STRUCTURAL ABNORMALITIES AND TREATMENT-RELATED PLASTICITY IN CLASSICAL TRIGEMINAL NEURALGIA

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Institute of Medical Science
University of Toronto

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Doctor of Philosophy

Institute of Medical Science
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Abstract

Classical trigeminal neuralgia (TN) is a unique neuropathic pain disorder characterized by highly intense electric shock-like attacks of unilateral facial pain. While TN is commonly associated with neurovascular compression of the trigeminal nerve at the root entry zone (REZ) its pathophysiology is not well understood. In conjunction with trigeminal nerve abnormalities, central gray matter and white matter (GM, WM) structure may be affected and/or contribute to the maintenance of TN. Surgical treatment for TN may produce analgesia by effectively normalizing these structural abnormalities. The main aim of this thesis is to determine if there are structural neural abnormalities in patients with TN and whether effective neurosurgical treatment can reverse these abnormalities. The specific aims were to determine: 1) if patients with TN have brain GM abnormalities; 2) if patients with TN have trigeminal nerve and/or brain WM
abnormalities based on multiple DTI-derived metrics; 3) if effective neurosurgical treatment for TN is associated with a reversal of the trigeminal REZ and cortical and subcortical GM abnormalities that we report in studies 1 and 2 of this thesis. In groups of TN patients with right-sided pain and healthy age- and sex-matched controls, magnetic resonance imaging revealed that patients had: 1) increased GM in the sensory thalamus, amygdala, periaqueductal gray, basal ganglia, contralateral primary somatosensory cortex, and frontal pole and less GM in the pregenual anterior cingulate cortex, insula, and orbitofrontal cortex; 2) abnormalities in the trigeminal REZ and cerebral WM tracts including the corpus callosum, cingulum, corona radiata, and superior longitudinal fasciculus; and 3) a reversal ventral anterior insula and trigeminal REZ abnormalities following effective treatment, with the degree of REZ normalization correlating with pain relief. Taken together, this thesis demonstrates for the first time both trigeminal nerve and brain abnormalities in patients with TN, and the impact of effective treatment on these abnormalities.
Acknowledgements

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Thank you to Nelson Gonçalves for bringing balance to my life. Palavras não podem expressar quanto o seu amor e apoio significa para mim. Obrigado pela tua paciência e compreensão durante estes períodos de estresse. Obrigado por cuidar de mim e ter certeza que eu não esquecesse de comer quando não era a minha prioridade. Este processo tornou-se mais fácil quando você entrou na minha vida.

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In loving memory of Lucille and Shabir Khan, Clifton and Vivian Ng-A-Kien, Gail Ferreira Ng-A-Kien, Rocky, and my Bailey boy. I miss you dearly.
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<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>Alternating current</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
</tr>
<tr>
<td>AD</td>
<td>Axial diffusivity</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
</tr>
<tr>
<td>AMH</td>
<td>A-fibre mechano-heat nociceptor</td>
</tr>
<tr>
<td>aMCC</td>
<td>Anterior midcingulate cortex</td>
</tr>
<tr>
<td>AMPA</td>
<td>$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>AMyg</td>
<td>Amygdala</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>B$_0$</td>
<td>Magnetic field</td>
</tr>
<tr>
<td>BA</td>
<td>Brodmann area</td>
</tr>
<tr>
<td>BG</td>
<td>Basal ganglia</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygen level dependent signal</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>Calcium ion</td>
</tr>
<tr>
<td>CL</td>
<td>Central lateral nucleus</td>
</tr>
<tr>
<td>CMH</td>
<td>C-fibre mechano-heat nociceptor</td>
</tr>
<tr>
<td>CLBP</td>
<td>Chronic low back pain</td>
</tr>
<tr>
<td>CMA</td>
<td>Cortical masticatory area</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CNV</td>
<td>Trigeminal nerve</td>
</tr>
<tr>
<td>CRPS</td>
<td>Complex regional pain syndrome</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CTA</td>
<td>Cortical thickness analysis</td>
</tr>
<tr>
<td>DCML</td>
<td>Dorsal column medial lemniscal pathway</td>
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</table>
DLPFC  Dorsolateral prefrontal cortex
dPCC  Dorsal posterior cingulate cortex
DRG  Dorsal root ganglia
DTI  Diffusion tensor imaging
DWI  Diffusion weighted imaging
EPI  Echo planar imaging
F  Precessional frequency
FA  Fractional anisotropy
fMRI  Functional magnetic resonance imaging
FSL  FMRIB Software Library
GABA  γ-aminobutyric acid
GKRS  Gamma Knife Radiosurgery
GLM  General linear model
GM  Gray matter
H⁺  Hydrogen ion
HT  Hypothalamus
IASP  International Association for the Study of Pain
IC  Insular cortex
ICHD-3  International Classification of Headache Disorders-3
IHS  International Headache Society
K⁺  Potassium ion
M1  Primary motor cortex
MCC  Midcingulate cortex
MD  Mean diffusivity
MNI  Montreal Neurological Institute
MRI  Magnetic resonance imaging
MS  Multiple sclerosis
MVD  Microvascular decompression surgery
M_z  Magnetization vector
Na  Sodium
NMDA  N-methyl-D-aspartate
NVC  Neurovascular compression
OFC  Orbitofrontal cortex
PB  Parabrachial nuclei
PCC  Posterior cingulate cortex
PCS  Pain Catastrophizing Scale
PET  Positron emission tomography
Pf  Parafascicular nucleus
pgACC  Pregenual anterior cingulate cortex
PFC  Prefrontal cortex
pMCC  Posterior midcingulate cortex
PNS  Peripheral nervous system
PPC  Posterior parietal cortex
REZ  Root entry zone
RF  Radiofrequency
RD  Radial diffusivity
RVM  Rostroventral medulla
S1  Primary somatosensory cortex
S2  Secondary somatosensory cortex
sgACC  Subgenual anterior cingulate cortex
SLF  Superior longitudinal fasciculus
SMA  Supplementary motor area
SNL  Spinal nerve ligation
STT  Spinotalamic tract
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>T₁</td>
<td>Relaxation time 1</td>
</tr>
<tr>
<td>T₂</td>
<td>Relaxation time 2</td>
</tr>
<tr>
<td>TBSS</td>
<td>Tract-based spatial statistics</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TMD</td>
<td>Temporomandibular disorder</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
</tr>
<tr>
<td>TN</td>
<td>Trigeminal neuralgia</td>
</tr>
<tr>
<td>TNP</td>
<td>Trigeminal neuropathic pain</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>TRPV1</td>
<td>Transient receptor potential V1</td>
</tr>
<tr>
<td>TTT</td>
<td>Trigeminothalamic tract</td>
</tr>
<tr>
<td>V₁</td>
<td>Ophthalmic branch of the trigeminal nerve</td>
</tr>
<tr>
<td>V₂</td>
<td>Mandibular branch of the trigeminal nerve</td>
</tr>
<tr>
<td>V₃</td>
<td>Maxillary branch of the trigeminal nerve</td>
</tr>
<tr>
<td>vAI</td>
<td>Ventral anterior insula</td>
</tr>
<tr>
<td>VBM</td>
<td>Voxel-based morphometry</td>
</tr>
<tr>
<td>VBSNC</td>
<td>Trigeminal brainstem sensory nuclear complex</td>
</tr>
<tr>
<td>VMpo</td>
<td>Posterior part of the ventral medial nucleus</td>
</tr>
<tr>
<td>VL</td>
<td>Ventral lateral nucleus</td>
</tr>
<tr>
<td>vlPFC</td>
<td>Ventral lateral prefrontal cortex</td>
</tr>
<tr>
<td>VP</td>
<td>Ventral posterior nuclei</td>
</tr>
<tr>
<td>vPCC</td>
<td>Ventral posterior cingulate cortex</td>
</tr>
<tr>
<td>VPI</td>
<td>Ventral posterior inferior nucleus</td>
</tr>
<tr>
<td>VPM</td>
<td>Ventral posterior medial nucleus</td>
</tr>
<tr>
<td>WDR</td>
<td>Wide–dynamic-range neuron</td>
</tr>
<tr>
<td>WM</td>
<td>White matter</td>
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Chapter 1
Introduction and General Aims

Idiopathic/classical trigeminal neuralgia (TN) is a unique neuropathic pain disorder characterized by highly intense electric shock-like attacks of unilateral facial pain. Although pain can occur spontaneously, it is frequently triggered by stimuli that are normally non-painful and movements of the face such as light touch, eating/chewing, shaving and draughts of wind [78,88,277]. As such, patients with TN may be unable to speak, eat, or be in constant fear of eliciting another pain attack [89]. This can be highly distressing and can negatively impact a patient’s quality of life and psychosocial status [89,441], leading them to seek pharmacological and/or surgical treatment options to achieve some degree of pain relief.

Classical TN is most commonly associated with neurovascular compression (NVC) of the trigeminal nerve at the root entry zone [328]. Unlike other trigeminal neuropathic pains, TN pain is unique in that it is not accompanied by major sensory loss [88]. Additionally, many patients respond well to treatments for TN, with a large proportion of patients having good or complete pain relief following treatment [328]. Studies of TN have increased substantially over the past century but there remains a poor understanding of its underlying pathophysiology, how it impacts the brain, and the effects of treatment.

One possibility is that long-term nociceptive input from the trigeminal nerve to the brain induces maladaptive plasticity in brain gray and white matter (GM, WM) regions involved in the multi-
dimensional experience of pain and pain modulation. Nocifensive behaviours adapted by patients with TN to prevent or lessen pain attacks may also influence brain regions involved in motor functions. Structural magnetic resonance imaging (MRI) and diffusion-weighted imaging (DWI) can be used to investigate brain GM and WM, respectively. Indeed, numerous studies have demonstrated abnormalities in GM regions involved in pain perception, its modulation, and motor function [124,296]. Several studies have also used diffusion tensor imaging (DTI) and found microstructural abnormalities in the cerebral WM tracts of chronic pain patients, many of which interconnect the GM regions involved in the multi-dimensional experience of pain [180,291,310,431]. Examining brain GM and WM abnormalities in TN provides an opportunity to study how neuropathic pain impacts the brain without the confounds of other sensory abnormalities.

Given that TN is frequently associated with NVC of the trigeminal nerve, previous structural imaging studies of TN have used DTI to examine trigeminal nerve abnormalities. The most commonly used DTI-derived metric to assess WM abnormalities is fractional anisotropy (FA), a quantitative biomarker of WM “integrity.” The results of the studies on patients with TN indicated that FA was significantly lower in the affected REZ of TN patients with NVC [174,200,259,283]. However, FA can decrease in a number of scenarios [3] and multiple DTI-derived metrics are available to better characterize microstructural abnormalities. Additionally, these other metrics have been linked to pathophysiological mechanisms such as demyelination, neuroinflammation, and edema [8,55]. Therefore, examining multiple DTI-derived metrics may be useful to noninvasively detect potential mechanisms contributing to TN pathophysiology.
Although medications are always the first line of treatment for TN [487], over time pain attacks may occur more frequently, be more sustained and less responsive to medications, leading patients to seek neurosurgical intervention for pain relief [328,489] [349]. Each year, approximately 8,000 patients undergo surgery for TN in the United States, with estimated costs exceeding $100 million [349]. Surgical treatments for TN can be highly effective, alleviating or substantially decreasing TN pain by more than 75% [265,268]. However, for some patients with TN, surgical treatments are ineffective in providing pain relief or the pain recurs. The mechanisms underlying the analgesic effects of surgical treatments for TN are not well understood, nor are the impacts of effective surgical treatment on brain structure.

Since the mechanisms underlying TN are not well understood, it is challenging to understand the downstream effects of surgical treatments for TN on the central nervous system. If TN is associated with structural brain GM and WM abnormalities in addition to trigeminal nerve abnormalities, then it is possible that effective surgical treatment results in pain-relief by normalizing these structural abnormalities.

Therefore, the mains aims of this thesis are:

1. To determine possible brain GM abnormalities in patients with TN.
2. To determine possible trigeminal nerve and/or brain WM abnormalities based on multiple DTI-derived metrics in patients with TN.
3. To determine if effective neurosurgical treatment for TN is associated with a reversal of the trigeminal REZ and cortical and subcortical GM abnormalities that were found in studies 1 and 2 of this thesis.
Chapter 2
Literature Review

2.1 What is Pain?

The International Association for the Study of Pain (IASP) defines pain as, “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” [303]. Importantly, pain is a complex multi-dimensional experience encompassing cognitive and affective, in addition to sensory domains. Under normal circumstances, pain can be thought of as an adaptive mechanism because it provides information about threat or injury to body tissues that supports healing. However, in certain circumstances pain can be maladaptive in that it is neither protective nor supports healing [97]. In this section, adaptive and maladaptive pains will be reviewed, with a particular focus on neuropathic pain. It will also include a brief description of pain and cognitive and affective interactions.

2.1.1 Adaptive Pain

Normally, pain warns the body of damaging stimuli and protects it from further damage. Thus, responses to this type of pain are adaptive as they are purposeful. In general, two types of pain are considered adaptive: nociceptive pain and inflammatory pain.
2.1.1.1 Nociceptive Pain

Nociceptive pain is crucial for warning the body about damaging or potentially damaging stimuli. As will be discussed in detail later in this chapter, noxious stimuli, detected by specialized peripheral receptors called nociceptors, convey information along thinly myelinated Aδ or unmyelinated C-fibre afferents into the central nervous system (CNS). Once in the CNS, specific tracts carry the nociceptive information to several brain regions involved in the multi-dimensional experience of pain. Importantly, nociceptive pain is not merely a submodality of touch as was historically thought [229]. It has both cognitive and affective components that are salient and/or unpleasant making the pain difficult to ignore. Nociceptive pain occurs in response to noxious stimuli including thermal, mechanical or chemical stimuli arising from superficial or deep origins (e.g., skin, muscle, bone, joints, viscera, vasculature) (Fig. 2-1) [97]. The important adaptive function of this type of pain is highlighted by the repeated injury and/or unintentional self-harm that frequently occurs in people with rare hereditary disorders who do not experience nociceptive pain [99,213]. Individuals with congenital insensitivity to pain can develop serious health complications if nociceptive stimuli go unnoticed and cause tissue damage that is not treated.
**Figure 2-1:** A schematic figure illustrating the pathway for nociceptive pain. Here, relevant stimuli activate peripheral nociceptors, which carry the nociceptive information to the central nervous system. Nociceptive pain warns the body of actual or potential tissue damage, which is an adaptive mechanism for survival. As such, this pain is considered adaptive. Nociceptive input then ascends the spinal cord and brainstem until it reaches the thalamus and other brain regions involved in the multi-dimensional experience of pain. Figure from [97] and reproduced with permission from Annual Reviews.

2.1.1.2 Inflammatory Pain

Inflammatory pain arising from tissue injury can also be described as adaptive. In general, while nociceptive pain functions to protect the body from tissue damage, inflammatory pain usually occurs as the body tries to heal itself and prevent further damage [97]. However, these pain types are often related. Inflammation can lead to a profound change in the responsiveness of the sensory system such that normally innocuous stimuli can now produce pain (allodynia) and
normally noxious stimuli can produce pain that is prolonged and exaggerated (hyperalgesia) [226,236]. These changes in sensitivity result from plasticity occurring either peripherally or centrally termed peripheral and central sensitization (or amplification), respectively (Fig. 2-2). When this occurs, the pain system can respond to both noxious and innocuous stimuli (producing alldynia) or produce amplified responses to noxious stimuli (producing hyperalgesia).

Importantly, inflammatory pain usually goes away with the resolution of the initial injury, but persists in some chronic disorders.

2.1.1.2.1 Peripheral Sensitization

Tissue injury results in the release of inflammatory mediators from the damaged cells. These mediators include different ions (K⁺, H⁺), histamine, bradykinin, 5-hydroxytryptamine, nitric oxide, and ATP [236]. Tissue injury also recruits immune cells, which release additional mediators such as cytokines and growth factors [236]. Some of the mediators can activate peripheral nociceptors directly resulting in spontaneous pain, while others act indirectly via the immune cells to release other pain-inducing agents [236]. These inflammatory mediators modify the response properties of the cells in the periphery, by activating intracellular transduction pathways, resulting in an increase in the production, transport, and membrane insertion of voltage-gated ion channels [97,236]. This results in increased membrane excitability and decreased thresholds for activation [97].
2.1.1.2.2 Central Sensitization

Sensitization can also occur centrally with the sustained or repetitive activation of primary afferent fibres [473]. In this case, CNS neurons (e.g., those in the dorsal horn of the spinal cord) change their responsiveness to sensory input such that they have amplified responses to noxious and innocuous stimuli. This change is triggered by intracellular cascades, which lead to facilitated excitatory transmission and depressed inhibition [473]. This can occur at one synapse (homosynaptic) or multiple synapses (heterosynaptic) [473]. Under inflammatory conditions, neuropeptides and neurotrophic factors such as substance P and brain-derived neurotropic factor are released from the terminals of the primary afferents [236]. This activates intracellular cascades with the net result being facilitated excitatory transmission produced by an exaggerated response to normal stimuli, an expansion of the receptive field size such that there is a spread of sensitivity to regions outside of the injured tissue, and a reduction in the threshold for activation of afferent inputs so that central neurons are responsive to even innocuous inputs [236,237,473]. Evidence suggests that many of these changes are mediated by N-methyl-D-aspartate (NMDA) receptors. There are also NMDA receptor-independent mechanisms for facilitating excitatory synaptic transmission, primarily via mechanisms involving a subset of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [189]. Under normal conditions, fast excitatory synaptic transmission in pain pathways involves the release of glutamate, which acts on AMPA receptors and is modulated by the activation of inhibitory neurons, which release glycine and/or γ-aminobutyric acid (GABA). Under inflammatory conditions, there is an influx of calcium ions via an AMPA receptor subtype that is permeable to calcium ions, and produces a lasting facilitation of synaptic transmission [189]. This pathway is considered NMDA-receptor-independent because calcium ions most frequently enter the postsynaptic neuron via NMDA
receptors. Also, there can be a long-term depression of GABA and glycine dorsal horn neurons, which have inhibitory effects on afferent input. This depression can occur with the activation of Aδ afferents, and requires NMDA receptor activation [376,473].

Figure 2-2: Inflammatory pain is typically considered an adaptive pain that occurs as the body tries to heal following tissue injury. It is characterized by a change in neuronal responsiveness such that normally innocuous stimuli can now elicit pain, and noxious stimuli produce exaggerated pain. These responses occur with plasticity that can take place peripherally and/or centrally. Tissue injury also recruits a number of immune cells (highlighted on this figure) that release inflammatory mediators, further contributing to this process. Figure from [97] and reproduced with permission from Annual Reviews.
2.1.2 Maladaptive Pain

When pain no longer serves to protect body tissues from damage and persists following the resolution of an injury, it is said to be maladaptive. Maladaptive pain can be broadly categorized as dysfunctional, which occurs in the absence of any identifiable noxious stimulus, inflammation, or damage to the nervous system (e.g., fibromyalgia), or neuropathic, which occurs following lesions to the nervous system caused by trauma, metabolic disease, chemicals, tumours, or other pathologies [97]. For the purposes of this thesis, this section will focus on neuropathic pain.

2.1.2.1 Neuropathic Pain

The IASP and the Neuropathic Pain Special Interest Group (NeuPSIG) of the IASP defines neuropathic pain as, “Pain caused by a lesion or disease of the somatosensory nervous system” [192,272]. Neuropathic pain has a prevalence of approximately 7-8% in the general population and can have a number of etiologies including disorders affecting the PNS or CNS (e.g., stroke, multiple sclerosis), trauma, or surgical procedures [19,59,452]. Importantly, neuropathic pain is not an inevitable consequence of a nervous system lesion. In fact, acute neural damage only transitions to neuropathic pain in a minority of individuals [97]. It has been reported that damage to relatively small nerves (e.g., the ilioinguinal nerve) results in neuropathic pain in approximately 5% of patients, whereas damage to a larger nerves (e.g., the sciatic nerve) results in neuropathic pain in about 30-60% of patients [227,235,285]. Having an understanding of why neuropathic pain develops in only some individuals has been a question of interest and in general it has been suggested that the main contributors involve imbalances between compensatory
reactions of the nervous system to damage, and a possible genetic predisposition that either enhances or protects against the development of neuropathic pain [97]. Specifically, maladaptive processes include ectopic impulse generation, ephaptic transmission, reduced inhibition, inappropriate connectivity, and neuro-immune interactions such as microglial activation [97]. Different pain symptoms may manifest depending on which of the maladaptive processes occur. Common symptoms, pathophysiological mechanisms, and reasons for treatment resistance will be discussed in the following sections.

2.1.2.1.1 Manifestation of Neuropathic Pain and Underlying Pathophysiology

While the conventional approach to treating and classifying neuropathic pain is based on etiology, others have suggested that clinical symptoms should be used, as these provide clues about the underlying pathophysiology [97,452]. Common manifestations of neuropathic pain include spontaneous pain, continuous pain (e.g., burning, pressure), paroxysmal pain (electric shock-like, stabbing pain), allodynia, paresthesias and dysesthesias, which are generally accompanied by sensory loss [97,452]. While the precise presentation of neuropathic pain symptoms can be variable between patients, the type of pain and degree of sensory loss are important clues about the underlying pathophysiology.

One feature of neuropathic pain is that the pain can appear to occur spontaneously, that is, in the absence of an identifiable noxious stimulus [97]. Spontaneous pain is the result of ectopic action potential generation that can occur in primary afferent neurons or neurons in the CNS. It should also be noted that the identity of a source of an evoking stimulus can be obscured leading to the
assumption that the pain is “spontaneous” and due to ectopic activity when in fact there may be a subtle underlying stimulus or process driving the seemingly spontaneous pain [41]. It is now well established that following peripheral nerve injury, spontaneous activity can be generated from unmyelinated C-fibres at multiple sites including the site of the injury, in the cell bodies of the injured cells in the dorsal root ganglia (DRG), or in neighbouring afferent fibres [11,97,452,476]. For example, in one study [469], a ventral rhizotomy procedure (L5 level) in rats primarily resulted in the degeneration of myelinated efferent fibres. Teased-fibre techniques were used to record electrophysiological data from single fibres and revealed that low-frequency spontaneous activity occurred in more than twice as many C-fibre afferents (25%) after ventral rhizotomy compared to the sham surgery (11%). Importantly, behavioural data showed a decrease in paw withdrawal thresholds (mechanical hyperalgesia) after the ventral rhizotomy, but not the sham procedure. The authors of this study concluded that degeneration of myelinated efferent fibres is sufficient to induce spontaneous C-fibre afferent activity related to behavioural signs of mechanical hyperalgesia [469]. While spontaneous activity occurs in nociceptors, it can also arise from the activity of low-threshold large myelinated fibres [76] onto central pain signaling neurons due to central sensitization (as discussed above). As with inflammatory pain, peripheral sensitization increases the excitability of the primary afferents such that they are more sensitive to both innocuous and noxious stimuli. One of the mechanisms underlying the generation of ectopic impulses and peripheral sensitization is changes in voltage-gated sodium channel expression [97,452]. Animal studies have shown that experimental nerve lesions (e.g., axotomy) result in abnormal sodium channel expression (e.g., Na\textsubscript{v1.3}, Na\textsubscript{v1.7}, Na\textsubscript{v1.8}, Na\textsubscript{v1.9), resulting in primary afferent hyperexcitability [106,135]. Further support for the role of sodium channels in neuropathic pain comes from clinical observations showing that medications that block
sodium channels (e.g., some antiepileptic medications) can provide neuropathic pain relief [328]. Spontaneous nerve activity is also associated with the upregulation of certain receptor proteins including the transient receptor potential V1 (TRPV1) [28].

In addition to occurring spontaneously, many neuropathic pains are constant and associated with allodynia, which may or may not be accompanied by sensory loss. Ongoing pain frequently occurs with CNS diseases such as MS or spinal cord injury, but can also occur with denervation or other peripheral injuries [452]. In many of these cases, the ongoing pain is described as burning in quality. With lesions that result in a disconnection between primary afferents and second-order neurons, the ongoing burning pain is often related to the spontaneous hyperactivity of the denervated second-order neurons [452]. Because many peripheral neuropathies affect all fibre types, including large myelinated Aβ fibres that carry tactile information, sensory loss commonly co-occurs with the pain symptoms.

Although pain is frequently spontaneous, it can also be triggered. When pain is evoked by a normally innocuous stimulus, this is termed allodynia. Allodynia can be thermal being evoked from warm or cool stimuli, static if it occurs with touch or pressure to the skin, or dynamic if it is elicited by light-moving tactile stimuli [28]. Dynamic mechanical allodynia is the most common type, with a prevalence of about 18-54% in neuropathic pain patients [58,246]. Peripheral nerve lesions can result in the ectopic activity of both nociceptive and non-nociceptive fibres. At points of demyelination, a compensatory mechanism is to overexpress voltage-gated sodium channels to enhance the probability of action potential transmission through this damaged region [452]. As
a result, both A fibres and C fibres sensitize, but Aβ fibres appear to be particularly important in the development of dynamic mechanical allodynia [452]. Repeated discharges of Aβ fibres can generate action potentials in C-fibres via functional cross-excitation or ephaptic transmission, transmitting nociceptive input to CNS neurons [10]. Ephaptic transmission is a critical mechanism underlying triggered pain from innocuous stimuli in general. Central sensitization is also critical in the maintenance of neuropathic pain. As previously discussed, synaptic facilitation via alterations in the synthesis of transmitters, neuromodulators, calcium channel density, and NMDA receptors account for many of these changes [97,473].

While neuropathic pain is frequently associated with constant ongoing pain, some neuropathic pains are characterized by paroxysmal pain; that is, pain that occurs in attacks. This pain is frequently described as being electric shock-like in character, as opposed to the burning pain described above. In many cases, this sharp paroxysmal pain is related to focal demyelination of non-nociceptive Aβ fibres [277,328,452]. This focal damage can result in bursts of spontaneous high-frequency discharges and the cross-excitation of nociceptive fibres [328,452].

Another mechanism that contributes to neuropathic pain is disinhibition. The dorsal horn contains several inhibitory interneurons that modulate primary afferent input using the neurotransmitters glycine and/or GABA [97]. These neurons receive input from higher brain regions via descending modulatory pathways [179]. It has been suggested that in neuropathic pain, these descending pathways are altered, such that there is an increase in the descending facilitation of nociceptive input [38]. In one study [67], it was demonstrated that this descending
facilitation contributed to the maintenance, but not initiation of neuropathic pain. Many other changes have been reported in the dorsal horn that may contribute to the persistence of neuropathic pain. These changes include the loss of spinal inhibitory interneurons, the release of BDNF from active microglia resulting in specific GABA receptors being depolarized instead of hyperpolarized when activated, and a change in the expression of µ opioid receptors [97,98].

Neuro-immune interactions also contribute to the development of neuropathic pain. Following nerve injury, macrophages play an important role in clearing cellular debris [97]. Macrophages are also central to Wallerian degeneration, a process whereby degeneration occurs distal to the axonal injury, and contribute to pain hypersensitivity [386]. In the CNS, microglia are massively activated in the dorsal horn shortly after peripheral nerve injury [39,386]. Microglia release many immune modulators that contribute to the induction and maintenance of neuropathic pain by altering neuronal function [90]. The molecular details are beyond the scope of this thesis.

2.1.2.5 Treatment Challenges

While over the past few decades there have been huge leaps in our understanding of the mechanisms underlying the development and maintenance of neuropathic pain, the treatment of neuropathic pain remains a challenge. In fact, a reduction of pain intensity by even 30% is considered a clinically meaningful result [28]. One reason is that neuropathic pains are heterogeneous in terms of the mechanisms underlying the pain and they frequently involve pathophysiological changes at multiple levels in both the CNS and PNS. While the suggested underlying mechanisms from the pain literature suggest certain drugs may theoretically be useful
in treating neuropathic pain, in practice they are often not effective, suggesting that there is still much to be learned about the pain mechanisms and drug actions [28]. Still, some pharmacological options are known to be at least initially effective in managing neuropathic pains (e.g., voltage-gated sodium channel blockers) [495]. Over time, however, patients often develop drug tolerance and doses may be increased producing unwanted side effects. In general, pain is highly variable between individuals. Individual differences in their analgesic responses to drugs due to genetics or other factors could account for interventions being effective for some individuals but not others. Additionally, the risk of developing neuropathic pain increases with age [380]. Many elderly patients have co-morbidities or are taking other drugs that limit their pharmacological treatment options. Other co-morbidities or health status may prevent some elderly patients from electing to undergo surgical treatment options for their pain. Pain is a complex experience and there are psychological, cognitive, and emotional components to neuropathic pain (discussed below) [28]. As such, these factors may need to be addressed in addition to managing pain symptoms directly. This interdisciplinary approach may include non-pharmacological treatment options such as cognitive behavioural therapy as therapeutic adjuncts [28]. In general, treatment regimens should be individualized such that a balance can be obtained between analgesia, side effects, comorbidities, and drug interactions [28].

2.1.3 Pain Interactions with Emotion

By definition, pain is a multi-dimensional experience that involves both sensory and affective components. As such, pain perception can be influenced by both the intensity of a noxious stimulus and one’s emotional state or mood. When in pain, it is not uncommon to experience a
range of emotions including fear, anxiety, and depression [102]. Although unpleasant, these attributes can motivate an individual to engage in certain behaviors that either stops or reduces the pain [70]. While pain can elicit certain emotions or a change in mood, experimental studies have also provided evidence suggesting emotional state can affect pain sensitivity. In these studies, emotion-altering stimuli presented to participants that included odors, videos, pictures, and music among other stimuli, could either enhance or decrease perceived pain depending on if the stimuli were negative or positive, respectively [129,273,372,459,465]. Furthermore, some of these studies demonstrated that it was specifically pain unpleasantness, and not pain intensity that was affected by the emotion-altering stimuli [273,459]. Many brain areas are involved in processing the affective-motivational components of pain and include limbic structures such as the anterior cingulate cortex (ACC), insula, and amygdala, in addition to other regions such as the prefrontal cortex and hypothalamus [468]. Importantly, these brain regions also have descending modulatory influences on incoming nociceptive input, via brainstem structures such as the periaqueductal gray and rostral ventromedial medulla, providing a physiological mechanism for the effects of mood on pain perception [70,102,468].

Clinical studies have reported that chronic pain is often comorbid with mood disorders such as depression and anxiety [299]. Indeed, it has been reported that depressive symptoms and global affective distress are significantly correlated with self-reported pain in chronic pain patients [181]. As the relationship between mood and pain in these clinical studies is correllational, it is not clear whether individuals with mood disorders have a predisposition to developing chronic pain, or if the toll of being in chronic pain causes individuals to develop depression and/or anxiety. As discussed above, these comorbidities may contribute to the difficulty in treating
patients with chronic pain and complicate our understanding of the pathophysiology of chronic pains. Although this relationship is complicated, studying chronic pain patients that are without some degree of negative affect would be studying a non-representative group, in most cases [133].

2.1.4 Pain Interactions with Cognition

Another part of the multi-dimensional experience of pain involves cognitive factors. Meichenbaum and Turk [300] were the first to propose that cognitive factors may affect pain perception. Under the cognitive-behavioural perspective, they suggested that the way individuals appraise pain might affect how they perceive it [169]. In this way, people with chronic pain might believe that they are limited in their ability to control their pain, which may result in maladaptive behaviours such as inactivity and/or an overreaction to nociceptive stimuli [169]. Certain beliefs about pain may lead to maladaptive coping, increased suffering, and further disability [169]. In one study [321], a modified thought-sampling procedure was used to evaluate migraine patients’ beliefs and pain symptoms before and after treatment. The results of this study indicated that patients with the lowest positive shift in appraisal of their headache-related thinking (e.g., maintained appraisals such as ‘There’s nothing I can do’) also had the least amount of pain relief following treatment [321]. Another cognitive factor that can greatly influence an individual’s perception of pain is catastrophizing [428]. Catastrophizing refers to extremely negative thoughts, where even minor problems are interpreted as catastrophes [169]. For example, an individual who scores high on catastrophizing might have thoughts such as, ‘No matter what I do, my pain doesn’t change anyway.’ One study reported that following cognitive-
behaviour therapy, decreased pain catastrophizing was significantly associated with increased pain tolerance, and decreased physical and psychosocial impairment [454]. This is closely related to the idea of coping, or the ability to deal with pain, adjust to it, or minimize the distress it causes. Coping strategies can involve behaviours that are either overt (e.g., resting, taking medications, relaxation techniques) or covert (e.g., distraction techniques) [169]. While poor coping strategies are closely associated with catastrophizing, adaptive coping techniques are related to decreased pain intensity ratings and increased pain tolerance [169].

Other cognitive factors such as attention and memory can also influence pain perception. Several studies have demonstrated that when individuals focus on pain, it can increase their perceived pain intensity [70]. It has been demonstrated that when participants intentionally direct their attention away from painful stimuli, there is activation of the superior parietal cortex, which is involved in attention [96]. Parts of the parietal cortex project to the primary and secondary somatosensory cortices (S1, S2), and the insula, which send projections to the brainstem or other brain regions, providing a possible pathway for the modulation of pain via attention [80,352]. Evidence also suggests that another crucial pathway plays a role in the attentional modulation of pain and includes prefrontal-cingulate projections to the brainstem [169]. Several neuroimaging studies have demonstrated that distraction away from pain results in decreased activation in brain areas involved in pain perception including the ACC, thalamus, and insula [26,448]. The modulation of ACC neurons by attention-demanding tasks has also been demonstrated using electrophysiological techniques [123]. In general, attention to pain may also affect how people remember things over time. For example, studies have demonstrated that chronic pain patients tend to selectively focus on negative and painful life events when probed on autobiographical
memory tasks [475]. Unconscious memories may also be affected. It has been suggested that chronic pain leads to somatosensory pain memories that manifest as enlarged representations in the S1 and other brain regions involved in pain processing [168,187]. Given that there is somatotopic organization in S1, cortical reorganization that expands a certain region may result in the enhanced responsivity to stimuli originating in the corresponding body area [167].

Taken together, this section of the thesis has described a number of cognitive and affective factors that influence pain perception and indicated how they can be abnormal in individuals who have chronic pain. While there are some similarities across different chronic pains, for the most part they are highly heterogeneous in terms of etiology and symptomology. As this thesis will focus on one neuropathic pain disorder trigeminal neuralgia, the next section will introduce the anatomy and physiology of the trigeminal system, pertaining to its function in pain and sensation.

2.2 Anatomy and Physiology of Trigeminal Pain and Sensation

The trigeminal nerve carries sensory including nociceptive information from oral and craniofacial regions to the brainstem, where it is relayed to the brain for perception. It also has a motor component innervating the muscles involved in mastication. Lesions of the trigeminal nerve and/or pathways can result in diverse sensory abnormalities, weakness to the muscles involved in mastication, and/or various pain syndromes. This section will provide a review of the
anatomy and physiology of the trigeminal system, which will be the foundation to later compare possible neurological and pathophysiological mechanisms underlying facial pain disorders, most notably, trigeminal neuralgia.

2.2.1 Peripheral Receptors and Afferent Fibres

One of the main roles of the trigeminal system is to relay sensory information from orofacial regions into the brain. Like the spinal systems innervating the body, sensory information from the trigeminal system is multimodal and includes discriminative touch, temperature, proprioception, and pain sensations, which are detected by specialized receptors in the target tissues. Additionally, the trigeminal system also innervates several unique target tissues including the cranial meninges, cornea, temporomandibular joint, tooth pulp, and oral and nasal mucosa [195].

There are four different types of sensory receptors, termed mehanoreceptors, located in the skin of the face that are involved in the detection of tactile information: Ruffini corpuscles, Meissner corpuscles, Merkel cell disks, and hair follicle receptors [407]. Although Pacinian corpuscles are also well-known mechanoreceptors involved in the detection of vibration and pressure, they appear to be absent in the skin of the face [221], and therefore will not be further discussed. Ruffini’s corpuscles, Meissner’s corpuscles, and Merkel’s disks are classified as low-threshold mechanoreceptors because they can be activated by weak mechanical stimuli [355]. Morphologically, these receptors have encapsulated nerve endings, which help to determine the type of stimuli the receptors will be responsive to. Sensory receptors can further be classified as
slowly adapting (Ruffini’s corpuscles and Merkel’s disks) or rapidly adapting (Meissner’s corpuscles) based on their rate of adaptation to sustained stimuli. Slowly adapting (tonic) receptors, continuously fire as long as a stimulus is present, while rapidly adapting (phasic) receptors respond only at the onset and frequently offset of stimulation [355]. The benefit of having receptors that adapt at different rates is that both dynamic and static qualities of a given stimulus can be encoded. While Meissner corpuscles and Merkel cell disks are located more superficially in the skin (cutaneous), Ruffini corpuscles are subcutaneous receptors that are particularly sensitive to stretching of the skin [281,407]. Merkel cell disks detect pressure on the skin and help to discriminate the textures of objects, and Meissner’s corpuscles are more sensitive to stroking and fluttering of the skin [407]. Another receptor type found in facial skin is hair follicle receptors, which are sensitive to the displacement of hair [281,407]. Along with the other cutaneous receptors, these hair cells have small receptive fields, providing better localization and discrimination of stimuli over subcutaneous (Ruffini corpuscle) receptors [281]. Together, these mechanoreceptors (including hair follicle cells) are described as detecting fine touch (as opposed to crude/coarse touch), vibration, and features such as the texture and shape of objects. They transmit information along rapidly conducting Aβ axons (large diameter, myelinated) [281], which when stimulated, do not usually produce a sensation of pain and are thus considered non-nociceptive neurons. In some cases of neuropathic pain, for example, Aβ axons can be involved in nociceptive signaling due to processes such as central sensitization and ephaptic transmission. As such, the mechanoreceptors described above that are typically associated with Aβ axons, may now elicit pain with the detection of low-threshold stimuli. For example, for many patients with TN, normally innocuous stimuli such as light touch to the face can elicit attacks of intense pain.
Other non-nociceptive mechanoreceptors are specialized for proprioception and provide information about forces arising from the body itself (e.g., joint or muscle position). One type of proprioceptor is the muscle spindle, which provides information about muscle length, or the degree to which it is being stretched [355]. Muscle spindles can either be slowly or rapidly adapting. While they are located in striated muscles throughout the body, their distribution varies. For example, large muscles that generate relatively coarse movements have few muscle spindles, while those that generate precise movements (e.g., extraocular muscles) have a greater number of muscle spindles. Other proprioceptors include Golgi tendon organs, which slowly adapt to detect changes in muscle tension, and joint receptors, which are rapidly adapting mechanoreceptors that provide information about joint position and movement [355].

The cutaneous receptors that have free nerve endings are sensitive to noxious (nociceptors), thermal (thermoreceptors), or crude tactile stimuli. It has been reported that the face has the highest density of free nerve endings [151]. The primary afferent neurons with free nerve endings are highly specialized and can be classified according to their fibre type: thinly myelinated Aδ fibres which are faster conducting (approximately 2-30 m/s) and the unmyelinated C-fibres that are more slowly conducting (< 2 m/s) [355,396]. Some of these primary afferents are non-nociceptive thermoreceptors that respond to changes in temperature that are perceived as either warm (35-45°C) (predominantly unmyelinated fibres) or cool (15-35°C) (predominantly thinly myelinated Aδ fibres) [113,114,115,121,379]. The remaining primary afferents are nociceptors classified as high threshold because they preferentially respond
to noxious stimuli regardless of the type of stimuli (thermal, mechanical, and/or chemical). Both C-fibres and A-fibres can transmit nociceptive information, but some preferentially respond to certain stimuli. C fibres that respond to mechanical and heat stimuli in the nociceptive range are called C-fibre mechano-heat nociceptors (CMHs) and are thought to be the most numerous [75, 256]. The CMH nociceptors typically respond to thermal stimuli greater than approximately 45°C, with responses increasing with increasing stimulus intensity [75]. Also, there are heat-responsive but mechanically insensitive C-fibres, the so-called, ‘silent nociceptors,’ which only develop mechanical sensitivity following injury [383]. In addition to these C-fibre nociceptors, there are two types of A-fibre mechano-heat nociceptors (type I AMHs and type II AMHs) [73]. These two nociceptors differ in their heat thresholds, with type I AMHs having very high thresholds (typically > 49°C), and type II AMHs having lower thresholds (approximately 43°C) [74, 150]. Also, some nociceptors respond to noxious stimuli other than mechanical or heat stimuli. Some C-fibre nociceptors respond to noxious thermal (heat and cold), mechanical, and chemical stimuli and as such are termed polymodal nociceptors [75], or HPC (heat, pinch, cold) cells [105]. Some A-fibre nociceptors have also been reported to be sensitive to noxious cold [113]. Perceptually, the differences in conduction velocity between these fibre types generally account for two categories of pain perception: first pain, which is sharp and immediate (mediated by Aδ fibres), and second pain, which is delayed and longer lasting (mediated by C fibres) [355].

Despite this variety, orofacial sensory receptors essentially work the same: stimuli that deform or otherwise stimulate the nerve endings of the receptors, result in a depolarizing current in the nerve ending, producing a receptor potential that can trigger action potentials. In this way, sensory transduction occurs, such that sensory information is converted to an electrical signal
that is carried via the fibres comprising the trigeminal nerve to the brainstem for further processing.

2.2.2 Trigeminal Nerve, Ganglion, and Roots

2.2.2.1 Major Branches of the Trigeminal Nerve

In the periphery, the trigeminal nerve is divided into three major branches that supply different regions of the face. These major branches are the ophthalmic (V1), maxillary (V2), and mandibular (V3) nerves, which then give rise to a number of smaller branches (Figure 2-3). While all three branches are sensory, V3 also has a motor component that innervates the muscles of mastication [270]. The anatomy of each of these major branches will be discussed.

![Figure 2-3](image.png)

**Figure 2-3:** The major peripheral branches of the trigeminal nerve. V1= ophthalmic branch, V2= maxillary branch, V3= mandibular branch. Figure from [270] (© 2005 by NOVEL collections).
2.2.2.1 Ophthalmic Branch (V1)

The V1 branch of the trigeminal nerve is the smallest of the three branches and mainly provides sensory innervation to several areas on the superior aspect of the face and cranial structures including the eyeball, forehead, upper eyelids, frontal sinus, lacrimal gland, and dura of the anterior cranial fossa [270]. It arises from the anterolateral aspect of the trigeminal ganglion and then enters the cavernous sinus inferiorly. The ophthalmic nerve also provides sensation from the eye muscles [270]. The ophthalmic branch further divides into three main branches: the lacrimal, frontal, and nasociliary nerves, all of which pass through the superior orbital fissure to enter the orbit. As its name suggests, the lacrimal nerve supplies the lacrimal gland. The frontal nerve further divides into smaller branches that supply the skin of the forehead closer to the midline, the upper eyelid, and parts of the frontal sinus. The nasociliary nerve gives rise to several small branches that innervate many regions of the eye including the iris, ciliary body, and cornea [270]. Other branches of the nasociliary nerve supply the mucous membrane on the anterior aspect of the nasal septum, the anterior portion of the lateral nasal wall, as well as the skin covering the tip of the nose. The V1 branch additionally contains autonomic fibres that are involved in lacrimation and pupil dilation.

2.2.2.1.2 Maxillary Branch (V2)

The V2 branch of the trigeminal nerve is also mainly sensory, providing innervation to the skin over the cheek, temple, lower eyelid, and part of the side of the nose via a number of smaller branches including the zygomaticofacial and zygomaticotemporal branches. Additionally, it
supplies portions of the nasal mucous membranes, teeth of the upper jaw, nasopharynx, maxillary sinus, soft palate, tonsil, and roof of the mouth via branches that include pterygopalatine and superior alveolar nerves [270]. It is one of the most commonly affected branches in some trigeminal neuropathic pains [230]. This branch arises from the central portion of the trigeminal ganglion and also passes through the cavernous sinus inferior to the V1 branch.

2.2.2.1.3 Mandibular Branch (V3)

The V3 branch of the trigeminal nerve has both sensory and motor roots from the inferior aspect of the trigeminal ganglion. The V3 branch courses through the foramen ovale, which is part of the sphenoid bone [270]. The V3 trunk gives off branches that supply the temporal dura mater and some muscles in the ear and soft palate, and divides into an anterior component that is mostly involved in motor functions, and a posterior component that is mostly sensory [270]. The anterior component supplies the medial and lateral pterygoid, masseter, and temporal muscles, which are involved in mastication. Injury to this anterior portion of V3 results in a flaccid paralysis of these muscles, which can be visualized during jaw retraction, as the jaw will deviate in the direction contralateral to the affected side [270]. The posterior component branches into three main nerves: the auriculotemporal, lingual, and inferior alveolar nerves. Together, they provide sensation to the many regions including the lateral surface of the ear, the parotid gland, the gums and teeth of the mandible, the anterior portion of the tongue, and the skin and mucous membranes of the lower lip and gums [270]. The inferior alveolar nerve itself also has a motor branch that innervates the mylohyoid muscle and the anterior belly of the digastric muscle, which are involved in swallowing and speaking. The V3 branch also contains autonomic fibres participating in salivation, and fibres from proprioceptors that provide information about jaw
position and participate in the jaw-jerk reflex, a stretch reflex that results in a brisk contraction when the mandible is tapped [464].

### 2.2.2.2 Trigeminal (Gasserian/Semilunar) Ganglion

The trigeminal (also known as Gasserian) ganglion contains the cell bodies of all of the trigeminal sensory axons, but the motor root does not enter the ganglion [270]. The trigeminal sensory neurons are pseudounipolar, with the anterior axons forming the three major peripheral branches of the trigeminal nerve, and the posterior branches forming the sensory root, which enters the lateral portion of the mid-pons [270,464]. It is analogous to the dorsal root ganglia of the spinal cord, and sits in Meckel’s cave, which is located on the superior surface of the petrous bone in the middle cranial fossa [270]. Neuroimaging and electrophysiological evidence suggests that there is somatotopic organization within the trigeminal ganglion, such that mechanoreceptive and nociceptive afferents from the V1 branch are found medially and anteriorly in the trigeminal ganglion, V2 afferents are caudal and lateral, and V3 afferents are in between [56], consistent with animal studies utilizing tracer methods [336]. Clinically, this somatotopy is taken into account for rhizotomy procedures used for the treatment of TN.

### 2.2.2.3 Trigeminal Root

The trigeminal nerve has both sensory and motor roots that enter and exit the pons, respectively. Microvascular conflicts are frequently observed at the sensory root of patients with some trigeminal neuropathic pains (discussed in detail in section 2.3.4.3) [117,138]. Anatomically, the sensory root or trigeminal root entry zone (REZ) primarily consists of CNS myelin produced by
oligodendrocytes. The CNS myelin at this location can be variable in length [298], but typically extends up to a few millimeters beyond the surface of the pons to a transition zone known as the Obersteiner-Redlich zone, where it transitions to peripheral myelin produced by Schwann cells [277,341]. The transition zone forms an arch shape in horizontal section (Fig 2-4). Thus, the REZ and transition zone are distinct anatomical sites [128,341]. While there appears to be a somatotopic organization of fibres in the trigeminal sensory root [342], evidence for a functional segregation between sensory modalities in this region remains somewhat controversial [118]. In one study, Pelletier and colleagues [342] determined that the trigeminal sensory root has three distinguishable, but overlapping somatotopic representations, but no separate regions contained fibres of a particular function. Still, some have argued that at the REZ, fibres change their direction to target their appropriate nuclei within the trigeminal brainstem sensory nuclear complex (discussed in detail below) [107,338]. Specifically, many Aβ afferent fibres carrying tactile information leave the trigeminal sensory root rostrally and enters the brainstem to be in the trajectory of the main sensory nucleus, while the majority of C fibres and some Aδ fibres carrying nociceptive, thermal, and crude touch information are situated more caudally so they can descend in the brainstem to be in the trajectory of the subnucleus caudalis (Fig 2-5) [118,396]. Therefore, while the trigeminal nerve is mixed in that it carries information pertaining to many functions, once in the brainstem, there is some functional segregation.
**Figure 2-4:** Photomicrograph of trigeminal nerve in horizontal section. The (a) lateral and (b) medial aspects of the trigeminal nerve are labeled and connected by Line A, which marks the line of the central-peripheral myelin junction point. The transition zone (c) can be viewed as the arch shaped zone extending from Line A, with the precise shape of the transition zone described by Line B. This photo is reproduced from [341] with permission from the Copyright Clearance Center (license number: 3524840251010).
Figure 2-5: A schematic illustration of trigeminal fibre distribution by type at the sensory root.

Rostrally, the predominant fibre type is A fibres, with C fibres being scarce. More caudally, the majority of fibres are C fibres. Note that this is a general pattern and does not mean that A fibres do not also project to more caudal nuclei also, for example. Also note the differentiated, but mixed somatotopy as indicated by V1, V2, and V3 proportions at different regions of the sensory root. A= A fibres, C= C fibres, V1= ophthalmic branch, V2= maxillary branch, V3= mandibular branch. This figure is adapted from [118] (permission to be included in the printing of Doctoral Dissertation granted as part of “Books on Demand” program from the Copyright Clearance Center).
2.2.3 Trigeminal Brainstem Nuclei

The trigeminal brainstem nuclei can be thought of as three separate units: the motor nucleus, the mesencephalic nucleus, and the trigeminal brainstem sensory nuclear complex (VBSNC), which is further subdivided into two nuclei [270,396]. Each of these units will be described below, however an emphasis will be made on the VBSNC for its role in sensory, including nociceptive transmission.

2.2.3.1 Trigeminal Motor Nucleus

The motor nucleus of the trigeminal nerve is located laterally in the mid-pons. This nucleus contains the cell bodies of the efferent motor fibres that are incorporated into the V3 branch of the trigeminal nerve to innervate the muscles involved in mastication. The motor nucleus sits in close proximity to the mesencephalic nucleus [464].

2.2.3.2 Mesencephalic Nucleus

The mesencephalic nucleus contains the cell bodies of primary afferents involved in proprioception [270]. These unipolar neurons are in close proximity to the trigeminal motor nucleus at their caudal extent and are primarily proprioceptive in function [270]. The receptor terminals of the neurons comprising the mesencephalic nucleus primarily respond to stretching of the muscles involved in mastication, but some are also located in the teeth, hard palate, and temporomandibular joint [464].
2.2.3.3 Trigeminal Brainstem Sensory Nuclear Complex (VBSNC)

The VBSNC is involved in relaying somatosensory information from the orofacial region. Specifically, it contains the cell bodies of second order neurons that receive input from the primary trigeminal afferent fibres in addition to other cranial (e.g., facial, glossopharyngeal, vagus) and upper cervical nerves contributing to sensation [396]. The majority of these fibres cross the midline and project to the contralateral thalamus [464]. The VBSNC is divided into the main (principal/chief) sensory nucleus and the spinal trigeminal nucleus (spinal tract nucleus), the latter of which is further divided into three subnuclei (oralis, interpolaris, and caudalis) [77,270,396,464]. While these divisions of the VBSNC can be distinguished, in general it is a relatively uniform complex with the exception of the most caudal subnucleus caudalis, which is a laminated structure much like the dorsal horn of the spinal cord [77,396]. As such, it is also referred to as the medullary dorsal horn [207,397].

The main sensory nucleus is the more rostral of the two nuclei and is positioned lateral to the motor nucleus. It is generally considered to be a relay for neural information related to the spatiotemporal properties of tactile sensations from the orofacial region [116,397], and accordingly, it primarily receives input from the larger myelinated Aβ afferents, which are associated with the mechanoreceptors (e.g., Meissners corpuscles, Merkel’s disks) described above [270]. Functionally and anatomically, the main sensory nucleus is considered to be analogous to the nucleus gracilis and nucleus cuneatus of the dorsal column medial lemniscal pathway, which carries tactile information from the body. Cells in the main sensory nucleus are classified as low-threshold mechanoreceptive (LTM) neurons and have relatively small receptive fields [77].
Neurons comprising the VBSNC can be functionally classified based on their receptive field properties. Therefore, LTM neurons respond to light tactile stimuli, but do not increase their discharge when noxious mechanical stimuli are applied [397]. They are differentiated from both wide-dynamic-range (WDR) neurons, which respond to light mechanical stimuli and increase their discharge rate as stimulus intensity increases to the noxious range, and nociceptive-specific (NS) neurons, which respond to noxious stimuli, but not low-threshold mechanical stimuli [353,397]. As such, WDR neurons receive inputs not only from Aβ afferents associated with mechanoreceptors (tactile stimuli), but also Aδ and/or C fibres, which are associated with free nerve endings and thermoreceptors to detect nociceptive stimuli and changes in temperature. Under normal conditions, NS neurons only receive afferents from Aδ and/or C fibres.

Somatotopy of oral and facial representations has been shown to be maintained in the main sensory nucleus, such that certain oral and facial regions synapse in a topographical manner [403].

The more caudal of the VBSNC nuclei is the spinal trigeminal nucleus; a long nucleus that extends to the second cervical root of the spinal cord and merges with the dorsal horn [270]. A large proportion of trigeminal afferent fibres descend in the spinal trigeminal tract, which is mainly composed of fibres related to nociceptive and thermoreceptive transmission (Aδ and C fibres) [270] and to a lesser extent facial touch [407], and synapse on one or more subnuclei of the spinal trigeminal nucleus [270,396]. The nucleus oralis is the most rostral of the subnuclei of the spinal trigeminal nucleus and is continuous with the subnucleus interpolaris inferiorly.
Together they are primarily involved in oral and dental sensation, but evidence suggests that they also receive some nociceptive input from orofacial structures [270]. Afferent fibres projecting to the most caudal of the subnuclei, the subnucleus caudalis, synapse in a particular manner: fibres from the perioral region of the face synapse rostrally, whereas fibres from more peripheral regions of the face synapse more caudally. As such, this creates a concentric “onionskin” somatotopic arrangement for the representation of facial pain in the brainstem, which is different from the dermatome distribution of the peripheral branches of the trigeminal nerve [270,396].

While the subnucleus caudalis contains all three neuron types (LTM, WDR, and NS), certain neurons predominate in specific layers of this laminated nucleus. In one study that conducted extracellular recordings of the activity of single neurons in the subnucleus caudalis of anaesthetized animals, it was reported that the majority of LTM neurons were located in layers III/IV and V/VI, WDR neurons were mainly in layers V/VI, and NS neurons were mainly located in layers I/II and V/VI [397]. Like the other nuclei in the VBSNC, the majority of these second order neurons cross the midline and project to the contralateral thalamus where they can be relayed to the appropriate cortical regions involved in the perception of pain and temperature.

**Other Nuclei**

Trigeminal afferents also project to targets other than the trigeminal sensory nuclei [292]. These targets include the cerebellum, nucleus solitarius, vestibular nucleus, hypoglossal nucleus, cuneate nucleus, and reticular formation, among other regions [77,215,292]. While the precise
oral and facial functions of these connections are not entirely understood [77], they likely allow
the integration of trigeminal afferent information with other systems.

2.2.4 Sensory Pathways and Supraspinal Projections

To this point, the anatomy of the trigeminal nerve and trigeminal brainstem nuclei have been
discussed, illustrating how sensory information from the face is detected and transmitted to
brainstem nuclei the CNS. In this section, ascending sensory pathways to supraspinal levels from
the body and face will be described and the major brain regions involved in the sensory
perception will be discussed.

2.2.4.1 Ascending Sensory Pathways

2.2.4.1.1 Spinothalamic and Trigeminothalamic Tracts

The spinothalamic tract (STT) or anterolateral pathway of the spinal cord carries nociceptive,
thermal, and crude touch information from the body [472]. The role of the STT in pain is well
established, but there remains a debate with regard to the contribution of certain components of
this tract, namely the lamina I NS neurons and lamina V WDR neurons [101,354]. Proponents of
one group (the pattern/intensity concept of pain sensation) claim that the lamina V WDR neurons
are “necessary and sufficient” for all types of pain sensation [354]. In contrast, others [101]
argue that WDR neurons cannot differentiate stimulus modality and therefore, lamina I NS
neurons which receive both Aδ and C afferents, encoding the distinct sensations of sharp (first)
pain and burning (2nd pain), respectively, are the neurons essential for nociceptive transmission.
Since the precise roles of NS and WDR neurons in pain remain under debate, the following section will combine the literature from both camps.

Nociceptive information from the body is transmitted along spinal nerves with their cell bodies in the dorsal root ganglia. The nociceptive afferents enter the spinal cord dorsally forming the Lissauer Tract and synapse on NS and WDR neurons, primarily in laminae I, but also in laminae II (substantia gelatinosa), and some of the Aδ fibres have collaterals terminating in lamina V of the dorsal horn [9,105,472]. The majority (~85%) of the second order neurons cross the midline in the ventral white commissure within one to two spinal cord segments forming the STT in the anterolateral quadrant of the spinal cord. These afferents project to the thalamus contralateral to side of nociceptive input [105]. Approximately 15% of STT fibres do not cross the midline and ascend to the ipsilateral thalamus. The fibres in the anterolateral STT have a somatotopic organization such that caudal body regions tend to be represented more laterally in the tract, and rostral body regions more medially [105]. This occurs since crossing STT axons initially enter the anterior funiculus of the spinal cord, then shift to the lateral funiculus as they ascend, as axons from more rostral body regions continually enter at the anterior funiculus [472]. Studies in primates have revealed that the STT ascends through the brainstem, where it is called the spinal lemniscus, and finally terminates in the thalamus at six projection sites (discussed in detail in next section): 1) the ventral posterior (VP) nuclei, 2) the posterior part of the ventral medial nucleus (VMpo), 3) the ventral lateral (VL) nucleus, 4) the central lateral (CL) nucleus, 5) the parafascicular (Pf) nucleus, 6) the dorsal medial nucleus, particularly the ventral caudal portion (MDvc) [105]. The thalamus relays the sensory information pertaining to both the sensory-discriminative (lateral nuclei) and affective-motivational (medial nuclei) dimensions of pain to
the appropriate cortical regions via thalamocortical projections. In addition to these thalamic projections, ascending STT afferents also have a number of spinobulbar projections, which are important for the integration of nociceptive and homeostatic activities, and also to a degree, the conscious experience of pain [105]. Specifically, some STT brainstem projections are to catecholamine cell groups that are known for integrating cardiorespiratory and autonomic functions [105]. Other brainstem projection sites include the reticular formation, the parabrachial nuclei, and the periaqueductal gray (PAG), which when stimulated can elicit cardiovascular changes, aversive behaviours, and importantly, nociceptive modulation [9,105].

A tract homologous to the STT, called the trigeminothalamic tract, carries nociceptive and thermal information from orofacial regions. The previous section of this thesis reviewed trigeminal nerve and brainstem anatomy, describing how some trigeminal nerve fibres enter the brainstem and synapse on the spinal trigeminal nuclei of the VBSNC. In particular, a large number of Aδ and C-fibres synapse on the cell bodies of WDR and NS neurons, many of which are located in the subnucleus caudalis of the VBSNC. Based on this observation and the findings of clinical studies that reported profound facial analgesia, but some spared tactile sensation with trigeminal tractotomy procedures, the subnucleus caudalis has come to be regarded as the integral trigeminal nucleus for nociceptive transmission from the face [395]. The majority of these second order neurons have axons that cross the midline and ascend in brainstem alongside the spinal lemniscus [105]. Although these trigeminal fibres are positioned immediately adjacent to the spinal lemniscus, they form their own tract sometimes referred to as the trigeminal lemniscus and terminate on VP medial (VPM) nucleus of the thalamus [464], among the other
previously mentioned thalamic nuclei involved in relaying nociceptive input. From the thalamus, the sensory information is then relayed to the appropriate cortical regions for perception.

2.2.4.1.2 Dorsal Column Medial Lemniscal and Trigeminal Somatic Sensory Pathways

The dorsal column medial lemniscal (DCML) pathway conveys sensory information regarding discriminative touch, vibration, and proprioception from the body. The primary spinal nerve afferents involved in this pathway are mainly large myelinated Aβ fibres that rapidly conduct (30-100 m/s) sensory information. Unlike the afferents relaying nociceptive information, the majority of the DCML afferents do not synapse in the dorsal horn. Instead, they ascend in the dorsal columns of the spinal cord and synapse on the dorsal column nuclei in the caudal medulla [355]. Although the majority of the primary afferents in this pathway immediately ascend in the dorsal columns, some collateral Aβ fibres synapse on LTM or WDR neurons in the spinal dorsal horn, primarily in laminae III-V, and participate in segmental reflexes, among other functions [355]. The ascending dorsal column fibres are somatotopically arranged such that the lower body is represented more medially in the posterior funiculus (fasciculus gracilis), while upper body fibres travel more laterally in the posterior funiculus (fasciculus cuneatus). There are two dorsal column nuclei that are named according to which fasciculus they receive input from: nucleus gracilis receives input from fasciculus gracilis and the more lateral nucleus cuneatus receives afferents from fasciculus cuneatus [355]. These nuclei contain the cell bodies of the second order neurons that cross the midline in the internal arcuate fasciculus and ascend to the thalamus via the medial lemniscus. Third order neurons specifically in the VPL nucleus of the thalamus relay the sensory information to the appropriate brain regions involved in the perception of these sensations, but mainly the S1 [355].
As with the trigeminothalamic tract that carries nociceptive input from the face, there is a separate pathway for non-noxious mechanosensory information from the face. As previously discussed, large myelinated trigeminal afferents enter the brainstem and synapse primarily on the main sensory nucleus of the VBSNC, the trigeminal homologue of the dorsal column nuclei [355]. Many of the second order neurons arising from the ventromedial aspect of the nucleus cross the midline and ascend in the trigeminal lemniscus, joining the fibres of the medial lemniscus to the contralateral VPM thalamus [270]. A smaller population of neurons from the dorsomedial aspect of the nucleus, with input from oral structures, ascends to the ipsilateral VPM thalamus [270,326]. The VPM thalamus then relays the mechanosensory information to specific regions of the somatosensory cortex (discussed below) [464].

2.2.4.2 Supraspinal Nociceptive and Pain Regions

Although it is now well established that pain perception is a multi-dimensional experience involving several cortical regions, it was once believed that pain was the only sensation that relied on thalamic, not cortical activity [198]. Over time, studies using anatomical, electrophysiological, and functional imaging approaches have helped to establish the cortical areas that receive input from the spinothalamic and trigeminothalamic tracts (STT and TTT) and contribute to the perception of pain [14,83,233]. In general, the conceptual framework used to account for the multi-dimensional experience of pain, divides the nociceptive system into lateral and medial components, with the lateral component (involving lateral thalamic nuclei) accounting for sensory-discriminative aspects of pain, and the medial component (involving
medial thalamic nuclei) accounting for the affective-motivational dimension of pain [301]. These thalamic nuclei then relay the nociceptive information to different cortical (and/or subcortical targets), with S1 and S2 being the main targets of the lateral system, and the insula and anterior cingulate cortex (ACC) being the main targets of the medial system (Fig. 2-6). While this dual model of pain processing is useful theoretically, pain processing is more complex. Other connections between these cortical areas directly receiving nociceptive input and other cortical or subcortical brain regions (e.g., prefrontal, premotor, parietal cortices, basal ganglia) exist, and are presumed to participate in cognitive-evaluative aspects of pain and pain modulation or in integrating nociceptive input with other sensory modalities (Fig. 2-7) [449,463]. The major regions of STT/TTT termination in the thalamus and cortex will be discussed and other regions that indirectly receive nociceptive information will be described.

**Figure 2-6:** The medial and lateral nociceptive systems. The lateral system is comprised of projections to the primary and secondary somatosensory cortices (S1 and SII) from the lateral thalamic nuclei (VPL/VPM, VPI) and carries information regarding the sensory-discriminative aspects of pain. The medial system is comprised of projections to the insula and anterior cingulate cortex (ACC) via thalamocortical projections from the medial thalamic nuclei (VMpo,
MDvc, Pf, CL), and is involved in processing the affective-motivational components of pain.
VPL= ventral posterolateral nucleus, VPM= ventral posteromedial nucleus, VPI= ventral posteroinferior nucleus, VMpo= ventromedial nucleus (posterior part), MDvc= medial dorsal nucleus (ventrocaudal part), Pf= parafascicular nucleus, CL= centrolateral nucleus. This figure has been reproduced from [450] with permission of the International Association for the Study of Pain® (IASP). The figure may not be reproduced for any other purpose without permission.

**Figure 2-7:** A schematic representation of the brain areas involved in pain perception including those receiving direct nociceptive input and those receive it indirectly. Abbreviations: S1: primary somatosensory cortex, M1: primary motor cortex, S2: secondary somatosensory cortex, ACC: anterior cingulate cortex, PCC: posterior cingulate cortex, SMA: supplementary motor area, PPC: posterior parietal cortex, PFC: prefrontal cortex, BG: basal ganglia, Amyg: amygdala, HT: hypothalamus, PB: parabrachial nuclei, PAG: periaqueductal gray. Adapted from [387] with permission from the Copyright Clearance Center (license number: 3524910950581).
2.2.4.2.1 Thalamus

As previously mentioned, there are a number of STT/TTT termination sites in the thalamus, including the VP complex, MDvc, VMpo, CL, VL, and Pf, nuclei [105,144]. The VP complex includes the VPL and VPM and is classically described as the thalamic termination site for the body and face, respectively [6,54]. In humans it is also frequently referred to as the ventrocaudal (Vc) nucleus [225]. These nuclei are somatotopically organized with nociceptive information from the face represented most medially, and the lower limb represented most laterally [144]. Inputs to the VP complex mainly originate from the contralateral dorsal horn laminae IV and V and are involved in relaying nociceptive input regarding the sensory-discriminative aspects of pain, which include features such as the location, intensity, and quality of the pain, to the primary somatosensory cortex (SI), areas 3b and 1 [61,143,301,450,470,471]. Nociceptive neurons in the VP thalamus are situated among mechanoreceptive neurons, which receive non-nociceptive inputs from the dorsal column medial lemniscal pathway [450]. Only approximately 10% of VP neurons are nociceptive, with the majority of these being WDR neurons [143]. Another region ventrally adjacent to the VPL and VPM is the VP inferior (VPI) nucleus. This nucleus receives nociceptive input from lamina I in addition to laminae IV and V and relays input to the secondary somatosensory cortex (SII) [143,144]. The VPI nucleus contains both WDR and NS neurons and also receives vestibular input [143].

The MD nucleus is part of the medial nociceptive system and as such is involved in the affective-motivational (suffering) dimensions of pain [47,472]. The MDvc, in particular, has been shown
to receive dense input from some lamina I STT cells in an anteroposterior topography, such that trigeminal inputs are most posterior [7]. The MDvc nucleus has a discrete population of NS neurons that have large, sometimes bilateral receptive fields [101,143]. These neurons project to the anterior cingulate cortex (ACC), part of the limbic system, unlike many of the other MD neurons that project to the prefrontal and orbitofrontal cortices [101,105]. Some medial thalamic neurons also project to the insular cortices and may have a role in interoception [100].

Another thalamic projection site for nociceptive input is to the VMpo nucleus. This nucleus is rudimentary to non-existent in non-primates, and only well-developed in humans [143]. It is located just posterior and inferior to the VP nucleus and primarily receives input from lamina I dorsal horn neurons [101]. These neurons project in a topographical manner to the dorsal aspect of the posterior insular cortex, and involved in the perception of several bodily “feelings” including pain, temperature, itch, and visceral sensations [143].

Two intralaminar nuclei, the CL and Pf, also receive nociceptive input. While the CL nucleus receives dense input from laminae V and VII dorsal horn neurons, the Pf receives only weak projections from laminae I and V [143]. The CL and Pf have large receptive fields and have been shown to respond to noxious heat [143]. The CL nucleus also receives dense projections from the cerebellum, substantia nigra, globus pallidus, and motor cortex, and along with the Pf nucleus, projects mainly to the basal ganglia and motor and parietal cortices [224]. Together, these intralaminar nuclei are thought to play a role in attention and motor related functions [144,374].
Lastly, the VL nucleus, which is located just rostral to the VP nucleus, receives moderately dense input from laminae V and VII dorsal horn neurons [105,143]. This nociceptive input converges with input from the cerebellum and projects to the motor cortex [425]. As such, it is likely involved in sensorimotor integration [105].

2.2.4.2.2 Direct Cortical Targets

While several cortical regions are implicated in the multidimensional experience of pain, only some receive direct nociceptive input from the thalamus, and are thus referred to as direct cortical targets. These areas are the S1 and S2, ACC and mid-cingulate cortex (MCC), and the insular cortex (IC) [69]. Each of these cortical areas will be described below.

The S1 cortex is located in the postcentral gyrus of the parietal lobe and consists of Brodmann areas (BA) 1, 2, 3a, and 3b [449]. It receives direct sensory, including nociceptive, input from the VP thalamus [402]. Areas 1 and 3b generally receive cutaneous tactile input, while areas 2 and 3a receive proprioceptive input [449]. While nociceptive neurons are rare in SI, they have been located in areas 1, 3a, and 3b, with many being spatially distinct from those involved in tactile processing [231,232,443,449]. It has been demonstrated that S1 nociceptive neurons have small receptive fields from the contralateral side of the body that are somatotopically organized [69]. Additionally, they are modulated by the intensity of the noxious stimuli, making these neurons particularly well-suited to code the sensory-discriminative aspects of pain [449]. Despite this anatomical evidence, the role of S1 in pain processing has been controversial. For example, human brain imaging studies have not consistently reported brain activations in S1 in response to
nociceptive stimuli [71,222]. Importantly, more recent evidence suggests that this apparent lack of S1 activation may be due to a number of other factors including difficulty detecting small focal S1 activations, which can be degraded by variations in sulcal anatomy when averaging across subjects in imaging studies, the modulation of S1 activity by cognitive factors such as attention, and the mixture of excitatory and inhibitory effects of nociceptive input to S1 [71]. Taken together, the vast majority of evidence supports a role for the S1 cortex in the sensory-discriminative aspects of pain.

The S2 cortex is located in the superior aspect of the lateral fissure where it makes up a large part of the parietal operculum [449]. S2 receives direct nociceptive input from the VPI nucleus of the thalamus, and it contains multiple somatotopic representations of the body suggesting that it may contain different subregions [165,424]. While the majority of neurons in S2 have contralateral receptive fields, many have bilateral receptive fields, fitting with neuroimaging and electrophysiological studies that have reported bilateral responses in S2 to a unilateral stimulus [449]. While S2 is often studied in the context of tactile representation, there is evidence showing that S2 receives nociceptive input and and therefore likely has a role in pain [16,262,449]. Specifically, S2 is thought to contribute to the sensory-discriminative aspects of pain perception. In a recent study by Lockwood and colleagues [271], it was shown that transcranial magnetic stimulation (TMS) delivered to S2 affected participants’ abilities to judge the pain intensity of a nociceptive laser stimulus delivered to the contralateral dorsal aspect of the hand. Importantly, more errors were made in the intensity discrimination task when TMS was applied to S2 relative to SI, suggesting a different role for these structures [271]. Additionally,
S2 has been implicated in cognitive-evaluative aspects of pain perception such as recognition, learning, and memory of painful events [384].

Another cortical area that receives direct nociceptive input from the thalamus is the insula, or IC. It is located deep in the lateral fissure and grossly divided into three short gyri anteriorly and two long gyri posteriorly [170]. The boundaries of the insula are the superior limiting sulcus superiorly, and the inferior limiting sulcus inferiorly [35]. The primate insular cortex can also be divided into three cytoarchitectural subregions based on the granularity in cortical layer IV, which is defined by the presence of small densely packed neurons [35,304]. There is a transition from granular cortex in the dorsal and posterior insula, to agranular cortex in the anterior and ventral insula. In between these regions, there is a large zone of dysgranular cortex [35]. The IC is involved in several functions including taste, somatosensation, and visceral sensation, and also participates in several autonomic, motor, and limbic functions, among others [20,170]. Given its many roles and vast connectivity, it is frequently described as being an integration site and for being crucial for interoception, or the subjective awareness of oneself based on the integration of these sensations/functions [100,170]. Although the IC is frequently described as having a role in affective-motivational components of pain (as mentioned above), there is evidence to suggest that there is a dual role for the insula in both the sensory-discriminative and affective-motivational dimensions of pain [61]. For example, the insula has been shown to encode both the intensity and laterality of thermal stimuli [45,62,94,104], in addition to participating in the affective dimensions of pain [61,413]. Therefore, its classification as belonging to either the medial or lateral pain systems is likely an over-simplification [446]. Afferent nociceptive input to S2 and the posterior insula is thought to be relayed rostrally to the anterior insula [422], where it
has reciprocal connections with various limbic structures including the amygdala and the cingulate cortex, which make it well-suited to participate in the affective dimensions of pain [20,170,305]. Indeed, it has been suggested that the insula can be functionally divided such that the anterior division is primarily associated with cognitive-affective processing while the posterior division is involved with sensory-discrimination [103,160,468]. In one functional MRI study, activation in the anterior insula was greater when participants had to rate their subjective intensity of the pain, in line with this region being involved in the cognitive evaluation of pain [245]. Much of the speculation for the anterior insula’s role in the affective component of pain comes from its close association with the ACC, a structure highly implicated in the evaluation of pain unpleasantness and emotion [103,357], but the anterior insula is also known to have a role in salience, pain intensity and anticipation, and negative emotions such as anxiety [468]. Some of these roles of the anterior insula may be disentangled by further subdividing it into ventral and dorsal components. The ventral anterior insula (vAI) is highly interconnected with multiple limbic and paralimbic regions including the pregenual ACC, periamygdaloid cortex, and the orbitofrontal cortex [305]. Thus it serves as a hub for the integration of limbic information, with the right vAI in particular participating in intense affective experiences [444]. On the other hand, stronger intrinsic connectivity of the dorsal anterior insula network is associated with better performance on tasks involving attention and processing speed [444]. The posterior insula receives direct nociceptive input from the VMpo thalamus in a somatotopic arrangement (face anterior, food posterior) that is orthogonal to the arrangement of S2 (face lateral, foot medial), making it suitable to play a role in the sensory-discriminative aspects of pain [335,449]. In one study, stimulation of the upper posterior part of the insular cortex elicited painful sensations in temporal lobe epilepsy patients undergoing depth stereotactic recording for pre-surgical
evaluation [335]. In this study, most of the insular sites that elicited painful sensations were in the right hemisphere and were associated with a strong affective component. This, alongside other studies that have reported a preferential increase in cerebral blood flow to the right ACC during nitroglycerin-induced cluster headache have led some to hypothesize that there may be a right-lateralized representation of pain in the brain, particularly for cognitive-affective aspects [206,335].

The cingulate cortex is a cortical region located just superior to the corpus callosum. It can be divided into anterior (ACC), mid (MCC), and posterior (PCC) regions with the ACC being further subdivided into the subgenual ACC (sgACC) and the pregenual ACC (pgACC), the MCC into the anterior MCC (aMCC) and posterior MCC (pMCC), and the PCC into dorsal PCC (dPCC) and ventral PCC (vPCC) based on regional microanatomy, connectivity, and physiological differences [461]. While this newer nomenclature has been widely adopted, classically, the cingulate was divided only into the ACC and PCC [461]. As such, some earlier studies used the term ACC to describe what is now classified as MCC. The cingulate cortex has been implicated in numerous functions spanning the cognitive, affective, and motor domains, its precise role in pain perception been difficult to pinpoint. The change in nomenclature to include the MCC has further complicated meta-analyses aimed at determining the function of cingulate subregions. It remains clear, however, that subregions of the ACC, and the aMCC play particularly important roles in pain [399,463]. Nociceptive input to the ACC from the MD and Pf thalamocortical projections has been established in monkeys [462] and confirmed in humans using both subdural and intradural recordings [212,263]. These nociceptive neurons have large, or even whole-body receptive fields and are therefore unlikely contributors to the sensory-
discriminative aspects of pain [408,479]. Fitting this concept, the medial pain and limbic systems converge in the cingulate gyrus [460], with evidence suggesting a particular role for the pgACC in pain unpleasantness. Indeed, one neuroimaging study reported that there was greater activation in the pgACC when patients were asked to assess the unpleasantness of a laser-evoked heat stimulus, compared to when they assessed the location of the stimulus, which activated MCC regions [252]. There is also evidence for a role of the pgACC in salience, and the regulation of emotional information, in general [124]. With regard to the aMCC, a recent neuroimaging meta-analysis reported that negative affect, pain, and cognitive control activate a common region within the aMCC [399]. Anatomically, this region of the cingulate cortex has reciprocal connections with frontoparietal regions involved in cognitive control and attention, and with insular regions implicated in pain and affect [399]. While the pgACC and aMCC are frequently discussed for their role in pain, more posterior divisions of the cingulate cortex, namely the pMCC and dPCC have also been implicated, however, these sites are not nociceptive-specific, and are also activated with innocuous electrical stimulation and movement-associated activity [43,210,323,463].

2.2.4.2.3 Other Supraspinal Regions Involved in Pain

In addition to the cortical regions described above, there is now a vast literature describing other brain regions that are involved in the perception of pain. These regions include the prefrontal (PFC) and orbitofrontal (OFC) cortices of the frontal lobe, the parietal cortex, cerebellum, basal ganglia, motor cortices, and amygdala [124,347]. Much of our knowledge about the pain-related activity of these brain areas comes from studies using non-invasive neuroimaging techniques and
anatomical studies describing the connectivity between these structures and those that receive direct nociceptive input from the thalamus.

In general, the role of the PFC has been attributed to cognitive functions such as those involving attention and executive processes [347]. Together with the posterior parietal cortex, another brain region known for its role in attention, these areas likely contribute to the localization and encoding of noxious stimuli [346]. While there is no evidence for direct nociceptive input to the PFC from the thalamus, the PFC has many connections with the cingulate cortex, and may receive nociceptive input via this route [449]. It is also frequently discussed for its role in descending modulation, likely via expectation or placebo [46,275]. While activation of the PFC has been reported in more than half of the studies examining acute pain in healthy individuals, it is reported even more frequently in studies of chronic pain, suggesting frontal lobe abnormalities may occur with chronic pain [15]. Another frontal region implicated in pain is the OFC; a region involved in the control and regulation of emotions [404], making it a likely contributor to the affective aspects of pain. Additionally, the OFC has many reciprocal connections with the amygdala, a limbic structure commonly discussed for its role in affective states (e.g., fear and anxiety) [127,297]. The amygdala contains nociceptive neurons [370] and is frequently reported as being active in both studies of experimental pain in healthy individuals and in chronic pain patients [409]. Additionally, there is evidence that the amygdala plays both facilitatory and inhibitory roles in pain modulation that are dependent on environmental conditions and affective states [320]. In contrast to the frontal lobe regions, there is some evidence to suggest that the posterior parietal cortex, just adjacent to SI, receives nociceptive input in monkeys [141]. In this study, the parietal neurons had bilateral receptive fields in the orofacial region and responded to
the visual presentation of noxious stimuli towards the face. This fits with the role of the posterior parietal cortex in the dorsal attention system for visuospatial processing, or as more currently described, for spatial information for action [184]. Other brain regions including the basal ganglia, cerebellum, and motor (e.g., primary and supplementary motor) cortices are thought to be involved in different aspects of motor responses to pain such as withdrawal reactions or restricting motor movements [280,340,347]. In recent years, evidence also suggests that these structures may play a role in sensory, cognitive, and/or emotional aspects of pain [315,423].

2.2.4.3 Cerebral White Matter Tracts

The supraspinal regions involved in pain perception communicate via several cerebral fibre tracts. These tracts are composed primarily of myelinated axons and associated neuroglia, and run in different directions. In general these fibres can be categorized as projection fibres, which connect cortical regions with deeper brain areas or the spinal cord, association fibres, which connect regions within a cerebral hemisphere, or commissural fibres, which connect regions between hemispheres. In this section, some of the major white matter tracts involved in the multi-dimensional experience of pain, its modulation, and motor function (Fig. 2-8) will be discussed.
**Figure 2-8:** A schematic illustration of some major white matter tracts connecting brain regions involved in the multi-dimensional experience of pain, its modulation (thalamo-cortical fibres, cingulum, uncinate fasciculus), and motor function (cortico-thalamic fibres, external/extreme capsules). The corpus callosum can also participate in these functions since it connects many homotopic brain regions interhemispherically. Figure adapted from [387] (with permission from the Copyright Clearance Center, License ID: 3524910950581).

### 2.2.4.3.1 Projection Fibres

Projection fibres consist of axons that connect the cortex with lower parts of the brain and spinal cord. Therefore, these fibres can either be afferent (corticipetal), relaying information to the cortex from lower structures, or efferent (corticofugal), which originate in the cortex and descend to deeper structures including the brainstem, diencephalon, basal ganglia, and spinal cord [63]. These fibres form the internal capsule, which in transverse section is a V-shaped structure with anterior limb fibres rostrally, the genu medially, and the posterior limb fibres caudally. The anterior and posterior limbs of the internal capsule consist of many fibres that are collectively
called thalamic radiations and include both thalamocortical and corticothalamic fibres. These fibres make reciprocal connections between the different lobes of the brain (frontal, parietal, temporal, and occipital) and specific thalamic nuclei [63]. As previously discussed, the VP thalamus in one of the major relays for sensory, including nociceptive, information from the face and body. It relays information to the cortex via the caudal portion of the posterior limb of the internal capsule in a somatotopic manner, with fibres originating from the head region being most rostral, upper body fibres being medial, and lower body fibres being the most caudal. Internal capsule fibres that are not thalamic radiations are mainly efferent tracts such as the corticospinal, corticobulbar, and corticoreticular tracts [63]. Efferent projections from the motor cortices descend in the internal capsule, with corticobulbar fibres located in the genu of the corpus callosum, and corticospinal fibres located in the adjacent rostral part of the posterior limb. As with the sensory fibres, there is somatotopic organization of these efferent fibres with those innervating head muscles being the most rostral (closest to the genu) [63]. Superior to the level of the thalamus, the fibres of the internal capsule fan out and form the corona radiata. Additionally, some of the fibres from the external capsule, which is located lateral to the lentiform and medial to the claustrum, initially travel with association fibres then branch off and descend to parts of the basal ganglia [381].

2.2.4.3.2 Association Fibres

Association fibres are confined to one hemisphere of the brain and classified as either short or long. Short association fibres, also known as U-fibres, are located superficially, just below layer VI of the cortex [381] and interconnect adjacent gyri or subregions within a gyrus [63]. Long association fibres interconnect brain regions, typically association regions, located within
different lobes of the brain [63]. The superior longitudinal fasciculus (SLF) is the largest of the long association fibres and is situated laterally in the hemisphere, immediately beneath the frontal, parietal, and temporal opercula [63]. This long C-shaped bundle arches around the lateral fissure and can be divided into four separate components that contain bi-directional fibres: SLF I, II, III, and the arcuate fasciculus [345]. Briefly, SLF I is the dorsal component and connects regions of the parietal and frontal lobes. SLF II is the major component, connecting the caudal-inferior parietal lobe (corresponding to the angular gyrus) to the dorsolateral frontal region [288]. SLF III is the ventral component, connecting the supramarginal gyrus and ventral premotor and prefrontal regions [288]. These regions connected by SLF I-III play important roles in attention, working memory, and regulating motor behaviours. The fourth component of the SLF, the arcuate fasciculus, runs contiguously with SLF II fibres connecting Broca’s area of the frontal lobe to Wernicke’s area of the tempo-parietal lobe [288]; crucial structures for the comprehension and production of language/speech.

Another long association bundle is the cingulum, which lies immediately beneath the cingulate cortex, on the medial aspect of the hemisphere. Like the SLF, the cingulum is a C-shaped bundle and is considered the association tract of the limbic lobe [63]. Cingulum fibres associated with subcallosal gyrus of the frontal lobe have projections to the parahippocampus gyrus and uncus of the temporal lobe. The cingulum has major roles in both emotion and memory processes.

The uncinate fasciculus, part of the inferior occipitofrontal fasciculus, interconnects the orbitofrontal cortices with the anterior aspect of the temporal lobe and insula [63]. This hook-
shaped bundle is classically considered part of the limbic system, and participates in emotional processing, memory, and social cognition among other functions [295].

The extreme capsule is located lateral to the claustrum and medial to the insular cortex caudally, and orbitofrontal cortex rostrally [381]. It connects several brain regions including the superior temporal gyrus, rostral insula, cingulate, and frontal cortices [289]. While the precise role of the extreme capsule remains elusive, its vast connections with the insular cortex, which is known to be involved in different aspects of pain processing, make it of interest in this thesis.

2.2.4.3.3 Commissural Fibres

While there are several smaller commissural bundles (e.g., posterior commissure, habenular commissure), the largest and most widely affected in neurological disorders is the corpus callosum. The corpus callosum interconnects the cerebral hemispheres, allowing for the rapid transfer and integration of sensory and motor information [63]. The nature of these connections is not entirely understood [52]. In general, the corpus callosum is topographically organized with anterior callosal fibres connecting frontal regions, including motor, anterior cingulate, and prefrontal cortices, and posterior fibres connecting parietal, temporal, and occipital brain areas, including somatosensory regions [159]. In transverse section, the enlarged anterior and posterior ends of the corpus callosum (the genu and splenium, respectively) can be visualized [63]. The genu gives rise to the forceps minor, connecting the orbital and prefrontal cortices [63]. The larger posterior end of the corpus callosum, the splenium, is composed of fibres of the forceps major connecting homotopic regions of the parietal, occipital, and temporal lobes [63]. The
fibres between the genu and splenium constitute the body of the corpus callosum and also interconnect regions of the frontal and parietal lobes.

### 2.2.5 Trigeminal Motor Pathways

In addition to the trigeminal pathways that convey sensory information, the trigeminal system also consists of pathways involved in masticatory movements [466]. In general, mastication, or chewing, involves coordination of the jaw, tongue, and some facial muscles [279]. As previously mentioned, the V3 branch of the trigeminal nerve contains a motor root that innervates the muscles of mastication (i.e., the medial and lateral pterygoid, masseter, and temporalis muscles), which allow the jaw to open and close. The cell bodies of these trigeminal efferent fibres are located in the trigeminal motor nucleus of the brainstem, in close proximity to the mesencephalic nucleus, which receives proprioceptive information from jaw muscles. Importantly, sensory feedback plays a significant role in certain motor functions such as optimizing bite force [214,257]. The motor nucleus receives inputs from a number of trigeminal sensory nuclei, including the mesencephalic nucleus, main sensory nucleus, the subnucleus oralis, in addition to local interneurons within the motor nucleus, itself [314]. With the exception of the projections from the mesencephalic nucleus, all projections to the motor nucleus from the trigeminal sensory nuclei are bilateral [314].

In the early 1970s, some critical experiments on decerebrate animals showed that mastication could be generated by the brainstem alone, and therefore, the concept of a masticatory central pattern generator was developed [131,314,466]. Although mastication is considered a
stereotyped movement, variability still occurs between cycles of chewing and this can likely be explained as modifications based on feedback from other subcortical and cortical regions [314]. Indeed, many subcortical regions project to the trigeminal motor complex and include the amygdala, hypothalamus, PAG, cerebellum, and basal ganglia [197,247]. Representations of the face including the jaw and tongue are found on cortical areas including S1 and (primary motor cortex) M1. In 1886, Ferrier first showed that stimulation of a cortical region called the cortical masticatory area (CMA) could elicit jaw movements [161]. In humans and primates, the CMA overlaps with lateral aspect of M1 [314]. While intracortical microstimulation of some cortical areas including the M1, S1, premotor cortex, insula, and ACC can elicit orofacial movements [314,318], only repetitive stimulation of the CMA results in rhythmic jaw movements. This stimulation results in the bilateral activation of jaw muscles via corticobulbar projections [327], such that there is a synchronous and coordinated activation of the jaw muscles on both sides of the face [151]. The cortical projections may directly or indirectly synapse on trigeminal motor neurons. It has been suggested that there are direct and indirect pathways to the trigeminal motoneurons [196]. The direct pathway involves excitatory inputs to the vicinity of the trigeminal motor nuclei (mostly the supratrigeminal premotor region), while the indirect pathway first synapses on the basal ganglia before reaching the premotor region of the trigeminal nuclei [314]. Since the majority of efferents from the basal ganglia use GABA as a neurotransmitter [481], the indirect pathway may be inhibitory.

Given the dual role of the trigeminal system in both sensory and motor functions, theories have been proposed to describe how sensory, and in particular pain sensations, affect jaw muscle activity have been proposed [340]. Importantly, pain and limitations in movement are common
symptoms in many facial pain disorders. One theory called the Integrated Pain Adaptation Model suggests that pain leads to alterations in muscular activity aimed at limiting movements of an affected muscle by redistributing function and load. This would, in the short term, protect the system from further injury and to support healing. However, prolonged muscular function plasticity can lead to more pain and further peripheral damage [340]. This theory has important clinical implications for studying chronic facial pain disorders, which often focus on the sensory/nociceptive aspects of the pathophysiology. It highlights the necessity to examine both sensory and motor, in addition to affective, cognitive, motivational, and emotional factors, among others, when trying to understand the overall pain disorder.

2.2.6 Antinociception and Descending Modulation

Descending pathways constitute a major mechanism in the control of nociceptive transmission, and it is now well established that both inhibitory and facilitatory mechanisms play a role [162,179,363]. In this section, the anatomy and mechanisms of the pain modulation system (Fig. 2-9) will be reviewed and the role of descending modulation in persistent pain will be discussed.
2.2.6.1 Anatomy and Mechanisms of Descending Modulation

Our understanding of the role of certain brainstem structures in pain modulation stems from the work of Reynolds who reported that abdominal surgery in a rat in the absence of anesthesia was feasible with focal electrical stimulation of the midbrain periaqueductal gray (PAG) [365]. In the years following this discovery, others demonstrated the existence of reversible binding sites for opioids and importantly, endogenous opioid peptides [211,343], which were important in establishing the concept of an endogenous mechanism for the modulation of pain [179]. The
PAG and its descending projections to the spinal cord dorsal horn were soon recognized as critical structures for pain inhibition, and others demonstrated that the microinjection of opioids into the PAG replicated the inhibitory effects that were achieved with electrical stimulation [179,478].

The PAG is a diverse structure involved in many functions other than pain modulation including fear, anxiety, and cardiovascular function [267]. However, its connectivity to other brainstem structures, specifically the rostroventral medulla (RVM) (including the medial nucleus raphe magnus) has been shown to be crucial in pain inhibition specifically [179]. For example, stimulus-induced inhibition from the PAG is blocked when the entire RVM is inactivated by local anesthetics [377]. As such, the RVM is considered to be the final common output for descending influences from the brain [351]. While many of these early studies examined the inhibitory influences of the PAG on nociceptive activity, it has since been clearly established that the PAG and RVM also exert descending facilitatory influences on spinal nociceptive processing, which may be crucial for maintaining chronic pain [67,351,363].

In the RVM, two types of neurons have been identified as pain modulatory neurons and are termed ON-cells, and OFF-cells [162]. A third type of neuron in the RVM has also been described, but these cells are neutral [351]. ON-cells are typically associated with the facilitation of nociceptive activity, and are characterized by a sudden increase in firing just prior to the initiation of a nocifensive response (e.g., tail or hind limb withdrawal). In contrast, OFF-cells are associated with the inhibition of nociceptive activity, and temporarily halt their firing just prior
to a nocifensive response [351,363]. These cells project to the spinal (or medullary) dorsal horn neurons where they exert their effects on the activity of the primary afferent neurons [30].

While these brainstem structures are significant contributors to pain modulation, it is also well-established that a number of other supraspinal sites in the brain, including regions of the prefrontal cortex, anterior cingulate cortex, insula, and amygdala, project to the PAG, and can in turn influence the activity of the PAG and RVM via cognitive and psychological factors such as attention, emotion, placebo, and anticipation [70,387,468]. Indeed, there is evidence to suggest that manipulating attentional states can alter the perceived intensity of pain sensations, while altering emotional states can alter the perceived unpleasantness of a pain stimulus [70]. This is an important point when considering chronic pain states, which are frequently associated with structural abnormalities in these brain regions [70].

### 2.2.6.2 Descending Modulation in Chronic Pains

In chronic pain disorders, it has been proposed that the descending pain modulatory system is altered such that there is either dysfunction in nociceptive inhibition, or enhancement in nociceptive facilitation [387]. Anatomical and pharmacological studies have suggested that the inhibitory and facilitatory pathways are distinct, and are likely both activated in conditions of acute pain [351]. However, in cases of persistent nociception, neuroplasticity may occur in the PAG, RVM, or from other regions in the descending system that produce a net facilitatory influence that contributes to chronic pain [351]. Previous work has shown that the behavioural expression of neuropathic pain depends on descending facilitatory systems arising from the
RVM, which may act to enhance the diminished afferent input from the injured fibres [138,162,351]. Indeed, animal studies using spinal transection manipulations have shown an elimination of the tactile hypersensitivity that is usually induced by nerve injury, suggesting that there is a significant contribution of descending modulatory regions in the expression of neuropathic pain [44,429]. Additionally, there is evidence that this enhanced facilitation is critical in the maintenance, but not necessarily the initiation of neuropathic pain [67]. For example, in an animal model of spinal nerve ligation (SNL), lesions to the RVM cells expressing μ-opioid receptors did not prevent the onset of SNL-induced tactile and thermal hypersensitivity, but it was associated with a reversal of these signs beginning four days post-SNL [67]. Thus, while it is important to have an understanding of the anatomy and physiology of sensory afferents and pathways in pain and chronic pains, it is also crucial to consider the influences of the descending modulatory systems.

2.3 Trigeminal Neuralgia

Lesions or disorders of the trigeminal system can result in chronic pain. One chronic facial pain disorder, trigeminal neuralgia (TN), has been described as one of the most severe pains that mankind suffers [469]. TN is a unique neuropathic pain characterized by highly intense electric shock-like attacks of unilateral facial pain. Patients with TN are usually pain-free between attacks, but at any time, a normally innocuous stimulus or movement can elicit pain [328]. TN pain is paroxysmal and patients are typically pain-free between attacks. Although pain can occur spontaneously, it is frequently triggered by normally non-painful stimuli and movements of the face such as light touch, eating/chewing, shaving and draughts of wind [78,88,277]. As such, TN
patients may be unable to speak, eat, or be in constant fear of eliciting another pain attack [89]. This can be highly distressing for patients, negatively impacting their quality of life, psychosocial status [89,441], and leaving them desperate to seek pharmacological and/or surgical treatments to achieve some degree of pain relief.

The quality of TN pain is highly intense, with patients using terms such terrifying, blinding, and torturing from the McGill Pain Questionnaire to describe their pain [302]. While TN pain may initially occur in bouts with relatively long periods of remission between them, the pain attacks tend to occur more frequently and be more sustained over time [66,171]. TN pain is usually unilateral, affecting only one side of the face. Although controversial, there have been some reports of TN occurring more frequently on the right side compared to the left side [364,440] (attributed to smaller foramen rotundum and ovale sizes on right side, through which the maxillary and mandibular branches of the trigeminal nerve pass through) [400], but not all studies have found this right-sided pain predominance [230]. There does appear to be a consensus regarding which branches of the trigeminal nerve are most commonly affected. Historically, it was noted that TN pain most frequently involved the maxillary or mandibular regions of the face [95]. In an epidemiological study by Katusic and colleagues (1990), clinical details of TN patients were analyzed over a 40-year period [230]. They reported that the most frequently affected division of the trigeminal nerve was the maxillary branch (35% of patients), followed by the mandibular branch (29% of patients), with both of these branches being affected in 19% of TN patients. Similar results have since been reported with the majority of studies showing that the maxillary and mandibular branches are the most commonly affected [25,78,258].
TN is most commonly associated with neurovascular compression of the trigeminal nerve at the root entry zone [328]. Unlike other neuropathic pains, TN pain is not accompanied by major sensory loss, as neurologic examinations are usually normal [88]. Additionally, many patients respond well to treatments for TN, with a large proportion of patients having good or complete pain relief following treatment [328]. Studies of TN have increased substantially over the past century but there remains a poor understanding of its underlying pathophysiology, how it impacts the brain, and the effects of treatment.

2.3.1 Historical Perspectives

There have been several descriptions of craniofacial pain throughout ancient history, but a clear description of TN was documented only over the past few centuries. This section will recount some of these reports, and review some of the key contributions to our current understanding of TN as its own clinical entity. Some early theories of TN etiology and treatment attempts will be briefly discussed.

The first clear documentation of TN patient was in 1671. It described the suffering of Johannes Laurentis Bausch, a well-known German physician and founder of the Imperial Leopoldian Academy of Natural Sciences [153]. Bausch had severe pain for 4 years, which was described as being sharp (lightning like), shooting, and in the distribution of the right maxillary region of his
face [95]. Over time, the pain became so severe that he was unable to speak and eat, which led to his eventual death due to malnutrition [95,140].

The first full description of TN by a physician and an account of its treatment were by John Locke [95,153]. In 1677, Locke wrote a series of letters to Dr. John Mapletoft describing the symptoms of his patient, the Countess of Northumberland, wife of the Ambassador to France [339]. He described her as having fits of “violent and exquisite torment” that could be triggered by opening her mouth or touching her gums, and could stop suddenly with varying time intervals between them. He also noted that the fits would make her twitch and draw her mouth to the right side. As the Countess previously had two teeth removed without any pain relief, Locke attempted a different approach, which included a thorough purging of the patient, which typically involved emptying the bowels [95,153].

TN as an explicit clinical entity was not established until 1756, and is credited to the French surgeon, Nicolaus André [95,153,339]. André coined the term “tic doloureux” to describe his patients because of the facial wincing and grimacing that accompanied their intense pain (tic = spasm, doloureux = painful). He believed that “vicious nervous liquids” irritated the affected nerve and resulted in shocks of pain. He also conceptualized the disorder in terms of convulsions [95], which was foundational for Trousseau’s central theories of TN etiology in the 1850s. Given that the pain most frequently presented in the maxillary region of his patients’ faces, his treatment approach involved the application of caustic substances to the infraorbital nerve, a terminal branch of the maxillary nerve, until it was destroyed [153]. In the 1750-60s, surgical
collaborators of André, including Maréchal, surgeon to Louis XIV, also made attempts to cut the infraorbital nerve to relieve TN pain, but these attempts were not successful and/or had to be aborted due to extensive hemorrhaging [95].

In 1773, the English physician, John Fothergill further characterized TN by describing that it predominantly affected older individuals and women [95]. Some of his other observations were that the pain came on suddenly and although short-lived, returned at irregular intervals. He also discussed that common triggers of the pain were eating, talking, and “the gentlest touch of a hand or a handkerchief” to the face [95]. Since two of his patients also had breast tumours, he suggested that TN might be related to a type of cancer [95]. As a treatment option, Fothergill suggested patients ingest Peruvian bark (bark from the cinchona tree) that contained quinine, as it was known to have analgesic effects [95].

In 1804, Fothergill’s great nephew, Samuel Fothergill, implicated lesions to the trigeminal nerve as being involved in TN, albeit in conjunction with the facial nerve. With their contributions to the understanding of this disorder, many started to refer to this pain disorder as Fothergill’s disease [95,153]. Implicating the trigeminal nerve in this disorder was an extension of work done by Thouret in 1782, who considered TN to be an affection of nerves in the face, and by Chaussier in 1802, who classified different forms of TN according to the divisions of the face that were affected [153].
In the 1820s, Charles Bell differentiated the functions of the trigeminal and facial nerves and localized TN pain solely to the trigeminal nerve [153]. This led to the eventual name change from tic douloureux and Fothergill’s disease to trigeminal neuralgia. At this time, treatment options included the administration of drugs such as stilbamadine (a systemic fungicide), which produced only short-term pain relief and were accompanied by a series of side effects including bilateral facial numbness, burning, paresthesias, and irreversible liver and renal damage [95]. Over the years new treatment approaches were developed which reflected both the advances made in technology and our understanding of TN pathophysiology (discussed in detail later in this chapter). While today TN is a well-known neuropathic pain, it can still be difficult to diagnose and treatments can be highly expensive, with patients often requiring multiple treatments.

2.3.2 Epidemiology and Cost

In one study conducted in the United States, it was reported that TN has a crude incidence rate of approximately 4.3 cases per 100,000 individuals [230], with the annual incidence being higher in women (5.9 cases per 100,000 women) compared to men (3.4 cases per 100,000 men) [230]. This translates to approximately 15,000 new TN patients being diagnosed in the United States alone, each year [349]. With greater awareness about TN and better diagnostic criteria, more recent studies have reported higher rates and suggest that the earlier reports underestimated its incidence [193,489].
TN typically affects more women than men and individuals over the age of 50 [78,88,442]. However, it can also affect younger adults or even children [277]. While TN typically occurs spontaneously, there is a family history for approximately 5% of patients [78,442], suggesting that TN may also have a genetic component [419].

With regard to treatments for TN, a prospective study by Pollock and Ecker (2005) [349] examined the cost-effectiveness of trigeminal neuralgia surgery, and determined that the cost (in American dollars) per pain-free year was $6,342, $8,174, and $8,269 for glycerol rhizotomy, microvascular decompression, and stereotactic radiosurgery, respectively. In addition to costs associated with treatment, TN pain directly impacts medical resource utilization. In a cross-sectional study aimed at examining the effects of TN pain on patient function, treatment patterns, and health resource utilization in six European countries [441], it was reported that 78% of patients visited their physician at least once in the preceding four weeks, and 45% visited two or more times. TN pain also had an impact on employment for 34% of patients, which included a decrease in scheduled work time, disability, or unemployment/early retirement [441]. Patients that remained employed reported missing on average four days of work in the prior month as a result of their TN pain [441]. It is clear that TN is a growing concern with a high degree of patient and economic burden.

2.3.3 Classification and Clinical Features

While some symptoms of TN can be slightly heterogeneous and/or change over time, there are some key features used to diagnose TN that make it unique from other neuropathic pains. In this
section, the common definitions of TN will be provided, and its classification and diagnostic criteria will be discussed.

2.3.3.1 Definition of Trigeminal Neuralgia

Current definitions of TN highlight many of the prominent features observed by medical practitioners centuries earlier. The International Association for the Study of Pain (IASP) defines TN as, “Sudden, usually unilateral, severe brief stabbing recurrent pains in the distribution of one or more branches of the Vth cranial nerve” [303]. The definition by the International Headache Society (IHS) is similar, but also acknowledges triggers and further details about the quality of the pain: “A disorder characterized by recurrent unilateral brief electric shock-like pains, abrupt in onset and termination, limited to the distribution of one or more divisions of the trigeminal nerve and triggered by innocuous stimuli” [1]. Both of these definitions describe classical (also referred to as idiopathic, typical, or primary TN). However, TN can also occur secondary to other disease processes, or be atypical in its symptomology. Having clear diagnostic criteria is critical to avoid misdiagnosis and unnecessary treatments. For example, since TN pain frequently occurs intraorally, it has been mistakenly classified as dental pain and healthy teeth have been unnecessarily extracted [88,95,364]. In the following section, the classification of TN as outlined by the IHS will be discussed in detail. These were the inclusion criteria used for patient selection in this thesis.
2.3.3.2 IHS Classification of Trigeminal Neuralgia

The IHS has extensively outlined the classification and diagnostic criteria for trigeminal neuralgia, alongside other headache disorders and cranial neuropathies and neuralgias in the International Classification of Headache Disorders-3 (ICHD-3) [165]. TN is described under Part 3, section 13: Painful cranial neuropathies and other facial pains. In addition to the definition of TN described above, the ICHD-3 notes that, TN can occur without any apparent cause or it can occur secondarily to another diagnosed disorder. Additionally, TN can be purely paroxysmal or be accompanied by persistent facial pain of moderate intensity.

2.3.3.2.1 Classical Trigeminal Neuralgia, Purely Paroxysmal

Classical TN, previously known as tic douloureux, occurs without any apparent cause other than neurovascular compression; hence it is also being referred to as idiopathic TN. The ICHD-3 outlines five diagnostic criteria required to achieve this diagnosis [165]:

A. At least three attacks of unilateral facial pain fulfilling criteria B and C

B. Occurring in one or more divisions of the trigeminal nerve, with no radiation beyond the trigeminal distribution

C. Pain has at least three of the following four characteristics:

1. recurring in paroxysmal attacks lasting from a fraction of a second to 2 minutes

2. severe intensity

3. electric shock-like, shooting, stabbing or sharp in quality

4. precipitated by innocuous stimuli to the affected side of the face

D. No clinically evident neurological deficit
E. Not better accounted for by another ICHD-3 diagnosis.

One of the most important features of this diagnosis is that the pain comes in attacks or paroxysms, and patients are asymptomatic between these paroxysms. The ICHD-3 does note that over time, the duration of the pain attacks may occur more frequently and be more sustained. An additional note is that some of the pain attacks may be or appear to be spontaneous, but to be diagnosed with classical TN, at least three attacks must by precipitated by innocuous stimuli. Lastly, if hypoesthesia or hypoalgesia are evident on clinical examination, axonal damage is likely and a diagnosis of trigeminal neuropathy may be more suitable. The ICHD-3 differentiates neuropathy from neuralgia by defining neuropathy as, “A disturbance of function or pathological change in a nerve or nerves…” and neuralgia as, “Pain in the distribution of a nerve or nerves” [165]. Therefore, while trigeminal neuropathies are also included in Part 3, section 13 of the ICHD-3 and can also result in pain in the distribution of the branches of the trigeminal nerve, it is associated with a structural lesion or systemic disease (e.g., acute Herpes zoster, trauma, multiple sclerosis, or a space-occupying lesion), neural damage/signs of trigeminal nerve dysfunction (e.g., hypoesthesia or sensory loss), and pain that is variable in quality and intensity. The ICHD-3 also uses the term symptomatic TN to refer to TN caused by structural lesions other than NVC.

2.3.3.2.2 Classical Trigeminal Neuralgia with Concomitant Persistent Facial Pain

Classical TN accompanied by background facial pain between attacks has previously been referred to as atypical TN, or TN type 2 [156]. While these patients also have recurrent attacks of
unilateral facial pain, they also have persistent facial pain of moderate intensity in their affected trigeminal distributions. For these individuals, processes such as central sensitization may account for some of their persistent pain and they typically respond poorly to treatment including neurosurgical interventions [165]. As a result, any TN patients with concomitant persistent facial pain were not included as participants in this thesis.

2.3.3.3 New Classification Scheme for TN and Related Facial Pains

Another classification system for TN and related pains has recently been proposed [156]. In this scheme, there are seven categories of TN and related facial pain syndromes as follows:

1. TN1 refers to classical TN as described by the IHS above.

2. TN2 describes facial pain that is aching, throbbing, or burning for more than 50% of the time and is constant in nature. There may be sharp paroxysmal pain in addition to this constant pain. Eller and colleagues [156] propose that TN1 transitions to TN2 over time, if not treated.

3. Neuropathic TN refers to pain in the distribution of the trigeminal nerve that occurs as a result of injury to the trigeminal system. Neuropathic TN includes patients that have had an unintentional injury to the trigeminal system (e.g., following facial trauma or oral surgery).

4. Trigeminal deafferentation pain also occurs with injury to the trigeminal system. However, these patients had surgery to intentionally injure the system (e.g., trigeminal rhizotomy or tractotomy).
5. Symptomatic TN describes patients that have TN secondary to MS.

6. Postherpetic TN refers to trigeminal pain resulting from an outbreak of facial herpes zoster. Allodynia and burning dysesthesia are usually accompanying symptoms.

7. Atypical facial pain describes patients that report facial pain in the context of a somatoform pain disorder. The pain is often bilateral, and spreads outside of the trigeminal distribution. It may also be accompanied by pain in other parts of the body.

2.3.4 TN Etiology and Pathogenesis

While our understanding of TN has drastically grown over the past few centuries, the etiology and pathophysiological mechanisms underlying TN are not well understood. In some cases, TN etiology is known because the pain is secondary to another disorder. However, in classical/idiopathic TN (the focus of this thesis), theories of etiology can be classified as either central or peripheral, with the most common peripheral theory involving neurovascular compression of the trigeminal nerve [406]. In the following section, the origin of secondary TN and theories of idiopathic TN origin will be discussed. A review of the literature examining TN pathogenesis and pathophysiological mechanisms will be provided.

2.3.4.1 Secondary TN- Known Etiology

In some cases, TN can occur secondarily to a known lesion or disease. In these cases, TN is classified as secondary or symptomatic. These patients frequently present with more atypical features such as persistent pain between paroxysms, and/or other sensory or motor deficits [328].
Approximately 2-5% of patients who have multiple sclerosis (MS) develop secondary TN [88]. These patients are typically younger than patients with classical TN and sometimes have bilateral pain and/or other neurological signs such as internuclear ophthalmoplegia [205]. The source of pain in TN due to MS is frequently a plaque of demyelination in the region of the trigeminal REZ or brainstem white matter [277].

TN can also occur secondary to benign cranial base tumours including vestibular schwannomas, meningiomas, and trigeminal schwannomas [86,209,277,350]. These tumours either arise from, or compress trigeminal nerve fibres, and have been reported to be the source of pain in approximately 1-6% of patients with facial pain [350]. A study of TN in the Mayo Clinic study found that pain occurred secondarily to tumour growth in approximately 9% of patients [86]. Like the TN patients with MS, patients with TN secondary to tumour growth tend to be younger, have persistent pain, and frequently develop neurologic deficits as the tumour grows [86]. Other less common sources of secondary TN include infiltrative disorders such as carcinomatous deposits within the trigeminal nerve or ganglion [92], fibrous bands across the petrous ridge, and epidermoid cysts [171,277]. Patients with TN secondary due a known lesion or disorder were not included in this thesis.

2.3.4.2 Theories of Central Etiology

The prominent theory of TN pathogenesis currently points to a peripheral etiology. However, earlier theories (see section 2.3.1) considered TN to be a disorder of unknown etiology that was
likely central in origin. In the 1850s, Trousseau observed that the painful paroxysms experienced by TN patients resembled a convulsive seizure he termed “epileptiform neuralgia” [451]. He suggested that the pain attacks experienced by TN patients were due to paroxysmal activity in the trigeminal system, similar to cerebral paroxysmal discharges experienced by epilepsy patients [173]. In the 1950s, Wilson suggested that the “epileptiform” discharge proposed by Trousseau was the result of impaired inhibitory mechanisms that were not effective in stopping the paroxysmal activity. Adding to this, Kugelberg and Lindblom conducted experiments that examined the effects of different stimuli applied to the trigger zones of patients. While rapid displacement of a hair was sufficient in triggering an attack sometimes, they found that in the majority of cases, a temporal summation of impulses was required to build up an excitatory state to elicit a pain attack [251]. Along with others, they also noted that the attack was self-sustained once it started as it could continue even after the stimulus was removed. Also, the pain attack was larger in magnitude than the stimulus, and it was followed by a refractory period during which time another attack could not be triggered. Taken together, these observations were considered support for TN being a CNS disorder [185,251]. In line with these observations and Trousseau’s theory of epileptiforme neuralgia, clinicians started to treat TN with the antiepileptic medication, carbamazepine, which is still one of the most frequently prescribed medications for TN [193]. Although antiepileptic medications are typically effective in managing TN pain initially, it is important to note that their effect is not selective for the brain, but can also affect peripheral nerves by slowing conduction velocities in both motor and sensory peripheral fibres [112,445]. While TN may have a central component and can secondarily affect the brain, there are several lines of evidence to suggest that the origin of TN and its pathogenesis is peripheral [328].
2.3.4.3 Neurovascular Compression Theory of TN

The most prevalent theory of classical TN etiology involves compression of the trigeminal nerve at the root entry zone (REZ) by aberrant blood vessels [277,328]. Vascular contact with the trigeminal nerve was first described in 1929 by Dandy, who observed that patients with TN frequently had grooves or bends in their trigeminal nerves made by offending vessels [110]. While not entirely understood, several lines of evidence have led to the proposition that over time this sustained static or pulsatile vascular compression leads to focal points of damage on the trigeminal nerve, resulting in the pain attacks characteristic of TN (discussed in detail below in section 2.3.4.4) [328].

Neurovascular compression (NVC)-associated chronic pain has also been reported in disorders other than TN. For example, glossopharyngeal neuralgia, a rare disorder characterized by severe paroxysmal pain in the ear, back of the throat, base of the tongue, or beneath the angle of the jaw, is commonly attributed to NVC at the REZ of the glossopharyngeal nerve [84]. Like TN, the pain is typically unilateral and precipitated by events such as swallowing, coughing, talking, or yawning [84]. In some cases, NVC of the median nerve by the median artery can result in secondary carpal tunnel syndrome (CTS) [22]. CTS is characterized by paresthesias in the median nerve territory of the hand, including burning sensations, and accompanied by radiating pain of the forearm, elbow, or shoulder [81]. In cases of “idiopathic” CTS, inflammation of the median nerve due to compression from the flexor retinaculum and/or surrounding tendons can stimulate angiogenesis, which in turn, exacerbates the inflammation [81]. While these new
vessels do not necessarily compress the median nerve as in TN, angiogenesis may secondarily exacerbate CTS pain by increasing inflammation, nerve swelling, and sensitization of the median nerve [81]. Another example of pain induced by vascular compression occurs in some cases of chronic sciatic pain [40]. In these cases, the source of the lumbar pain, which radiated into the buttock and leg, was compression of the sciatic nerve by varicotic gluteal veins [40].

Some have argued that neurovascular contact may not sufficiently explain TN etiology as blood vessels are often seen in the vicinity of the trigeminal nerve on routine autopsy, or on MRI scans of asymptomatic patients without any history of the disorder [306,328]. Still, an overwhelming majority of patients with TN (80-90%) do have NVC at their trigeminal nerves ipsilateral to their side of pain [277,378]. Furthermore, NVC at the unaffected trigeminal nerve or in healthy individuals without TN (asymptomatic NVC), occurs less frequently and is less severe than at the affected trigeminal nerve in TN (symptomatic NVC) [12,13,287,378]. For example, an MRI study of NVC in TN [276], reported that NVC occurred on the unaffected trigeminal nerve in 55% of patients, but that none of the vascular contacts were severe enough to produce nerve dislocation or distortion. In contrast, nerve dislocation or distortion occurred 32% of the time at the affected trigeminal nerve, suggesting that NVC associated with TN is more severe than in asymptomatic patients [276].

The severity of NVC in patients with TN has been categorized into four categories based on the extent of nerve circumference in contact with the vessel as a proxy of severity: severe (NVC with the vessel covering >20% of the trigeminal nerve circumference); moderate (NVC with vessel
covering <20% of the nerve circumference); simple (the vessel slightly touches the trigeminal nerve); none (no contact between any vessel and the trigeminal nerve) (Figure 2-10) [378]. In patients undergoing surgery for TN, some NVC of the affected trigeminal nerve was detected in nearly all patients with 50% of patients having severe compression, 33% having moderate compression, and 10% having simple contact. In contrast, only a third of these patients showed any NVC on their contralateral (unaffected) nerves, which were mostly of the simple contact form [378]. Similarly, only a third of healthy individuals were found to have NVC, mostly of the simple contact form [378]. These observations in healthy individuals and at the unaffected trigeminal nerves must be interpreted with caution as they rely on neuroimaging data, which have limited resolution. The degree of contact was not confirmed operatively. Other studies have also shown that TN is associated with more severe compression of the trigeminal nerve, and also atrophy of the affected trigeminal nerve, likely due to NVC [13,157,258,411]. Erbay and colleagues [157] reported that the mean diameter and cross-sectional area of the trigeminal nerve on the symptomatic side in TN is significantly smaller compared to the asymptomatic side based on MRI visualizations. The authors proposed that the atrophy was likely secondary to structural abnormalities including axonal loss and demyelination that result from NVC [157]. Furthermore, a recent study by Antonini and group [13] reported that trigeminal NVC as detected by MRI is likely to be symptomatic when it is accompanied by anatomical nerve changes including nerve atrophy. Taken together these studies provide evidence that severe NVC accompanied by structural changes to the trigeminal nerve occurs on the symptomatic side of patients with TN and support a peripheral theory of TN etiology.
Figure 2-10: Schematic illustration of the severity analysis of the neurovascular compression (NVC). Severity was based on the extent of the nerve circumference in contact with the vessel and classified into four groups: severe (NVC with the vessel covering >20% of the trigeminal nerve circumference); moderate (NVC with vessel covering <20% of the nerve circumference); simple (the vessel slightly touches the trigeminal nerve); none (no contact between any vessel and the trigeminal nerve) (not shown). CN V= trigeminal nerve. This figure is from [378] and is used with permission from the American Society of Neuroradiology (license number: 3532540245767).

In addition to these findings, others have examined the type of offending vessels involved and precise location of the NVC along the trigeminal nerve to determine if these factors are associated with TN and possibly its etiology. One study that prospectively examined 579 patients undergoing microvascular decompression surgery for the treatment of TN found that one or
several offending vessels could be identified in 97% of patients, and that most (88%) cases involved the superior cerebellar artery (SCA) the NVC occurred at the REZ in half of these cases [411]. Other studies have reported similar outcomes, with the SCA being the most frequently reported offending vessel [64,276], followed by the anterior inferior cerebellar artery, and less frequently the posterior inferior cerebellar artery, vertebral artery, basilar artery, or a vein [27,64,276,411]. Figure 2-11 provides examples of various types of NVC as seen through the posterior approach of the cerebellopontine angle in TN patients undergoing microvascular decompression surgery [411]. Anatomically, these blood vessels are in close proximity to the trigeminal nerve, but do not usually compress it. With increasing age, however, arteries tend to elongate [217]. The result can be redundant arterial loops and/or veins that compress cranial nerve root entry zones at the cerebellopontine angle [111,217], fitting with TN patients primarily affecting older individuals. Although NVC can occur anywhere along the length of the symptomatic trigeminal nerve, compression proximal to the brainstem, particularly at the REZ, has been reported to be the most commonly affected region, associated with greater TN severity, and a higher risk of pain recurrence following surgery [27,265,306,411,439]. Additionally, a number of pathological changes specifically at the trigeminal REZ have been reported, as this region of the nerve appears to be less resistant to compression [128].
Figure 2-11: Examples of types of neurovascular compression (compression sites indicated by arrows) as seen through the posterior approach of the right cerebellopontine angle: superior cerebellar artery in supero-medial (upper left) and supero-lateral (upper right) position; anterior inferior cerebellar artery inferiorty compressing the trigeminal root entry zone at the pons (lower left); satellite trigeminal veins embedded in the trigeminal nerve tissue (lower right). CN V= trigeminal nerve. This figure has been adapted from [411] with permission (license number: 35249320707276).

Lastly, evidence to support the NVC theory of TN etiology comes from studies examining pain and trigeminal nerve function before and after effective treatment. Specifically, there is evidence for functional abnormalities of the affected trigeminal nerves of TN patients [108,260,330] that
resolves with successful decompression surgery and that is associated with immediate pain relief [27,260,265,328,364]. In one study by Leandri and colleagues [260], ten patients with TN affecting the maxillary region of their face and with NVC at the trigeminal REZ as identified by MRI and MR angiography, underwent microvascular decompression surgery. Using electrophysiological methods, trigeminal nerve conduction was measured before, during, and after surgery by stimulating the infraorbital nerve and recording both early scalp evoked potentials and direct REZ responses. The authors noted that the scalp responses reflected a volley of incoming signals passing through three main components along the trigeminal pathway: W1 (just before the Gasserion ganglion), W2 (the retro-Gasserian root), and W3 (inside the brainstem, just after the REZ). Before treatment, all patients had an altered scalp response consisting of a delayed or absent W3. The authors attributed this finding to a conduction impairment at the REZ, fitting with their observations of the NVC location [260]. Immediately following the decompression, seven patients (70%) showed a recovery, or normalization of the scalp response. Similarly, direct recordings from the REZ showed a reduction in latency of the response following surgery, which paralleled the reduction in latency of the peak W3 component from the scalp recording. This study provided the first neurophysiological evidence showing the immediate effect of decompression of the trigeminal nerve at the REZ, which included normalization of trigeminal evoked potentials and pain relief for the patients, further supporting a peripheral etiology of TN.

2.3.4.4 Pathophysiology of Trigeminal Neuralgia

The pathophysiological mechanisms underlying TN are not entirely understood and some of the theories surrounding TN pathophysiology remain controversial. As classical TN etiology is most
frequently associated with NVC at the trigeminal nerve, several studies have conducted ultrastructural and immunohistochemical analyses of the trigeminal nerves of TN patients and demonstrated pathological changes, most notably demyelination [137,201,234,278,294,328,359]. These pathological findings have led to hypotheses that test how trigeminal nerve alterations can account for characteristic features of TN such as paroxysmal pain that can either occur spontaneously or be triggered by normally non-painful stimuli [137,358]. In this section, studies examining trigeminal nerve pathology in TN will be reviewed, and some of the most prevalent theories of TN pathophysiology aimed at describing its characteristic symptomology and response to treatment will be discussed.

2.3.4.1.1 Trigeminal Nerve Pathology

For some patients undergoing surgical procedures for TN, trigeminal nerve biopsy specimens have been extracted and submitted to detailed micro/ultrastructural analyses and immunohistochemistry. The most prevalent finding is focal demyelination of the symptomatic trigeminal nerve [137,201,234,294,359]. While some early studies reported demyelination among other morphological changes along the length of the trigeminal nerve and ganglion [37,234], Hilton and colleagues [201] were the first to demonstrate that the structural abnormalities occurred at the site of the NVC. In their study, a biopsy of the trigeminal nerve root from a patient without any prior surgical history for TN was examined. The biopsy included an abnormal area of tissue that was compressed by a vessel and adjacent healthy fibres. Their main finding was demyelination at the site of the compression, with several of the demyelinated axons being in close proximity to each other [201]. Similarly, in other studies examining TN patient biopsy specimens of the trigeminal root, regions of the most severe NVC had few
remaining axons, and nearly all that remained were demyelinated and close together [137,359]. In addition, regions adjacent to the NVC had more surviving axons, but dysmyelination (axons with a disrupted residual myelin sheath) was evident [137]. Axonal loss and demyelination of the trigeminal nerve are consistent with studies that have described atrophy of the symptomatic trigeminal nerve of TN patients [13,157,258,411]. Importantly, the site of the NVC is frequently the REZ.

Anatomically, myelinated axons at the REZ are primarily associated with CNS myelin produced by oligodendrocytes. CNS myelin surrounding the axons can vary in length (Figure 2-12) [298], but typically extends up to a few millimeters beyond the surface of the pons to a transition zone known as the Obersteiner-Redlich line, where it transitions to peripheral myelin produced by Schwann cells [277,341]. Immunohistochemical studies have demonstrated NVC-induced focal demyelination that largely affects the CNS myelin at the trigeminal REZ [294]. Although retrograde pathophysiological changes along the length of the axon can occur with focal injury [87,137], abnormalities of peripheral myelin are less consistently reported in TN and are less drastic when they are observed [294]. The notion that CNS myelin is primarily disrupted at the REZ of TN patients is consistent with peripheral myelin being significantly more resistant to compression and damage [185]. Therefore, the REZ is likely a key region in TN pathophysiology. This provides the rationale for why vascular contact more distally along the trigeminal nerve can occur in individuals without any history of TN [194,328].
Figure 2-12: Diagram showing that the dorsal root entry zone of the trigeminal nerve can be variable in length and may extend to distal portions of the nerve. This figure is from [298] and reproduced with permission from the Journal of Neurosurgery.

2.3.4.4.2 Pathophysiological Mechanisms- “The Ignition Hypothesis”

Lack of a good animal model of TN that reproduces its key clinical features has limited testing theories of the pathophysiology of the disease. For example, lesions to the trigeminal nerve, root, and ganglion result in what appears to be painful facial neuropathies that do not closely resemble TN symptoms [136]. Much of what is known about TN pathology comes from trigeminal nerve biopsies taken from patients during surgical procedures for TN, as described above. Hypotheses aimed at explaining the mechanisms of TN pathophysiology must account for both the pathological changes associated with NVC of the trigeminal REZ and the unique symptoms of TN pain, including pain that is paroxysmal, spontaneous, and triggered by normally non-painful stimuli such as light touch. Two candidate pathophysiological mechanisms that are frequently discussed are ectopic or spontaneous firing of trigeminal afferent fibres, and ephaptic transmission between fibres mediating light touch and those involved with nociception.
One theory that has gained a lot of recognition for blending these ideas to account for TN symptomology is the ignition hypothesis [358], which was more recently updated to account for advances made in our understanding of nerve pathophysiology [136]. This theory proposes that TN pain results from an abnormal generation of sensory impulses of trigeminal fibres generated from regions of demyelination (or other structural abnormalities) referred to as pacemaker sites [136]. Several studies have provided evidence for the hyperexcitability of sensory neurons such that they can be spontaneously active following injury [65,72,136,277,360]. Other neurons may be silent (i.e., not spontaneously generating impulses), but are described as having a hair-trigger threshold such that a momentary stimulation can produce a burst of firing lasting several seconds. This phenomenon is referred to as afterdischarge [136,138,269]. Additionally, afterdischarge bursts can recruit neighbouring neurons, which can in turn recruit more neighbours via neuron-to-neuron coupling. The ignition hypothesis proposes that this recruitment of a population of neurons demonstrating afterdischarge underlies the paroxysmal explosion of pain characteristic of TN [136].

Importantly, many of the myelinated axons that become focally demyelinated with compression injuries convey non-nociceptive sensory information. Thus, a mechanism must also be in place to recruit nociceptors in this process so that the paroxysm felt by TN patients is painful. The ignition hypothesis discusses another mechanism that may explain the recruitment of nociceptors termed “crossed afterdischarge” [136,358]. In an intact peripheral nerve, it has generally been presumed that each primary sensory neuron independently conveys signals, such that they have no effect on neighbouring neurons until they reach the spinal cord [10]. This type of isolated signaling can occur because in the periphery, cells are isolated from one another by layers of insulating material (e.g., myelin, mesaxon, and satellite cell processes) [136]. In fact, it has been
shown that a small proportion of primary afferent neurons in intact dorsal root ganglia (DRG) fire spontaneously [139] and have small effects on neighbouring cells, but these effects are typically subthreshold for spike initiation [10,139]. In cases where there is nerve damage such as demyelination, and the cellular insulation is broken down, the axons of sensory neurons come in closer contact with each other at these sites, and when one neuron fires, it can now depolarize previously silent neighbours [136,358]. When this cross-excitation occurs between individual axon pairs that become electrically coupled, it is termed ephaptic crosstalk/transmission [390]. In the TN literature, it has been discussed that ephaptic crosstalk between fibres mediating light touch (Aβ fibres) and those involved in nociception (Aδ and c fibres), may account for TN pain attacks that are triggered by brief triggered stimuli, such as light touch, to the face [10,136,277,328]. In one study that examined electrophysiological recordings in excised rat DRG, the authors found that the spike activity of myelinated axons triggered by peripheral nerve stimulation could produce a depolarization of unmyelinated neighbouring c-neurons that shared the same ganglion [10]. Rappaport and Devor (1994) [358] further note that “crossed afterdischarge” can occur, which is cross-talk between a population of neurons that do not have to necessarily be in close apposition to each other. They describe crossed afterdischarage as a non-synaptic, non-ephaptic coupling, whereby impulse activity in a group of afferents evokes the non-synaptic release of potassium ions (K⁺) into the interstitial space that can stimulate neighbouring neurons by diffusion [136,358].

One more feature that the ignition hypothesis attempts to account for is the stop mechanism of TN pain. Classical TN is characterized by short, paroxysmal bursts of pain, with patients being pain-free between attacks. Additionally, there is a refractory period immediately following an
attack, during which time another attack of pain cannot be elicited [136,251]. Devor and colleagues [136] point out that the self-sustained activity of neurons eliciting a “hair-trigger” phenomenon response is transient. They describe the stop mechanism of TN pain attacks to involve an influx of calcium ions (Ca$^{2+}$) during depolarization, followed by an opening of Ca$^{2+}$-activated K$^+$ channels and an efflux of K$^+$ ions through these channels causing the cells to hyperpolarize and the firing to cease. This post-burst hyperpolarization may occur for a minute or more, and may account for the refractory period following a TN pain attack [136]. In periods of pain remission or with effective treatment, long-term processes such as remyelination or a decrease in the hyperexcitable state of the neurons may be involved, as they could bring the trigger level below threshold for extended periods [136]. While the precise mechanisms underlying the pain relief experienced by TN patients following medical and surgical intervention remains elusive, it remains clear that pain is significantly reduced or entirely relieved, at least for a period of time, for many of these individuals.

### 2.3.5 Current Treatments

There are currently several treatment approaches available for the management of TN. Although medications are always the first line of treatment [487], over time pain attacks may occur more frequently, be more sustained, and be less responsive to medications, leading patients to seek neurosurgical intervention for pain relief [328,489] [349]. Each year, approximately 8,000 patients undergo surgery for TN in the United States, with estimated costs exceeding $100 million [349]. Surgeries for TN are generally categorized as ablative (destructive to the nerve) or non-ablative (preserving the nerve), with microvascular decompression (MVD) surgery being the
only generally accepted non-ablative technique [487]. Common ablative techniques include percutaneous trigeminal rhizotomies and focal radiosurgery such as Gamma Knife radiosurgery (GKRS) [203]. Additionally, unlike other severe chronic pain conditions, surgical treatments for TN can be highly effective in alleviating or substantially decreasing TN pain by more than 75% [265,268]. In this section, medical and surgical treatments for TN will be described, and the clinical outcomes from these procedures will be reviewed. A particular focus on MVD and GKRS procedures will be made, as patients who underwent these two surgical interventions were included in this thesis.

### 2.3.5.1 Medications

Based on Trousseau’s theories of TN resembling convulsive seizures, in 1942 Bergouignan introduced the anticonvulsant medication, phenytoin, as a treatment for TN. Phenytoin has been shown to be initially effective for approximately 60% of TN patients, however, the effectiveness declines to approximately 25% in the long-term [89]. Additionally, patients frequently experience a variety of unpleasant side effects including drowsiness, dizziness, and ataxia [89,95,173]. In the early 1960s, Blom [51] demonstrated greater effects on TN pain with the antiepileptic medication carbamazepine, which is still the main drug prescribed for the management of TN pain today [89,95,173]. Since carbamazepine has a good initial effect on pain in approximately 70-80% of TN patients, it has been considered the gold standard to which other drugs have been compared in clinical trials [89,328]. It has been shown to be superior to placebo in multiple clinical studies [328]. Carbamazepine’s mechanism of analgesia is not really understood, however it is thought to be related to the blockade of voltage-sensitive sodium channels resulting in the stabilization of hyperexcitable neurons, inhibition of repetitive firing,
and/or reduced neural signal propagation [89,495]. Although carbamazepine can take effect within a few days [486], in the long-term patients can develop resistance or there can be problems with tolerability [436]. In some cases, the loss of effectiveness can be compensated for by adding other voltage-sensitive sodium channel blockers such as lamotrigine [328,495]. In one study, lamotrigine was found to be superior to placebo in patients with TN refractory to carbamazepine [488]. Still, side effects such as tiredness and poor concentration may be too much for patients, or undesirable drug interactions may occur [349,489]. Other medical options include oxcarbazepine, which is keto-analog of carbamazepine and is more favourable in terms of side-effects, gabapentin, which although was created to mimic GABA, likely also takes it effect by binding to voltage-gated calcium channels, pregabalin, which can be used for TN patients that are unresponsive to carbamazepine, and baclofen, which is a GABA$_B$ receptor agonist, among several others [89,486]. If TN pain is no longer controlled by medications or the side effects become overbearing, surgical procedures can be considered. Due to the severity of the condition and invasiveness of many of the surgeries for TN, the studies examining patient outcome were not typically placebo-controlled.

2.3.5.2 Percutaneous Trigeminal Rhizotomies

Percutaneous rhizotomies are considered ablative procedures as they are aimed at partially lesioning a portion of a nerve. Several rhizotomy techniques are available for the management of TN and include glycerol rhizotomy, thermal/radiofrequency thermocoagulation, and balloon compression [203,344]. While each technique uses a different means to damage the trigeminal nerve, one commonality is a percutaneous penetration of the foramen ovale to the trigeminal cistern with the guidance of fluoroscopy and sometimes computerized tomography.
neuronavigation [203,242]. The ideal outcome of a percutaneous trigeminal rhizotomy procedure is the destruction of sensory fibres carrying nociceptive information (Aδ and c fibres) and preservation of those carrying touch information (Aβ fibres). However, this is not practical and with damage to touch fibres, sensory alterations occur [203]. These alterations include sensory loss (facial numbness), dysesthesia, and corneal anesthesia and keratitis (due to ophthalmic nerve damage) [89]. There appears to be a trade-off between pain and sensory loss, such that the greater the sensory loss, the less the likelihood of early pain recurrence [328,344].

The mechanism of action of percutaneous procedures has been related to the pathophysiology described in the ignition hypothesis [358]. Specifically, it is thought these procedures lower the likelihood of ectopic firing and ephaptic afterdischarge by reducing the population of the hyperexcitable trigeminal neurons. Pain relief and sensory abnormalities occur because both touch and nociceptive neurons are damaged, and percutaneous lesions are non-focal and non-specific. The nerve damage may also result in an accumulation of cellular debris and gliosis that eventually clear, allowing surviving neurons to once again be in close apposition to each other, and fire abnormally, which would coincide with pain recurrence [358].

While a relatively high proportion of patients (in the range of 90%) achieve pain relief immediately following surgery, pain recurrence is frequent in the longer term [89,203,242,388,455]. The rates of TN pain recurrence from the literature are variable, and depend on factors such as type of rhizotomy, timeframe of evaluation, need for concomitant medications, and amount of pain control achieved [242]. One prospective study examining the
outcome and recurrence rates of patients undergoing radiofrequency (RF) thermal rhizotomies for TN reported that pain recurrence occurred for 27% of patients (including those that required additional surgery and those that were medically managed), however this rate encompassed a large range of time (long-term follow-up was > 6 months post-surgery to 68 months post-surgery) [388]. Other studies have reported pain recurrence rates ranging from 10-72% depending on the type of rhizotomy, with RF thermocoagulation procedures having the lowest mean recurrence rates, and glycerol rhizotomies having the highest [89,274,344]. While longer-term pain recurrence may be higher than other surgical options for TN, percutaneous rhizotomies are a valuable treatment option for individuals who are in need of immediate pain relief, but not good candidates for microvascular decompression surgery.

2.3.5.3 Microvascular Decompression

Although Dandy was the first to observe vessels compressing the trigeminal nerves of TN patients, the first reports of decompressing the trigeminal nerve for TN were not until the 1950s [95,178,432,433]. These early decompression approaches had some beneficial effects for patients; however, they were performed without the aid of magnification and had high rates of morbidity and mortality [298]. In 1967 Jannetta began using an operating microscope during this procedure, allowing him to better visualize the cranial nerves, which significantly improved the decompression technique [216] making it the treatment of choice for TN today [442].

MVD surgery relies on the careful positioning of the patient using a head-fixation device. Under general anesthesia, a retrosigmoid craniotomy approach is used, such that a small piece of bone
from the skull is removed via a small incision behind the ear (Figure 2-13) [298]. The posterior fossa and specifically the cerebellopontine angle can be then approached, and using microscopic magnification, the location of the trigeminal nerve can be identified based on its relationships with the tentorium cerebelli and the petrosal sinus complex [203]. Once the trigeminal REZ is inspected and the offending vessel isolated, nerve decompression is done by dissecting the arachnoid tissue around the trigeminal nerve and blood vessel and placing pieces of shredded Teflon© between them (Figure 2-14) [203,298].

**Figure 2-13:** Drawing of bone exposure via incision behind ear during microvascular decompression surgery. This figure is from [298] and reproduced with permission from the Journal of Neurosurgery.
Figure 2-14: Diagram of dissection and decompression of the trigeminal nerve. A. Neurovascular contact between the trigeminal nerve (V) and an offending vessel with the arachnoid mater present. Prior to decompressing the trigeminal nerve, the arachnoid mater must be dissected. B. After the arachnoid is removed, the vascular loop can be mobilized and the Teflon© can be inserted between the vessel and nerve. This figure is from [298] and reproduced with permission from the Journal of Neurosurgery.

Due to the highly invasive nature of this procedure, MVD surgery does not come without potential risks such as hemorrhage, cerebrospinal fluid leakage, and damage to other cranial nerves. However, these complications occur in only <3% of cases [298] and patients experience long lasting pain relief compared to other surgeries for TN [203]. In general, approximately 90% of patients obtain pain relief immediately after surgery, with 80% still being pain-free at the one-year follow-up [203]. Approximately 75% of patients are still pain-free three years post-surgery, and by 10 years after surgery, 70% of patients are pain-free and off medications [203]. Others have reported that following MVD, the pain recurrence rate is approximately 4% per year, which
plateaus to 1-2% by year six [27]. In contrast, the ablative procedures have a recurrence rate of about 50% after 3-5 years, which increases linearly onwards [489].

The mechanism of pain relief following MVD can be explained in terms of the ignition hypothesis [136]. In cases of TN associated with vascular compression, focal demyelination at the trigeminal REZ can occur and cause sensory neurons to become hyperexcitable. MVD likely has an immediate effect on pain because it displaces the presumed source of the pain, allowing neuronal activity to normalize. Long-term pain relief may involve slower processes such as remyelination and further trigeminal root recovery [136].

2.3.5.4 Gamma Knife Radiosurgery

Gamma Knife radiosurgery (GKRS) is the most common type of focal radiosurgery used for the treatment of TN [203]. It is the least invasive of the intracranial TN treatment options and therefore a less risky option for both younger and elderly patients, and patients with multiple comorbidities [89,203]. GKRS was first used by Leksell in 1971 to treat TN by lesioning the trigeminal ganglion and adjacent root [261], but is now also used to treat a variety of lesions including tumours or blood vessel abnormalities such as arteriovenous malformations. With advances made in our understanding of TN pathophysiology, the target to treat TN has moved to a portion of the trigeminal nerve distal to the REZ, or the REZ itself [95,185]. Since this target in this case is very small, having a diameter of approximately 3mm, highly focused radiation and detailed pre-treatment planning using magnetic resonance images are keys to ensuring that critical surrounding structures (e.g., the brainstem) are avoided.
The Gamma Knife system is composed of three parts: the radiation unit, the stereotactic frame, and the computerized dose planning system (Lindquist, 1995). There are approximately 200 radioactive cobalt-60 (\(^{60}\text{Co}\)) sources (depending on the machine) that are focused on the trigeminal target using a collimator helmet and a stereotactic frame, which is fitted to patients under local anesthesia (Figure 2-15) [88,182,185,186,266,492]. Patients typically receive a dose between 70-90 Gy to the 100% isodose line using the smallest (4 mm) collimator in a single fraction [203,328]. Unlike the percutaneous procedures and MVD, the effects of GKRS are typically delayed, with the majority of patients experiencing pain relief approximately 4-6 weeks post-radiosurgery [418]. Potential risks of this procedure include sensory abnormalities such as facial numbness, paresthesias and dyesthesias in 3-54% of patients [89,166] and similarly to percutaneous procedures, this occurrence appears to be associated with pain recurrence [203]. Estimates of the proportion of TN patients with pain relief following GKRS are variable depending on time frame post-surgery and the scales used to measure patient pain status. It has been reported that at the one-year follow-up, approximately 70-80% of patients have good/excellent pain relief [31,186,203,243,328,331,401,412], with approximately 50% of patients being entirely pain-free and without medications [401]. It has also been reported that pain improvement is maintained in about 70% of TN patients, three years post-GKRS [203,401], but drops to 30% of patients ten years post-treatment [244].
Figure 2-15: A schematic diagram illustrating the general concept of Gamma Knife radiosurgery. Gamma rays are emitted from several radioactive cobalt sources focused on a target using a collimator (spherical) helmet and a stereotactic frame (not shown). This figure is reproduced from [182]. Copyright 2008, Dave Klemm.

The mechanism of pain-relief from GKRS is not well understood. As the dosage used for TN radiosurgery is sub-necrotic (<100 Gy) [494], GKRS may produce analgesia by injuring trigeminal fibres just enough to impede the aberrant signaling of these neurons. Corroborating this idea and in line with the ignition hypothesis, it has been found that radiation doses in the range used for TN radiosurgery can decrease the excitability of cells by at least partially blocking sodium and nerve conduction [185]. While the bulk of the radiation is delivered to a distal trigeminal target, a small amount of radiation also reaches the REZ directly. Some evidence suggests that pain outcomes are better when the isocenter is more proximal to the REZ [60,186]. Corroborating this, one study examined the effects of increasing the GKRS treatment target to include a larger section of the affected trigeminal nerve of TN patients [166]. The results
indicated that increasing the treatment volume did not significantly improve pain relief and may increase complications associated with this procedure [166].

2.4 Structural Brain Imaging

Prior to any surgical intervention for TN, patients undergo brain imaging, especially magnetic resonance imaging (MRI), with close attention paid to the posterior fossa to rule out other potential causes of TN such as tumours or vascular malformations. Typically, MRI is also part of patient follow-up. Not only is brain imaging useful for clinical purposes, but also several MRI techniques are available to non-invasively examine brain structure and function in clinical populations. In this section, the basic principles of MRI will be reviewed and some of the commonly used methods for assessing brain gray matter and white matter structure will be discussed.

2.4.1 MRI

2.4.1.1 Magnetic Resonance

Magnetic resonance (MR) uses strong magnets and radiofrequency pulses to collect information about atomic nuclei within tissues of the body [458]. Unlike other forms of imaging, it does not require the use of ionizing radiation to obtain images. For clinical imaging and some research applications, the MR signal is most frequently derived from hydrogen nuclei, with magnetic moments that under normal circumstances are randomly distributed such that there is no net
magnetism of tissue. However, when surrounded by a strong external magnetic field (B₀), the nuclei change their orientation to be either parallel or antiparallel to the magnetic field. As parallel alignment is the lower energy state between these two orientations, slightly more nuclei take this orientation. A net magnetization vector (Mz) aligned to the external magnet results from the difference between these two populations of nuclei. While individual nuclei take on a certain orientation, they are not static, but precess around the direction of the external field. The frequency of this precession is given by the Larmor equation/frequency:

\[ F = \gamma B₀/2\pi \]

where F is the precessional frequency, \( \gamma \) is the gyromagnetic ratio of the nucleus, and B₀ is the strength of the magnetic field [458]. Importantly, the net Mz from nuclei in the magnet in its equilibrium state does not produce a measurable signal. To obtain information from the precessing spins of the nuclei, the direction of the net Mz must be altered by exciting the spins using radiofrequency (RF) pulses at the Larmor (resonance) frequency. When this happens, the net Mz flips from the positive z-axis (aligned with the main magnetic field B₀ generated by scanners) to the transverse plane (perpendicular to the Mz), where it rotates around B₀ at the Larmor frequency. This rotating transverse magnetization will induce an alternating current (AC) that can be measured using a receiver coil placed around the patient. After the RF transmitter is turned off, the hydrogen protons will seek an equilibrium state, that is, the low energy state. Over time, the transverse magnetization decays as the longitudinal magnetization increases toward its pre-excitation value. The times required for the transverse and longitudinal magnetizations to return to their equilibrium states are called relaxation times: T₁ and T₂ [458]. T₁ relaxation describes the longitudinal relaxation, or the realignment of protons to the external magnetic field. It is defined as the time required for the system to recover 63% of its equilibrium value after
being exposed to a 90° RF pulse. Importantly, various tissues have different T1 values, which can be coded into a gray scale when MR data are reconstructed into image space via Fourier transformation. The second relaxation time, T2, is always shorter than the T1 time [458]. T2 describes transverse or spin-spin relaxation. The RF pulse results in the synchronized precessing of proton spins in the transverse plane. As time passes, the spins begin to dephase due to differences in the Larmor frequency by random local magnetic inhomogeneities, spin-spin interaction, and inhomogeneity of the main static magnetic field (B0) [458]. The T2 relaxation time is the time it takes for dephasing to decay to 37% of its original value, which again is variable between tissue types.

**2.4.1.2 Image Formation and MR Signals**

As previously mentioned, RF pulses generate a signal that is captured by a receiver coil housed within the magnet. To create an image, the MR signal must contain information about the location of the hydrogen protons so it can be spatially encoded. This is done in three steps: slice selection, frequency encoding, and phase encoding [458]. Briefly, to select an imaging slice, a magnetic gradient is added along the main magnetic field creating a gradient of precessional frequencies from the patient’s head to toe. Applying a narrow band of frequencies will therefore excite a relatively thin slice of spins representing a cross section through the body [458]. With a change in excitation frequency, another parallel slice can be acquired. Each slice can be further subdivided into small voxels by applying magnetic field gradients in the x and y directions that assign specific frequency and phase signatures to the protons in the slice. This raw data or ‘k-space’ data can be reconstructed into image space using Fourier transformation.
The signal intensity in each voxel will be dependent on the relaxation times of the protons and the proton density of the tissue in that voxel. One RF excitation builds one line in k-space. Most images have 256 lines in k-space and therefore the time it takes to acquire an image with this resolution is 256 times the time it takes to acquire the signal for one line, which can vary from 1 msec to several seconds. There are many new ways to acquire data in k-space that have moved away from acquiring one line of data at a time that have revolutionized image acquisition including echo planar techniques enabling ultrafast imaging (e.g., [485]).

MRI can visualize differences in proton density, $T_1$, and $T_2$ of different tissue types. This multi-parametric capability offers superior soft tissue contrast compared to uni-parametric modalities such as computerized tomography [458]. One way to modify image contrast is to vary how often RF pulses are applied, known as the repetition time (TR). For example, the contrast of an MR image will mainly be influenced by $T_1$ if the TR is shorter than the time necessary for total longitudinal relaxation. On the other hand, if the TR is long, then the contrast will be dependent primarily on the differences in $T_2$ if the echo time (TE) is also long [458]. A second RF pulse can be applied at a time TE (echo time) that can reverse the direction of precession of water protons to generate a spin echo. This is done to eliminate phase errors that accumulate in proton precession caused by static magnetic field inhomogeneities [458]. If TR is long, but TE is short, then the image will not be dependent on $T_1$ or $T_2$ but instead on the tissue’s proton density. Lastly, the resolution of an image depends on factors such as field of view, pixel size, and slice thickness. The pixel size (i.e., a 2-dimension picture element) of an image is determined by the
size of k-space, which is comprised of the number of frequency-encoding (128 or 256 on x-axis) and phase-encoding (128 or 256 on y-axis) steps, as previously discussed. Once the pixel size and slice thickness are known, the volume of each pixel, called a voxel (i.e., a 3-dimensional element), can be determined. While high-resolution MR images typically mean smaller voxel size, the signal to noise ratio is a limiting factor because if voxels become too small, they will not contain enough protons to produce a measurable signal [458].

2.4.2 Gray Matter Imaging

Noninvasive techniques that can be used to study GM abnormalities across different patient populations is crucial to developing a better understanding of these disease processes. Given the sensitivity of T1-weighted imaging in detecting differences between tissue types, structural analyses of brain gray matter (GM) are typically done based on T1-weighted MR scans (Figure 2-16). To do this, data are first preprocessed to distinguish and classify brain tissues into GM, white matter (WM), and cerebrospinal fluid (CSF) components. Then, several automated and semi-automated techniques are available to measure GM, the two most common methods being voxel-based morphometry (VBM) (http://www.fmrib.ox.ac.uk/fsl/) [18] and cortical thickness analysis (CTA) (http://surfer.nmr.mgh.harvard.edu/) [163]. While VBM can assess both subcortical and cortical GM density or volume, CTA was developed to measure the thickness of cortical GM on a submillimeter scale [163]. This is crucial since the cortex is highly convoluted and measuring thickness based on slice data or volume measurements often result in overestimations of GM [163]. Since many disease processes involve subtle cortical changes, the submillimeter accuracy of CTA is favourable. Therefore, in this thesis, subcortical GM analyses
were carried out using VBM and cortical GM analyses were done using CTA. Each of these techniques is described in detail below.

![Image of high-resolution T1-weighted MR scan](image.png)

**Figure 2-16:** Example of high-resolution T1-weighted MR scan in axial (left), coronal (middle), and sagittal (right) views.

### 2.4.2.1 Voxel-Based Morphometry

A detailed description of the methods used for VBM analyses has previously been published (see [18]). In its simplest form, VBM involves the voxel-wise comparison of the local concentration (i.e., density or volume) of GM between two groups of subjects (e.g., patients and controls). Whether the VBM result is in density or volume depends on which steps are taken and can vary between imaging software. For this thesis, VBM as implemented in the FMRIB’s Software Library (FSL) was used and is specifically described in the general methods (chapter 4.3.2.2). As such, our results are reported in units of volume (described below). VBM differs from deformation-based and tensor-based morphometry techniques, as these techniques primarily deal with differences in brain shape, not with differences in the local composition of brain tissue after
images have been normalized [18]. Prior to examining subcortical GM differences between groups, a series of pre-processing steps are completed (Figure 2-17 summarizes the main steps). These steps include the removal of non-brain tissues (e.g., skull) from an image of the whole head as this tissue is not required for analyses, spatial normalization of all scans to a standard space, and the classification and segmentation of tissue types. Spatial normalization involves transforming each subject’s data into the same stereotactic space. In order to do this, images are registered to a template image (e.g., the MNI 152 standard space), usually using an affine transformation (i.e., linear, preserving proportions). A second step is done to account for global nonlinear shape differences. Importantly, the goal of spatial normalization is not to precisely align every GM structure exactly, as all images would then be identical and group differences could not be obtained. Instead, the aim of this process is to allow for the detection of regional differences in the density or volume of GM at a local scale, after accounting for global shape differences [18]. After images are spatially normalized, they are segmented into different tissue types (GM, WM, and CSF) based on the MR signal intensity of the voxels. In T1-weighted images, the signal intensity of CSF is dark, the signal intensity of WM is bright, and the signal intensity of GM lies between these two extremes. Once the voxels attributed to GM based on signal intensity have been identified and categorized, the images are smoothed using an isotropic Gaussian kernel. When images are smoothed, the intensity of each voxel is replaced by the weighted average of the surrounding voxels (how far around the voxel depends on the size of the smoothing kernel), which essentially blurs the image. Smoothing is important since normalization is an imperfect process and some inaccuracies may occur. Smoothing makes the data more normally distributed, increasing the validity of parametric tests, which is an important assumption of VBM [467]. However, excessive smoothing can diminish the ability to accurately
identify local differences in brain GM. As previously mentioned, VBM results can be reported as differences in either density or volume. If analyses are conducted on data after the smoothing step, they are said to be unmodulated and the results are reported as differences in GM ‘density’ [18]. In some cases, images undergo an additional step called modulation, whereby the signal intensity of each voxel is modified to take into account how much it expanded or contracted when it was registered to standard space [154]. Mathematically, this degree of expansion or contraction, or the magnitude of local deformations, is specified by the Jacobian determinant. Thus, modulation involves the voxel-wise multiplication of the Jacobian determinant to keep the total amount of GM constant regardless of expansion or contraction, allowing for differences in GM ‘volume’, rather than GM density, to be reported.

**Figure 2-17**: Summary of the main preprocessing steps for VBM analyses. Figure modified from: http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM.
The output from a VBM analysis is a statistical parametric map that shows regions where GM density or volume differs between the groups of interest. The statistical analyses are typically done using the general linear model (GLM), which is flexible enough to allow for many different tests including group comparisons or the relationship between GM and covariates of interest (e.g., pain duration). Standard parametric tests ($t$ tests and $F$ tests) are used to test the hypotheses as long as the data are independent and normally distributed. The voxel-wise statistical parametric maps that are produced contain the results of many dependent statistical comparisons. Therefore, multiple comparison corrections should be carried out to rule out the possibility that any of the findings were due to chance [18].

### 2.4.2.2 Cortical Thickness Analysis

The human cerebral cortex of humans is highly folded with its thickness varying between 1 and 4.5 mm across regions [163]. Given the convoluted nature of the cortex, manual techniques for estimating cortical thickness are labour intensive and volume measurements tend to be inaccurate or insufficient in detecting subtle cortical changes [163]. Thus, cortical thickness analysis (CTA) was developed to provide an automated means to accurately measure the thickness of the cerebral cortex with submillimeter accuracy. Like VBM, images are aligned to a stereotactic space and tissues are classified as GM, WM, and CSF. The normalization processes between VBM and CTA are similar in that subjects are aligned in a common space for group analyses. However, VBM uses a 3-dimensional volume coordinate of a voxel to best match the intensity of the voxel across subjects, whereas CTA uses a 2D coordinate of a vertex (described below) to best match curvature across subjects [188]. To measure cortical thickness, the border between the WM and the GM, known as the *white surface* (Figure 2-18, top panel), is identified to
provide an estimate of the inner boundary of the cortex using a triangular tessellation mesh over this surface [109]. This inner border is constructed first because resolution limitations innate to MRI make it difficult to directly compute the outer border between the GM and the meningeal tissue/CSF, known as the pial surface [163] (Figure 2-18, bottom panel). Once the inner cortical surface is identified, it is refined for submillimeter accuracy and deformed outward to the pial surface [163]. The surface model itself is mesh of triangles, with the corners of each triangle being a vertex [188]. Thickness can then be computed as the perpendicular distance measured between the inner and outer cortical boundaries at every vertex in each hemisphere. Each vertex is given an X, Y, and Z coordinate so that different morphometric measures can be obtained. Like VBM, the thickness data are smoothed using a Gaussian kernel [163] and a GLM is used to evaluate group differences in cortical thickness. Additionally, other metrics such as curvature, or how sharply the cortex is folded at each point, can be obtained using the CTA method [188].
Multiple comparisons can be an issue when running CTA analyses. Thus, permutation-based nonparametric statistics (e.g., Monte Carlo simulations) can be used to account for this problem that is implicit to methods using voxel-by-voxel (or vertex by vertex) hypothesis testing [322]. One benefit of using a nonparametric testing is that there are only a few assumptions for validity, unlike parametric testing. Monte Carlo simulations run iterations to calculate the probability of obtaining a false positive (alpha) in an entire 3-dimensional image. In doing this, the minimum cluster size (or number of contiguous vertices) required to reach significance while controlling for a particular alpha level can be obtained.
2.4.3 White Matter Imaging

WM analyses are typically based on MR-diffusion weighted imaging (DWI), which is sensitized to the random Brownian motion of hydrogen protons, mainly in water molecules [220,313]. To measure diffusion using MRI, phase differences between gradient pulses are used to detect water motion. Multiple images are collected so that the signal can be sensitized to diffusion in many different directions, building up multiple measurements for each voxel in the brain [220]. The pulse sequence most commonly used for DWI (the pulsed-gradient, spin echo sequence with echo planar imaging (EPI) readout) uses a pair of gradient pulses placed on either side of the 180° refocusing plane [8]. The first gradient pulse dephases the magnetization and the second pulse rephases it [8,313]. This refocusing is only perfect if the water molecules have not moved/diffused between the two pulses as the phases induced by the pulses will cancel each other out and there will be no signal attenuation [8]. In cases where diffusion occurs in the direction of an applied gradient, the net motion will result in the signal phase to change by different amount for each pulse. Since MRI signals are proportional to the sum of magnetization components from all water molecules within a voxel, phase dispersion from diffusion will result in the attenuation of the signal (i.e., the more phase dispersion, the darker the voxel) [8].

The diffusion coefficient of pure water at 20°C is approximately 2.0 x 10⁻³ mm²/s, but can increase at higher temperatures [8]. The diffusion of water in body tissues occurs inside, outside, around, and through cellular structures [8]. Structural barriers such as cellular membranes can hinder diffusion, causing water molecules to take tortuous paths limit their displacement. This
hindrance may be lessened or exacerbated in pathological conditions [8]. The ability to measure the diffusion of water molecules in different tissue types is useful because some tissues such as WM, have structures that restrict diffusion primarily along one axis. Therefore in WM, diffusion is more restricted across an axon than along it due to structural barriers such as myelin, axonal membranes, microtubules, and neurofilaments [36]. Interestingly, it has been shown that diffusion imaging on live and fixed brains provides similar results, suggesting that water molecules still move in postmortem/fixed brains (unless frozen), and neuroanatomical information derived from diffusion imaging is primarily of static anatomy and is less influenced by physiology [313]. To obtain meaningful measures from diffusion scans, mathematical models can be fit to each voxel to derive information about the directionality of the diffusion, and presumably underlying tissue microstructure within that voxel. The most common model is the diffusion tensor model.

2.4.3.1 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) involves fitting a tensor, which is an ellipsoid shaped mathematical model, at each brain voxel of a diffusion MR scan. The tensor is characterized by three orthogonal eigenvectors and their associated eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) (Figure 2-19a). By determining the eigenvector associated with the largest eigenvalue in each voxel, fibre orientation can be visually represented on a DTI image using different colours. The most commonly used direction scheme assigns the colour green to fibres in the anterior-posterior orientation, red to fibres in the left-right orientation, and blue to fibres that are oriented in the superior-inferior direction [337] (Figure 2-20).
**Figure 2-19:** Diffusion tensor imaging involves fitting a tensor model, defined by three orthogonal eigenvectors and their associated eigenvalues ($\lambda_1, \lambda_2, \lambda_3$), to every voxel in the brain (A). Using the tensor model, the parameter fractional anisotropy (FA) is used to describe the degree of anisotropy within a voxel. FA can range from zero to one, with values closest to zero indicating that diffusion is highly isotropic (spherical tensor shape), and values closer to one meaning diffusion is highly anisotropic (B). This figure is from [220] and reproduced with permission from Annual Reviews.

**Figure 2-20:** Example of colour-encoded fibre orientation maps in axial (left), coronal (middle), and sagittal (right) views.
In general, the shape of the tensor carries information about the three-dimensional character of the water molecules’ diffusion [220]. As previously mentioned, diffusion between tissue types can vary due to structural barriers innate to certain tissues that can hinder diffusion. These variations are reflected in the shape of the tensor derived from a voxel of that tissue type. For example, the shape of a tensor derived from a voxel within the CSF would be roughly spherical in shape, indicating that diffusion is not hindered and molecules can flow equally in all directions. In this case, diffusion is said to be isotropic. In contrast, there are several barriers to diffusion in WM bundles making diffusion along the length of the axis greater than across it. Diffusion is said to be anisotropic when this occurs and the shape of the tensor is less spherical. Within the tensor model, the degree of anisotropy is captured by the parameter fractional anisotropy (FA) (discussed in more detail below). FA can range from zero (completely isotropic) to one (completely anisotropic) (Figure 2-19b). FA values in healthy WM can be as low as 0.1, but approach 1.0 in areas of tightly packed fibre bundles such as the corpus callosum [220]. This variation can be caused by a number of factors including crossing WM fibers, image noise, artifacts (e.g., misregistration of diffusion images, eddy currents, motion), and/or partial volume effects (i.e., signal mixing of GM, WM, and CSF) [8]. These influences can lower FA measures. Therefore, a threshold (e.g., FA > 0.2) is commonly used as one way of controlling these confounding factors. Although FA can be used to characterize WM properties, it is not the only measure. Measuring FA in combination with other DTI-metrics can provide more information regarding the shape of the tensor and potentially provide a means to noninvasively capture certain pathophysiological processes [8,36].
2.4.3.2 Measures of Tissue Microstructure

Currently, the most widely used measure of anisotropy is FA, which was first described by Basser and Pierpaoli [33] with the following equation:

\[
FA = \sqrt{\frac{1}{2} \cdot \frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}{(\lambda_1)^2 + (\lambda_2)^2 + (\lambda_3)^2}}
\]

Importantly, FA as a single scalar measure does not fully describe tensor shape. Other measures include axial diffusivity (AD), which corresponds to the main diffusion direction, or diffusion along the length of the axon ($\lambda_1$), radial diffusivity (RD), which is diffusion perpendicular to the main diffusion direction (average of $\lambda_2$ and $\lambda_3$), and mean diffusivity (MD), which measures the local magnitude of diffusion regardless of direction (average of $\lambda_1$, $\lambda_2$, and $\lambda_3$). Although FA measurements represent a robust method for assessing the degree of directionality of diffusion, this ratio measure is a function of changes in diffusion in these other metrics. Therefore, if diffusion changes along the length of the tensor ($\lambda_1$) were proportional to those perpendicular to the tensor (RD), then FA would remain relatively unchanged even though abnormalities in diffusion may exist [3]. Additionally, these other measures of tissue microstructure, namely RD and MD have been linked to specific pathophysiological mechanisms [8,55]. This is because the diffusion of water is particularly sensitive to changes in the architecture of cellular membranes that can occur under certain pathological conditions. While reduced FA has been reported across a broad spectrum of disorders [8], the direction of MD, RD, and AD changes are more variable depending on the precise underlying pathophysiology. For example, in a mouse model of demyelination, the absence of myelin was reported to increase RD, while having a minimal effect on AD [420]. Others have reported that AD may be a more specific marker for axonal
damage [8]. Additionally, inflammation and edema appear to primarily increase MD, while lowering FA [8]. Therefore, these measures can provide a noninvasive means to detect certain pathophysiological mechanisms that may be contributing to changes in FA.

Other non-pathological factors can also influence DTI-derived metric values. For example, DTI is known to be particularly susceptible to artifacts that can arise due to eddy currents and subject head motion [8]. Prior to any WM analyses, corrections should be made to remove these potential distortions. Other factors that can influence DTI-metric values include partial volume between voxels containing more than one tissue type (e.g., signal mixing of GM and WM, which would result in lower FA than if the voxel contained only WM), and regions of crossing WM tracts [8]. In cases where a voxel contains crossing fibres, FA can be significantly reduced without any WM abnormalities.

### 2.4.3.3 Tract-Based Spatial Statistics

DTI was originally used to examine WM within individual subjects/patients. However, it was soon realized that an understanding of WM organization across populations in health and disease required the ability to do between group analyses. The first studies of group effects used a VBM-style approach, whereby each subject’s FA image was registered to standard space so that voxelwise statistics could be used to determine regions that differed between groups or regions that correlated with certain covariates of interest [416]. This approach had some benefits but concerns soon emerged regarding the interpretability of the method. For example, once subjects were aligned in standard space, there was uncertainty to whether any given voxel would contain data from the same part of the WM tract across subjects [416]. Another concern regarded that
extent to which the WM data should be smoothed, as variations in smoothing kernels can greatly affect the results, for example, by increasing partial volume effects. In order to address this concern, Tract-Based Spatial Statistics (TBSS) was developed [416]. TBSS is a fully automated method that allows for the whole-brain comparison of WM tracts between groups while overcoming some of the limitations of the VBM-style approaches. Prior to using TBSS, scans are typically pre-processed to correct for artifacts created by motion or eddy-currents. As each diffusion scan contains multiple 3D images encoding different directions and at least one image without diffusion weighting (B₀ image), another preprocessing step involves linearly registering all images to each other, prior to estimating diffusion-related measures [416]. The diffusion tensor model is then fit to the diffusion data so that tensor eigenvalues can be calculated and DTI-metrics such as FA can be derived for each subject [32,33]. Each subject’s FA data is then projected on to the mean FA skeleton using a nonlinear registration [416]. In this step, each subject’s data needs to be aligned to make local comparison possible across subjects, but without warping too much because otherwise individual topology will be lost. To do this, a nonlinear approach based on free-form deformations and B-splines is utilized [373]. Since registration is better when the registration target is a single FA image compared to a blurrier group image, a single subject’s FA image is used for all nonlinear registration [416]. The subject chosen for alignment is the “most typical” subject of the entire group (i.e., the subject that minimizes the amount of warping required for all other subjects to align to it) [416]. After all subjects’ FA images are aligned to the most typical target, the entire aligned dataset is registered using an affine transform onto the 1 x 1 x 1 mm³ MNI152 standard template [416]. The transformed images are then averaged to create a mean FA skeleton is created, which corresponds to the tracts common to all subjects. The skeleton is “thinned” such that the voxel with the highest FA
is the center of the tract. Further thresholding of the mean FA value (usually by 0.2) excludes voxels that likely include GM and/or CSF [416]. Once this mean FA skeleton is created, each subject’s aligned FA image is projected onto the mean skeleton using a nonlinear registration. From here, the data can be submitted to voxelwise cross-subject statistics for each point on the common skeleton. The simplest approach is to use a GLM across subjects to process each skeleton voxel independently. However, permutation-based approaches are more commonly used as they are part of common software packages, providing strong control over family-wise errors (false positives) and do not have any assumptions about the normality of the data [322]. Additionally, the FA nonlinear registration can be applied to MD, RD, and AD maps, which are then projected onto the mean FA skeleton.

2.5 Neuroimaging of Pain

Over the past few decades, neuroimaging has greatly enhanced our understanding of brain regions involved in the multi-dimensional experience of acute pain. Brain imaging has also demonstrated that many of the brain regions involved in acute pain function abnormally and/or have abnormal structure in patients with chronic pains. This section reviews the studies that have used MRI techniques to assess brain responses to acute pain, as well as those that have demonstrated abnormalities in brain function and structure in chronic pains. Additionally, some of the cellular mechanisms underlying MR-detectable abnormalities in brain structure and the effects of effective treatment on brain abnormalities associated with chronic pain will be discussed.
2.5.1 Acute Pain

Many studies of acute pain have used neuroimaging techniques to examine brain responses related to the presentation of a nociceptive stimulus in healthy individuals. A common neuroimaging technique to indirectly measure brain activity is functional MRI (fMRI). In particular, fMRI measures the blood oxygen level dependent (BOLD) signals that are related to the proportion of oxy- to deoxy- hemoglobin in the blood [332]. BOLD contrast occurs since oxyhemoglobin is normally diamagnetic. However, when it gives up its oxygen, it becomes paramagnetic, which produces a difference in magnetic susceptibility between the blood vessel and tissue surrounding it [332]. Therefore, physiological events (e.g., increases in neural activity) that change the oxy- to deoxy- hemoglobin ratio can be noninvasively detected as an accentuated BOLD response [332]. With increased neuronal activity, there is also an increased demand for oxygen by neurons. In response to this demand, there is an increase in blood flow to these regions of higher activity. Blood oxygenation changes with the delivery oxygen to these neurons. Numerous studies have taken a stimulus-evoked fMRI approach to examine how acute nociceptive stimuli evoke changes in brain activity [124]. In general, these studies show that nociceptive stimuli evoke BOLD changes in several brain regions including the primary and secondary somatosensory cortices (S1, S2), the anterior and mid-cingulate cortices (ACC, MCC), insula, prefrontal cortex (PFC), primary and supplementary motor areas (M1, SMA), thalamus, basal ganglia, cerebellum, and brainstem [15,124,152,347,446]. As previously discussed, some of these brain areas receive nociceptive input directly, while others receive it indirectly. The input/output organization of these brain areas suggest that in addition to their putative role in pain, they also contribute to cognitive, affective, or motor responses that are not
necessarily specific to pain. This is important because the idea of a ‘pain matrix’ has been described in the literature, which refers to the network of brain regions consistently reported as being involved in acute nociceptive processing (e.g., [446,447]). While it has been argued that there is to some degree, a macroscopic ‘cerebral signature’ for central pain processing [447], others have suggested that this cannot be the case since at the macroscopic level of fMRI, nociceptive and non-nociceptive somatosensory stimuli, auditory stimuli, and visual stimuli elicit highly similar responses in the thalamus, S2, insula, and ACC (regions of the so-called ‘pain matrix’) [316]. Additionally, others have shown that the exact location of the activity within these brain regions can differ depending on the type of nociceptive stimulus used (e.g., cutaneous or visceral), the intensity of the stimulus, or individual factors such as differences in cognitive states during an experiment [15,426]. Therefore, while neuroimaging studies of pain consistently report patterns of brain activity in response to acute nociceptive stimuli, the precise role of these brain regions is a topic of debate because they are not specific to pain processing.

2.5.2 Chronic Pain

2.5.2.1 Gray Matter Abnormalities

In addition to functional abnormalities, several studies have identified structural abnormalities in the brain GM of patients with chronic pain using VBM and CTA techniques. The detailed results of these studies have been reviewed by others [124,296]. In general, the most common GM regions found to be abnormal in chronic pain include many of the regions activated in functional imaging studies of acute pain such as the PFC, insula, ACC, MCC, thalamus, S1, S2, basal ganglia, amygdala, and brainstem [124]. Such GM abnormalities have been reported across
many types of chronic pain including chronic back pain [17,394], fibromyalgia [68,248,282], irritable bowel syndrome [50,125,392], phantom-limb pain [147], complex regional pain syndrome [180], ankylosing spondylitis [477] and disorders affecting the trigeminal system such as migraine [366,382,457], trigeminal neuropathic pain [117,190], and temporomandibular disorder (TMD) [183,308,482]. While these studies have reported abnormal GM in patients compared to healthy controls, the direction of the GM abnormalities were not always in the same direction between patient groups even when comparing disorders affecting the trigeminal system. For example, in S1, some TMD patients were reported to have thicker cortex compared to controls [308], while in trigeminal neuropathic pain patients, S1 was reported to be thinner than controls’ [190]. Differences in the direction of abnormality may be related to the precise symptomology of each pain disorder [307], as it is following peripheral nerve injury, for example [437]. In this study, patients that had complete peripheral nerve transection and repair surgery at least 1.5 years prior to study enrolment participated in an MR imaging session and completed a sensory and motor assessment. Compared to healthy controls, patients had thinner GM multiple regions including the S1 and S2 cortex. Importantly, GM in these sensory regions were negatively correlated with measures of sensory recovery (i.e., vibration and mechanical detection thresholds), demonstrating a clear link between function and structure [437]. Similar GM variations may occur with differences in pain characteristics. However, some abnormalities appear to be common across most chronic pains, include less anterior insula GM in conjunction with less GM in the cingulate cortex and dorsolateral prefrontal cortices [124,199,296]. It has been suggested that these areas reflect pain chronicity in general [199], and may be associated with negative affect and changes in pain modulation [468], as opposed to precise pain symptoms.
While these GM abnormalities are evident in patients across many types of chronic pain, it is difficult to determine if these abnormalities occurred as a result of the patients being in chronic pain, or if they were pre-existing prior to the onset of the disorder. Some previous studies of chronic pain have found correlations between pain duration and brain GM, such that the longer pain duration, the greater the GM abnormality [50,308,482]. This suggests that long-term pain may drive some of the neuroplasticity in GM. Additionally, the literature examining pain-cognition interactions and personality suggest that individual differences in these factors can affect pain perception and possibly predispose certain individuals to developing chronic pain. In one study, healthy individuals exhibited one of two types of behavioural outcomes when asked to perform an attention-demanding cognitive task (Stroop task) in the presence of absence of a concurrently applied pain stimulus [393]. For one group of subjects (P-group, for pain dominates), pain impaired Stroop task performance, but for the other group (A-group, for attention dominates), participant task performance improved with pain. Interestingly, fMRI revealed that cognitive engagement in the Stroop task attenuated S1, S2, and anterior insula responses only in the A-group. Follow-up studies have further demonstrated structural differences between A-type and P-type individuals such that P-type individuals have increased GM in regions such as the insula, MCC, OFC, thalamus, and supplementary motor cortex [158]. Additionally, in a recent study that examined the behavioural and neural aspects of spontaneous disengagement of attention from pain, it was demonstrated that individuals that have a tendency to mind wander away from pain have stronger structural and functional connectivity between default mode and antinociceptive brain networks [250]. These results suggest that inherent differences in brain structure and function may allow some individuals to cope better when in pain, while making others more likely to develop chronic pain.
Personality factors can also influence an individual’s response to pain. For example, pain catastrophizing, defined as “an exaggerated negative mental set brought to bear during actual or anticipated pain experience” can be assessed using the Pain Catastrophizing Scale (PCS), which is comprised of rumination, magnification, and helplessness subscales [427]. It has been demonstrated that individuals that score high on pain catastrophizing do not cope well with pain and may have a greater likelihood of developing chronic pain [391,398]. In one study, PCS scores strongly correlated with DLPFC thinning in patients with chronic pain due to irritable bowel syndrome [50].

While abnormalities in GM structure may be pre-existing, there is some experimental evidence to suggest that GM abnormalities may at least be partially pain driven. In one study examining the GM correlates of acute pain, healthy participants received 20 minutes of noxious stimulation to the left forearm over eight consecutive days [438]. They had MRI scans on sessions one and eight. The results of a VBM analysis comparing these two time-points revealed that GM significantly increased in the MCC and contralateral S1 after patients had the painful repetitive stimulation. Moreover, this GM increase was maintained three weeks later, but receded by the one-year follow-up [438]. Therefore, prolonged nociceptive stimulation could induce MR-detectable structural changes to brain GM in regions involved in pain processing that were maintained for a period following the cessation of nociceptive input. Additionally, other studies (discussed in chapter 2.5.3) have demonstrated that some GM abnormalities are reversible.
following effective treatment for chronic pain. This suggests that pain is partially driving the GM abnormalities, as the abnormalities normalize when patients are no longer in pain.

2.5.2.2 White Matter Abnormalities

2.5.2.2.1 Cerebral WM

In addition to widespread GM abnormalities, several studies have also reported abnormalities in the cerebral WM tracts of chronic pain patients, many of which interconnect the GM regions involved in the multi-dimensional experience of pain. Many of the more recent studies examining WM in chronic pain populations have used the TBSS method for between group comparisons with healthy control participants [180,291,310,431]. WM abnormalities in the form of lower FA have been reported in several chronic pain including migraine [367,431], temporomandibular disorder [310], complex regional pain syndrome (CRPS) [180], fibromyalgia [282], and persisting back pain [291]. However, higher FA has also been reported in patients with irritable bowel syndrome (IBS) [85]. The precise location of these abnormalities is variable between chronic pains and may be related to various factors such as disease duration or pain symptomology. For example, in one study that examined migraine patients with and without aura, it was reported that only patients that had migraine with auras had lower FA in their optic radiations, bilaterally [367]. Other studies have reported lower FA in WM adjacent to GM regions involved in experience of pain such as S1, M1, the cingulum, corpus callosum, and prefrontal WM regions [180,310,431]. In one study, significant negative correlations were found between FA in the WM adjacent to the ventrolateral PFC (vlPFC) and thalamus and pain intensity, suggesting a lower the FA is associated with the greater the pain intensity [310].
Additionally, this study further characterized the WM abnormalities by showing that the regions of lower FA also had higher MD and RD, but not AD compared to control participants. In another study, patients with sub-acute back pain were longitudinally followed so that patients whose back pain persisted were compared to those who recovered from their pain [291]. The patients whose back pain persisted had significantly lower FA in parts of the left superior longitudinal fasciculus and anterior limb of the internal capsule compared to those whose pain recovered, suggesting that these abnormalities occur with disease chronicity [291]. Given the unique pain symptoms of TN, it remains to be determined if cerebral WM abnormalities also occur in these patients.

2.5.2.2.2 Trigeminal Nerve

The most prevalent theory of TN etiology involves neurovascular compression (NVC) of the trigeminal nerve at the root entry zone (REZ) and associated damage. This has motivated a handful of studies to examine structural abnormalities in the trigeminal nerves of patients with TN using DTI derived metrics [174,200,259,283]. These studies extracted FA from the trigeminal nerves of patients and healthy control participants, with the majority of the studies [200,259,283] focusing on trigeminal REZ values. The results indicated that FA was significantly lower in the affected REZ of TN patients with NVC, however, FA can decrease in a number of scenarios [3]. While a few of these studies [174,259,283] also measured apparent diffusion coefficient (ADC), a quantitative measure of water motility in a voxel that is independent of orientation [348], the results were inconsistent, with only one study reporting differences between the affected and unaffected REZ values of TN patients [259].
As previously discussed, FA is often used as a quantitative biomarker of white matter (WM) “integrity.” However, other DTI metrics such as mean, radial, and axial diffusivities (MD, RD, AD) provide more information about the shape of the tensor in addition to factors underlying WM microstructure and pathology and could provide useful information as to why FA is decreased in TN. Another limitation of the aforementioned studies is the low number of diffusion gradients collected. For example, two of these studies collected diffusion data with only six gradient-directions [174,200], one study with 15 gradient-directions [283], and the other with 32 gradient-directions [259]. While only six noncollinear directions are required to estimate the diffusion tensor, many more images are usually required to boost the signal intensity-to-noise ratio (SNR) [317]. The rationale for sampling more directions is that it reduces orientational dependence and increases the accuracy and precision of estimating tensor-derived parameters such as FA [317]. For example, in one study that used Monte Carlo computer simulations, it was determined that at least 20 unique directions were necessary for a robust estimation of anisotropy [223]. Of the studies on TN patients described above, only one met this criterion [259]. Therefore, while some studies have used FA and ADC to examine structural abnormalities in the trigeminal nerves of TN patients, the interpretation of these findings is difficult, since other metrics were not examined and limitations were evident with the acquisition of the diffusion data.
2.5.2.3 Cellular Mechanisms Underlying MR-Detectable Differences in Brain Structure

While structural imaging techniques are sensitive enough to detect brain differences in GM and WM, they cannot directly inform us about the cellular events underlying the observed changes. There are a number of candidate mechanisms at the cellular and molecular level that may help explain the MR-detectable GM and WM abnormalities reported in chronic pains. Importantly, only a few histological studies have directly examined the mechanisms underlying MR-detectable structural changes in the brain, and these studies focused on learning (e.g., [53,264]). This is relevant since not all differences detected in patient populations compared to healthy controls are necessarily a direct consequence of pathophysiological mechanisms (as discussed in chapter 2.4.3). While abnormalities in MR measures are frequently reported in patient populations and are associated with certain pathophysiological mechanisms such as demyelination, inflammation, and edema [8,36], differences in structure may also reflect skill, knowledge, or cognitive specialization [491], which are relevant when considering the cognitive and learning aspects of pain.

One of the first studies to demonstrate a relationship between GM structure and cognitive specialization reported that expert taxi drivers had larger hippocampal volumes compared to control participants [284]. In this case, the larger hippocampal volumes were considered to be the result of experience-dependent plasticity that occurred to accommodate the extensive spatial maps these taxi drivers relied on to facilitate their navigation. Longitudinal studies have also demonstrated both GM [146,149] and WM [385] changes in brain structure following training on
a particular task (e.g., juggling), as indicated by increased GM density and higher FA following the training period.

Additionally, individual variations in brain structure may be pre-existing. For example, in WM, genetic factors can explain approximately 75-90% of the variation in FA in some large fibre bundles, particularly in the frontal and parietal lobes; however, the corpus callosum is one fibre bundle that appears to be more readily influenced by environmental factors [91]. So while structural brain abnormalities in patients with chronic pain may reflect central pathophysiological processes, they may also be due to pre-existing abnormalities, or other processes that can influence the cognitive and learning aspects of pain. These processes likely involve various mechanisms and different cell types, but ultimately, the only way to directly link imaging measures and the mechanisms underlying their changes is to conduct histological studies.

Potential cellular mechanisms underlying structural differences as detected by MRI have been broadly divided into two categories: neuronal and non-neuronal mechanisms [491]. In GM, neuronal mechanisms may include changes in neuronal size or number, synaptogenesis, dendritic branching, axon sprouting, synaptic pruning, neuronal cell death, while non-neuronal mechanisms include alterations in vasculature, or the size or numbers of glial cells (Fig. 2-21) [53,491]. It has been hypothesized that neurogenesis may account for observed increases in GM. Since neurogenesis is known to occur in the hippocampus of adults, an attractive hypothesis is that experience-dependent plasticity results in the production of more hippocampal neurons to
facilitate learning. In one study examining GM plasticity, mice were trained on different versions of a water maze task and MR-volume measurements were used to assess structural differences between groups [264]. The results indicated that the mice that relied on spatial cues to perform the task had hippocampal volumes increases, while the mice that had a cued version of the task had growth in the striatum. Histological analyses revealed that these volume increases correlated with GAP-43 (growth-associated protein-43) staining, a marker for axonal growth cones [264]. Importantly, no correlations were found between volume increases and neuronal size or number, suggesting that the MRI volume increases occurred with the remodeling of neuronal processes and not neurogenesis [264]. In general, it is unlikely that neurogenesis has a large role in MR-detected changes outside of the hippocampus, since in the adult brain, the generation of new neurons is limited to the dentate gyrus of the hippocampal formation and the subventricular zone and its projection to the olfactory bulb [175] [491]. Instead, other mechanisms such as changes in gliogenesis, synaptogenesis, dendritic arborization or more generally changes in the complexity of neuropil, and vascular changes may contribute to observed GM differences outside of these two regions. Some histological studies have reported changes in synaptogenesis and dendritic spine morphology with motor skill learning [238,241]. Others have demonstrated that synaptic changes persist for several weeks following motor training, but initial astrocytic growth declines in the absence of training [239]. These findings demonstrate that different cells respond differently to experience and that the responses can either persist for a certain period or be transient. Motor learning in the context of pain is important, since pain often results in limitations of movement or nocifensive behaviours to prevent pain or worsen it. For example, TN patients often restrict facial movements or chew with only one side of their mouth to avoid triggering pain. Like other motor skills, patients may get better at these nocifensive behaviours
over time. Increased GM density has been associated with cognitive specialization, as discussed above, which were considered to be the result of experience-dependent plasticity (e.g., dendritic branching or synaptogenesis) to accommodate the acquisition of the new task. There is also some evidence to support the contribution of vasculature changes, including interactions between neurons, capillaries, and glia, to MR-detectable GM differences, but these effects may be related to activity changes with training on a particular task and further research in this area is warranted [491].

The precise cellular mechanisms underlying pain-related MR-detectable GM changes are not yet established. With regard to cortical thinning, one possibility, as described in other patient populations, is that there are focal losses of cortical inhibitory interneurons, making these regions hyperactive [421]. Evidence to support alterations in cortical inhibition comes from studies using transcranial magnetic stimulation (TMS) on patients with chronic pain [29]. By applying magnetic pulses to the cortex of chronic pain patients and measuring peripheral muscle activity, TMS studies have demonstrated changes in cortical inhibition mediated by GABA receptors [29]. Although these studies have focused on the motor cortex, it is possible that similar mechanisms are taking place in other cortical regions and will be an avenue of future research [29]. Another possibility is that thinner cortex reflects pruning due to use-dependent synapse elimination, as associated with the development of cognitive abilities and behavior [435]. In this scenario, thinner cortex in regions such as the ACC, PFC, and insula could indicate an enhanced or more efficient system for processing the cognitive-affective dimension of pain, for example. Similarly, greater GM thickness or volume may occur because a given region is functioning ineffectively (i.e., no synaptic pruning), or because the region is functioning in overdrive to
process or counter-balance the barrage of nociceptive activity to the brain in chronic pains (e.g., more glial cells and blood vessels to accommodate increased activity).

**Figure 2-21:** Candidate cellular mechanisms underlying MR-detectable GM increases can be neuronal and include axon sprouting, dendritic branching and synaptogenesis, and neurogenesis (top panel) or non-neuronal such as alterations to glial cells and/or blood vessels (bottom panel). This figure is adapted from [491] and used with permission (license number: 3530371385572).

In WM, differences in fibre organization such as packing density, axonal sprouting/branching, axon diameter/number, crossing fibres, and myelin remodeling are potential neuronal
mechanisms underlying changes to WM microstructure, while other factors include altered astrocyte morphology and angiogenesis (Fig. 2-22) [36,55,491].

**Figure 2-22:** Mechanisms underlying WM changes include alterations in fibre organization (e.g., axon diameter, sprouting, branching, packing density), myelin formation, or changes in myelin thickness and morphology (top panel). Other non-neuronal factors include astrocyte changes and angiogenesis (bottom panel). This figure is adapted from [491] and used with permission (license number: 3530371385572).
Experiments using diffusion imaging methods to examine WM have proposed that changes in myelin could account for some of the differences observed in their studies. Altered myelin could provide a mechanism for the regulation of impulse conduction velocities in nociceptive transmission. Histological evidence to support the influence myelination on DTI-derived parameters comes from a study by Song and colleagues [420]. In this study, an experimental mouse model of demyelination was used to demonstrate that increased radial diffusivity (RD) was associated with the severity of demyelination in the corpus callosum. In addition, RD decreases were subsequently observed with remyelination [420]. Several other mechanisms underlying WM plasticity could also affect diffusion imaging measures. For example, axonal sprouting has been shown to occur following learning after the induction of long-term potentiation [4], and the pruning of axons can also occur to refine functional circuits [491]. Since some diffusion measures, such as axial diffusivity, are influenced by how tightly packed axons are within a voxel, differences in axonal sprouting or pruning could affect this metric. Similarly, there is evidence in support of the possible rebranching of fibres with experience [491]. Any changes in the route of fibre bundles can affect the directional preference of water diffusion and thus diffusion imaging measures.

In summary, only a handful of studies have directly examined the cellular mechanisms underlying macrostructural changes obtained using neuroimaging methods. Clearly there is a need for more integrative research between groups studying human populations using MRI and those taking histological approaches in animal models. While some candidate mechanisms can be extrapolated based on anatomical knowledge and findings from other literatures, an important question regarding the permanence of this plasticity, particularly in patient populations, remain.
In the next section, the effects of successful treatment for chronic pain on MR-detectable structural abnormalities will be discussed, showing that the maladaptive plasticity that has occurred in these cases is at least partially reversible.

2.5.3 Treatment Effects on Structural Brain Abnormalities in Chronic Pains

As described in the previous section, brain imaging has repeatedly demonstrated GM and WM differences in patients with chronic pain compared to healthy control subjects in regions that participate in the multi-dimensional experience of pain. However, an ongoing debate continues as to whether these abnormalities are pre-existing or if they develop as a consequence of being in chronic pain. Recent clinical studies (see below) have now reported at least a partial reversal of brain structural abnormalities following effective treatment [191,368,369,394], providing evidence that abnormalities in patient with chronic pain, in part arise as a consequence of being in pain.

Rodriguez-Raecke and colleagues [368] conducted one of the first studies to examine the effects of treatment on structural abnormalities in chronic pain patients. In this study, 32 patients with longstanding chronic pain due to hip osteoarthritis underwent MRI before total hip replacement surgery. Consistent with previous structural imaging studies of chronic pain, these patients had less GM in the ACC, insula, frontal regions (orbitofrontal cortex and dorsolateral prefrontal cortex (OFC, DLPFC)), amygdala, and brainstem, compared to controls. Following treatment, a
subset of 10 patients that were completely pain-free following surgery had a repeat MRI at approximately six weeks and four months post-surgery. While no significant changes in GM occurred from baseline to the first follow-up scan, significant GM increases in the ACC, DLPFC, insula, amygdala, and brainstem (towards the level of healthy controls) occurred when the first and second scans were compared to the four month follow-up. The authors concluded that pre-treatment GM abnormalities likely resulted from prolonged nociceptive input to the brain due to the primary hip osteoarthritis that altered brain morphology and/or cytoarchitecture. They reasoned that because the patients scanned at follow-up were completely pain-free, meaning there would no longer be a barrage of nociceptive input, and the brain regions would be able to normalize [368]. In a follow-up to this study, Rodriguez-Raecke longitudinally examined a total of 20 patients with chronic pain due to hip osteoarthritis that had scans up to one year post-surgery [369]. Similarly, they reported that patients had less GM compared to controls in the ACC, insula, and prefrontal cortices. When the patients were pain-free after recovery from surgery, a significant GM increase in the ACC, insula, and pars orbitalis of patients occurred. They concluded that GM abnormalities are not cause of chronic pain, but are secondary to the disease process [369].

A third study of patients with painful hip osteoarthritis used whole-brain VBM to examine GM [191]. Patients were scanned before and approximately nine months following hip arthroplasty. Before surgery patients had significantly less GM in the thalamus bilaterally, and significantly more GM in regions including the amygdala and insula, compared to controls [191]. After surgery, the thalamus was the only GM region to normalize towards the level found in healthy
controls. Importantly, this “reversal” of decreased thalamic GM volume was associated with decreased pain and increased function.

Another study used VBM to investigate 32 patients with posttraumatic headache 14 days and three months following whiplash injury [329]. Of these patients, 12 developed chronic headache at the three-month time point and underwent a third MRI one year-post-injury. At three months post-injury, the patients who developed chronic headache had decreased GM in the ACC and DLPFC. These abnormalities resolved at one year post-injury, when patients were no longer having headaches.

While the preceding studies used a VBM-approach, a more recent study [394] used CTA to examine 18 patients with chronic low back pain (CLBP) before and approximately six months after spine surgery or facet joint injections. The results indicated that before treatment, patients with CLBP had significantly thinner cortex in many regions including the left DLPFC, bilateral anterior insula, primary somatosensory cortex, and right ACC, compared to controls. Following treatment, 11 patients were classified as responders, as they had significant reductions in pain following treatment. After a strict correction for multiple comparisons, the left DLPFC was the only region found with significant thickening in patients towards the level of healthy controls. Functionally, activity in left DLPFC of patients during an attention-demanding task was abnormal before treatment, but normalized following treatment [394]. While the pre- to post-treatment thickness changes were not significant, increased thickness in the primary motor cortex was associated with reduced physical disability, and increased anterior insula thickness was
associated with reduced pain. Taken together, these data indicate that both structural and functional brain abnormalities in chronic pain patients are at least partially reversible following effective treatment for chronic pain, indicating that some of the cellular mechanisms underlying these MR-detectable changes occur secondary to the disease process and are not permanent.
Chapter 3
Rationale, Specific Aims, and Hypotheses

The general aim of this thesis was to determine if patients with TN have structural brain and trigeminal nerve abnormalities compared to healthy control participants, and if these abnormalities normalize following effective surgical treatment for TN. To determine this, MRI-based studies were conducted to assess gray and white matter abnormalities before and after surgery for TN. The rationale, specific aims, and hypotheses for each of these studies are provided below.

3.1 Study I: Cortical and Subcortical Gray Matter Abnormalities in Idiopathic Trigeminal Neuralgia

As described in Chapter 2, TN is characterized by highly intense pain in the distribution of one or more branches of the trigeminal nerve and is commonly associated with NVC of the trigeminal nerve. As such, TN is classified as a trigeminal neuropathic pain in contrast to a trigeminal neuropathy, which is associated with more constant pain and numbness of the skin or mucosal membranes in the distribution of the trigeminal nerve, and/or muscle weakness in the muscles involved in mastication [414]. Patients with chronic pains other than TN can have abnormal GM in brain regions associated with the sensory-discriminative and cognitive-affective dimensions of pain, pain modulation, emotion, and motor function [50,124,190,308], the latter of which may be related to alterations in muscular activity aimed at limiting movements to protect the system from further injury and support healing [280,340]. These GM abnormalities may be
pre-existing, or they may reflect maladaptive plasticity arising from long-term pain and/or nociceptive input to the brain [296], impaired pain modulation [387], and/or compensatory/nocifensive motor behaviors to avoid triggering pain [340]. In contrast to other neuropathic pains (e.g., trigeminal neuropathic pain), TN patients are without significant sensory abnormalities that may contribute to some of the GM abnormalities reported in other neuropathic pains. Examining GM abnormalities in TN provides an opportunity to study how neuropathic pain impacts central GM, without the confounds of other sensory abnormalities. Therefore, the main aim of this study was to determine if brain abnormalities occur in the brains of patients with TN and if they become progressively worse over time.

**Main Study Aim:** To determine if patients with TN have brain GM abnormalities.

**Specific Aims:**

1. To determine if TN is associated with GM abnormalities in brain regions, particularly those involved in the multi-dimensional experience of pain and its modulation
2. To determine the relationship between GM abnormalities and TN pain duration

**Specific Hypotheses:**

1. Compared to healthy controls TN patients will have:
a) GM increases in areas associated with pain perception and emotion (e.g., thalamus, primary and secondary somatosensory cortex (S1, S2), amygdala, anterior and mid-cingulate cortex (ACC; MCC), insula and prefrontal cortex (PFC);

b) GM decreases in areas associated with pain modulation (e.g., periaqueductal gray (PAG));

c) GM increases in motor regions (e.g., primary motor cortex (M1), basal ganglia); and
d) GM abnormalities that progress due to pain chronicity

3.2 Study II: Abnormal Trigeminal Nerve and Brain White Matter Microstructure in Idiopathic Trigeminal Neuralgia

The most prevalent theory of TN etiology is that neurovascular compression (NVC) of the trigeminal nerve at the root entry zone (REZ) [216,328], the location where the trigeminal nerve enters the brainstem, results in damage or focal myelin loss [137,201,278,293] which can disrupt normal nociceptive transmission. Although gross NVC is not always apparent with standard imaging, MRI-based diffusion tensor imaging (DTI) has been shown to be a useful tool for examining the trigeminal system in great detail [202,456]. Clinical studies using DTI have identified microstructural abnormalities, including decreased fractional anisotropy (FA), in the affected REZ of TN patients with NVC [200,259,283]. Although FA is often used as a quantitative biomarker of white matter (WM) “integrity,” other DTI metrics such as mean, radial, and axial diffusivities (MD, RD, AD) provide insight into factors underlying WM microstructure and pathology. Additionally, there are several cerebral WM tracts that allow for the transmission
of nociceptive information to GM structures involved in the many aspects of pain perception. It is not known if cerebral WM abnormalities occur in TN, a neuropathic pain disorder without significant sensory abnormalities, as they do following peripheral nerve injury and in other neuropathic pains that are often accompanied by abnormalities such as numbness [126,358].

**Main Study Aim:** To determine whether patients with TN have trigeminal nerve and/or brain WM abnormalities based on multiple DTI-derived metrics.

**Specific Aims:**

1. To better characterize trigeminal REZ abnormalities in TN patients by extracting the diffusion metrics MD, RD, and AD in addition to FA
2. To determine if abnormalities also occur in the brain WM of TN patients compared to healthy controls as measured by FA, MD, RD, and AD.

**Specific Hypotheses:**

1. In addition to abnormalities in FA, TN patients will also have abnormalities in MD, RD, and AD at their affected trigeminal REZ compared to their unaffected REZ and to values from healthy control participants. Specifically, higher MD, RD, and AD will characterize these abnormalities, which have been linked to pathophysiological mechanisms such as edema, demyelination, neuroinflammation (MD, RD), and nerve compression (AD) [8].
2. Patients with TN will have WM abnormalities in cerebral tracts involved in relaying information between GM structures involved in the sensory, cognitive-affective, and modulatory aspects of pain and motor function, many of which were identified as abnormal in Study 1. The WM abnormalities will be characterized by lower FA and higher MD, RD, and AD.

3.3 Study III: Brain Plasticity Following Effective Surgical Treatment for Idiopathic Trigeminal Neuralgia

Idiopathic TN is a severe neuropathic facial pain disorder commonly associated with neurovascular compression at the trigeminal REZ. It is a unique neuropathic pain in that patients are typically pain-free between attacks and without major sensory abnormalities. However, over time pain attacks may occur more frequently, be more sustained, and less responsive to medications leading patients to seek neurosurgical intervention for pain relief [328,489]. Unlike most severe chronic pain conditions, neurosurgical treatment of TN can be highly effective in reducing pain intensity by more than 75% in many patients [265,268]. Surgical treatments for TN target the trigeminal nerve at different anatomical sites with the goal of displacing the offending vessel compressing the trigeminal nerve at the REZ, as in microvascular decompression (MVD) surgery, or partially injuring the affected trigeminal nerve distal to the REZ to reduce nociceptive signaling, as in Gamma Knife radiosurgery (GKRS) [487,489]. In Studies 1 and 2 of this thesis, we identified several GM and WM abnormalities in the brains and trigeminal nerves of TN patients. While effective surgical treatment can reverse GM
abnormalities in some chronic pains [191,368,369,394], it remains to be determined if effective surgical treatment for TN depends on resolving structural abnormalities in the trigeminal nerve and/or GM of these patients.

**Main Study Aim:** To determine if effective neurosurgical treatment for TN is associated with a reversal of the trigeminal REZ and cortical and subcortical GM abnormalities that we report in Studies 1 and 2 of this thesis [132,134].

**Specific Aims:**

1. To determine if surgical treatment for TN is associated with a reversal of trigeminal REZ abnormalities such that they normalize towards the level of healthy controls.

2. To determine if the GM abnormalities we previously identified in TN patients normalize towards the level of healthy controls following effective surgical treatment for TN.

3. To determine if the degree of normalization of REZ and GM abnormalities is associated with the degree of pain relief achieved following surgery.

**Specific Hypotheses:**

1. Surgical treatment for TN will be associated with a reversal of REZ abnormalities towards the level of healthy controls for patients that have effective treatment only.

2. The GM abnormalities in TN patients that have effective surgical treatment will also normalize towards the levels of healthy controls.
3. The degree of normalization of the REZ and GM abnormalities will be associated with the degree of pain relief achieved, such that the greater the normalization, the greater the pain relief.
Chapter 4
General Methods

4.1 Overview of Project

The studies that comprise this thesis were based on brain imaging and clinical data obtained from patients seen at the Toronto Western Hospital for idiopathic TN. Patients with TN had a pre-treatment (baseline) brain imaging session, including structural MRI sequences to assess gray matter (study I) and white matter (study II). For study III, patients were included only if they had undergone brain imaging of gray and white matter both pre- and post- surgical treatment. For all studies, patient demographics and clinical details were obtained via retrospective chart reviews. All healthy control participants, recruited as part of a larger ongoing prospective study on healthy aging, had MRI sequences to assess gray and white matter structure at one time point.

4.2 Participants and Recruitment

Under the approval of the University Health Network Research Ethics Board, patient charts were reviewed to determine eligibility for this study. Patients were included if they had pre-treatment (baseline) MRI scans and met our inclusion and diagnostic criteria for TN as established by the International Headache Society Classification [165]. These criteria were: (1) unilateral (right-sided) pain, (2) intense, sharp, superficial or stabbing paroxysmal facial pain precipitated from trigger areas or by trigger factors; stereotyped attacks for each patient, (3) no clinically evident neurological or sensory deficit or not attributed to another disorder, and (4) no previous surgical procedures for TN. Patient demographic and clinical details were also obtained via chart reviews.
Recruitment of healthy control participants and the procedure whereby their consent was obtained was also approved by the University Health Network Research Ethics Board. Control participants were recruited as part of an ongoing study examining brain structure and healthy aging across the lifespan. Controls were excluded if they had any history of chronic pain, neurological or psychiatric disorder, and/or any contraindication to MRI scanning (e.g., claustrophobia, pacemaker, metallic foreign body). Each healthy control participant provided written informed consent.

4.3 MR Imaging

4.3.1 Data Acquisition

All patients had a pre-treatment imaging session that lasted approximately one hour. In addition to other scans acquired for clinical purposes, the imaging session included: 1) a high-resolution T1-weighted whole brain anatomical scan, and 2) a diffusion weighted imaging (DWI) scan. Controls participants underwent MRI including T1-weighted and DWI scans. For a subset of patients, the precise imaging session that was done at baseline (including the DWI scan) was repeated approximately 3-6 months post-treatment (Study III).

All brain imaging data were acquired using a 3T GE Signa HDx MRI system fitted with an eight-channel phased array head coil. Participants were placed in a supine position in the MRI
scanner. For comfort, participants were given earplugs to reduce scanner noise and also a “panic button,” which could be used to alert staff if they felt uncomfortable or needed to stop the scan.

For gray matter analyses, T1-weighted 3D FSPGR axial images were obtained for the entire head: (0.9 x 0.9 x 1.0 mm$^3$ voxels derived from a 256 x 256 matrix and field of view of 24 cm (controls) or 22 cm (patients), echo time= 5 ms, repetition time= 12 ms, inversion time= 300 ms).

For white matter analyses, 60-direction diffusion-weighted magnetic resonance images (DWI) were acquired for all participants using the following protocol: spin echo EPI sequence; in-plane voxel size 0.94 x 0.94; slice thickness= 3 mm; TE= 86.6 ms; TR= 12,000 ms; 1 B$_0$; b-value= 1,000 s/mm$^2$; matrix= 256 x 256; 1 excitation; ASSET.

4.3.2 Gray Matter Analyses

Two types of analyses were used to examine brain gray matter (GM). Cortical GM was measured using cortical thickness analysis (CTA) and subcortical GM was examined using a voxel-based morphometry (VBM) technique. For all GM analyses, group comparisons were restricted to masks of brain regions based on a priori hypotheses.

4.3.2.1 Cortical Thickness Analysis

The CTA was done using the FreeSurfer software v. 4.5.0 (http://surfer.nmr.mgh.harvard.edu/).
This surface-based pipeline automatically generates models of cortical GM with submillimeter accuracy [163], and consisted of several pre-processing stages that allow for group comparison. At the first stage, anatomical brain images of all participants were aligned to a standard space [109]. Specifically, an affine registration was used to align the T1-weighted scans of all participants to the Talairach atlas [434]. Next, images underwent intensity normalization to classify voxels into different tissue types (GM, white matter, cerebrospinal fluid). Intensity normalization is critical, as T1-weighted images are often corrupted by magnetic susceptibility artifacts and RF-field inhomogeneities, which can lead to differences in intensity and contrast across images, even within the same tissue type [109]. Thus, this step corrects for these intensity variations (termed “bias fields”), by measuring variations in white matter (WM) intensity to derive an estimated bias field, and then dividing the intensity of each voxel by this bias field to remove its effect [109]. The intensity-normalized images were then skull-stripped using a deformable template model [109,389] and cutting planes were computed to separate the left and right cerebral hemispheres, and the cortical from subcortical structures [109]. WM voxels were used to estimate the GM/WM boundary, which was covered with a triangular tessellation and deformed outward to the GM/cerebrospinal fluid (CSF) boundary. Thickness was computed as the distance measured between these boundaries at every point in each hemisphere (Figure 4-1). Data were smoothed with a 6mm full-width half-maximum (FWHM) spatial smoothing kernel, and submitted to statistical analysis to test for group differences.
Figure 4-1: Cortical thickness measurements were done by measuring the distance perpendicular from the GM/WM boundary (yellow line) to the GM/CSF boundary (red line), at each point of both hemispheres. This figure is reproduced from [163]. Copyright (2000) National Academy of Sciences, USA.

4.3.2.2 Voxel-Based Morphometry

The VBM [18] in FMRIB’s Software Library (FSL) v. 4.1.8 (http://www.fmrib.ox.ac.uk/fsl/) was used to examine subcortical GM. First, raw DICOM files were converted to NIfTI-1, since FSL requires files to be in this format. This was done using the dcm2nii function, part of the MRIcron software package (http://www.mccauslandcenter.sc.edu/mricro/mricron/dcm2nii.html). Next, the skull and other non-brain tissues were removed from T1-weighted images using the Brain Extraction Tool (BET) [415]. GM was segmented using the FMRIB’s Automated Segmentation Tool (FAST4) [493]. The GM tissue was aligned to the Montreal Neurologic Institute (MNI) 152 standard 2 mm template using a linear registration tool [218]. Images were averaged creating a study-specific template, to which GM images were non-linearly re-registered. As some brains were either contracted or expanded in this process, Jacobian modulation was used, which
involves scaling the voxel concentration by the amount of contraction or expansion that was required, so that the total amount of GM remains the same as in original images. This allowed for group differences in GM to be reported as normalized volumes. The modulated images were then smoothed with an isotropic Gaussian kernel with a sigma of 2mm (FWHM= 4.6mm). For consistency with the CTA, VBM results were converted from MNI to Talairach coordinates using the Yale Nonlinear MNI to Talairach Conversion Algorithm [255] implemented on the BioImage Suite 2.0 website (http://www.bioimagesuite.org/Mni2Tal/).

4.3.3 White Matter Analysis

For all WM analyses, the DWI scans underwent standard preprocessing steps using the FMRIB Diffusion Toolbox (FDT), part of FSL v.4.1.8 (http://www.fmrib.ox.ac.uk/fsl). To use this toolbox, scans were required to be in NIfTI-1 format, so the diffusion-weighted DICOM images could be converted using the dcm2nii function, part of the MRIcron software package (http://www.mccauslandcenter.sc.edu/mricro/mricron/dcm2nii.html). All diffusion data were corrected for eddy current-induced distortions and participant head movement artifacts. This was done by using an affine registration to align the images to a non-diffusion reference volume (b0) collected at the beginning of each scan. Next, brains were extracted from non-brain tissues using the Brain Extraction Tool [415] and FA maps were created for each subject by fitting a tensor model to the raw diffusion data using DTIFIT in the FDT toolbox. As FA changes can occur in multiple scenarios, non-FA (RD, AD, and MD) maps were also created to provide more information about the shape of the tensor and possible pathophysiological mechanisms (e.g., demyelination, neuroinflammation, and edema) underlying abnormalities on these metrics.
4.3.3.1 Root Entry Zone Microstructural Analysis

For each participant, values for all DTI-derived metrics (FA, RD, AD, and MD) were extracted from both the left and right trigeminal nerves at the root entry zone (REZ). The REZ is the cisternal part of the trigeminal nerve just as it enters the pons [228]. It is an important region because it is believed that TN pain results from focal damage to the myelin at the REZ by neurovascular compression. The trigeminal nerves were identified in the axial plane using color orientation maps created by overlaying the principle eigenvector image (V1) over the FA map. The trigeminal nerves were visualized in the pontine cisternal space for each patient. To obtain the DTI-derived values, masks four voxels in size were manually placed at each REZ (for mask location, refer to chapter 6, Fig. 6-2 A) in the axial plane, and the metrics were extracted and submitted to statistical analysis.

4.3.3.2 Whole Brain Group WM Analysis using Tract-Based Spatial Statistics

Tract-Based Spatial Statistics (TBSS) [416] was used to compare brain FA between the TN and healthy control groups (results in Chapter 6). Following preprocessing, FA data underwent nonlinear registration to a 1 x 1 x 1 mm FA map in standard space (FMRIB58_FA) using a b-spline representation of the registration warp field [373]. The mean FA image created (thresholded at 0.2) was thinned to create a mean FA skeleton that represented the centers of all tracts common to the group (Figure 4-2). Each subject's aligned FA data were projected onto this skeleton and the resulting data underwent voxelwise cross-subject statistics. In the areas where
FA was determined to be significantly different in patients compared to controls, a region of interest analysis was used to test for focal WM alterations in RD, AD, and MD. The non-FA images used for TBSS were created by applying the FA nonlinear registration to the additional parametric maps, and projecting them onto the original mean FA skeleton. WM tracts with significant microstructural abnormalities were identified using the JHU ICBM-DTI-81 white-matter labels atlas [312] and the JHU White Matter Tractography Atlas [208], both included in FSL.

**Figure 4-2:** For TBSS, a mean skeleton (1 voxel in thickness) of the white matter tracts common to the group was created. An example of this skeleton is shown in axial (left), coronal (centre), and sagittal (right) views.
5.1 INTRODUCTION

Idiopathic trigeminal neuralgia (TN) is a chronic neuropathic pain disorder characterized by recurring highly intense electric shock-like pain in the distribution of one or more branches of the trigeminal nerve. The pain is usually unilateral, occurring either spontaneously or precipitated by innocuous sensory stimuli and movements. Although some sensory abnormalities have been reported in TN using detailed quantitative sensory testing [286,410], there is typically no major sensory loss detectable with bedside clinical tests, as observed in other neuropathic pains [286,405]. For example, in a large multi-center study examining somatosensory abnormalities in different neuropathic pain syndromes, typical sensory abnormalities in TN were
mechanical hyperalgesia without sensory loss; an uncommon combination in all other syndromes examined [1].

Many theories have been proposed to explain the unique pain characteristics of idiopathic TN. In general, theories of TN focus on either central or peripheral nervous system pathogenesis [406]. Given that patients with TN are typically initially responsive to anticonvulsant medications, it has been suggested that the pain paroxysms characteristic of TN originate in the central nervous system and are analogous to epileptic seizures in brainstem trigeminal structures [4,358]. Alternatively, proponents of a peripheral etiology suggest that TN results from damage to the trigeminal nerve or its myelin, with the most prevalent theory proposing neurovascular compression of the trigeminal nerve root entry zone (REZ) by a nearby arterial branch [405]. Although neurovascular contact (NVC) or compression of the trigeminal nerve is frequently observed, a significant number of idiopathic TN cases do not demonstrate clear evidence of neurovascular compression [306]. As the putative pathogenesis of TN is unique, so are its clinical features. While tumors or other structural abnormalities can result in the perception of a trigeminal neuropathic pain, characterized by persistent pain and sensory changes, idiopathic TN patients are mostly pain-free between attacks and without major sensory abnormalities [430]. Since NVC or compression alone does not fully explain this unique syndrome, central abnormalities may significantly contribute to TN pain [72,172].

Patients with chronic pains other than TN can show abnormal gray matter (GM) in brain regions associated with pain and its modulation, sensory-discriminative and cognitive-affective
dimensions of pain, emotion, and motor function [50,190,308], the latter of which may be related to alterations in muscular activity aimed at limiting movements to protect the system from further injury and support healing [280,340]. Therefore, GM abnormalities may reflect maladaptive plasticity arising from long-term pain and/or nociceptive input to the brain [296], impaired pain modulation [387], and/or compensatory/nocifensive motor behaviors to avoid triggering pain [340]. Importantly, while most chronic pain syndromes are also accompanied by prominent sensory deficits, TN is predominantly characterized by pain, but not major sensory loss [286]. This provides a unique window to our understanding of how pain impacts central GM. GM abnormalities may be dynamic, progressive, and pain-driven over time [50,308,482]. Based on the described studies and in consideration of the unique features of neuropathic pain in TN, we hypothesized that compared to healthy controls TN patients would have GM (i) increases in areas associated with pain perception and emotion (e.g., thalamus, primary and secondary somatosensory cortex (S1, S2), amygdala, anterior and mid-cingulate cortex (ACC; MCC), insula and prefrontal cortex (PFC), (ii) decreases in areas associated with pain modulation (e.g., periaqueductal gray (PAG)), (iii) increases in motor regions (e.g., primary motor cortex (M1), basal ganglia), and (iv) abnormalities that progress due to pain chronicity.

5.2 METHODS

5.2.1 Ethics Statement

The University Health Network Research Ethics Board approved this retrospective study of idiopathic TN patients. There was no active participation in this study by patients, as patient data
were analyzed retrospectively. Additionally our Research Ethics Board provides study approval, but does not require individual patient consent for retrospective studies. All scans were anonymized prior to analysis and stored in secure databases. Recruitment of healthy control subjects and the procedure whereby their consent was obtained was also approved by the University Health Network Research Ethics Board. Each healthy control participant provided written informed consent.

5.2.2 Participants

Twenty-four right-handed idiopathic TN patients from the Toronto Western Hospital seen between May 2008 and February 2011 were included in the study. Inclusion criteria were: (1) unilateral (right-sided) pain involving at least the maxillary or mandibular (V2 or V3) branches of the trigeminal nerve, (2) intense, sharp, superficial or stabbing paroxysmal facial pain precipitated from trigger areas or by trigger factors; stereotyped attacks for each patient; no clinically evident neurological or sensory deficit or not attributed to another disorder [165], and (3) no previous surgical procedures for TN. Demographic and clinical details for patients were obtained via retrospective chart reviews. Patients were sex-matched to a cohort of 24 healthy pain-free control participants and the mean ages between the groups were not statistically different ($p= 0.81$, see results). At the individual level, all but 2 control participants were within 3 years of the patients.
5.2.3 Imaging Protocol

Brain imaging data were acquired using a 3T GE Signa HDx MRI system fitted with an eight-channel phased array head coil. T1-weighted 3D FSPGR axial images were obtained from the top of the head to the upper cervical levels of C1-C2 (0.9 x 0.9 x 1.0 mm³ voxels derived from a 256 x 256 matrix and field of view of 24cm (controls) or 22cm (patients), echo time= 5 ms, repetition time= 12 ms, inversion time= 300 ms).

5.2.4 Cortical Thickness Analysis

To examine cortical GM, cortical thickness analysis (CTA) was performed using FreeSurfer software v. 4.5.0 (http://surfer.nmr.mgh.harvard.edu/); methods have been described in detail elsewhere [163]. Briefly, T1-weighted scans were registered to the Talairach atlas [434]. Images underwent intensity normalization to identify tissue types, and the skulls were removed. White matter (WM) voxels were used to estimate the GM/WM boundary, which was deformed outward to the GM/cerebrospinal fluid (CSF) boundary. Thickness was computed as the distance measured between these boundaries at every point in each hemisphere. A general linear model (GLM) was used to evaluate group differences, with age included as a variable of no interest. We restricted analyses to a mask including our hypothesized regions of interest (ROI) (Fig 5-1, A-D) created with a predefined cortical parcellation scheme (aparc2005) implemented in FreeSurfer [164]. Data were smoothed with a 6mm full-width half-maximum (FWHM) spatial smoothing kernel, and thresholded at a corrected $p< 0.05$ (from a combination of an uncorrected image-wide $p$-value of 0.01, and a cluster threshold of 183 and 188 contiguous vertices for left and right hemispheres, respectively, based on Monte Carlo simulations with 5000 iterations (AlphaSim,
implemented in Analysis of Functional NeuroImages (AFNI) (http://afni.nimh.nih.gov/afni), as done previously [50,308,437].

5.2.5 Voxel-Based Morphometry

To examine subcortical GM group differences, we used voxel-based morphometry (VBM) [18] in FMRIB’s Software Library v. 4.1.8 (http://www.fmrib.ox.ac.uk/fsl/). Non-brain tissues were removed from structural images using the Brain Extraction Tool (BET) [415]. Tissue-type segmentation classified tissue using FMRIB’s Automated Segmentation Tool (FAST4) [493]. The GM-classified tissue was aligned to the Montreal Neurologic Institute (MNI) 152 standard 2 mm template (voxel size= 2 x 2 x 2 mm) using a linear registration tool [218]. Images were averaged creating a study-specific template, to which GM images were non-linearly re-registered. Jacobian modulation allowed group differences in GM to be reported as normalized volumes. The modulated images were then smoothed with an isotropic Gaussian kernel with a sigma of 2mm (FWHM= 4.6mm). Using the Harvard-Oxford subcortical structural atlas, a mask was constructed to restrict analyses to the thalamus, amygdala, hippocampus, basal ganglia, and brainstem (Fig 5-1, E-G). A voxelwise GLM was applied with age as a variable of no interest. Statistical significance was determined using permutation-based non-parametric testing and thresholded at $p<0.05$ using Randomise, implemented in FSL, corrected for multiple comparisons with threshold-free cluster enhancement [417]. For consistency with the CTA, VBM results were converted from MNI to Talairach coordinates using the Yale Nonlinear MNI to Talairach Conversion Algorithm [255] implemented on the BioImage Suite 2.0 website (http://www.bioimagesuite.org/Mni2Tal/).
We performed a secondary analysis to determine if prolonged nociceptive activity (i.e., pain duration) was related to GM changes. In this analysis, TN duration was treated as a regressor of interest. Analyses were restricted to the CTA and VBM masks described above. The same statistical correction methods were used to determine significant correlations between GM and pain duration.

5.3 RESULTS

5.3.1 Patient Demographics

Patient demographic information is shown in Table 5-1. The TN group (n= 24) was comprised of 15 women and 9 men (mean age ± SD: 48.5 ± 12.7 years) who had right-sided TN pain for 6.3 ± 3.0 years with a mean age at pain onset of 43.0 ± 12.9 years. The majority of patients were on medications for TN at the time of scan acquisition, with the most common being carbamazepine (Table 5-1). The healthy control group consisted of 15 women and 9 men with a mean age ± SD of 47.6 ± 12.3 years, which was not significantly different (p= 0.81) from the patient group.

5.3.2 Regions of Abnormal Cortical Thickness in TN Patients

All CTA results are provided in Table 5-2 and prominent findings are illustrated in Figure 5-2. Compared to controls, TN patients had 14-16% greater cortical thickness within three regions: the left (contralateral to side of pain) S1 including the putative face area (Fig 5-2 D, inferior S1
cluster), frontal pole (FP) (BA 10) bilaterally, and M1 bilaterally. CTA also revealed several
cortical regions that were 10-19% thinner compared to controls. The most prominent areas of
thinning were located bilaterally in the pregenual ACC (pgACC) (BA 24) and the ventral
orbitofrontal cortex (OFC) (BA 11) (Fig 5-2, A & C). Cortical thinning was also observed in the
right dorsal posterior insula (dpINS), ventral anterior insula (aINS) (Fig 5-2 B), and posterior
cingulate cortex (PCC).

5.3.3 Larger Subcortical Volumes in TN Patients

Subcortical regions that had significantly different GM volumes in the TN patients compared to
controls are listed in Table 5-3. Although the ventral pons had significantly less GM volume in
the TN group compared to controls, for all other subcortical regions that were significantly
different between groups, patients had larger volumes. The TN group had greater GM volumes
than controls bilaterally in the several thalamic regions that spanned the medial dorsal (MD)
nucleus, the ventral posteromedial (VPM) nucleus (Fig 5-3 A), the pulvinar, and the ventral
lateral (VL) nucleus. The TN group also had greater GM than controls in the right amygdala (Fig
5-3 B), an increase of 13%, and in a cluster spanning the nucleus accumbens (NAc), caudate, and
anterior putamen (19% increase) (Fig 5-3 C). Bilaterally, a substantially greater volume of the
posterior putamen (average increase of 38%) and in the PAG (increase of 27%) (Fig 5-3, D & E)
was observed in the TN group compared to the control group.
5.3.4 No Correlation between Gray Matter Abnormalities and Pain Duration

TN pain duration was not significantly related to cortical thickness or subcortical volume for any region within the CTA and VBM masks, nor within areas of GM abnormality when specifically examined ($p > 0.05$).
**Table 5-1**: TN patient demographic information

<table>
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<th>Sex</th>
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<th>Pain Duration (years)</th>
<th>Age at Pain Onset</th>
<th>Medication</th>
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</tr>
<tr>
<td>R17</td>
<td>M</td>
<td>24</td>
<td>V3</td>
<td>2</td>
<td>22</td>
<td>CBZ, TCA-N</td>
</tr>
<tr>
<td>R18</td>
<td>M</td>
<td>38</td>
<td>V2</td>
<td>2</td>
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</tr>
<tr>
<td>R19</td>
<td>F</td>
<td>42</td>
<td>V2, V3</td>
<td>2</td>
<td>40</td>
<td>CBZ, GBP</td>
</tr>
<tr>
<td>R20</td>
<td>F</td>
<td>52</td>
<td>V1, V2</td>
<td>3</td>
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<td>CBZ</td>
</tr>
<tr>
<td>R21</td>
<td>F</td>
<td>27</td>
<td>V2, V3</td>
<td>13</td>
<td>14</td>
<td>CBZ, GBP</td>
</tr>
<tr>
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<td>V3</td>
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<td>50</td>
<td>CBZ</td>
</tr>
<tr>
<td>R23</td>
<td>M</td>
<td>62</td>
<td>V1, V2, V3</td>
<td>3</td>
<td>59</td>
<td>PGB</td>
</tr>
<tr>
<td>R24</td>
<td>F</td>
<td>60</td>
<td>V2</td>
<td>6</td>
<td>54</td>
<td>CBZ</td>
</tr>
</tbody>
</table>

Note: -- indicates information not available

Abbreviations: Pain Dist: pain distribution, referring to the peripheral branches of the trigeminal nerve (V1: ophthalmic branch; V2: maxillary branch; V3: mandibular branch); CBZ: carbamazepine; PGB: pregabalin; GBP: gabapentin; TCA: tricyclic antidepressant; VPA: valproic acid; TCA-N: nortriptyline
Table 5-2: Cortical thickness abnormalities in TN

<table>
<thead>
<tr>
<th>Hemis</th>
<th>Region</th>
<th>BA</th>
<th>Peak Coordinates (TAL)</th>
<th># Vert</th>
<th>Peak T-Score</th>
<th>% Change (cf. Controls)</th>
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<tbody>
<tr>
<td>Right</td>
<td>FP</td>
<td>10</td>
<td>X Y Z</td>
<td>770</td>
<td>9.12</td>
<td>16.0 incr</td>
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<tr>
<td></td>
<td>OFC</td>
<td>11</td>
<td>14 20 -13</td>
<td>418</td>
<td>4.86</td>
<td>-17.2 decr</td>
</tr>
<tr>
<td></td>
<td>pgACC</td>
<td>24</td>
<td>8 29 -9</td>
<td>203</td>
<td>2.57</td>
<td>-13.2 decr</td>
</tr>
<tr>
<td></td>
<td>PCC</td>
<td>23</td>
<td>7 -39 30</td>
<td>305</td>
<td>2.85</td>
<td>-9.5 decr</td>
</tr>
<tr>
<td></td>
<td>S. temp</td>
<td>42</td>
<td>39 -31 12</td>
<td>295</td>
<td>3.55</td>
<td>-12.3 decr</td>
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<tr>
<td></td>
<td>dpINS</td>
<td>32</td>
<td>-20 16</td>
<td>362</td>
<td>4.46</td>
<td>-12.9 decr</td>
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<tr>
<td></td>
<td>aINS</td>
<td>41</td>
<td>-10 -12</td>
<td>206</td>
<td>3.15</td>
<td>-12.3 decr</td>
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<tr>
<td></td>
<td>M1</td>
<td>4</td>
<td>25 -13 64</td>
<td>205</td>
<td>3.32</td>
<td>12.1 incr</td>
</tr>
<tr>
<td>Left</td>
<td>FP</td>
<td>10</td>
<td>-32 52 -11</td>
<td>680</td>
<td>6.59</td>
<td>15.8 incr</td>
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<tr>
<td></td>
<td>OFC</td>
<td>11</td>
<td>-14 20 -14</td>
<td>490</td>
<td>6.38</td>
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<td>pgACC</td>
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<td>261</td>
<td>3.41</td>
<td>-18.8 decr</td>
</tr>
<tr>
<td></td>
<td>S. temp</td>
<td>42</td>
<td>-40 -32 7</td>
<td>232</td>
<td>5.50</td>
<td>-12.6 decr</td>
</tr>
<tr>
<td></td>
<td>S1</td>
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<td>-54 -17 34</td>
<td>232</td>
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<td>15.5 incr</td>
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<tr>
<td></td>
<td>S1</td>
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<td>-47 -19 55</td>
<td>400</td>
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<td>14.2 incr</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>4</td>
<td>-35 -11 55</td>
<td>223</td>
<td>4.06</td>
<td>13.7 incr</td>
</tr>
</tbody>
</table>

Abbreviations: TN: trigeminal neuralgia; Hemis: hemisphere; BA: Brodmann Area; TAL: Talairach; # Vert: number of vertices; ipsi: ipsilateral to side of pain; contra: contralateral to side of pain; GM: gray matter; OFC: orbitofrontal cortex; PCC: posterior cingulate cortex; pgACC: pregenual anterior cingulate cortex; S. temp: superior temporal cortex; MI: primary motor cortex; S1: primary somatosensory cortex; FP: frontal pole; dpINS: dorsal posterior insula; aINS:
anterior insula; % Change: percent thickness change; Change *cf.* Controls: direction of change compared to controls.
Table 5-3: Subcortical volume abnormalities in TN

<table>
<thead>
<tr>
<th>Region</th>
<th>Peak Coordinates (TAL)</th>
<th># Voxels</th>
<th>Peak T-Score</th>
<th>% Change</th>
<th>Change (cf. Controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X  Y  Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>5  -9  3</td>
<td>518</td>
<td>5.52</td>
<td>18.4</td>
<td>incr</td>
</tr>
<tr>
<td>R NAc/Caud/ Putamen</td>
<td>7  5  -5</td>
<td>89</td>
<td>4.83</td>
<td>18.6</td>
<td>incr</td>
</tr>
<tr>
<td>L Post. Putamen</td>
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<td>64</td>
<td>7.42</td>
<td>41.2</td>
<td>incr</td>
</tr>
<tr>
<td>R Post Putamen</td>
<td>29  -21  5</td>
<td>60</td>
<td>5.00</td>
<td>34.2</td>
<td>incr</td>
</tr>
<tr>
<td>R Amygdala</td>
<td>23  -5  -12</td>
<td>19</td>
<td>4.92</td>
<td>13.2</td>
<td>incr</td>
</tr>
<tr>
<td>PAG</td>
<td>4  -33  -6</td>
<td>19</td>
<td>5.93</td>
<td>27.3</td>
<td>incr</td>
</tr>
<tr>
<td>Pons</td>
<td>9  -26  -34</td>
<td>561</td>
<td>4.49</td>
<td>-84.9</td>
<td>decr</td>
</tr>
</tbody>
</table>

Abbreviations: TN: trigeminal neuralgia; TAL: Talairach; # Voxels: number of voxels; R: right; L: left; NAc: nucleus accumbens; Post: posterior; PAG: periaqueductal gray; % Change: percent thickness change; Change cf. Controls: direction of change compared to controls.

Note- voxel size for VBM analysis= 2 x 2 x 2 mm.
Figure 5-1: Analyses were restricted to gray matter masks. CTA and VBM gray matter analyses were restricted to masks. (A-D) Cortical thickness masks for the left (A & B) and right (C & D) hemispheres, including lateral (A & C) and medial (B & D) views. (E-G) VBM masks of subcortical structures in coronal (E), sagittal (F) and axial (G) views. This figure has been reproduced from [131]. Copyright 2013 DeSouza et al.
**Figure 5-2:** Cortical thickness abnormalities in trigeminal neuralgia patients. CTA revealed significant group differences in several cortical brain regions. Red clusters indicate thinner cortex in patients compared to controls ($p<0.05$, corrected). Prominent findings of cortical thinning in TN are shown in panels A-C, including graphs of mean cortical thickness values ± SEM (in mm): controls (black bars), patients (red bars). Areas of cortical thickening in TN are highlighted in panel D. Thinner cortex in TN was observed in: (A) the bilateral pgACC; graph illustrates thickness for left cluster; (B) the right insular cortex including the dpINS and the ventral aINS, and (C) the bilateral ventral OFC; graph illustrates thickness for right OFC cluster. TN patients had thicker cortex in (D) the bilateral FP and M1, and contralateral (left) S1; graphs illustrate thickness for right FP cluster and the inferior S1 cluster (putative face area).

Abbreviations: LH= left hemisphere; RH= right hemisphere; R= right; L= left; pgACC= pregenual anterior cingulate cortex; PCC= posterior cingulate cortex; aINS= anterior insula; dpINS= dorsal posterior insula; OFC= orbitofrontal cortex; FP= frontal pole; M1= primary motor cortex; S1= primary somatosensory cortex. This figure has been reproduced from [131].

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Figure 5-3: Regions of greater subcortical volume in patients with trigeminal neuralgia. VBM analysis revealed significant group differences in several subcortical brain regions. Significant
results ($p<0.05$, corrected) are displayed on the MNI152 (2 mm) T1 brain template. Blue clusters indicate greater GM volume in patients compared to controls. Graphs of normalized GM volumes ± SEM are shown to the right of brain images: controls (black bars), patients (red bars). Increased patient GM volume was observed in: (A) the sensory thalamus, including the MD and VPM thalamus bilaterally; (B) the right amygdala; (C) a cluster spanning the right nucleus accumbens, anterior putamen and caudate; (D) the posterior putamen bilaterally; (E) the PAG (green box shows magnified region). Abbreviations: R= right; L= left; MD= medial dorsal nucleus (thalamus); VPM= ventral posterior medial nucleus (thalamus); NAc= nucleus accumbens; PAG= periaqueductal gray. This figure has been reproduced from [131]. Copyright 2013 DeSouza et al.
5.4 DISCUSSION

In this study, we provide novel evidence that TN, a neuropathic pain thought to arise from a peripheral event, characterized primarily by pain and the absence of major sensory loss is associated with pronounced alterations in brain GM. These abnormalities occur in neuroanatomical regions that contribute to sensory-discriminative and cognitive-affective dimensions of pain, pain modulation, and motor function. Specifically, we identified that compared to controls, TN patients have: (1) greater GM in the thalamus, contralateral S1 (putative face area), amygdala, FP, PAG, M1, and basal ganglia (including the putamen and NAc), and (2) cortical thinning in the OFC, pgACC, and insula. Abnormalities in these brain regions have been reported in other chronic pain disorders [124]. However, in previous studies, the contributions of sensory loss and pain are often intertwined. Our findings raise the possibility that the CNS contributes to the development and/or maintenance of TN pain and help advance our knowledge of how a peripheral event is associated with central gray changes.

Potential mechanisms underlying MR-detectable GM differences have been broadly divided into two categories: neuronal and non-neuronal mechanisms, including alterations in vasculature, the size or numbers of glia and/or dendritic spines/branches [53,491]. Evidence for the activity-dependent alteration of these structures has been demonstrated as a result of experience and learning, with the majority of studies reporting non-neuronal mechanisms as the primary contributor to GM differences in regions outside of the hippocampus [491]. GM abnormalities in TN patients may also occur via these mechanisms, elicited by experience-dependent factors such as changes in nociceptive input to the CNS, affect, and learned compensatory/nocifensive motor
behaviors. Alternatively, it is possible that some individuals are more susceptible to developing chronic pain because of pre-existing GM abnormalities [124]. This possibility cannot explicitly be tested in a cross-sectional study. In the current study, we did not find any evidence to support this possibility based on assessing disease duration-related GM abnormalities. However, we cannot totally rule out the contribution of some pre-existing functional or structural vulnerability as was found in other chronic pain studies [309].

Consistent with our *a priori* hypothesis, we found that TN patients had more GM in the thalamic nuclei compared to controls. Although we included patients with only right-sided pain, the greater thalamic volume was found bilaterally. This bilateral finding is in line with a previous functional MRI study on TN patients that showed bilateral thalamic activity when unilateral stimuli were used to evoke pain attacks [57]. Also, certain thalamic neurons, including MD neurons, have been shown to have large, bilateral receptive fields [101], which may in part account for this finding. Previous studies of chronic facial pain have reported mixed findings with regard to patient thalamic volume relative to controls [190,308,482]. This variability may be due to the heterogeneity of pain symptomology between chronic facial pains [307]. For example, patients with trigeminal neuropathic pain have constant pain accompanied by numbness [328]. Indeed, one study examining different chronic facial pains found that only trigeminal neuropathy patients had significantly smaller thalamic volume compared to controls [190]. It is possible that this difference reflects a system that is steadily receiving nociceptive, but not discriminative touch input, fitting with trigeminal neuropathy symptomology. However, a barrage of nociceptive input, in combination with mostly intact discriminative touch sensations (i.e., no numbness), may induce GM increases in the medial and lateral thalamic nuclei of TN patients.
Similarly, this theory may account for our finding of a thicker putative face S1 in TN. The barrage of nociceptive input to the thalamus from the trigeminal nerve may in turn lead to activity-dependent plasticity in S1 [473] via thalamocortical projections. Indeed, repeated noxious stimulation in healthy individuals can increase S1 GM volume [438]. With pain chronicity, thicker S1 cortex, particularly of the contralateral putative face region, may reflect enhanced intensity and localization of nociceptive information from the face. Increased S1 GM has previously been reported in chronic pain disorders involving the trigeminal system including migraine, temporomandibular disorder (TMD), and trigeminal neuropathic pain [117,119,308].

We further observed that TN patients had thinner cortex in the pgACC, aINS, and OFC compared to controls, a finding that has been reported in numerous chronic pain conditions [124]. These regions have been implicated in aspects of the cognitive-affective dimension of pain as well as other functions including attention, salience, interoception, and top-down mechanisms such as placebo analgesia, and pain modulation [387]. The pgACC is thought to contribute to pain unpleasantness, salience, and the regulation of emotional information [124], while the aINS has been implicated in salience as well as pain intensity and anticipation, and negative emotions such as anxiety [468]. The role of the OFC includes sensory, pain, and emotion regulation [490]. Less GM in these regions may then reflect the high degree of pain unpleasantness and/or emotional responses to TN or chronic pain in general, as similar findings have been reported in several chronic pain populations [124].
The mechanism of pain-related cortical thinning is not yet established. One possibility, as described in other patient populations, is that there are focal losses of cortical inhibitory interneurons, making these regions hyperactive [421]. In the chronic pain literature, evidence to support alterations in cortical inhibition comes from studies using transcranial magnetic stimulation (TMS) on patients with chronic pain [29]. By applying magnetic pulses to the cortex of chronic pain patients and measuring peripheral muscle activity, TMS studies have demonstrated changes in cortical inhibition mediated by gamma aminobutyric acid (GABA) receptors [29]. Although these studies have focused on the motor cortex, it is possible that similar mechanisms are taking place in other cortical regions and will be an avenue of future research [29]. An alternate explanation is that thinner cortex reflects pruning due to use-dependent synapse elimination, as associated with the development of cognitive abilities and behavior [435]. Thus, thinner cortex in these regions could indicate an enhanced or more efficient system for processing the affective dimension of pain.

Interestingly, the FP was thicker in TN patients; a finding also reported in TMD [308]. The precise function of the FP is not well understood, but it has been implicated in a number of complex cognitive functions including multitasking, monitoring, and evaluating expected outcomes [453]. It is possible that the thicker cortex in the FP may occur because there is a greater cognitive load associated with having TN. Future behavioral studies are thus needed to determine the role of cognitive factors on the structural abnormalities reported in the current study.
Similar to findings in migraine [366], another paroxysmal pain disorder, we report greater PAG volume in TN patients. It has been proposed that the descending pain modulatory system is altered in chronic pain such that there is either dysfunction in nociceptive inhibition, or enhancement in nociceptive facilitation [387]. Previous animal research has shown that PAG stimulation inhibits trigeminal nociceptive signals, suggesting that PAG abnormalities may lead to the disinhibition of trigeminal afferents [240]. Greater PAG volume may reflect activity-dependent increases in modulation because the system is ineffective at inhibiting nociceptive signals, or because there is a need to counter-balance the barrage of nociceptive input from the trigeminal nerve. Additionally, pain modulation can be influenced by several psychological factors such as attention, emotion, placebo, and anticipation via connections to cortical regions including the ACC, insula, and PFC and subcortical regions such as the amygdala [387]. This combined with a larger PAG volume may reflect differences in the psychological modulation of TN pain. In our study, we show these regions to be abnormal in TN patients. Additionally, and consistent with our results, there is right-lateralized noxious-evoked amygdala activity, possibly related to the emotional response to pain and pain modulation [219]. Although evidence for pain-related lateralization is controversial, right hemispheric lateralization using functional neuroimaging techniques has also been documented in several brain areas, regardless of the side of stimulation [93].

Consistent with our hypothesis, we report increased GM in motor regions. One characteristic of TN is that normally non-painful movements can elicit attacks of pain, so some patients attempt to restrict facial movements. This restriction has been observed in other facial pains such as TMD [340]. The Pain Adaptation Model [280,340] proposes a framework for nocifensive behaviors.
Specifically, pain leads to alterations in muscular activity aimed at limiting movements of an affected muscle by redistributing function and load. In the short term, this protects the system from further injury to support healing, but prolonged muscular alteration can lead to more pain and further peripheral damage. Greater GM volumes in these structures may represent compensatory/nocifensive strategies employed by TN patients to prevent and/or decrease their pain. Since facial movements such as talking and chewing are characteristically bilateral, the bilateral putamen and motor thalamus findings may represent bilateral compensatory motor behaviours. Additionally, larger basal ganglia volumes have been reported bilaterally in patients with migraine, an interesting finding given that migraine pain, like TN, can be paroxysmal involving the trigeminal nerve [290]. Importantly, M1 findings may also reflect alterations to the motor root of the trigeminal nerve and/or abnormal pain modulation, given the use of motor cortex stimulation as a treatment for neuropathic pain [177].

Some basal ganglia structures that were larger in TN patients have also been implicated in non-motor functions. For example, evidence suggests a role for the putamen in the sensory aspects of pain [423]. TN patients also had larger NAc volumes, which may reflect greater efforts to evaluate ongoing pain and/or predict future outcomes regarding pain [23].

Some previous studies of chronic pain have found correlations between pain duration and brain GM [50,308], suggesting that long term pain drives neuroplasticity [438]. We did not find evidence for this relationship in our TN patients. However, TN is quite different since it consists of repetitive paroxysmal pain rather than the sustained ongoing pain characteristic of other
neuropathic pains. Additionally, TN patients are heterogeneous with regard to the number of pain attacks they have per day, and thus the use of pain duration may not be an adequate index of total pain over time for the purposes of assessing pain-driven brain changes in this patient group. Future longitudinal studies are needed to explore this factor.

Additionally, nearly all of our patients were taking medication for TN, with the most common being the anticonvulsant, carbamazepine. Although the precise impact of these medications on brain morphology is not known, it is possible that they influence structural abnormalities. Future studies are needed to examine the effects of these drugs on brain GM.

Taken together, our study demonstrates for the first time, that patients with idiopathic TN have prominent GM abnormalities in brain regions involved in sensory-discriminative and cognitive-affective dimensions of pain, pain modulation, and motor function. Unlike other facial pains, TN patients have paroxysmal pain, frequently triggered by innocuous stimuli, and are without major sensory loss. The GM abnormalities reported in the current study likely reflect this unique symptomology. Understanding the contribution of central GM abnormalities in this population is a crucial step towards elucidating the mechanisms underlying this unique neuropathic pain.
Chapter 6
STUDY II: Abnormal Trigeminal Nerve and Brain White Matter Microstructure in Idiopathic Trigeminal Neuralgia

This study was published in *Pain*.

DeSouza DD, Hodaie M, Davis KD. (2014). Abnormal Trigeminal Nerve Microstructure and Brain White Matter in Idiopathic Trigeminal Neuralgia. *Pain*, 155(1): 37-44) has been reproduced with permission from the International Association for the Study of Pain ® (IASP). It may not be reproduced for any other purposes without permission.

6.1 INTRODUCTION

Idiopathic trigeminal neuralgia (TN) is a severe neuropathic pain disorder affecting the trigeminal nerve. It is characterized by intense, lancinating attacks of facial pain, typically triggered by normally non-painful stimuli or movements. Unlike many other neuropathic pains, TN patients usually do not exhibit major sensory loss, and are pain-free between pain attacks [328,358]. As the disorder progresses, pain attacks may become more frequent and the pain more sustained [277].

Previous research on TN has mainly focused on gross trigeminal nerve abnormalities, but little is known about the nature of such presumed nerve damage. The most prevalent theory is that TN is associated with neurovascular compression (NVC) of the trigeminal nerve at the root entry zone
The REZ is a transition zone between central and peripheral nervous system (CNS and PNS) myelin and where the trigeminal nerve is believed to be the most vulnerable to vascular compression [474]. Over time, the nerve-vessel contact results in damage or focal myelin loss [137,201,278,293], which can disrupt normal nociceptive transmission. Although gross NVC is not always apparent with standard imaging, MRI-based diffusion tensor imaging (DTI) has been shown to be a useful tool for examining the trigeminal system in great detail [202,456]. Clinical studies using DTI have identified microstructural abnormalities, including decreased fractional anisotropy (FA), in the affected REZ of TN patients with NVC [200,259,283]. Although FA is often used as a quantitative biomarker of white matter (WM) “integrity,” other DTI metrics provide insight into factors underlying WM microstructure and pathology.

Previous studies have shown white matter (WM) abnormalities in the brain following peripheral nerve injury and in chronic pain [126,358]. Importantly, many of these patients also had other significant sensory abnormalities including numbness. It is not understood how TN, without major sensory loss, impacts brain WM. Therefore, we examined WM abnormalities in TN to provide insight into the mechanisms involved in neuropathic pain, without the confound of other sensory abnormalities. Our study aim was to examine both trigeminal nerve and brain WM using multiple DTI-derived metrics to test the hypothesis that TN is associated with both nerve and brain WM abnormalities.
6.2 METHODS

6.2.1 Participants

Under the University Health Network Research Ethics Board approval, retrospective MR analyses were carried out in 18 patients with right-sided TN, seen at the Toronto Western Hospital, and 18 healthy control participants. All healthy control participants provided written informed consent (note that our Research Ethics Board does not require this for retrospective analyses of patient data as was analyzed here). For each patient, demographic and clinical details were obtained via retrospective chart reviews. Inclusion criteria were: unilateral (right-sided) pain in the distribution of one or more peripheral branches of the trigeminal nerve; intense, sharp, superficial or stabbing paroxysmal pain precipitated from trigger areas or by trigger factors; stereotyped attacks for each patient; no clinically evident neurological or sensory deficit or not attributed to another disorder [165], and no previous surgical procedures for TN.

6.2.2 Image Acquisition

Using a 3T GE MRI system with an eight-channel phased array head coil, 60-direction diffusion-weighted magnetic resonance images (DWI) were acquired for all participants using the following protocol: spin echo EPI sequence; in-plane voxel size 0.94 x 0.94; slice thickness= 3 mm; TE= 86.6 ms; TR= 12,000 ms; 1 $B_0$; b-value= 1,000 s/mm$^2$; matrix= 256 x 256; 1 excitation; ASSET.
6.2.3 DTI-Derived Metrics

DTI techniques use DWI scans, which are sensitized to the diffusion of water molecules [220], to characterize WM microstructure. This property is useful since in healthy WM, diffusion is more restricted across an axon than along it due to structural barriers such as myelin, axonal membranes, microtubules, and neurofilaments [36]. A mathematical model, typically a tensor/ellipsoid, characterized by its three orthogonal eigenvectors and their associated eigenvalues ($\lambda_1, \lambda_2, \lambda_3$), can be applied to each brain voxel to provide information about the three-dimensional character of the water molecules’ diffusion [220]. The most commonly used DTI-derived metric is FA [32], which ranges from 0 (completely isotropic) meaning water molecules can diffuse equally in all directions (e.g., cerebrospinal fluid), to 1 (completely anisotropic), meaning the diffusion of water is hindered (e.g., along axons in WM). Other metrics, highlighted in Figure 6-1 include axial diffusivity (AD), which corresponds to the main diffusion direction, or diffusion along the length of the axon ($\lambda_1$), radial diffusivity (RD), which is diffusion perpendicular to the main diffusion direction (average of $\lambda_2$ and $\lambda_3$), and mean diffusivity (MD), which measures the local magnitude of diffusion regardless of direction (average of $\lambda_1, \lambda_2, \lambda_3$). Although FA measurements represent a robust method for assessing the degree of directionality of diffusion, this ratio measure is a function of changes in diffusion in AD and RD. Therefore, if diffusion changes along the length of the tensor ($\lambda_1$) were proportional to those perpendicular to the tensor (RD), then FA would remain relatively unchanged even though abnormalities in diffusion may exist [3]. Additionally, these other measures of tissue microstructure, namely RD and MD have been linked to pathophysiological mechanisms such as demyelination, neuroinflammation, and edema [8,55].
6.2.4 Preprocessing

Prior to analyses, all scans underwent standard preprocessing steps using the FMRIB Diffusion Toolbox (FDT), part of FSL v.4.1.8 (http://www.fmrib.ox.ac.uk/fsl). Briefly, diffusion data were corrected for eddy-current and movement artifacts. Brains were extracted from non-brain tissues using the Brain Extraction Tool [415] and FA and non-FA (RD, AD, and MD) images were created for each subject by fitting a tensor model to the raw diffusion data using DTIFIT in the FDT toolbox.

6.2.5 Trigeminal REZ Analysis

For each participant, values for all DTI-derived metrics (FA, RD, AD, and MD) were extracted from both the left and right trigeminal nerves at the REZ. The trigeminal nerves were identified in the axial plane using color orientation maps created by overlaying the principle eigenvector image (V1) over the FA map. The trigeminal nerves were clearly visualized in the pontine cisternal space for each patient. To obtain the DTI-derived values, masks four voxels in size were manually placed at each REZ (Fig 6-2 A) in the axial plane. The REZ is defined as the cisternal part of the trigeminal nerve just as it enters the pons [228]. Between-group comparisons were carried out to compare patient and healthy control REZ values using independent samples t-tests. Also, to assess for abnormalities within the patient group, mean REZ values for the left and right trigeminal nerves of each patient were compared using paired t-tests. All trigeminal REZ analyses were done in SPSS v.21 and determined to be significant at the $p<0.05$ level.
6.2.6 Tract-Based Spatial Statistics

Tract-Based Spatial Statistics (TBSS) [416] was used to compare brain FA between the TN and healthy control groups. Following preprocessing, FA data underwent nonlinear registration to a 1 x 1 x 1 mm FA map in standard space (FMRIB58_FA) using a b-spline representation of the registration warp field [373]. The mean FA image created (thresholded at 0.2) was thinned to create a mean FA skeleton that represented the centers of all tracts common to the group. Each subject's aligned FA data were projected onto this skeleton and the resulting data underwent voxelwise cross-subject statistics. Permutation-based non-parametric testing was carried out using Randomise with Threshold-Free Cluster Enhancement (part of FSL), which inherently tests for multiple comparisons. Thus, all data were tested for significance at $p < 0.05$, corrected. In the areas where FA was determined to be significantly different in patients compared to controls, a region of interest analysis was used to test for focal WM alterations in RD, AD, and MD. The non-FA images used for TBSS were created by applying the FA nonlinear registration to the additional parametric maps, and projecting them onto the original mean FA skeleton. Since all significant TBSS results were displayed on the one-voxel thick skeleton, significant clusters were thickened for visualization purposes using “tbss_fill,” part of FSL. WM tracts with significant microstructural abnormalities were identified using the JHU ICBM-DTI-81 white-matter labels atlas [312] and the JHU White Matter Tractography Atlas [208], both included in FSL.
6.3 RESULTS

6.3.1 Patient Demographics

Patient demographic details are provided in Table 6-1. The TN group consisted of 18 patients (11 women, 7 men; mean age ± SD: 54.1 ± 17.0 years) with right-sided pain. Patients were on medications for TN pain at the time of their scan acquisition, with the most common one being the antiepileptic medication, carbamazepine. The healthy control group also consisted of 11 women, 7 men; mean age ± SD of 49.6 ± 12.7 years, which was not significantly different from the patient group ($p=0.38$).

6.3.2 Abnormal Microstructure at Trigeminal Root Entry Zone

The FA at the REZ of patients’ affected (right) trigeminal nerves was 22% lower than their unaffected side and 27% lower than that measured in the healthy controls ($p<0.05$) (Fig 6-2 B). FA in the unaffected (left) REZ of patients was not significantly different from controls ($p=0.23$). Furthermore, the three other DTI-derived measures of WM microstructure (RD, AD, MD) were all significantly greater in patients, compared to controls ($p<0.05$) (Fig 6-2, C-E). This effect was observed for both the nerves on the affected (by 29-52%) and the unaffected (by 31-37%) sides. There were no statistically significant differences between the patients’ affected and unaffected sides for AD or MD ($p>0.05$), although there was a trend towards significance for RD ($p=0.055$).
6.3.3 Brain White Matter Abnormalities in Corpus Callosum, Cingulum, Corona Radiata, and Superior Longitudinal Fasciculus

In general, there were widespread regions of brain WM abnormalities in the TN patients with lower FA, and higher RD and MD (p<0.05, corrected) compared to the healthy controls (Fig 6-3). AD was also significantly higher in the brains of patients; however these findings were not as widespread as FA, RD, and MD abnormalities.

The main WM regions of interest that showed prominent abnormalities are highlighted in Figure 6-4. Specifically, FA was lower (by 9%) in the corpus callosum (genu, body, and splenium) of patients, while RD and MD were 7-16% higher (p<0.05, corrected) (Fig 6-4, A-C). AD was also higher (by 5%) in the corpus callosum; however, this finding was limited to the splenium (Fig 6-4 D). Additionally, patterns of lower FA and higher RD and MD were found in the cingulum and the posterior corona radiata (bilaterally) of patients (Fig 6-4, A-C). WM abnormalities were also found in the left superior longitudinal fasciculus (SLF) of patients, contralateral to their side of pain, as characterized by lower FA and higher RD (p<0.05, corrected) (Fig 6-4, A & C). The SLF abnormalities were predominantly localized to the parietal and temporal SLF.
**Table 6-1**: TN patient demographic details

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
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<th>Pain Distribution</th>
<th>Medication</th>
</tr>
</thead>
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<td>55</td>
<td>V1/V2</td>
<td>GBP</td>
</tr>
<tr>
<td>R2</td>
<td>F</td>
<td>44</td>
<td>V2</td>
<td>CBZ</td>
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<td>CBZ</td>
</tr>
<tr>
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<td>F</td>
<td>42</td>
<td>V2/V3</td>
<td>GBP; CBZ</td>
</tr>
<tr>
<td>R5</td>
<td>F</td>
<td>76</td>
<td>V1/V2</td>
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<td>CBZ</td>
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<td>79</td>
<td>V1/V2/V3</td>
<td>CBZ</td>
</tr>
</tbody>
</table>

Abbreviations: Pain distribution refers to the peripheral branches of the trigeminal nerve (V1: ophthalmic branch; V2: maxillary branch; V3: mandibular branch); CBZ: carbamazepine; GBP: gabapentin; PGB: pregabalin.
Figure 6-1: Schematic representation of DTI-derived metrics and tensor changes that can occur with lower FA. Four DTI-derived metrics derived from the eigenvalues of the tensor model were examined. Schematic representations of how these metrics were calculated and their formulas are shown in Panel (A), with AD being diffusion along the length of the axon ($\lambda_1$) (left), RD being diffusion perpendicular to the length of the axon (average of $\lambda_2$ and $\lambda_3$) (center), and mean diffusivity (MD), being the magnitude of diffusion regardless of direction (average of $\lambda_1$, $\lambda_2$, and $\lambda_3$) (right). Panel (B) shows the tensor model and the formula for FA (top). Also illustrated are
two scenarios where FA has decreased, but the shape of the tensors are different due to differences in the other three DTI-derived metrics (shown in chart). The first scenario is when FA, AD, and MD decrease, but RD remains stable (bottom left), and the second is when FA decreases, but AD, RD, and MD increase (bottom right) (e.g., TMD and TN). For additional potential scenarios of changes to the tensor, see reference [2]. Abbreviations: FA= fractional anisotropy; RD= radial diffusivity; MD= mean diffusivity; AD= axial diffusivity. This figure has been reproduced from [129] with permission of the International Association for the Study of Pain ® (IASP). The figures may NOT be reproduced for any other purpose without permission.
Figure 6-2: Microstructural abnormalities in the nerves of TN patients. TN patients had abnormal WM microstructure in their trigeminal nerves (* indicates significance of $p<0.05$).

Axial images were used to locate the trigeminal nerves as they entered the pons. Panel (A) shows an example of an axial T1-weighted image with the trigeminal nerves visible in the cisternal space surrounding the pons. On DTI scans, masks four voxels in size were manually placed at the REZ of each patient and control (red box shows magnified region of pons and trigeminal nerves with masks circled at REZ). Graphs of the extracted DTI-derived values ± SEM are provided: controls (black bars), patients (red bars). Compared to controls, patients had significantly lower FA in their affected trigeminal nerve (B), higher RD (C), MD (D), and AD (E) in both their affected and unaffected nerves (units in mm$^2$/s). There was also a trend ($p=0.055$) towards higher RD in the patients’ affected compared to unaffected sides (red bars, panel...
(C)). Please note that affected side refers to the right trigeminal REZ (side of pain for patients) and the unaffected side refers to the left trigeminal REZ (pain-free side for patients).

Abbreviations: REZ = root entry zone; CN V = trigeminal nerve; FA = fractional anisotropy; RD = radial diffusivity; MD = mean diffusivity; AD = axial diffusivity. This figure has been reproduced from [129] with permission of the International Association for the Study of Pain ® (IASP). The figures may NOT be reproduced for any other purpose without permission.
Figure 6-3: Abnormalities in brain white matter microstructure using multiple DTI metrics.

Group TBSS results revealed widespread regions of brain WM abnormalities in TN patients compared to healthy controls for all four of the DTI-metrics examined ($p < 0.05$, corrected). Blue clusters indicate patients $<$ controls (FA) and red clusters indicate patients $>$ controls (MD, RD,
and AD). Abbreviations: FA = fractional anisotropy; RD = radial diffusivity; MD = mean diffusivity; AD = axial diffusivity. Note that the brain images at x = 0 appear darker due to the presence of the falx cerebri. This figure has been reproduced from [129] with permission of the International Association for the Study of Pain ® (IASP). The figures may NOT be reproduced for any other purpose without permission.
Figure 6-4: Abnormal brain WM in the corpus callosum, cingulum, posterior corona radiata, and superior longitudinal fasciculus. The main regions of brain WM abnormalities from the group TBSS results ($p<0.05$, corrected) are highlighted for each DTI-metric, with graphs of the extracted DTI-derived values ± SEM to the right: controls (black bars), patients (red bars). MD, RD, and AD units in mm$^2$/s. Blue clusters indicate patients< controls and red clusters indicate patients> controls. Abbreviations: pCR= posterior corona radiata; CC= corpus callosum; CB= cingulum bundle; SLF= superior longitudinal fasciculus; FA= fractional anisotropy; RD= radial diffusivity; MD= mean diffusivity; AD= axial diffusivity. This figure has been reproduced from [129] with permission of the International Association for the Study of Pain ® (IASP). The figures may NOT be reproduced for any other purpose without permission.
6.4 DISCUSSION

This is the first study to demonstrate that patients with idiopathic TN not only have WM abnormalities in their trigeminal nerves, but also in their CNS WM. The brain WM abnormalities were located in tracts connecting regions implicated in the multi-dimensional experience of pain, attention, and motor function. These WM abnormalities, characterized by lower FA, and higher RD, MD, and AD, suggest not only disrupted nerve/tract organization, but also other pathological features such as neuroinflammation, and edema of the nerve/axons.

6.4.1 Trigeminal Root Entry Zone Abnormalities

Although the precise mechanism of TN pathophysiology is not entirely understood, the most prevalent theory involves alterations to the trigeminal nerve, usually by vascular compression [358]. Structurally, these alterations can include NVC-induced focal demyelination of the WM fibres in the trigeminal REZ [358], resulting in the abnormal firing of trigeminal nerve afferents. Consistent with this theory and with previous studies examining FA at the trigeminal REZ [200,259,283], we report lower FA at the affected trigeminal REZ of TN patients, compared to their unaffected side and to healthy controls. Our findings are consistent with a decrease in WM “integrity” by NVC only at the affected trigeminal REZ.

A decrease in FA can occur in multiple scenarios [3] (Fig 1B). For the affected REZ of TN patients, a disproportionately greater increase in RD and MD over AD could account for the
significantly lower FA. This ratio may reflect focal injury and/or demyelination from NVC at the 
REZ of the affected nerve, but not the unaffected nerve, or general microstructural disintegration 
of the affected nerve.

MD and AD were not significantly different between the TN patients’ affected and unaffected 
sides, although there was a trend towards greater RD in the affected trigeminal REZ, in line with 
the theory that NVC results in focal demyelination of the affected side [420]. Interestingly, all 
three measures, on both the affected and unaffected sides, were different compared to controls. 
Therefore, subtle microstructural abnormalities may occur in the trigeminal system as a whole in 
this patient group. One possibility is that individuals who develop TN are more susceptible to 
chronic pain because of pre-existing abnormalities in their WM, possibly explaining why some 
individuals with NVC of the trigeminal nerve never develop TN. With pre-existing trigeminal 
nerve abnormalities (e.g., higher MD, AD, and/or RD) [204,277,440], vascular compression may 
be sufficient to result in TN pain, and additionally lower FA. The current study cannot test this 
scenario, however, it remains possible that there are individual differences in susceptibility to 
develop chronic pain [122,204]

An alternate explanation is that these bilateral abnormalities reflect compensatory motor 
behaviors. The trigeminal nerve contains a motor branch that innervates the muscles of 
mastication. As normally non-painful movements such as chewing, are well-known triggers of 
TN pain [42], patients often restrict facial movements. Given the bilateral innervation in this 
system, nocifensive behaviors, including limiting jaw movements, may result in abnormalities to 
both trigeminal nerves or to CNS WM along motor pathways.
6.4.2 Abnormalities in WM connecting Brain Regions Involved in the Multi-Dimensional Experience of Pain and Motor Function

Although TN is known to involve trigeminal nerve dysfunction, other theories of TN propose central nervous system (CNS) pathology [406]. Central nervous system plasticity can occur following peripheral nerve injury [126,437]. In line with other studies of neuropathic pain, we recently reported cortical and subcortical brain gray matter (GM) abnormalities in TN patients in regions involved in the sensory, cognitive-affective, and modulatory aspects of pain and motor function [134]. Many of these brain areas are anatomically and/or functionally connected. We report several areas of abnormal brain WM in TN patients, mainly marked by lower FA and higher RD and MD in the corpus callosum, cingulum, posterior corona radiata, and superior longitudinal fasciculus. These tracts connect brain regions known to participate in the multi-dimensional experience of pain, attention, and motor function, and may be contributing to the unique symptoms of TN pain and/or compensatory motor behaviors.

The corpus callosum interconnects the cerebral hemispheres, allowing for the rapid transfer and integration of sensory and motor information [63]. However, the nature of these connections is not well understood [52]. The corpus callosum is topographically organized with anterior callosal fibres connecting frontal regions, including motor, anterior cingulate, and prefrontal cortices, and posterior fibres connecting parietal, temporal, and occipital brain areas, including somatosensory regions [159]. The WM abnormalities in this study spanned the length of the corpus callosum, suggesting that there may be differences in how TN patients integrate
cognitive, sensory, and motor information. Corroborating this, we have previously shown abnormal GM thickness in the prefrontal, sensory, and motor cortices of TN patients [134]. While abnormal sensory integration may result from differences in the amount of nociceptive information entering the CNS from the trigeminal system, abnormal differences in the corpus callosal fibres connecting motor cortices may reflect the restriction of facial movement by TN patients to prevent or lessen pain attacks. Abnormal sensory integration is also suggested by our findings of WM abnormalities observed in the posterior corona radiata (adjacent to the posterior parietal cortex). The parietal cortex is known for its role in multisensory integration, which aids in the detection, localization, and reaction to external stimuli [21].

Microstructural abnormalities in the form of lower FA in brain WM have been reported in other chronic pains, [120,180,310,367,431] and studies of chronic pain affecting the trigeminal system have reported lower FA in the corpus callosum [310,483,484]. Of the studies that examined multiple DTI metrics [310,483], differences in these metrics were not consistent, despite lower FA in these cases. Patients with temporomandibular disorder (TMD) had higher RD and MD in regions of lower FA [310]; however, migraine patients had lower MD and AD, but unchanged RD.[483]. As various pathological processes affect these metrics differently [55], this highlights the need to examine other DTI-derived metrics in addition to FA, in order to gain insight into possible differences in pathophysiological mechanisms between chronic pains. As TN and TMD patients share a similar pattern of microstructural abnormality in the corpus callosum, similar mechanisms may be involved in these patients groups that are not shared with migraine patients.
Additionally, we found abnormal WM microstructure bilaterally in the cingulum bundle (adjacent to the midcingulate cortex) of patients. Abnormal cingulum WM has previously been reported in TMD [310]. The cingulum is thought to be involved in the affective dimension of pain, and cingulotomy procedures have successfully been used to provide pain relief for patients with intractable pain [480]. Abnormalities in cingulum WM may reflect the high degree of pain unpleasantness associated with having TN. TN patients also had abnormal WM in the SLF underlying the left temporoparietal cortex. The temporoparietal junction (TPJ) is a component of the ventral attention network, a right-lateralized system activated by salient stimuli including prolonged pain [249]. However, the left TPJ, in conjunction with the frontal lobes, is also thought to play a role in attention [362] and higher-order cognitive processing [375]. Together, abnormal cingulum and SLF microstructure may reflect abnormal processing of the cognitive-affective dimension of pain in TN patients.

6.4.3 Mechanisms Underlying White Matter Microstructural Abnormalities

As in GM, the mechanisms underlying experience-dependent changes to WM microstructure have been shown to have an influence on neuroimaging measures [55,491]. Candidate mechanisms underlying MR-detectable axonal plasticity include changes in fibre organization such as packing density, axonal sprouting/branching, axon size/number, crossing fibres, and myelin remodeling [36,55,491]. Other factors such as astrocyte morphology and angiogenesis have also been shown to influence MR-detectable changes in WM [491]. In our study, we show that TN patients have lower FA in their affected trigeminal nerves at the REZ and brain WM,
compared to healthy controls. Lower FA in the affected REZ of TN patients may be related to
NVC-induced axonal damage, which may include less fibre organization and/or nerve atrophy
[157,278,293]. Also, lower FA in the brain WM of TN patients may correspond to less fibre
organization, including more axonal sprouting/branching, more crossing fibres, or larger axons
[491].

Our finding of increased MD and RD may be linked to NVC-induced focal demyelination of the
trigeminal REZ [293], neuroinflammatory processes, and/or edema [33,36]. Injury to the
trigeminal REZ may involve neuroinflammation and edema as part of the injury response.
Additionally, neuropathic pain is typically associated with prolonged nociceptive activity to the
central nervous system. This could lead to central sensitization [253,473], a process involving
central neuroinflammatory processes and edema. Indeed, evidence that chronic pain may induce
neuroinflammation in the brain and in turn influence brain WM has been reported [124],
suggesting that these mechanisms may also contribute to the RD and MD abnormalities in the
brain WM of TN patients.

One caveat of note is that all study patients were on medications for TN pain, the most common
being the antiepileptic drug, carbamazepine. The effects of antiepileptics on brain structure are
not well understood, but there is evidence to suggest they influence immune activity [34,49].
Therefore, it is possible that these medications contributed to changes in the DTI-derived metrics
associated with inflammation and/or edema.
7.1 INTRODUCTION

Classical trigeminal neuralgia (TN) is a severe neuropathic pain characterized by highly intense electric shock-like attacks of unilateral facial pain [203]. Over time, the pain may occur more frequently and be less responsive to medications leading patients to seek neurosurgical intervention for pain relief [328,489]. Neurosurgical treatment of TN can be highly effective in reducing pain intensity by more than 75% in many patients [265,268]. It is not known whether these treatments work by directly resolving structural abnormalities in the trigeminal nerve, brain, or another indirect mechanism.

Surgical treatment for TN targets the trigeminal nerve at different anatomical sites with the goal of displacing the offending vessel compressing the trigeminal nerve root entry zone (REZ), as in microvascular decompression (MVD) surgery, or partially injuring the trigeminal nerve distal to the REZ to reduce nociceptive signaling, as in Gamma Knife radiosurgery (GKRS) [487,489]. Estimates of the proportion of patients with good or complete pain relief following surgery are approximately 70-80% and 85-90% for GKRS and MVD, respectively.
With such high response rates, TN patients are ideal for studying the neural correlates of pain relief following neurosurgical interventions.

TN has been associated with neurovascular compression (NVC) of the trigeminal nerve at or near the dorsal root entry zone (REZ); the location where the trigeminal nerve exits the brainstem [216,489]. However, the pathophysiology largely remains elusive. There is histological evidence for NVC-induced focal demyelination at the REZ causing the abnormal firing of trigeminal nerve afferents [137,277,328,358]. Corroborating this, MRI-based diffusion tensor imaging (DTI) studies have shown microstructural REZ abnormalities characterized by lower fractional anisotropy (FA) [132,200,259,283], and as we recently reported, higher mean, radial, and axial diffusivities (MD, RD, and AD) [132]. These metrics have been linked to pathophysiological mechanisms such as demyelination and edema [8,33,36], making DTI a useful tool to noninvasively examine trigeminal nerve microstructure and pathology.

In addition to nerve abnormalities, we have identified brain gray and white matter (GM, WM) abnormalities in patients with TN in structures involved in pain perception, pain modulation, and motor function [132,134], including the thalamus, basal ganglia, amygdala, periaqueductal gray, somatosensory, motor, insular, cingulate, and prefrontal cortices, cingulum and corpus callosum. These structures have been described as being abnormal in peripheral nerve injury and chronic pain [124,126,132,296,310]. Although individual predisposing factors may influence the development of chronic pain and CNS plasticity [199,204,277], GM abnormalities may occur
consequentially to chronic pain. For example, it has been demonstrated that some GM abnormalities are reversible with effective treatment [191,368,369,394].

In this study we determined the effects of neurosurgical treatment on trigeminal nerve and brain structure. Regardless of surgery type, our aim was to use structural MRI to determine if effective treatment for TN is associated with a reversal of the trigeminal REZ and GM abnormalities that we previously reported [132,134]. Therefore, we compared a group of TN patients before and after treatment to a cohort of age-matched healthy participants. We hypothesized that effective treatment would be associated with a normalization of both nerve and brain abnormalities.

7.2 METHODS

7.2.1 Participants and Study Design

A total of 39 participants were included in the study: 25 patients who underwent GKRS or MVD neurosurgical procedures at the Toronto Western Hospital for the treatment of classical TN and 14 healthy age cohort-matched controls. Patients were included in the study if they had right-sided pain, met the criteria for classical TN as outlined by The International Classification of Headache Disorders [165], and had both pre- and post-treatment high resolution anatomical MRI scans within approximately two to six months of treatment (months ± SD: 5.8 ± 4). Since pain-relief typically occurs sooner following MVD compared to GKRS and there can be acute effects following surgery, no follow-up scans were acquired within first two months post-surgery.
Therefore, follow-up MR scans were obtained at a time that coincided with when patients experienced pain-relief, but were not confounded by the acute effects of surgery. Only patients with right-sided pain were included to avoid flipping the scans of patients to control for side of pain. A subset (n= 14) of the patients also had diffusion-weighted scans for white matter (WM) analysis. Patient demographic and clinical details were obtained via retrospective chart reviews and are presented in Table 7-1. Control participants were recruited as part of an on-going study examining brain structure and aging in healthy individuals and had an MRI at a single time-point. All control subjects had both high-resolution anatomical and diffusion-weighted scans. Participants were excluded if they had any other neurological or sensory disorder or TN secondary to multiple sclerosis, tumors, or a structural lesion other than vascular compression. The University Health Network Research Ethics Board approved this study and all healthy control participants provided consent.

7.2.2 Neurosurgical Treatments

Fifteen patients were treated with GKRS (Leksell Gamma Knife 4C unit © or Leksell Perfexion unit©, Stockholm, Sweden) as we have previously described [202]. Briefly, patients received 80 Gy to the 100% isodose line using a 4 mm collimator in a single fraction. The target was just distal to the trigeminal REZ, so that the 20% isodose line remained outside of the brainstem, in this way keeping the dose to the brainstem to less than 15Gy. The remaining ten patients had MVD surgery. A detailed description of this procedure has been described elsewhere [203,331]. Briefly, a retrosigmoid craniotomy approach was used. Under general anesthesia, the posterior fossa and specifically the cerebellopontine angle were approached. Using microscopic
magnification, the location of the trigeminal nerve was identified based on its relationships with the tentorium cerebelli and the petrosal sinus complex. The trigeminal REZ was inspected and decompressed of offending blood vessels using pieces of shredded Teflon©.

Patients provided verbal ratings of their pain intensity before and after surgery, on a scale from 0 (no pain) to 10 (most intense pain imaginable). Since neurosurgical treatment for TN tends to result in either excellent outcomes or minimal pain relief, we set a cut-off of at least a 75% reduction in preoperative pain to distinguish effective from ineffective treatment. This cut-off has previously been used as an indicator of good surgical outcome for TN [265].

7.2.3 Image Acquisition

Brain imaging data were acquired on a 3T GE Signa HDx MRI system with an eight-channel phased array head coil. Patients were scanned twice (pre- and post-surgery), and control subjects were scanned once. For all participants, high resolution T1-weighted 3D FSPGR axial images were obtained: in-plane voxel size= 0.9 × 0.9; slice thickness= 1 mm; matrix= 256×256; field of view= 24 cm (controls) or 22 cm (patients); echo time (TE)= 5 ms; repetition time (TR)= 12 ms; inversion time= 300 ms.

For the subset of 14 TN patients and all control participants, 60-direction diffusion-weighted magnetic resonance images were also acquired with the following protocol: spin echo EPI
sequence; in-plane voxel size= 0.94 x 0.94; slice thickness= 3 mm; TE= 86.6 ms; TR= 12,000 ms; 1 B0; b-value= 1,000 s/mm²; matrix= 256 x 256; 1 excitation; ASSET.

### 7.2.4 Gray Matter Analysis

Cortical and subcortical GM analyses were restricted to masks of regions of GM abnormalities that we previously reported in right-sided TN patients [134] (Fig. 7-1). Most of the patients (16/25) had not previously been examined for GM abnormalities and nine patients were included from our previous study. Specifically, cortical regions of interest (ROIs) included the right ventral anterior insula (vAI) and posterior cingulate cortex (PCC), the left primary somatosensory cortex (S1), and bilateral primary motor cortex (M1), posterior insula, frontal pole (FP), anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC) (Fig. 7-1A-B). As each ROI was examined separately and treatment effects on each GM region were considered independently, the statistical values for the longitudinal GM analyses are reported as uncorrected values.

Cortical GM was analyzed using cortical thickness analysis (CTA) in FreeSurfer v. 4.5.0 (http://surfer.nmr.mgh.harvard.edu/) [163] as we previously described [134]. At each time-point (before and after treatment), we used FreeSurfer’s standard preprocessing pipeline, which included the registration of patient and control T1-weighted anatomical scans to the Talairach atlas [434], skull stripping, and tissue classification into GM, WM, and cerebrospinal fluid (CSF). Data were smoothed with a 6 mm full-width half-maximum (FWHM) smoothing kernel.
and cortical thickness was measured as the distance between the GM/WM and GM/CSF boundaries at each point within each ROI.

Subcortical GM was assessed using voxel-based morphometry (VBM) in FMRIB’s Software Library (FSL) v. 4.1.8 (http://www.fmrib.ox.ac.uk/fsl/) as we previously described [134]. Comparisons were restricted to ROIs that included bilateral regions of the thalamus, basal ganglia, PAG, and right amygdala (Fig. 7-1C). For all participants, skulls were stripped using the Brain Extraction Tool (BET) [415] and tissues types were classified using FMRIB’s Automated Segmentation Tool [493]. Each scan was then aligned to the Montreal Neurological Institute (MNI) 152 standard 2 mm template. A study-specific template was also created, to which the GM images were non-linearly re-registered. Images then underwent Jacobian modulation to normalize GM volumes and were smoothed with an isotropic Gaussian kernel (FWHM= 4.6 mm). Cortical thickness and subcortical volume data for each participant were extracted from the masks and submitted to statistical analysis.

### 7.2.5 Trigeminal REZ Metric Analysis

The FA, MD, RD, and AD values were extracted bilaterally from the trigeminal REZ in patients with diffusion weighted imaging (DWI) scans, as we have done previously [132]. FA is generally regarded as a proxy for WM organization or “integrity” but can decrease in multiple scenarios [3,132]. Mathematically, FA is a ratio of the other diffusion metrics and cannot fully describe the tensor. As proportional changes in MD, RD, and AD can produce the same values of FA, subtle abnormalities in these metrics may be detected even when abnormal FA is not [3,8].
Additionally, MD, RD, and AD have been linked to pathophysiological mechanisms such as
demyelination, edema, and neuroinflammation [8,33,36] and may provide insight into possible
pathology underlying microstructural abnormalities in patients with TN. DWI scans were
preprocessed using the FMRIB Diffusion Toolbox (FDT) in FSL v. 4.1.8, and scans were eddy-
current and motion-corrected, and skull-stripped using the brain extraction tool (BET) [415]. For
each subject, a tensor model was fit to the raw diffusion data using DTIFIT in the FDT toolbox.
Data were extracted bilaterally from each trigeminal nerve at the REZ from the individual FA,
MD, RD, and AD maps. The trigeminal nerves were visualized in the axial plane using color
orientation maps created by overlaying the principle eigenvector image over the FA map. In this
view, a mask spanning four voxels was placed at each REZ at the border of the basilar pons and
trigeminal nerve as it entered the brainstem (Fig 7-2A).

7.2.6 Statistical Analysis

For TN patients, within-subject comparisons were made to compare pre- and post-treatment GM
and REZ metrics using paired samples t-tests. The pre- to post- treatment differences expressed
as a percent change were submitted to a bivariate correlation analysis to determine if there was a
relationship between the degree of change over time and the degree of pain relief. Data from the
effective and ineffective treatment groups were compared with a repeated measures analysis of
variance (ANOVA) that included group (effective vs. ineffective treatment group), as a between-
subject factor, and time (pre-treatment vs. post-treatment), as a within-subject factor. For the
REZ analysis, side (affected vs. unaffected REZ) was also included as a within-subject factor.
Data from patients were also compared to age-matched healthy participants using independent
samples t-tests. All statistical analyses were computed in SPSS Statistics v.21 and determined significant at the $p < 0.05$ level.

**Figure 7-1:** Masks of abnormal brain gray matter regions in patients with trigeminal neuralgia. Gray matter (GM) analyses were restricted to masks of brain regions that were previously determined to be abnormal in TN patients [134]. Masks include regions where patient GM > controls (yellow), and patient GM < controls (green). (A) Cortical masks for the left hemisphere.
shown on lateral (left), medial (right) and inferior (bottom) views. (B) Cortical masks for the right hemisphere shown on lateral (left), medial (right) and inferior (bottom) views. A green box highlights a magnified and slightly rotated view of the ventral anterior insula mask, as it could not be visualized through the lateral fissure in the lateral view. (C) Subcortical masks shown on axial views. Abbreviations: M1= primary motor cortex; S1= primary somatosensory cortex; FP= frontal pole; OFC= orbitofrontal cortex; pINS= posterior insula; pgACC= pregenual anterior cingulate cortex; vAI= ventral anterior insula; PCC= posterior cingulate cortex; Nac/C= nucleus accumbens/caudate nucleus; Amg= amygdala; PAG= periaqueductal gray; MD/VPM= dorsal medial/ventral posterior medial nuclei of the thalamus; Put= putamen; LH= left hemisphere; RH= right hemisphere

7.3 RESULTS

7.3.1 TN Pain Before and After Treatment

Patient demographics and treatment effects on pain and brain structure are provided in Tables 7-1 and 7-2, respectively. Twenty-five patients (15 women, 10 men; mean age ± SD: 57.6 ± 11.5 years) with right-sided TN and 14 healthy control participants (9 women, 5 men; mean age ± SD: 51.7 ± 10.9 years) were included in the GM analyses. Only patients with right-sided pain were included to avoid flipping the scans of patients to control for side of pain. Each patient group was compared to their own cohort of controls selected from the cohort of controls described above, such that the mean ages between patient and control groups were within approximately 4 years
of each other. Treatment was considered effective for 60% (15 of 25) of patients (10 women, 5 men; mean age ± SD: 54.6 ± 13.0 years; mean % pain relief: 98.5 ± 5.7) and ineffective for the remaining 40% (10 of 25) of patients (5 women, 5 men; mean age ± SD: 62.0 ± 7.4 years; mean % pain relief: 30.5 ± 25.2) (effective treatment controls: 9 women, 3 men; mean age ± SD: 50.7 ± 11.3) (ineffective treatment controls: 4 women, 4 men; mean age ± SD: 57.8 ± 7.6). The effective treatment group consisted of 6 patients who had GKRS and 9 patients who had MVD. The ineffective treatment group consisted of 9 patients who had GKRS and 1 patient who had MVD. Overall, MVD was an effective pain treatment for 83% of patients and GKRS was an effective pain treatment for 53% of patients. The effective and ineffective treatment groups were similar in terms of pain distribution and medications, but pain duration was longer for patients who had ineffective vs. effective treatment ($p=0.02$) (see Table 7-1).

Of the 14 TN patients that had DWI scans, eight (5 women, 3 men; mean age ± SD: 48.5 ± 13.4; mean % pain relief: 97.3 ± 7.8) had effective treatment and six (3 women, 3 men; mean age ± SD: 64.7 ± 4.6; mean % pain relief: 30.8 ± 28.0) had ineffective treatment. In the effective treatment group, 75% (n=6) of patients had MVD surgery while the remaining 25% (n= 2) had GKRS. The majority of patients in the ineffective treatment group (83%; n= 5) had GKRS and only one patient had MVD surgery. The ineffective treatment group was older than the effective treatment group for the REZ analyses ($p=0.02$), so each patient group was age- and sex-matched to their own control groups for comparison (effective treatment controls: 5 women, 3 men; mean age ± SD: 49.6 ± 12.6) (ineffective treatment controls: 3 women, 3 men; mean age ± SD: 60.3 ± 6.3).
7.3.2 Brain and Trigeminal Nerve Abnormalities in TN Before Treatment

Compared to controls, the CTA and VBM analyses revealed that before treatment, the effective treatment group had: 1) 9.0% thinning in the right vAI, 10.4% thinning in the bilateral posterior insula, 15% thinning in the OFC, 9.1% thinning in the ACC, and 8.4% thinning in the right PCC ($p < 0.05$), 2) 14.4% thicker cortex in the M1 bilaterally, left S1 (13.6%), and the FP cortex bilaterally (15.4%) ($p < 0.05$), and 3) greater subcortical volume in the thalamus (15.4%), basal ganglia (posterior putamen bilaterally: 33.5%; right head of caudate/nucleus accumbens: 14.9%), PAG (32.4%), and right amygdala (6.4%) ($p < 0.05$). These findings are consistent with our previous findings of GM abnormalities in TN [134]. The ineffective treatment group had a similar pattern of GM abnormalities pre-treatment: 1) 9.0% thinning cortex in the right vAI, 7.7% thinning bilaterally in the posterior insula, 15.2% thinning in the left OFC, 6.4% thinning in the right ACC, and 10.2% thinning in the right PCC ($p < 0.05$), 2) thicker cortex in the M1 bilaterally (16.0%), left S1 (12.1%), and the FP cortex bilaterally (18.5%) ($p < 0.05$), and (3) greater subcortical volumes in the posterior putamen bilaterally (33.8%), and the PAG (24.1%) ($p < 0.05$). The ineffective treatment group also had greater subcortical volume in the thalamus (8.4%), right amygdala (5.8%), and right head of caudate/nucleus accumbens (8.9%) compared to controls; however, these differences were not statistically significant ($p > 0.05$).

Prior to treatment, there were marked differences in the DTI-derived metrics extracted from the trigeminal REZ of patients compared to controls. Within the effective treatment group, FA in the
affected REZ was significantly lower pre-treatment compared to the unaffected side (-10.4% difference) \( (p=0.02) \) and to healthy controls (-14.7%) \( (p=0.03) \) (Fig. 7-2B). Additionally, MD, RD, and AD were significantly higher in patients bilaterally (by 20.3% - 35.2%) \( (p<0.05) \) (Fig. 7-3A-C), consistent with our previous study [132]. Within the ineffective treatment group, FA in the affected REZ was also significantly lower pre-treatment compared to controls (by 31.1%) \( (p=0.01) \) and to the unaffected side (by 22.3%) \( (p=0.04) \) (Fig 7-2C). The pre-treatment FA value at the affected REZ was also lower than pre-treatment FA at the affected REZ of patients that had effective treatment, however, this difference was not statistically significant \( (p=0.10) \). MD, RD, and AD values were significantly higher at the affected REZ pre-treatment compared to the unaffected side (by 9.6% - 31.0%) (Fig. 7-3D-F) and also compared to controls for MD and RD (by 27.6% and 45.5%, respectively) \( (p<0.05) \). Bilateral abnormalities were not evident.

### 7.3.3 Normalization of Insula Thickness Following Treatment

Following treatment, the right vAI was the only GM region to normalize by significantly increasing in thickness (mean % increase ± SD: 3.3 ± 7.2, see Table 7-2), such that it was no longer significantly thinner than controls (Fig. 7-4). The increase in vAI thickness occurred for both the effective and ineffective treatment groups, but within-subject comparisons showed that the effect was only significant in the effective treatment group \( (p<0.05) \). Further statistical analyses did not reveal any effects of group or time. No other regional GM changes were found post-treatment compared to pre-treatment.
7.3.4 Resolution of REZ Abnormalities after Effective Pain Treatment

The percent changes for each DTI-derived metric, from pre-treatment to post-treatment, are shown in Table 7-2. Following effective treatment, the FA abnormality in the affected trigeminal REZ resolved such that FA increased and was no longer significantly different from the unaffected side or from controls (Fig. 7-2B). Additionally, the bilateral MD, RD, and AD abnormalities resolved (decreased by following treatment) and were no longer significantly higher than control values (Fig. 7-3A-C). One exception was left post-treatment AD, which was lower than pre-treatment values, but still slightly higher than controls ($p< 0.05$). The ineffective treatment group showed a different pattern of REZ abnormality, which did not reverse following treatment. Following surgery, affected REZ FA remained significantly lower compared to controls and the unaffected side (Fig. 7-2C). Additionally, the MD, RD, and AD abnormalities at the affected REZ of the ineffective treatment group patients persisted post-treatment (Fig. 7-3D-F), and AD at the affected REZ increased further to become significantly different from controls post-treatment ($p= 0.02$) (Fig. 7-3F).

A repeated measures ANOVA indicated that the affected and unaffected REZ values differed depending on which group patients belonged to (effective vs. ineffective treatment). A significant group x side interaction occurred for FA ($F= 6.17, p= 0.03$), MD ($F= 12.47, p= 0.005$), RD ($F= 10.42, p= 0.008$), and AD ($F= 13.10, p= 0.004$), with MD ($F= 5.18, p= 0.04$) and AD ($F= 5.34, p= 0.04$) also showing a main effect of side (Fig. 7-5). To test for significant group effects on these DTI measures, a one-way ANOVA was also conducted and revealed that the
effective and ineffective treatment groups had significantly different AD values pre-treatment at the unaffected REZ (F= 5.81, p= 0.03) and FA values post-treatment at the affected REZ (F= 6.66, p= 0.02). No other significant differences between patient groups were obtained.

### 7.3.5 Relationship between Treatment-Induced Structural Changes and Pain Relief

The relationship between the change in DTI metrics and pain relief after treatment is shown in Fig. 7-6A-D. The percent pain relief after treatment was significantly correlated (bivariate correlation analysis) with the pre- to post-treatment differences in each DTI metric as follows: MD (r= -0.535, p= 0.024), RD (r= -0.512, p= 0.031), and AD (r= -0.539, p= 0.023). These associations indicate that more pain relief is associated with greater resolution of MD, and RD, and AD abnormalities following treatment (Fig. 7-6B-D). Additionally, there was a trend towards a positive correlation between pain relief and proportion of FA change following treatment (r= 0.434, p= 0.061), suggesting that patients with the greatest amount of pain relief have larger increases in FA towards the level of controls, at the REZ (Fig. 7-6A). No significant correlations between proportion of DTI metric change in the unaffected nerve and percent pain relief were found for any of the metrics examined (p> 0.05).

In contrast to the nerve findings, there was no significant correlation between the percent change in vAI GM thickness and percent pain relief pre- to post-treatment (r= 0.022, p= 0.92).
Table 7-1: Demographic and Clinical Variables

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Sex</th>
<th>Age</th>
<th>Pain Dist.</th>
<th>Pain Dur. (Yrs)</th>
<th>Tx Type</th>
<th>Scans</th>
<th>Pain Int. (Pre-Tx)</th>
<th>Pain Int. (Post-Tx)</th>
<th>Pain Int. (%) ↓</th>
<th>Meds</th>
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<td>3</td>
<td>70</td>
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</table>

Note: -- indicates information not available

Abbreviations: Pain Dist.= pain distribution (refers to peripheral branches of the trigeminal nerve (V1: ophthalmic branch; V2: maxillary branch; V3: mandibular branch)); Pt.= patient; Pain Dur. (Yrs)= pain duration (years); Tx= treatment; GK= Gamma Knife radiosurgery; MVD= microvascular decompression; T1= T1-weighted scan (for gray matter analysis); DW= diffusion-weighted scan (for trigeminal nerve analysis); Pain Int. (Pre-Tx)= pain intensity (pre-treatment);
Pain Int. (Post-Tx)= pain intensity (post-treatment); Pain Int. (% ↓)= pain intensity (% decrease);

* indicates patients classified as having effective treatment; CBZ= carbamazepine;

GBP=gabapentin; PGB= pregabalin; BCF= baclofen; DLX= duloxetine.
### Table 7-2: Treatment Effects on Pain and Brain Structure

<table>
<thead>
<tr>
<th>Measure</th>
<th>Treatment Effects(^)</th>
<th>% Change Effective Tx</th>
<th>% Change Ineffective Tx</th>
</tr>
</thead>
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<td><strong>Pain</strong></td>
<td><strong>Pain Intensity</strong></td>
<td>(- 98.5 \pm 5.7^*) (n= 15)</td>
<td>(- 30.5 \pm 25.2) (n= 10)</td>
</tr>
<tr>
<td><strong>Gray Matter: vAI</strong></td>
<td><strong>Cortical Thickness</strong></td>
<td>(3.3 \pm 7.23^*) (n= 15)</td>
<td>(2.0 \pm 10.11) (n= 10)</td>
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<tr>
<td><strong>White Matter: Affected REZ</strong></td>
<td><strong>FA</strong></td>
<td>(10.6 \pm 10.1^*) (n= 8)</td>
<td>(- 3.6 \pm 36.6) (n= 6)</td>
</tr>
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<td></td>
<td><strong>MD</strong></td>
<td>(- 13.3 \pm 24.7) (n= 8)</td>
<td>(10.4 \pm 18.5) (n= 6)</td>
</tr>
<tr>
<td></td>
<td><strong>RD</strong></td>
<td>(- 17.2 \pm 28.3) (n= 8)</td>
<td>(8.0 \pm 25.3) (n= 6)</td>
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<td></td>
<td><strong>AD</strong></td>
<td>(- 9.7 \pm 22.6) (n= 8)</td>
<td>(12.5 \pm 11.8^*) (n= 6)</td>
</tr>
<tr>
<td><strong>White Matter: Unaffected REZ</strong></td>
<td><strong>FA</strong></td>
<td>(4.5 \pm 10.1) (n= 8)</td>
<td>(2.9 \pm 11.5) (n= 6)</td>
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<td></td>
<td><strong>AD</strong></td>
<td>(- 14.1 \pm 19.8) (n= 8)</td>
<td>(- 8.4 \pm 14.9) (n= 6)</td>
</tr>
</tbody>
</table>

Note: \(^*\) Values represent means ± standard deviation and (-) values indicate a mean decrease in the measure post-treatment, compared to pre-treatment. * Indicates significant change (\(p< 0.05\)).
Abbreviations: Tx= treatment; vAI= ventral anterior insula; REZ= trigeminal root entry zone;
FA= fractional anisotropy; MD= mean diffusivity; RD= radial diffusivity; AD= axial diffusivity.
**Figure 7-2:** Abnormal trigeminal root entry zone FA normalizes following effective treatment for TN. (A) An axial DTI scan at the level of the pons highlighting the locations of the REZ masks (in white circles) used to extract each DTI-derived metric. Bar graphs of mean FA values ± SEM are shown for controls (black bars), and for the unaffected (gray) and affected (white) REZ of patients, pre- and post- treatment. Individual patient data are also shown at both time points, with patients who received Gamma Knife radiosurgery in green, and those who received MVD in purple. (B) For the effective treatment group, patients had lower FA at the affected REZ compared to controls and to the unaffected REZ. Effective treatment was associated with an increase in FA towards the level of controls. (C) For the ineffective treatment group, significantly lower pre-treatment FA values at the affected REZ persist in these patients following treatment, compared to the unaffected side and to controls. Abbreviations: Tx= treatment; REZ= trigeminal root entry zone; GKRS= Gamma Knife radiosurgery; MVD= microvascular decompression; Pre-
tx= patients pre-treatment; Post-tx= patients post-treatment; Unaff (L) Side= unaffected (left) side; Aff (R) Side= affected (right) side.
Figure 7-3: Trigeminal abnormalities characterized by higher MD, RD, and AD also normalize following effective TN treatment. The results for the effective (A-C) and the ineffective (D-F) treatment groups are displayed. Bar graphs of the mean MD, RD, and AD values ± SEM are
shown for controls (black bars), and for the unaffected (gray) and affected (white) REZ of patients, pre- and post- treatment. Individual patient data are also shown at both time points, with patients who received Gamma Knife radiosurgery in green, and those who received MVD in purple. For the effective treatment group (left panel): Bilateral increases at the REZ of TN patients were evident pre-treatment for (A) MD, (B) RD, and (C) AD. Effective treatment decreased these metrics bilaterally (except for left AD). For the ineffective treatment group (right panel): Pre-treatment abnormalities at the affected REZ of the ineffective treatment group were evident in the form of higher (D) MD, (E) RD, and (F) AD, compared to controls and to the unaffected side. These abnormalities were maintained, or even worsened following treatment. Asterisks indicate significance at the $p< 0.05$ level. Abbreviations: Tx= treatment; REZ= trigeminal root entry zone; GKRS= Gamma Knife radiosurgery; MVD= microvascular decompression; Pre-tx= patients pre-treatment; Post-tx= patients post-treatment; Unaff (L) Side= unaffected (left) side; Aff (R) Side= affected (right) side.
**Figure 7-4:** Gray matter analyses revealed that the right ventral anterior insula (vAI) was only brain region to normalize in patients following effective treatment. (A) Mask of the right vAI region that cortical thickness data were extracted from (green cluster on inflated brain for visualization purposes). Bar graphs of mean cortical thickness values ± SEM (in mm) are shown for controls (black bars) and patients (white bars). Individual patient data are also shown pre- and post-treatment, with patients who received gamma Knife radiosurgery in green, and MVD in purple. (B) Plot showing resolution of vAI abnormalities in patients who had effective treatment. Pre-treatment, patients had significantly thinner vAI thickness compared to controls. Following
treatment, vAI thickness significantly increased so that it was no longer different from controls. (C) vAI data for ineffective treatment group. Pre-treatment, patients also had significantly thinner vAI thickness compared to controls. Although there was a slight increase in patient vAI thickness following treatment, these values were not significantly different from the patients’ pre-treatment thickness values. Asterisks indicate significance at the $p<0.05$ level.

Abbreviations: vAI= ventral anterior insula; GKRS= Gamma Knife radiosurgery; MVD= microvascular decompression; Tx= treatment.
**Figure 7-5:** A repeated measures ANOVA further characterized patient REZ differences between the effective and ineffective treatment groups. Control values were not included in this analysis, but are shown (black) for comparison purposes. Data plotted are mean DTI metric values ± SEM for the effective (blue) and ineffective (red) treatment groups, before and after surgery (MD, RD, and AD units in mm²/s). A significant group x side interaction was found for all metrics examined (A-D), indicating that the affected and unaffected REZ values differed depending on which group patients belonged to. Additionally, a main effect of side occurred for MD (B) and AD (D). All results shown were significant at the $p<0.05$ level. Abbreviations: Tx= treatment; Pre-Tx= pre-treatment; Post-Tx= post-treatment.
**Figure 7-6:** Correlational analyses show that the proportion of change of DTI metrics was associated with the degree of pain relief following treatment. There was a trend towards a positive correlation between proportion of FA change and percent pain relief, such that the greater the pain relief, the greater the percent % in FA towards the level of healthy controls (A). Furthermore, significant negative correlations were found between percent pain relief and (B) MD, (C) RD, and (D) AD, suggesting that the greater the pain relief, the greater the normalization (% decrease) in MD, RD, and AD. Asterisks indicate significance at the $p<0.05$ level. Abbreviations: AD= axial diffusivity; MD= mean diffusivity; RD= radial diffusivity; FA=
7.4 DISCUSSION

For the first time, we demonstrate that structural MRI and DTI-derived metrics can detect structural abnormalities and plasticity of the trigeminal nerve and brain in response to effective treatment for TN pain. Specifically, structural abnormalities at the trigeminal REZ and vAI cortex reversed towards the level of healthy controls following effective, but not ineffective, neurosurgical treatment and changes in the nerve were associated with the degree of postsurgical pain relief.

7.4.1 Pain Relief Following Effective Treatment

We classified a proportion of the TN patients as having had effective treatment based on a stringent criteria of at least a 75% reduction in pain intensity, which has previously been used [265]. Consistent with previous studies [203,328,331,412], 90% of TN patients achieved effective treatment from MVD surgery. However, fewer patients had effective treatment from GKRS than noted by others (53% vs. 70-80%, respectively) [31,203,243,328,331,412]; partially due to our 75% criteria. The majority of previous studies have used broader indices such as the Barrow Neurological Institute pain scale [155].
7.4.2 Normalization of REZ Microstructural Abnormalities following Effective Surgery Only

Consistent with previous studies [132,200,259,283], we report lower FA at the affected trigeminal REZ of TN patients pre-treatment that significantly increased towards healthy control FA values following effective but not ineffective treatment. Although one study previously showed a decrease in FA following GKRS, FA was extracted from the radiosurgical target distal to the REZ [202]. Prior to treatment, we also found higher MD, RD, and AD bilaterally in patients that had effective treatment, consistent with our previous study [132]. MVD and GKRS reduced these metrics towards the level of healthy controls. Subtle bilateral diffusion abnormalities may be pre-existing in some TN patients, but as effective treatment resolves these abnormalities, they may be associated with pain. Our correlational results indicate that pain relief is linked to MD, RD, and AD normalization following effective treatment at the affected trigeminal REZ only. Contralateral REZ normalization following effective treatment may have occurred secondarily to systemic changes in the trigeminal system as a whole, including other sensory and/or motor behaviors involving bilateral projections.

The affected REZ values resolved following effective treatment, but either stayed the same or worsened following ineffective treatment. Bilateral abnormalities in MD, RD, and AD were not evident in the ineffective treatment group at baseline, suggesting that patients who did not respond to neurosurgical treatment may represent a different subset of TN patients [66]. Differences between patient groups at baseline were also evident, although only AD at the unaffected REZ was significantly different. Therefore, the success of surgical treatment for TN
may be related to the severity of pre-treatment DTI abnormalities. In our study, patients whose treatment was ineffective had also had longer pain duration. This could indicate that they were at a different stage of the disease, making them less responsive to treatments targeting the trigeminal nerve [66], consistent with the finding that TN patients with longer pain durations have a higher likelihood of being treated multiple times [185]. The REZ abnormalities from the affected trigeminal nerve did not resolve in the ineffective treatment group following surgery, corroborating a role for these abnormalities in TN pain and/or pathology. The majority of ineffective responses occurred after GKRS, and the treatment itself may have been ineffective. There is a range of biological responses to radiation [325], which may account for differences in the effectiveness of radiosurgery in the current study. It is also possible that some minimal threshold of REZ normalization is required for patients to experience pain relief. Although the current study cannot definitively determine this threshold, knowing this would be of great clinical significance in determining if the treatment itself was ineffective, and/or if patients could benefit from an additional procedure.

7.4.3 Significance of Trigeminal REZ in TN and Proposed Mechanisms of Pain Relief

MVD and GKRS alleviate pain via different mechanisms and target different anatomical sites on the trigeminal nerve [487,489]. We extracted DTI-metrics only from the REZ. Although both treatments effectively impacted REZ microstructure, the precise mechanisms underlying the analgesic effect of these surgeries and their possible influences on the REZ are not well understood. Anatomically the myelinated axons at the REZ are primarily associated with central
nervous system (CNS) myelin produced by oligodendrocytes. CNS myelin extends beyond the surface of the pons, where it transitions to peripheral myelin produced by Schwann cells [277,341]. Previous studies performing ultrastructural and immunohistochemical analyses of the trigeminal nerve in patients with TN have consistently shown focal abnormalities at the REZ including demyelination [137,277,294]. In contrast, peripheral myelin is significantly more resistant to damage [185] and abnormalities of peripheral myelin are less consistently reported in TN [294].

While microvascular decompression presumably influences REZ abnormalities by removing the source of the compression and pathophysiological sequelae at this location, the mechanisms underlying the effects of GKRS on the REZ are less clear. A prominent theory of TN pathogenesis suggests that NVC-induced demyelination of trigeminal axons results in ephaptic transmission between damaged myelinated and unmyelinated (nociceptive) fibres and ectopic firing, accounting for both touch-induced and spontaneous pain attacks [137]. The net result is a spread of excitatory stimulation between neurons into the CNS. GKRS targets a portion of the trigeminal nerve distal to the REZ to limit the amount of radiation delivered to the brainstem. As the dosage used for TN radiosurgery is sub-necrotic (<100 Gy), GKRS may produce analgesia by injuring trigeminal fibres just enough to impede the aberrant signaling of these neurons. Studies examining the effects of radiation on peripheral nerves have found that radiation doses in the range used for TN radiosurgery can decrease the excitability of cells by partially blocking sodium and nerve conduction [185]. GKRS effects are typically delayed, with patients experiencing pain relief approximately 4-6 weeks post-radiosurgery [418]. Over time, the axonal injury induced by GKRS would resolve and be accompanied by remyelination and normalization
of membrane channels including those at the REZ [185]. While most radiation is delivered to a distal trigeminal target, some reaches the REZ directly. Some evidence suggests that pain outcomes are better when the isocenter is closer to the REZ [60,186]. Similarly, we found that the largest amount of pain relief occurred in patients with the greatest normalization of REZ abnormalities at the affected nerve. Since GKRS does not involve the alleviation of NVC at the REZ, it is possible that over time NVC-induced injury and TN pain may recur, consistent with reports of pain recurrence following GKRS.

7.4.4 vAI was only GM Region to Normalize with Effective Treatment

Using an ROI approach to examine GM regions we previously reported as abnormal in TN [134], we found that the vAI was the only GM region to normalize following effective treatment. Specifically, vAI thickness increased post-treatment so that it was no longer significantly thinner than controls. Effective TN treatment is likely associated with decreased nociceptive input from trigeminal afferents to the central nervous system. Thus, vAI normalization may have occurred secondarily in response to resolution of the REZ abnormalities. Long lasting activity-dependent changes in synaptic plasticity between neurons may occur [2], and be associated with structural GM changes. The resolution of GM abnormalities following successful treatment for chronic pain has previously been reported [191,368,369,394]. Specifically, increased right AI thickness has been shown [368,394] and associated with changes in pain relief [394]. Thinner AI cortex in conjunction with thinner cingulate and dorsolateral prefrontal cortices has been reported across numerous chronic pain conditions [124,199,296]. These areas may reflect pain chronicity in
general [199], and be associated with negative affect and changes in pain modulation [468]. Functional MRI studies have corroborated the role of the vAI insula in affect, and the dorsal AI in salience detection [82,130,254]. With effective treatment for TN, vAI normalization may reflect the cessation of the affective components of pain or other cognitive functions associated with the multi-dimensional experience of pain. Interestingly, other GM regions frequently implicated in pain did not normalize with effective treatment. This brings to question their role in pain, as opposed to other cognitive processes such as attention and salience [145].

7.4.5 Conclusions
A resolution of trigeminal REZ and vAI cortex abnormalities occur following effective neurosurgical treatment for TN. These findings may reflect the alleviation of pain, and/or other sensory, motor, and affective changes occurring with successful treatment. The reversal of REZ abnormalities, as detected by DTI, was associated with pain relief regardless of treatment type. As the DTI metrics examined in the current study have been linked to disrupted WM organization and pathophysiological mechanisms such as demyelination, successful treatment may effectively resolve these issues to contribute to pain relief. As conventional imaging techniques are incapable of detecting these subtle abnormalities, DTI may be a crucial adjunct to conventional imaging. Studying patients with TN to determine whether there has been adequate normalization of DTI metrics could bring us closer to personalizing treatment and permit a better means for the prognosis and assessment of treatment response. For the first time, this would allow for a noninvasive tool capable of objectively measuring the effectiveness of pain treatment.
Chapter 8
General Discussion

8.1 Novel Aspects and Summary of Results

This is the first comprehensive study of both gray and white matter (GM and WM) structural abnormalities in idiopathic TN, and the first to investigate the effects of neurosurgical treatment on these structural abnormalities. The main findings of this thesis are:

1. Compared to healthy controls, TN patients have both cortical and subcortical GM abnormalities in several regions involved in the multi-dimensional experience of pain and associated cognitive and motor functions, characterized by: a) thinner cortex in the pgACC, ventral anterior and posterior insula, and OFC; b) thicker cortex in contralateral S1 (putative face area), M1, and FP; and c) greater subcortical volumes in the thalamus, amygdala, PAG, and basal ganglia (including the putamen and NAc);

2. Patients with TN have WM abnormalities in their trigeminal nerve REZ compared to controls, characterized by lower FA at the affected REZ and higher MD, RD, and AD bilaterally, suggesting disrupted nerve organization and pathological features such as demyelination, neuroinflammation, and edema limited to the REZ;

3. Patients with TN have WM abnormalities in tracts connecting brain regions implicated in the multi-dimensional experience of pain, attention, and motor function, mainly marked by lower FA and higher RD and MD in the corpus callosum, cingulum, posterior corona radiata, and superior longitudinal fasciculus, suggesting disrupted tract organization and pathological features such as neuroinflammation, and edema of cerebral axons;
4. Effective neurosurgical treatment for TN is accompanied by normalization of structural abnormalities at the affected trigeminal REZ and right ventral anterior insula (vAI). The normalization of REZ DTI metrics other than FA were more strongly associated with pain relief indicating that these subtle measures of diffusion, including MD and RD, may be more sensitive in detecting microstructural changes associated with effective treatment in TN. The resolution of vAI thickness abnormalities, on the other hand, may reflect other affective, sensory, and/or cognitive changes associated with treatment success, as following treatment, these changes were not associated with the proportion of pain relief achieved.

Taken together, these studies have demonstrated that idiopathic TN is associated with both brain and trigeminal nerve abnormalities at the REZ. The pattern of abnormalities may reflect TN symptomology, characterized by electric shock-like paroxysmal pain without major sensory loss. Although TN brain abnormalities were widespread, effective treatment was associated with the normalization of only trigeminal REZ and vAI abnormalities with the REZ changes being directly associated with pain relief. Thus, structural abnormalities that may have developed due to chronic pain or alternatively were pre-existing. However, the treatment effects suggest that abnormalities may at least be partially pain-driven. These possibilities will be discussed in more detail below.
8.2 Unique Pain, Unique Brain? Structural GM Abnormalities and Trigeminal Neuralgia Symptomology

Many chronic pain populations have been shown to have structural abnormalities in brain areas involved in both ascending and descending pain pathways and motor functions. Some of these abnormalities are consistent between chronic pains and may reflect the high degree of unpleasantness and/or negative affect from being in chronic pain, in general. However, the direction of the abnormalities (i.e., increases, decreases) varies between chronic pains in some brain areas. One possible explanation is that structural GM abnormalities reflect specific pain and/or sensory symptoms. Thus, here I consider the possibility that the structural GM abnormalities found in TN patients reflect specific TN symptomology.

Many of the GM abnormalities we report in the TN patients were consistent with findings in other chronic pain populations. For example, TN patients had thinner cortex in the pgACC, anterior insula (vAI specifically), and OFC compared to controls, a finding that has been reported in numerous chronic pain conditions [124]. These regions have been implicated in aspects of the cognitive-affective dimension of pain as well as other functions including attention, salience, interoception, and top-down mechanisms such as placebo analgesia, and pain modulation [387]. Less GM in these regions may then reflect the high degree of pain unpleasantness and/or emotional responses to TN or chronic pain in general, as similar findings are consistently reported between chronic pain populations [124]. In support of this finding, we report that abnormalities in the vAI normalize following effective surgical treatment for TN, but were not associated with changes in pain intensity. Therefore, the resolution of this abnormality
may instead reflect other affective, sensory, and/or cognitive changes associated with treatment success. Future studies should examine this possibility by examining the relationship between vAI normalization following treatment and changes in reports of mood, sensation, and/or other behavioural measures.

Within the ascending pathway, nociceptive information is transmitted from the spinal cord/brainstem to specific nuclei in the thalamus, which then relays the information to the cortex for pain perception and cognitive functions related to experiencing pain. Therefore, studies of chronic pain often examine the thalamus, as it is a critical node in the pain pathway. However, there are mixed findings regarding abnormalities of thalamic GM in patients with chronic facial pain [190,308,482]. This variability may be due to the heterogeneity of pain symptomology between chronic facial pains [307]. In one study [190]), the aim was to determine whether abnormalities in brain GM structure and biochemistry are evident in both patients with trigeminal neuropathic pain (TNP) and TMD, with the rationale being that brain abnormalities may differ between chronic pain disorders that are neuropathic (e.g., TNP) versus non-neuropathic (e.g., TMD). The authors discussed that neuropathic pain can result from either central or peripheral somatosensory system damage, while TMDs frequently result from peripheral nociceptor activation [190]. The VBM results indicated that compared to controls, smaller thalamic volumes were evident for patients with trigeminal neuropathy, but not TMD. Furthermore, magnetic resonance spectroscopy revealed that TNP patients also had a reduction in the N-acetylaspartate/creatine ratio, a biochemical marker of neuronal loss or dysfunction in the same region of the thalamus. It was concluded that since the pathogenesis underlying TNP and TMD are fundamentally different, these differences might be generated and/or maintained
by structural abnormalities in brain regions including the thalamus [190]. In our study we report that TN patients had larger thalamic volumes compared to controls. Pain is one prominent feature that typically accompanies trigeminal neuropathy, but it is also characterized by numbness of the skin or mucosal membranes in the distribution of the trigeminal nerve, and/or muscle weakness in the muscles involved in mastication [414]. This is in contrast to TN, which is characterized by paroxysmal pain without major sensory impairment or motor weakness[328,414]. Thus, it is possible that this difference in the direction of thalamic GM abnormality between patients with TNP and TN reflects differences in these symptoms such that the thalamus of TNP patients is steadily receiving trigeminal nociceptive, but lessened discriminative touch input (due to numbness). However, as TN patients are without major sensory loss, a barrage of trigeminal nociceptive input, in combination with mostly intact discriminative touch sensations (i.e., no numbness) may induce GM increases in the medial and lateral thalamic nuclei of TN patients.

Similarly, in our study, patients with TN had thicker S1 cortex (putative face region) compared to controls, but thinner face S1 cortex has previously been reported in patients with TNP, relative to controls [117]. Symptoms between patients with different types of facial pain may also account for differences in the direction of GM abnormalities in S1. S1 is a somatotopically organized cortical area, with some evidence supporting its role in the sensory aspects of pain perception [71,324]. Differences in the amount of nociceptive and sensory input to the thalamus between facial pain populations may lead to activity-dependent plasticity in S1 [473] via thalamocortical projections. A relationship between S1 thickness and function (as measured by the BOLD signal during evoked pain) has previously been demonstrated in patients with TNP
The results of the study indicated that relative to controls, patients with TNP had thinner face S1 cortex, bilaterally [117]. Furthermore, this region of S1 thinning overlapped with S1 functional activations that occurred when patients experienced allodynic pain elicited by brushing the affected facial region. Importantly, patients with TNP frequently have continuous pain, large allodynic regions, and prominent sensory loss, but patients with TN have paroxysmal pain that lasts only seconds to minutes, limited trigger zones that can initiate a pain attack, and no major sensory loss [328]. Since the literature now supports a role for S1 in the sensory aspects of pain perception, it is possible that pain and sensory qualities are reflected as differences in the direction of S1 abnormality between facial pain groups.

We also report that TN patients had thicker M1 cortex and greater posterior putamen volumes bilaterally, compared to controls. This was found in addition to a region of greater basal ganglia volume on the right side, spanning the caudate and NAc. While some of these structures may have multiple roles in the sensory and evaluative aspects of pain [423] [23], the M1 and basal ganglia are primarily known for their role in motor function. Importantly, common triggers of TN pain include talking and chewing. As such, TN patients often use compensatory/nocifensive strategies such as limiting facial movement to avoid triggering pain attacks. The Integrated Pain Adaptation Model [280,340] proposes a framework for nocifensive behaviors. Specifically, the model suggests that pain leads to alterations in muscular activity aimed at limiting movements of an affected muscle by redistributing function and load. In the short term, this protects the system from further injury to support healing, but prolonged muscular alteration can lead to more pain and further peripheral damage. As facial movements such as talking and chewing are characteristically bilateral, the bilateral putamen and M1 findings may reflect this strategy
employed by TN patients to limit and/or prevent pain attacks. Since this model describes how pain and motor function are interrelated, it helps to account for why GM regions classically involved in motor functions might be abnormal in patients with TN. Therefore, if a patient with TN has pain attacks triggered by talking, they may adopt the strategy of restricting the degree to which they open and close their jaws when talking, or move their mouths more slowly to avoid pain. This altered pattern of muscle activation may in turn influence the sensory-motor system via connections in the peripheral and central nervous systems [340], which may be reflected as brain GM abnormalities in motor regions such as M1 and the basal ganglia.

In addition to sensory and motor abnormalities, TN patients were also found to have increased GM volumes of the right amygdala and PAG, compared to control participants. The amygdala is well known for its role in emotion processing, fear conditioning [127], and the emotional-affective dimension of pain [176,320]. There is also evidence for right-lateralized noxious-evoked amygdala activity, possibly related to the emotional response to pain [219], consistent with our GM finding, which was restricted to the right amygdala. As the amygdala receives nociceptive inputs from the dorsal horn via parabrachial area of the brainstem [319], increased right amygdala volume in patients with TN may reflect negative emotional responses due to the barrage of nociceptive input from the trigeminal nerve.

The amygdala also appears to play a role in pain modulation via emotional and attentional processes [320,468]. The top-down modulation of pain via these mechanisms can occur via projections to the PAG, which we also report as having increased volume in TN patients. The
descending modulation of pain can either be inhibitory or facilitatory [334]. Therefore, abnormal amygdala and PAG abnormalities may reflect an abnormal pain modulation system that is either ineffective at inhibiting trigeminal nociceptive inputs, or one that facilitates these trigeminal nociceptive inputs.

The amygdala has also been implicated in the pathophysiology of anxiety disorders [127,361]. One characteristic of TN pain is that it can occur spontaneously or be triggered by normally non-painful stimuli or movements. As such, many TN patients experience elevated levels of anxiety because they are unsure when they may experience an attack of pain, or they fear that doing certain activities will trigger an attack of pain. While this increased anxiety is frequently described anecdotally, one study demonstrated that TN patients had significantly higher levels of depression and anxiety when compared to patients from other hospital clinics, as measured by the Hospital Anxiety Depression (HAD) Scale [79]. Increased amygdala volumes have been reported in childhood anxiety, with machine learning algorithms being able to reliably predict levels of childhood anxiety based on amygdala morphometry [356]. High levels of childhood anxiety were also associated with increased functional connectivity between the amygdala and distributed brain systems involved in attention and emotional regulation [356]. Additionally, there is evidence to suggest that impaired prefrontal functioning in combination with up-regulated amygdala responsivity may contribute to anxiety in adults [48]. In contrast to other chronic pains whereby patients experience more constant pain, the element of not knowing when a pain attack might occur may elicit additional fear or anxiety in TN patients, which may be reflected as enlarged amygdala volumes. In contrast, decreased amygdala GM has been reported
in other chronic pains such as migraine and fibroymyalgia [68,457]. Future studies aimed at better characterizing the psychological and affective aspects of TN are warranted.

8.3 Abnormalities in WM Reveal Potential

Pathophysiological Mechanisms Underlying TN

In addition to the GM abnormalities in patients with TN discussed above, these studies also identified structural abnormalities in the trigeminal nerves and brain WM. The brain WM abnormalities were located in tracts connecting regions implicated in the multi-dimensional experience of pain, attention, and motor function. These WM abnormalities, characterized by lower FA, and higher RD, MD, and AD, suggest not only disrupted nerve/tract organization, but also other pathological features such as neuroinflammation, and edema of the nerve/axons.

WM abnormalities have been reported in the brains of numerous neurological populations, with decreased FA being the most commonly reported finding. Additionally, WM tracts, including the corpus callosum, are abnormal across several patient populations in general. In our study, we used DTI-derived metrics in addition to FA to better characterize the microstructural abnormalities because decreased FA can result from different scenarios. These other metrics, MD, RD, and AD, have been linked to pathophysiological mechanisms such as demyelination, edema, and neuroinflammation. The use of multiple metrics might aid in determining potential pathophysiological differences between patient populations in the same brain areas that report decreased FA. For example, we reported abnormalities in the corpus callosum of TN patients as
demonstrated by lower FA, and higher MD, RD, and AD. Decreased FA in the corpus callosum of patients with clinical depression have also been reported, however, FA was characterized by higher RD and MD, but preserved AD in this case [5]. Therefore, the abnormal diffusion measures in the WM tracts common to both mood disorders and chronic pain disorders may be different due the differences in etiology and pathophysiology between the groups, even though they share some common clinical features such as negative affect [133].

8.4 Importance of the Trigeminal REZ in TN and Pain Relief Following Effective Treatment

The term ‘idiopathic’ was originally used to describe classical TN since a cause for this severe facial pain could not be discerned at the time. In 1929, Dandy observed that TN patients frequently had grooves or bends in their trigeminal nerves made by offending vessels [110]. In line with this NVC theory of TN, the results of this thesis indicate that DTI can be used to identify abnormalities limited to the trigeminal REZ that are likely related to the underlying pathophysiology of TN, and further question the use of the term ‘idiopathic’ to describe TN. We reported that TN patients had lower FA at the affected trigeminal REZ, compared to their unaffected side and to healthy controls, which is consistent with a decrease in WM “integrity” by NVC only at the affected trigeminal REZ. In some additional analyses that were not published in these studies, DTI metrics were extracted from a control trigeminal site distal to the REZ and significant differences between affected and unaffected or control nerves were not obtained. While others have previously demonstrated lower FA at the affected REZ [200,259,283], a
decrease in FA can occur in multiple scenarios [3]. For the affected REZ of TN patients, a disproportionately greater increase in RD and MD over AD can account for the significantly lower FA. This ratio may reflect focal injury and/or demyelination from NVC at the REZ of the affected nerve, but not the unaffected nerve, or general microstructural disintegration of the affected nerve. Interestingly, MD, RD, and AD measures were abnormal at both the affected and unaffected REZ, compared to controls. Therefore, subtle microstructural abnormalities may occur in the trigeminal system as a whole in this patient group. While it is possible that some of these subtle bilateral abnormalities are pre-existing in patients, making them more susceptible to developing TN, an alternate hypothesis is that these bilateral abnormalities reflect compensatory motor behaviors. Given the bilateral innervation in this system, nocifensive behaviors, including limiting jaw movements, may result in abnormalities to both trigeminal nerves or to CNS WM along motor pathways.

Importantly, effective surgical treatment for TN was associated not only with the normalization of trigeminal abnormalities at the affected REZ, but also at the unaffected REZ. Our correlational results indicated that the proportion of MD, RD, and AD normalization in TN patients following effective treatment was associated with the degree of pain relief at the affected trigeminal REZ only. Therefore, contralateral REZ normalization in the effective treatment group may have occurred secondarily to systemic changes in the trigeminal system as a whole, including other or other sensory and/or motor behaviors involving bilateral projections. While some of the bilateral abnormalities in TN patients may be pre-existing, this finding suggests that they are at least partially due to TN, as they resolve following effective treatment.
8.5 Study Limitations

There were some limitations in this thesis that should be considered when interpreting the results. Limitations specific to each study were discussed in the corresponding chapters.

1. The majority of TN patients included in the study were on medications, most commonly the anti-epileptic medication carbamazepine, at the time of their MR scans (which included pre- and post-treatment scans for some individuals). Although the precise impact of these medications on brain morphology is not known, it is possible that they influence structural abnormalities. For example, medications may influence immune activity [34,49], which may in turn affect MR-derived measures associated with inflammation and/or edema. Future studies are needed to examine the effects of these drugs on brain structure.

2. There were practical issues related to clinical imaging scheduling that imposed limits on scan time. These limitations impacted the choice of MRI protocols. For example, a very lengthy DTI scan was not possible and so the shorter DTI sequence created images that were not iso-voxel (i.e., voxels had different dimensions in the x, y, and z planes). It has been suggested that diffusion-weighted scans ideally be collected with iso-voxel resolution because diffusion anisotropy measures of WM may be affected by voxel size [333]. In one study, FA measures extracted from non iso-voxels were underestimated in areas of crossing fibers [333]. However, it should be noted that the in this thesis, the patients and control subjects were scanned using the same protocol. Therefore any biases in diffusion metrics would be expected to have been equal.
between groups. Still, to ensure that the measurements are as accurate as possible, future studies should acquire DTI data with iso-voxel dimensions.

3. The study did not control for potential psychological comorbidities such as depression and anxiety. It is well documented that mood disorders are associated with structural and functional brain abnormalities in regions that are also implicated in the cognitive, affective, and emotional aspects of pain [148]. Indeed several patients with chronic pain also have comorbid depression and anxiety. As such, mood is certainly an important factor to consider when studying any chronic pain population. An attempt to study chronic pain patients that are without some degree of negative affect would be studying a non-representative group [133]. Therefore, future research aimed at better understanding this complex relationship is required.

4. TN patients included in the treatment study underwent one of two different surgical procedures, MVD or GKRS. As previously described, MVD and GKRS alleviate pain via different mechanisms and target different anatomical sites on the trigeminal nerve. The goal of MVD is to alleviate the compression on the trigeminal nerve, usually at the REZ, caused by an offending vessel. GKRS targets the cisternal portion of the trigeminal nerve slightly distal to the REZ in order to partially damage the affected trigeminal nerve, possibly by decreasing abnormal afferent inputs [487,489]. Regardless of treatment type, patient REZ abnormalities normalized following effective treatment, highlighting the importance of this region in TN pathophysiology. With that said, it is difficult to interpret the mechanism of pain relief in a mixed treatment group, as different treatments may affect structures along pain pathways differently. This may be the
reason that the only GM region to normalize following effective treatment was the vAI, in contrast to other studies that have reported more widespread normalization following effective treatment [368,369,394]. Additionally, patients in our study were only followed-up at one time-point within approximately six months post-surgery. In a study examining the effects of acute repetitive painful stimulation on GM in healthy individuals, it was reported that the resultant GM increases to the MCC and contralateral S1 cortex were maintained at three weeks post-study, but receded by the one-year follow-up [438]. Although this study was conducted using acute stimulation in healthy individuals, it is possible that in our study, another time further post-surgery is required to detect the normalization of structural abnormalities in the brain GM of TN patients. Having a larger sample size is also warranted to increase the generalizability of the findings.

8.6 Future Directions

In addition to addressing the limitations listed above, future studies could build on the findings of this thesis to better elucidate the mechanisms underlying TN pathophysiology and effective treatment as follows:

1. Future studies should incorporate additional behavioural measures (e.g., quantitative sensory testing, motor testing, depression, anxiety, and personality scales) so that the hypothesized reasons for the direction of the GM abnormalities can be tested.
2. Future studies should also consider the degree of NVC that occurs in patients with TN. One way to do this is to categorize degrees of NVC according to the method outlined by Satoh and colleagues: severe (NVC with the vessel covering >20% of the trigeminal nerve circumference); moderate (NVC with vessel covering <20% of the nerve circumference); simple (the vessel slightly touches the trigeminal nerve); none (no contact between any vessel and the trigeminal nerve) [378]. In this way, relationships between the degree of NVC and nerve/brain abnormalities could be examined. Additionally, the impact of NVC severity treatment outcome could be assessed.

3. Studies are needed to examine the cellular mechanisms underlying MR-detectable structural abnormalities in chronic pain. To examine this specifically in TN, an appropriate animal model of TN is needed to ensure that the sharp and paroxysmal nature of TN pain could be mimicked, unlike current animal models of neuropathic pain [311].

4. Functional imaging studies using resting state connectivity approaches would be valuable in the future to examine how TN changes brain networks. Several studies have now demonstrated disrupted brain resting state networks using fMRI in chronic pain populations. This has yet to be explored in TN patients. If TN patients were also to present with resting state functional abnormalities before treatment, effective surgical treatment may influence functional changes that precede structural changes. One study examining back pain patients as they transitioned from acute to chronic pain and reported decreased brain GM that was preceded by initially greater corticostriatal functional connectivity [24]. In other words, altered functional connectivity
predicted pain persistence in back pain patients before GM changes could [24]. This technique may be useful in helping to determine why pain persists for some TN patients following treatment.

5. Other functional imaging methods such as positron emission tomography (PET) could provide insight into the molecular mechanisms of TN, for example, by investigating mu-opioid receptor availability in vivo. In patients with trigeminal neuropathic pain, clinical pain has been associated with alterations in the endogenous mu-opioid system, which may similarly occur in TN [142].

6. Multivariate statistical techniques for neuroimaging are now available. Specifically, multivariate pattern analyses (MVPA) that are based on machine learning techniques can identify spatial and/or temporal patterns of large datasets that best discriminate between two or more conditions (e.g., acute pain patients that will recover versus acute pain patients that will develop chronic pain) [371]. One potential application of this technique would be to differentiate potential subtypes of TN. For example, there is some debate in the literature as to whether TN with and without concomitant facial pain are actually separate disorders. It has been suggested that they may represent different degrees of injury to the trigeminal nerve, and therefore be part of a continuous spectrum rather than discrete diagnoses [66]. Another potential use for MVPA is to determine if certain patients would have greater benefit from one surgery type over another, which could potentially prevent patients from having to undergo multiple surgeries.
8.7 Conclusions

In summary, this thesis provides novel evidence for structural abnormalities in the trigeminal nerve and brain of TN patients in regions involved in the multi-dimension experience of pain, pain modulation, and motor function. The precise pattern of abnormalities may be reflective of symptomology specific to TN and may implicate certain pathophysiological mechanisms such as neuroinflammation and/or edema in this disorder. Additionally, effective surgical treatment for TN normalized structural abnormalities at the trigeminal REZ and insular cortex, with the REZ normalization being specifically associated with pain relief. These findings demonstrate the malleability of the brain and trigeminal nerve and may reflect the alleviation of pain, and/or other sensory, motor, and affective changes that occur with successful treatment.
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