoch to capture usual pain, against the need to keep the time frame short enough to maximize recall accuracy.

VARIABLE NAME: **Date of onset**

DESCRIPTION: This variable specifies the date this particular pain problem started, i.e. the worst, second worst or third worst pain problem.

CODES: YYYY/MM/DD

COMMENTS: If the day of the month is unknown, record 99. If the month of the year is unknown, record 99. The year should be given as an approximation if it is not known.

VARIABLE NAME: **Are you using or receiving any <u>treatment</u> for your pain problem?**

DESCRIPTION: This variable documents any treatment the patient is using or receiving for any pain.

CODES: No
Yes

COMMENTS: By “treatment” is meant any prescribed or non-prescribed medical, surgical, psychological, or physical treatment that the patient is using or receiving *for pain that has been present the last seven days* to alleviate his/her pain/pains. This variable may include chronic and intermittent drug treatment, physical therapy, relaxation training, nerve blocks etc.

INTERNATIONAL SPINAL CORD INJURY PAIN BASIC DATA SET

DATA COLLECTION FORM – Version 2.0

Date of data collection: YYYY/MM/DD

Have you had any pain during the last seven days including today:

□ No □ Yes

If yes:

Please note that the time period during the last week applies to all pain interference questions.

In general, how much has pain interfered with your day-to-day activities in the last week? No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8 - □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your overall mood in the last week?
No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8 - □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your ability to get a good night's sleep? No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8 - □ 9 - □ 10 Extreme interference

How many different pain problems do you have?

□ 1; □ 2; □ 3; □ 4; □ ≥5

Please describe your three worst pain problems:

Worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders throat neck shoulder				**Nociceptive** □ Musculoskeletal □ Visceral □ Other
Arms/hands upper arm elbow forearm wrist hand/fingers				**Neuropathic** □ At-level SCI □ Below-level SCI □ Other □ **Other**
Frontal torso/genitals chest abdomen pelvis/genitalia				□ **Unknown**
Back upper back lower back				**Intensity** and **duration of pain:** **Average pain intensity in the last week:** 0 = no pain; 10 = pain as bad as you can imagine □ 0; □ 1; □ 2; □ 3; □ 4; □ 5; □ 6; □ 7; □ 8; □ 9; □ 10
Buttocks/hips buttocks hip anus				
Upper leg/thigh				**Date of onset:** YYYY/MM/DD
Lower legs/feet knee shin calf ankle foot/toes				**Are you using or receiving any <u>treatment</u> for your pain problem:** □ No □ Yes

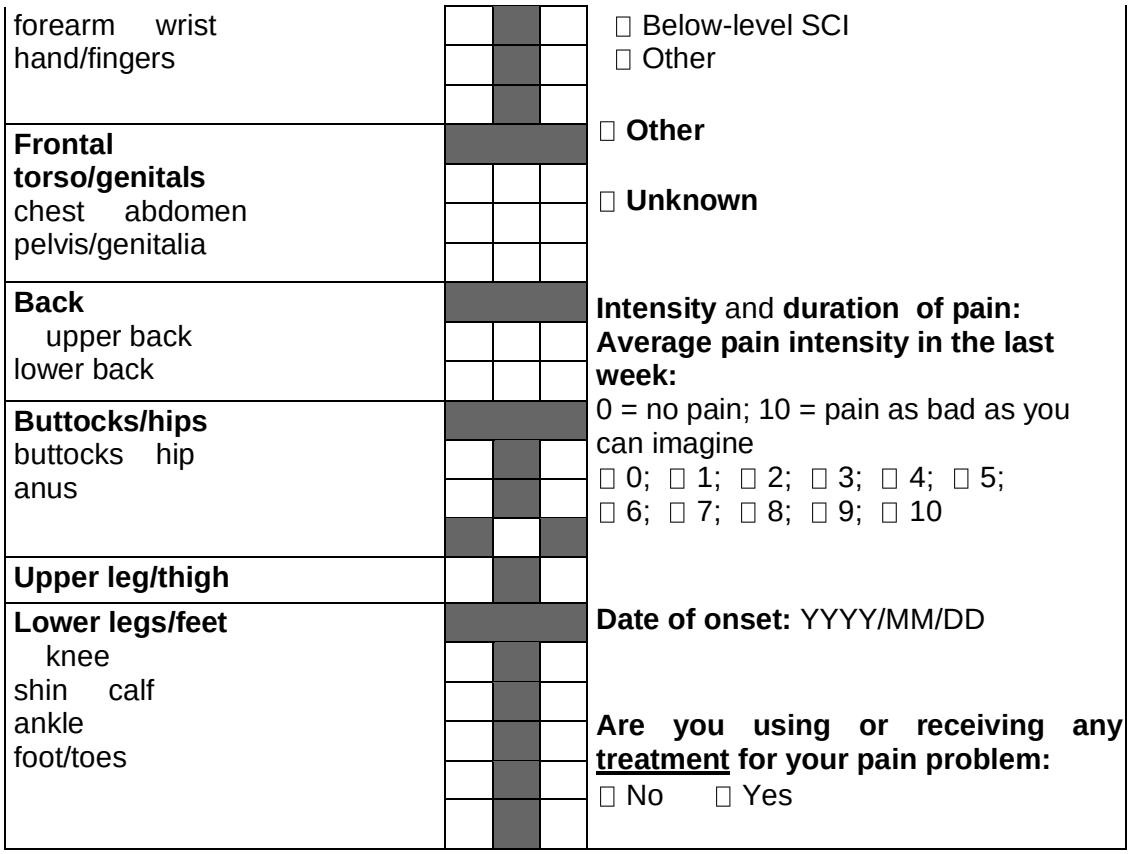
Second worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				

throat neck shoulder		**Nociceptive** □ Musculoskeletal □ Visceral □ Other
Arms/hands upper arm elbow forearm wrist hand/fingers		**Neuropathic** □ At-level SCI □ Below-level SCI □ Other
Frontal torso/genitals chest abdomen pelvis/genitalia		□ **Other** □ **Unknown**
Back upper back lower back		**Intensity** and **duration of pain:** **Average pain intensity in the last week:** 0 = no pain; 10 = pain as bad as you can imagine □ 0; □ 1; □ 2; □ 3; □ 4; □ 5; □ 6; □ 7; □ 8; □ 9; □ 10
Buttocks/hips buttocks hip anus		
Upper leg/thigh		
Lower legs/feet knee shin calf ankle foot/toes		**Date of onset:** YYYY/MM/DD **Are you using or receiving any <u>treatment</u> for your pain problem:** □ No □ Yes

Third worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders throat neck shoulder				**Nociceptive** □ Musculoskeletal □ Visceral □ Other
Arms/hands upper arm elbow				**Neuropathic** □ At-level SCI



Training case 1

Date: May 26, 2008

This is a 34 year old man with a C6 AIS B cervical injury after a diving accident in 2000. He experiences two different pains, one in the legs and the other in the center of the abdomen. The pain in his abdomen started shortly about 6 years after his SCI and is the most problematic problem of the two. He describes this pain as “cramping” and “shooting” with an average intensity of 7/10. The pain occurs daily, but is intermittent, with periods of pain “flares” followed by periods of being free from the abdominal pain. Although the hour-long pain flares are usually worse in the afternoon compared to the morning, evening, or nighttime, they seem to be related to constipation. He has tried opioids and antidepressants but does not recall the names or doses, and they did not help. He has not tried anticonvulsants. Currently, he takes no medication for this pain.

The second pain located in his legs from his thighs down to his toes is perceived as “sharp,” “aching,” and “squeezing.” This pain began 1 to 3 months after injury. The intensity of this pain is 1/10 on average, but may increase to 10/10 for brief periods (up to 5 minutes at a time). This pain is present only in relation to severe spasms, but occurs up to 10 times a day. There is no consistent temporal pattern to this pain; it tends to occur throughout the day with no time period being better or worse. He is taking baclofen for this pain and reports that this medication is very helpful.

He does not feel that pain affects his overall day-to-day activities and upon inquiry he rates interference with activities as very low, perhaps 1/10. He also does not feel that his mood is affected and rates the influence of mood as 0/10. He does, however, mention that he frequently wakes up but that this is not related to his pain, and he rates sleep interference as 0/10.

Note: *In an assessment situation these questions and the endpoints are read verbatim to the patient and he or she answers the question by choosing the appropriate number. Please also note that this training case is not a real case. Furthermore, the treatments used in these cases do not reflect recommendations by the Pain dataset committee but are merely examples of common treatments used to relieve pain in this population.*

INTERNATIONAL SPINAL CORD INJURY PAIN BASIC DATA SET – FORM -

Version 2.0 CASE 1

Date of data collection: 2008/05/26

Have you had any pain during the last 7 days including today: □ No **X Yes**

If yes:

Please note that the time period during the <u>last week</u> applies to all pain interference questions.

In general, how much has pain interfered with your day-to-day activities in the last week? No interference □ 0 - **X 1** - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8- □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your overall mood in the last week?

No interference **X 0** - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8- □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your ability to get a good night's sleep? No interference **X 0** - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8- □ 9 - □ 10 Extreme interference

How many different pain problems do you have?
□ 1; X 2; □ 3; □ 4; □ >5

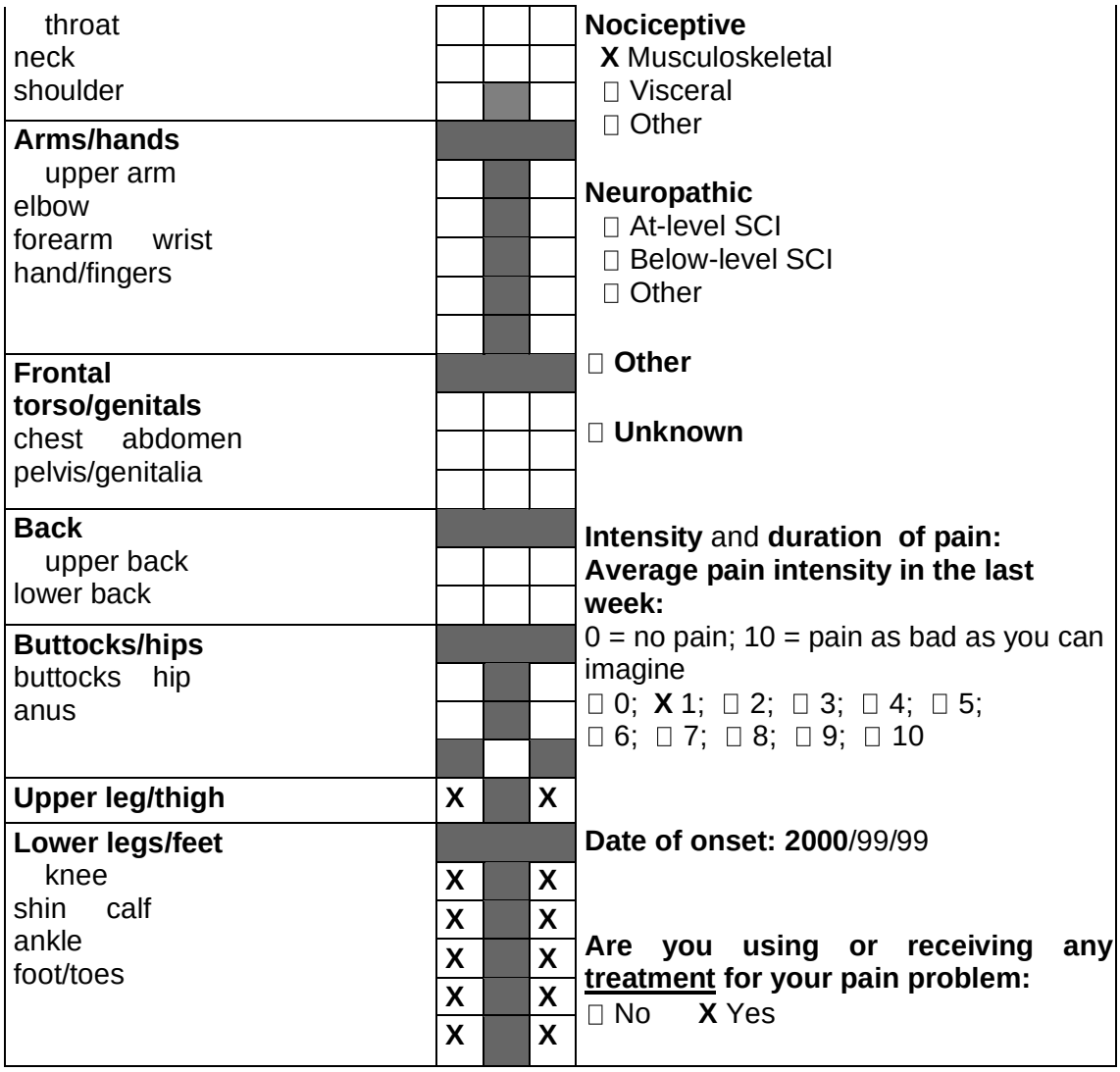
Please describe your <u>three</u> worst pain problems:

Worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				
throat				**Nociceptive**
neck				□ Musculoskeletal
shoulder				**X** Visceral
				□ Other
Arms/hands				
upper arm				**Neuropathic**
elbow				□ At-level SCI
forearm wrist				□ Below-level SCI
hand/fingers				□ Other
				□ **Other**
Frontal torso/genitals				
chest abdomen				□ **Unknown**
pelvis/genitalia		**X**		
Back				**Intensity** and **duration of pain:**
upper back				**Average pain intensity in the last week:**
lower back				0 = no pain; 10 = pain as bad as you can imagine
Buttocks/hips				
buttocks hip				□ 0; □ 1; □ 2; □ 3; □ 4; □ 5;
anus				□ 6; **X** 7; □ 8; □ 9; □ 10
Upper leg/thigh				**Date of onset: 2006/**99/99
Lower legs/feet				
knee				
shin calf				**Are you using or receiving any <u>treatment</u> for your pain problem:**
ankle				
foot/toes				□ No **X** Yes

Second worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				



Training case 2

Date: October 26, 2008

This is a 25 year old woman with a C5 AIS A spinal cord injury following a traffic accident Aug 25 2005. She experiences three different kinds of pains, one located in the arms and hands, a second pain located in the buttocks and upper legs, and a third pain located in the shoulders.

She feels that the pain that she experiences in her arms and hands (upper arms through fingers) is the worst because it has a particularly unpleasant electric quality. It began within a month after her injury. She describes the pain in her arms as very intense, rating it as 8/10, on average. Light touching of the

skin, touch by clothes and taking a shower trigger an intense electric burning pain. She has this pain every day on a continuous basis, although this pain is worse in the afternoon compared to the morning or evening. The pain gets a little better when she lies down or when she is thinking about something else. She takes an anticonvulsant medication and applies topical patches including a local anesthetic for this pain with partial benefit.

She describes the pain in the upper legs and buttocks as “burning,” “pricking” and “pulsating.” This pain started about one year after injury. This pain is also very intense; she rates it as a 7/10, on average. The pain is always present, independent of movements or muscle spasms, but usually is more severe in the evening as compared to the morning or afternoon. The anticonvulsant she is taking has no effect on this pain problem.

The pain in the shoulders is aching and started about two years after injury and is not quite as intense as the other two pains. This pain is usually only present in the afternoon and evening after workout or after periods of prolonged wheelchair propulsion or working at the computer. In the last week, pain was present for a total of 5 days. It usually lasts a couple of hours, and resolves after rest. She rates it as a 4/10, on average. She takes paracetamol or NSAIDS for this pain once or twice per week; she finds both of these medications somewhat helpful for the shoulder pain.

She reports that her pain interferes with her activities every day and she rates this interference as 8/10. Similarly, she also mentions that her pain makes her feel sad on a daily basis and she rates it as 7/10 interfering significantly with mood. She wakes up several times every night because of pain and this is a very difficult problem for her. She rates it as 10/10.

Note: *In an assessment situation these questions and the endpoints are read verbatim to the patient and he or she answers the question by choosing the appropriate number. Please also note that this training case is not a real case. Furthermore, the treatments used in these cases do not reflect recommendations by the Pain dataset committee but are merely examples of common treatments used to relieve pain in this population.*

INTERNATIONAL SPINAL CORD INJURY PAIN BASIC DATA SET – FORM -

Version 2.0 CASE 2

Date of data collection: 2008/10/26

Have you had any pain during the last 7 days including today: □ No **X Yes**

If yes:

Please note that the time period during the last week applies to all pain interference questions.

In general, how much has pain interfered with your day-to-day activities in the last week? No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - **X 8**- □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your overall mood in the past week?
No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - **X 7** - □ 8 - □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your ability to get a good night's sleep? No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8 - □ 9 - **X 10** Extreme interference

How many different pain problems do you have?
□ 1; □2; X 3; □ 4; □ >5

Please describe your three worst pain problems:

Worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				
throat				**Nociceptive**
neck				□ Musculoskeletal
shoulder				□ Visceral
				□ Other
Arms/hands				
upper arm	**X**		**X**	**Neuropathic**
elbow	**X**		**X**	**X** At-level SCI
forearm wrist	**X**		**X**	□ Below-level SCI
hand/fingers	**X**		**X**	□ Other
	X		**X**	
				□ **Other**
Frontal torso/genitals				
chest abdomen				□ **Unknown**
pelvis/genitalia				
Back				**Intensity** and **duration of pain:**
upper back				**Average pain intensity in the last week:**
lower back				0 = no pain; 10 = pain as bad as you can imagine
Buttocks/hips				□ 0; □ 1; □ 2; □ 3; □ 4; □ 5;
buttocks hip				□ 6; □ 7; **X** 8; □ 9; □ 10
anus				
Upper leg/thigh				**Date of onset: 2005/09/99**
Lower legs/feet				
knee				
shin calf				**Are you using or receiving any <u>treatment</u> for your pain problem:**
ankle				□ No **X** Yes
foot/toes				

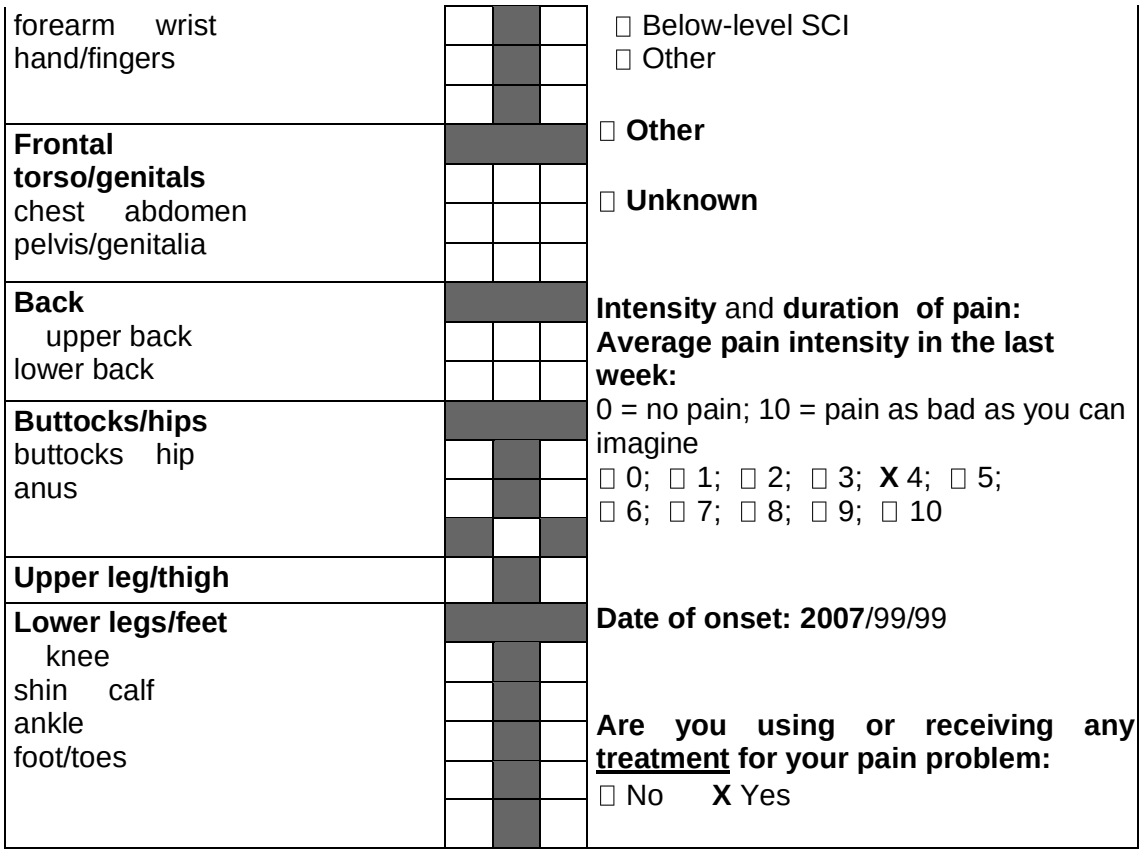
Second worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				

throat				**Nociceptive**
neck				□ Musculoskeletal
shoulder				□ Visceral
Arms/hands				□ Other
upper arm				**Neuropathic**
elbow				□ At-level SCI
forearm wrist				**X** Below-level SCI
hand/fingers				□ Other
Frontal torso/genitals				□ **Other**
chest abdomen				□ **Unknown**
pelvis/genitalia				
Back				**Intensity** and **duration of pain:**
upper back				**Average pain intensity in the last week:**
lower back				0 = no pain; 10 = pain as bad as you can imagine
Buttocks/hips				□ 0; □ 1; □ 2; □ 3; □ 4; □ 5;
buttocks hip	**X**		**X**	□ 6; **X** 7; □ 8; □ 9; □ 10
anus				
Upper leg/thigh	**X**		**X**	
Lower legs/feet				**Date of onset: 2006/08**/99
knee				
shin calf				**Are you using or receiving any <u>treatment</u> for your pain problem:**
ankle				□ No **X** Yes*
foot/toes				*She is taking an anticonvulsant although this medication is not effective for this pain.

Third worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				**Nociceptive**
throat				**X** Musculoskeletal
neck				□ Visceral
shoulder	**X**		**X**	□ Other
Arms/hands				**Neuropathic**
upper arm				□ At-level SCI
elbow				



Training case 3

Date: September 3, 2008

This is a 20 year old female who sustained a T10 AIS A spinal cord injury on July 8, 2004. She has a one level zone of partial preservation of light touch and pinprick sensation. She underwent a three level posterior decompression with fusion and instrumentation at the time of injury. She experiences two different types of pain of which a daily “sharp” attack-like lower back pain triggered by flexion of the spine is the worst. This pain came on insidiously over the last year and she cannot identify an inciting event. She describes this pain as very intense and brief, lasting less than one minute at a time and she rates it at an average of 8/10. It is most intense in the morning, afternoon and evening and is not present when she lays flat in bed at night. On physical exam, she exhibits tenderness to palpation over the low back both centrally and adjacent to the midline in paraspinal muscles. Portions of the hardware can be palpated over her low back. Opioid medication is somewhat effective in decreasing the severity of the pain, although it does not take it away completely.

In addition, she has a second pain that she describes as a constant pressure and as a “tight girdle” that is felt about the lower abdomen. This pain has been present since approximately 4 weeks after injury and does not vary in intensity. This pain is constant and rated at 4/10. The opioid medication does not relieve this pain.

She describes that pain does not really affect her day-to-day activities since she has to “get things done.” She rates the pain interference with activities as 1/10. She does, however, mention that pain affects her mood to a moderate degree and rates the pain interference as 5/10 since she does not feel that way every day. Sleep is also interrupted by pain and she rates sleep interference as 5/10.

Note: *In an assessment situation these questions and the endpoints are read verbatim to the patient and he or she answers the question by choosing the appropriate number. Please also note that this training case is not a real case. Furthermore, the treatments used in these cases do not reflect recommendations by the Pain dataset committee but are merely examples of common treatments used to relieve pain in this population.*

INTERNATIONAL SPINAL CORD INJURY PAIN BASIC DATA SET – FORM - Version 2.0 CASE 3

Date of data collection: 2008/09/03

Have you had any pain during the last 7 days including today: □ No **X Yes**

If yes:

Please note that the time period during the <u>last week</u> applies to all pain interference questions.

In general, how much has pain interfered with your day-to-day activities in the last week? No interference □ 0 - **X 1** - □ 2 - □ 3 - □ 4 - □ 5 - □ 6 - □ 7 - □ 8 - □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your overall mood in the past week?
No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - **X 5** - □ 6 - □ 7 - □ 8 - □ 9 - □ 10 Extreme interference

In general, how much has pain interfered with your ability to get a good night's sleep? No interference □ 0 - □ 1 - □ 2 - □ 3 - □ 4 - **X 5** - □ 6 - □ 7 - □ 8 - □ 9 - □ 10 Extreme interference

How many different pain problems do you have?
□ **1; X 2;** □ **3;** □ **4;** □ **>5**

Please describe your <u>three</u> worst pain problems:

Worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				
throat				**Nociceptive**
neck				**X** Musculoskeletal
shoulder				□ Visceral
				□ Other
Arms/hands				
upper arm				**Neuropathic**
elbow				□ At-level SCI
forearm wrist				□ Below-level SCI
hand/fingers				□ Other
				□ **Other**
Frontal torso/genitals				
chest abdomen				□ **Unknown**
pelvis/genitalia				
Back				**Intensity** and **duration of pain:**
upper back				**Average pain intensity in the last week:**
lower back	**X**	**X**	**X**	0 = no pain; 10 = pain as bad as you can imagine
Buttocks/hips				□ 0; □ 1; □ 2; □ 3; □ 4; □ 5;
buttocks hip				□ 6; □ 7; **X** 8; □ 9; □ 10
anus				
Upper leg/thigh				**Date of onset: 2007**/99/99
Lower legs/feet				

knee				
shin calf				**Are you using or receiving any <u>treatment</u> for your pain problem:**
ankle				
foot/toes				□ No **X** Yes

Second worst pain problem:

Pain locations /sites (can be more than one, so check all that apply): right (R), midline (M), or left (L)	**R**	**M**	**L**	**Type of pain** **Intensity** and **duration of pain** **Treatment of pain**
Head				**Type of pain (check one):**
Neck/shoulders				**Nociceptive**
throat				□ Musculoskeletal
neck				□ Visceral
shoulder				□ Other
Arms/hands				**Neuropathic**
upper arm				**X** At-level SCI
elbow				□ Below-level SCI
forearm wrist				□ Other
hand/fingers				
				□ **Other**
Frontal torso/genitals				□ **Unknown**
chest abdomen				
pelvis/genitalia	**X**	**X**	**X**	
Back				**Intensity** and **duration of pain:** **Average pain intensity in the last week:** 0 = no pain; 10 = pain as bad as you can imagine □ 0; □ 1; □ 2; □ 3; **X** 4; □ 5; □ 6; □ 7; □ 8; □ 9; □ 10
upper back				
lower back				
Buttocks/hips				
buttocks hip				
anus				
Upper leg/thigh				**Date of onset: 2004/08/08**
Lower legs/feet				
knee				
shin calf				**Are you using or receiving any <u>treatment</u> for your pain problem:**
ankle				
foot/toes				□ No **X** Yes*
				*the opioid medication she is taking has no effect on this pain.

Appendix B: MRI Protocols

Structural T1-weighted 3D GRAPPA

SIEMENS MAGNETOM TrioTim syngo MR B19

\\USER\HEAD\MORSE EXOSKELETON\MORSE EXO\SAG 3D T1 GRAPPA

TA: 5:17 PAT: 2 Voxel size: 1.0×1.0×1.0 mm Rel. SNR: 1.00 SIEMENS: gre

Properties	
Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	single

Routine	
Slab group 1	
Slabs	1
Dist. factor	20 %
Position	L0.0 A21.1 H44.1
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
Slice oversampling	0.0 %
Slices per slab	192
FoV read	256 mm
FoV phase	100.0 %
Slice thickness	1.00 mm
TR	20 ms
TE	4.92 ms
Averages	1
Concatenations	1
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast	
MTC	Off
Magn. preparation	None
Flip angle	25 deg
Fat suppr.	None
Water suppr.	None
SWI	Off
Averaging mode	Short term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution	
Base resolution	256
Phase resolution	100 %
Slice resolution	80 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Interpolation	Off
PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	24
Matrix Coil Mode	Auto (Triple)
Reference scan mode	Integrated
Image Filter	Off
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Normalize	Off
B1 filter	Off
Raw filter	Off
Elliptical filter	Off

Geometry	
Multi-slice mode	Interleaved
Series	Interleaved
Saturation mode	Standard
Special sat.	None
Tim CT mode	Off

System	
Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	REF
Table position	H
Table position	22 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
Auto Coil Select	Default
Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
R >> L	350 mm
A >> P	263 mm
F >> H	350 mm

Physio	
1st Signal/Mode	None
Segments	1
Dark blood	Off
Resp. control	Off

Inline	
Subtract	Off
Liver registration	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off

3/+

Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On
Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off

Sequence

Introduction	On
Dimension	3D
Elliptical scanning	On
Phase stabilisation	Off
Asymmetric echo	Off
Contrasts	1
Bandwidth	190 Hz/Px
Flow comp.	No
Allowed delay	0 s
RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On

Resting-state fMRI EPI

SIEMENS MAGNETOM TrioTim syngo MR B19

\\USER\HEAD\MORSE EXOSKELETON\MORSE EXO\fMRI_RESTING_STATE

TA: 6:18 PAT: Off Voxel size: 3.0×3.0×3.0 mm Rel. SNR: 1.00 SIEMENS: ep2d_pace

Properties	
Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine	
Slice group 1	
Slices	47
Dist. factor	0 %
Position	L0.0 A20.0 H67.7
Orientation	T > C-8.9
Phase enc. dir.	P >> A
Rotation	180.00 deg
Phase oversampling	0 %
FoV read	216 mm
FoV phase	100.0 %
Slice thickness	3.0 mm
TR	3000 ms
TE	30 ms
Averages	1
Concatenations	1
Filter	Raw filter
Coil elements	HEA;HEP

Contrast	
MTC	Off
Flip angle	85 deg
Fat suppr.	Fat sat.
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	124
Delay in TR	0 ms
Multiple series	Off

Resolution	
Base resolution	72
Phase resolution	100 %
Phase partial Fourier	Off
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Distortion Corr.	Off
Prescan Normalize	Off
Raw filter	On
Intensity	Weak
Slope	25
Elliptical filter	Off
Hamming	Off

Geometry	
Multi-slice mode	Interleaved
Series	Interleaved

Special sat.	None

System	
Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Sum of Squares
Auto Coil Select	Off
Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	L0.0 A20.0 H67.7
Orientation	T > C-8.9
Rotation	180.00 deg
R >> L	216 mm
A >> P	216 mm
F >> H	141 mm

Physio	
1st Signal/Mode	None

BOLD	
GLM Statistics	Off
Dynamic t-maps	Off
Starting ignore meas	0
Ignore after transition	0
Model transition states	Off
Temp. highpass filter	Off
Threshold	4.00
Paradigm size	3
Meas[1]	Baseline
Meas[2]	Baseline
Meas[3]	Active
Motion correction	Off
Spatial filter	Off

Sequence	
Introduction	Off
Bandwidth	2240 Hz/Px
Free echo spacing	Off
Echo spacing	0.51 ms
EPI factor	72
RF pulse type	Normal
Gradient mode	Fast*

11/+

Appendix C: Copyright Permissions

Chapter 1:

Figure 1.1
Original Publication: Gibbs K, Beaufort A, Stein A, Leung TM, Sison C, Bloom O. Assessment of pain symptoms and quality of life using the International Spinal Cord Injury Data Sets in persons with chronic spinal cord injury. *Spinal Cord Ser Cases*. 2019;5:32. doi:10.1038/s41394-019-0178-8
Link to copyright information: https://creativecommons.org/licenses/by/4.0

Figure 1.2
Original Publication: Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Res*. Sep 4 2015;1619:1-11. doi:10.1016/j.brainres.2014.12.045

Link to copyright information: https://creativecommons.org/licenses/by-nc-nd/4.0/

Figure 1.3
Original Publication: Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2:17023-. doi:10.1038/sigtrans.2017.23

Link to copyright information: https://creativecommons.org/licenses/by/4.0/

Figures 1.6 and 1.7
Original Publication: Cooper MA, Kluding PM, Wright DE. Emerging Relationships between Exercise, Sensory Nerves, and Neuropathic Pain. *Front Neurosci*. 2016;10:372. doi:10.3389/fnins.2016.00372

Chapter 2:

Original Publication: Kowalski JL, Nguyen N, Battaglino RA, Falci SP, Charlifue S, Morse LR. miR-338-5p Levels and Cigarette Smoking are Associated With Neuropathic Pain Severity in Individuals With Spinal Cord Injury: Preliminary Findings From a Genome-Wide microRNA Expression Profiling Screen. *Arch Phys Med Rehabil*. Apr 2022;103(4):738-746. doi:10.1016/j.apmr.2021.09.005

Copyright information:

Dear Jesse Kowalski,

Thank you for your query.

Please note that, as one of the authors of this article, you retain the right to reuse it in your thesis/dissertation. You do not require formal permission to do so. You are permitted to post this Elsevier article online if it is embedded within your thesis. You are also permitted to post your Author Accepted Manuscript online.

However posting of the final published article is prohibited.

*"As per our Sharing Policy, authors are permitted to post the Accepted version of their article on their institutional repository – as long as it is for **internal institutional use only.***

It can only be shared publicly on that site once the journal-specific embargo period has lapsed. For a list of embargo periods please see: Embargo List.

You are not permitted to post the Published Journal Article (PJA) on the repository."

Please feel free to contact me if you have any queries.

Kind regards,

Kaveri Thakuria
Senior Copyrights Coordinator

ELSEVIER | HCM - Health Content Management

Visit Elsevier Permissions

From: Administrator
Date: Sunday, June 05, 2022 03:27 AM GMT

Dear Customer

Thank you for contacting Elsevier's Permissions Helpdesk.

This is an automated acknowledgement to confirm we have received your query. Ticket number 220605-003732 has been opened on your behalf and we aim to respond within two business days.

Regards,

Permissions Helpdesk

From: Jesse Kowalski
Date: Sunday, June 05, 2022 03:27 AM GMT

Hello, I am the first author of a publication I need to include in my doctoral dissertation. Do I need to request copyright permissions to include this publication in my dissertation document which will be made publicly available online through the University of Minnesota's Library? (https://doi.org/10.1016/j.apmr.2021.09.005)

Thank you,
Jesse