Structural Brain Abnormalities in Temporomandibular Disorders

by

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Abstract

Temporomandibular disorders (TMD) are a family of prevalent chronic pain disorders affecting masticatory muscles and/or the temporomandibular joint. There is no unequivocally recognized peripheral aetiology for idiopathic TMD. The central nervous system (CNS) may initiate and/or maintain the pain in idiopathic TMD due to sustained or long-term nociceptive input that induces maladaptive brain plasticity, and/or to inherent personality-related factors that may reduce the brain's capacity to modulate nociceptive activity. The main aim of this thesis is to determine whether there are structural neural abnormalities in patients with TMD, and whether these abnormalities are related to TMD pain characteristics, or to neuroticism. The specific aims are to delineate in TMD: (1) gray matter (GM) brain abnormalities and the contribution of pain and neuroticism to abnormalities; (2) the contribution of abnormal brain GM aging in focal cortical regions associated with nociceptive processes; and (3) abnormalities in brain white matter and trigeminal nerve and the contribution of pain. In groups of 17 female patients with TMD and 17 age- and sex- matched controls, magnetic resonance imaging revealed that patients with TMD had: (1) thicker cortex in the somatosensory, ventrolateral prefrontal and frontal polar cortices
than controls, (2) cortical thickness in motor and cognitive areas that was negatively related to pain intensity, orbitofrontal cortical thickness that was negatively correlated to pain unpleasantness, and thalamic GM volume correlated to TMD duration, (3) an abnormal relationship between neuroticism and orbitofrontal cortical thickness, (4) abnormal GM aging in nociceptive, modulatory and motor areas, (5) widespread abnormalities in white matter tracts in the brain related to sensory, motor and cognitive functions, (6) reduced trigeminal nerve integrity related to pain duration, and (7) abnormal connectivity in cognitive and modulatory brain regions. In sum, this thesis demonstrates for the first time abnormalities in both peripheral nerve and CNS in patients with TMD.
Acknowledgments

This thesis is dedicated to my father, who instilled in me a deep appreciation for learning, an insatiable curiosity to understand the world, the drive to do so, and the humbleness to realize that I may not understand everything. I owe my successes to his unwavering love and support.

I am grateful to Dr. Karen Davis for her mentorship. As a supervisor, Karen has a unique understanding of her students’ needs and addressing them. Her training is unparalleled: the emphasis and careful study of the body of literature is truly humbling. She has a deep understanding of the scientific method, and her meticulous methodology maintains a level of rigour and a high standard of quality in the work produced in her lab. Karen’s greatest skill is transferring this deep appreciation to her students. Her patience and flexibility allow students to find themselves a niche within the field, and to develop an expertise. Her rigour ensures that the questions are addressed appropriately, and that studies are well-conducted. The greatest thing I will take away from Karen’s lab is a strong sense of integrity. This is the greatest gift a young scientist can be given – Thank you, Karen.

I am also very grateful to my committee members, Drs. Adrian Crawley, Barry Sessle and Howard Tenenbaum, for their guidance and direction. They have helped me tremendously in the development of the questions posed in this thesis, through helping me navigate the literature, and understand complex concepts. I would like to especially thank Adrian for all of the time he spent with me outside the lab, and the friendship we have developed. I would also like to thank Dr. Mary Pat McAndrews – an unofficial committee member who has provided me with much feedback, support and was always ready to lend an ear.

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guidance, leadership and support. They also helped us stay on track and helped us develop a deeper understanding of science. I am very grateful for time and the many conversations we had together.

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During my training, the lab had a turnover, and the Davis lab was full of new faces and colleagues. Drs. Nathalie Erpelding and Tim Salomons have been phenomenal colleagues and are great friends. Both have helped me academically, and have become some of my closest friends. Qi Wu, Danielle DeSouza, Aaron Kucyi, Gang Wang, and Ruma Goswami are great additions to the lab, full of ideas. It’s been a real pleasure working alongside such brilliant minds for the past five years, and I will take away many pleasant memories.

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I would like to thank my friends for their distractions, their support and their love. Your encouragement got me through this!

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Human beings are members of a whole,
In creation of one essence and soul.
If one member is afflicted with pain,
Other members uneasy will remain.
If you've no sympathy for human pain,
The name of human you cannot retain!

-Sa’adi, Golestan, 1258
It is a shame that we possess such insufficient knowledge concerning the character of pain – those symptoms which represent the essential part of all bodily suffering of man.

-A. Goldscheider, *Ueber den Schmerz*, 1894
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<th>Description</th>
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<tbody>
<tr>
<td>AAOP</td>
<td>American Association of Orofacial Pain</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
</tr>
<tr>
<td>aMCC</td>
<td>Anterior mid-cingulate cortex</td>
</tr>
<tr>
<td>AMH</td>
<td>Aδ-mechano-heat sensitive afferent</td>
</tr>
<tr>
<td>Amyg</td>
<td>Amygdala</td>
</tr>
<tr>
<td>ASSET</td>
<td>Array Spatial Sensitivity Encoding Technique</td>
</tr>
<tr>
<td>B₀</td>
<td>Magnetic field</td>
</tr>
<tr>
<td>BA</td>
<td>Brodmann’s Area</td>
</tr>
<tr>
<td>BCE</td>
<td>Before Common Era</td>
</tr>
<tr>
<td>BET</td>
<td>Brain Extraction Tool</td>
</tr>
<tr>
<td>BG</td>
<td>Basal ganglia</td>
</tr>
<tr>
<td>Cb</td>
<td>Cerebellum</td>
</tr>
<tr>
<td>CBP</td>
<td>Chronic back pain</td>
</tr>
<tr>
<td>cCMA</td>
<td>Caudal cingulate motor area</td>
</tr>
<tr>
<td>CH</td>
<td>Cluster headache</td>
</tr>
<tr>
<td>CL</td>
<td>Centrolateral nucleus of the thalamus</td>
</tr>
<tr>
<td>CM</td>
<td>Centromedian nucleus of the thalamus</td>
</tr>
<tr>
<td>CMA</td>
<td>Cingulate motor area</td>
</tr>
<tr>
<td>CMH</td>
<td>C-mechano-heat sensitive afferent</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CNV</td>
<td>Cranial nerve five (trigeminal nerve)</td>
</tr>
<tr>
<td>CNVII</td>
<td>Facial nerve</td>
</tr>
<tr>
<td>CNIX</td>
<td>Glossopharyngeal nerve</td>
</tr>
<tr>
<td>CNX</td>
<td>Vagus nerve</td>
</tr>
<tr>
<td>CPP</td>
<td>Chronic pelvic pain</td>
</tr>
<tr>
<td>CPTH</td>
<td>Chronic posttraumatic headache</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>CTTH</td>
<td>Chronic tension-type headache</td>
</tr>
<tr>
<td>DBM</td>
<td>Deformation-based morphometry</td>
</tr>
<tr>
<td>DCML</td>
<td>Dorsal-column medial-leminiscal pathway</td>
</tr>
<tr>
<td>dIPFC</td>
<td>Dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DLPT</td>
<td>Dorsolateral pontomesencephalic tegmentum</td>
</tr>
<tr>
<td>dmPFC</td>
<td>Dorsomedial prefrontal cortex</td>
</tr>
<tr>
<td>DNIC</td>
<td>Diffuse noxious inhibitory controls</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>dODF</td>
<td>Diffusion orientation density functions</td>
</tr>
<tr>
<td>dPCC</td>
<td>Dorsal posterior cingulate cortex</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion-weighted imaging</td>
</tr>
<tr>
<td>EC/ExC</td>
<td>External/Extreme capsules</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>FDT</td>
<td>FSL Diffusion Toolbox</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FMS</td>
<td>Fibromyalgia</td>
</tr>
<tr>
<td>fODF</td>
<td>Fibre orientation density functions</td>
</tr>
<tr>
<td>FSL</td>
<td>FMRIB's Software Library</td>
</tr>
<tr>
<td>FSPGR</td>
<td>Fast spoiled gradient echo</td>
</tr>
<tr>
<td>FWE</td>
<td>Family-wise error</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-width half-maximum</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>GMV</td>
<td>Gray matter volume</td>
</tr>
<tr>
<td>GP</td>
<td>Globus pallidus</td>
</tr>
<tr>
<td>HC</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Hip OA</td>
<td>Hip osteoarthritis</td>
</tr>
<tr>
<td>HPC</td>
<td>Heat-pinch-cold</td>
</tr>
<tr>
<td>HT</td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>HTM</td>
<td>High-threshold mechanoreceptors</td>
</tr>
<tr>
<td>HypH</td>
<td>Hypnic headache</td>
</tr>
<tr>
<td>IASP</td>
<td>International Association for the Study of Pain</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>IC</td>
<td>Internal capsule</td>
</tr>
<tr>
<td>IC_AL</td>
<td>Anterior limb of the internal capsule</td>
</tr>
<tr>
<td>ICBM</td>
<td>International Consortium for Brain Mapping</td>
</tr>
<tr>
<td>iTL</td>
<td>Inferior temporal lobe</td>
</tr>
<tr>
<td>LC</td>
<td>Locus coeruleus</td>
</tr>
<tr>
<td>LEP</td>
<td>Laser-evoked potentials</td>
</tr>
<tr>
<td>LTM</td>
<td>Low-threshold mechanoreceptors</td>
</tr>
<tr>
<td>M1</td>
<td>Primary motor cortex</td>
</tr>
<tr>
<td>MCC</td>
<td>Mid-cingulate cortex</td>
</tr>
<tr>
<td>MCS</td>
<td>Motor cortex stimulation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MD</td>
<td>Mean diffusivity</td>
</tr>
<tr>
<td>MD</td>
<td>Medial dorsal thalamus</td>
</tr>
<tr>
<td>MDvc</td>
<td>Ventral caudal portion of the medial dorsal nucleus of the thalamus</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
</tr>
<tr>
<td>MeT</td>
<td>Mesencephalic nucleus of the trigeminal nerve</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>MOH</td>
<td>Medication-overuse headache</td>
</tr>
<tr>
<td>mPFC</td>
<td>Medial prefrontal cortex</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MSN</td>
<td>Main sensory nucleus</td>
</tr>
<tr>
<td>NC</td>
<td>Caudate nucleus</td>
</tr>
<tr>
<td>NEO-FFI</td>
<td>NEO-Five Factor Inventory</td>
</tr>
<tr>
<td>NRM</td>
<td>Nucleus raphe magnus</td>
</tr>
<tr>
<td>NS</td>
<td>Nociceptive-specific</td>
</tr>
<tr>
<td>ODF</td>
<td>Orientation density functions</td>
</tr>
<tr>
<td>OFC</td>
<td>Orbitofrontal cortex</td>
</tr>
<tr>
<td>PAG</td>
<td>Periaqueductal gray</td>
</tr>
<tr>
<td>PCS</td>
<td>Pain catastrophizing scale</td>
</tr>
<tr>
<td>PDF</td>
<td>Probability density function</td>
</tr>
<tr>
<td>PET</td>
<td>Positron-emission tomography</td>
</tr>
<tr>
<td>Pf</td>
<td>Parafascicular nucleus of the thalamus</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>pgACC</td>
<td>Pregenual anterior cingulate cortex</td>
</tr>
<tr>
<td>PHG</td>
<td>Parahippocampal gyrus</td>
</tr>
<tr>
<td>PIFP</td>
<td>Persistent idiopathic facial pain</td>
</tr>
<tr>
<td>plIC</td>
<td>Posterior limb of the internal capsule</td>
</tr>
<tr>
<td>PMC</td>
<td>Premotor cortex</td>
</tr>
<tr>
<td>pMCC</td>
<td>Posterior mid-cingulate cortex</td>
</tr>
<tr>
<td>PMv</td>
<td>Ventral premotor cortex</td>
</tr>
<tr>
<td>PNS</td>
<td>Peripheral nervous system</td>
</tr>
<tr>
<td>PO</td>
<td>Posterior nucleus of the thalamus</td>
</tr>
<tr>
<td>PPC</td>
<td>Posterior parietal cortex</td>
</tr>
<tr>
<td>pTL</td>
<td>Posterior temporal lobe</td>
</tr>
<tr>
<td>Put</td>
<td>Putamen</td>
</tr>
<tr>
<td>PVD</td>
<td>Provoked vestibulodynia</td>
</tr>
<tr>
<td>QST</td>
<td>Quantitative sensory testing</td>
</tr>
<tr>
<td>RA</td>
<td>Relative anisotropy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>rCBF</td>
<td>Regional cerebral blood flow</td>
</tr>
<tr>
<td>rCMA</td>
<td>Rostral cingulate motor area</td>
</tr>
<tr>
<td>RCZ</td>
<td>Rostral caudal zone</td>
</tr>
<tr>
<td>RD</td>
<td>Radial diffusivity</td>
</tr>
<tr>
<td>rf</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>RF</td>
<td>Reticular formation of the brainstem</td>
</tr>
<tr>
<td>RFT</td>
<td>Random field theory</td>
</tr>
<tr>
<td>Rheum Arth</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RVM</td>
<td>Rostral ventromedial medulla</td>
</tr>
<tr>
<td>S</td>
<td>Signal</td>
</tr>
<tr>
<td>S1</td>
<td>Primary somatosensory cortex</td>
</tr>
<tr>
<td>S2</td>
<td>Secondary somatosensory cortex</td>
</tr>
<tr>
<td>SC</td>
<td>Subcoeruleus</td>
</tr>
<tr>
<td>sgACC</td>
<td>Subgenual cingulate cortex</td>
</tr>
<tr>
<td>SM</td>
<td>Submedian nucleus of the thalamus</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary motor area</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical parametric mapping</td>
</tr>
<tr>
<td>STG</td>
<td>Superior temporal gyrus</td>
</tr>
<tr>
<td>STN</td>
<td>Spinal trigeminal nucleus</td>
</tr>
<tr>
<td>STT</td>
<td>Spinothalamic tract</td>
</tr>
<tr>
<td>SVC</td>
<td>Small-volume correction</td>
</tr>
<tr>
<td>T1</td>
<td>Spin-lattice relaxation time or Magnetization time</td>
</tr>
<tr>
<td>T2</td>
<td>Spin-spin relaxation time or Signal decay time</td>
</tr>
<tr>
<td>TBSS</td>
<td>Tract-based spatial statistics</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>Thal</td>
<td>Thalamus</td>
</tr>
<tr>
<td>TI</td>
<td>Inversion time</td>
</tr>
<tr>
<td>TIV</td>
<td>Total intracranial volume</td>
</tr>
<tr>
<td>TL</td>
<td>Temporal lobe</td>
</tr>
<tr>
<td>TMD</td>
<td>Temporomandibular disorder</td>
</tr>
<tr>
<td>TMD-RDC</td>
<td>Temporomandibular disorder research diagnostic criteria</td>
</tr>
<tr>
<td>TMJ</td>
<td>Temporomandibular joint</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>TTT</td>
<td>Trigeminothalamic tract</td>
</tr>
<tr>
<td>uODF</td>
<td>Uncertainty orientation density functions</td>
</tr>
<tr>
<td>Symbol</td>
<td>Term</td>
</tr>
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<td>--------</td>
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</tr>
<tr>
<td>V₁</td>
<td>Ophthalmic branch of the trigeminal nerve</td>
</tr>
<tr>
<td>V₁₁</td>
<td>Primary visual cortex</td>
</tr>
<tr>
<td>V₂</td>
<td>Maxillary branch of the trigeminal nerve</td>
</tr>
<tr>
<td>V₃</td>
<td>Mandibular branch of the trigeminal nerve</td>
</tr>
<tr>
<td>VBM</td>
<td>Voxel-based morphometry</td>
</tr>
<tr>
<td>VBSNC</td>
<td>Trigeminal brainstem nuclear complex</td>
</tr>
<tr>
<td>V₅c</td>
<td>Subnucleus caudalis</td>
</tr>
<tr>
<td>V₅i</td>
<td>Subnucleus interpolaris</td>
</tr>
<tr>
<td>VL</td>
<td>Ventrolateral nucleus of the thalamus</td>
</tr>
<tr>
<td>vLPFC</td>
<td>Ventrolateral prefrontal cortex</td>
</tr>
<tr>
<td>vmPFC</td>
<td>Ventromedial prefrontal cortex</td>
</tr>
<tr>
<td>vmPO</td>
<td>Posterior region of the ventromedial nucleus of the thalamus</td>
</tr>
<tr>
<td>V₀</td>
<td>Subnucleus oralis</td>
</tr>
<tr>
<td>vPCC</td>
<td>Ventral posterior cingulate cortex</td>
</tr>
<tr>
<td>VPI</td>
<td>Ventroposteriorinferior nucleus of the thalamus</td>
</tr>
<tr>
<td>VPL</td>
<td>Ventroposterolateral nucleus of the thalamus</td>
</tr>
<tr>
<td>VPM</td>
<td>Ventroposteriomedial nucleus of the thalamus</td>
</tr>
<tr>
<td>vSTR</td>
<td>Ventral striatum</td>
</tr>
<tr>
<td>WDR</td>
<td>Wide-dynamic range</td>
</tr>
<tr>
<td>γ</td>
<td>Gyromagnetic ratio</td>
</tr>
<tr>
<td>λ₁</td>
<td>Longitudinal diffusivity</td>
</tr>
<tr>
<td>ρ</td>
<td>Proton density</td>
</tr>
<tr>
<td>τ</td>
<td>Diffusion time</td>
</tr>
<tr>
<td>ω₀</td>
<td>Precession rate</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction and General Aims

Temporomandibular disorders (TMD) comprise clinical problems involving the structures of and around the temporomandibular joint (TMJ), the masticatory musculature, or both [1,418]. TMD represent the most common orofacial chronic pain disorder [285], and are primarily characterized by spontaneous pain, or pain associated with jaw function, which can affect mandibular range of motion and produce TMJ sounds during jaw function. Pain from TMD can arise from the muscles of mastication, the TMJ, or both, and, in general, when combined (i.e., muscular and TMJ pain) the chief complaint tends to be muscular in nature [402].

There have not yet been any comprehensive epidemiological studies of TMD in the Canadian population, and so this thesis will refer to data from the United States. TMD are estimated to affect between 3-20 percent of the United States’ adult population [269,539,540,562]. Of these, only a small proportion of persons suffering from TMD pain seek treatment, and are seen at tertiary and quaternary medical facilities, such as pain clinics [269,350]. It has been estimated that TMD cost $4 billion annually due to lost-wages and medical treatment. TMD 1.5-9 times more prevalent in women [113,201,284,285,312,721] and a nationwide epidemiological study in the United States reported that 84% of persons with TMD were women [284].

In some cases, there is no clear aetiological evidence for TMD pain, and the pain is considered idiopathic [283,286,287,653]. In this group of patients, it has been suggested that abnormal function in the central nervous system (CNS) may initiate or maintain TMD pain [757]. One line of evidence for abnormal CNS function is that TMD symptomatology, including persistent pain, allodynia, and hyperalgesia occurs not only in the orofacial region, but also in other body sites [305,314,390,565,756,883]. Patients with TMD also show greater temporal summation of pain to repetitive noxious heat stimuli [563], and dysfunctional diffuse noxious inhibitory controls (DNIC) [113,491,756]. These centrally-mediated processes provide further evidence for CNS dysfunction in TMD and provide evidence for the involvement of ascending nociceptive pathways and/or descending pain-modulatory pathways [515]. Additionally, patients with TMD can exhibit cognitive [370,379,380] and motor dysfunction [837] possibly related to abnormalities in brain regions associated with these functions [799,800,839,928].
There are two main routes by which the CNS may contribute to the development and/or maintenance of chronic pain conditions such as TMD. One possibility is that long-term nociceptive input into the brain induces maladaptive brain plasticity, which may play a role in maintaining pain [12,213,576,950]. This maladaptive plasticity suggests that patients with TMD may be unable to habituate to increased nociceptive activity, which may be related to a reduced capacity of the brain to dampen pain by descending (top-down) controls [95].

The second route by which the CNS may contribute to the development and/or maintenance of chronic pain relates to inherent personality-related factors that reduce the brain’s capacity to modulate nociceptive input. This poor control of pain might lead to the development of vulnerability towards the development of chronic pain. For example, there is evidence that neuroticism may be associated with pain-related suffering [408], pain sensitivity [177,373,907], nerve injury outcomes as well as neuropathic pain [848], inhibition of negative thoughts [178], and the development of TMD [315].

Structural magnetic resonance imaging (MRI) and diffusion-weighted imaging (DWI) can be used to investigate the structure of CNS gray and white matter, respectively. Indeed, many structural MRI studies have demonstrated differences in gray matter in populations of patients with chronic pain associated with pain-related characteristics (e.g., intensity, unpleasantness, or duration) (see [98,206,303,356,575,764,772]). Furthermore, structural MRI studies have reported accelerated loss of gray matter in the brains of patients with chronic pain [27,502]. These age-related changes in chronic pain may be related to the cumulative effect of pain over time (i.e., pain duration) or other pre-existing factors that may alter the normal pattern of aging in TMD. In addition to abnormalities in central gray matter found in the presence of chronic pain, previous studies examining measures of white matter integrity in other clinical conditions with sensory abnormalities and/or chronic pain reported abnormalities in white matter tracts related to sensory, modulatory and cognitive functions [156,356,558,847]. Furthermore, these studies reported white matter abnormalities correlated with clinical findings in the patients. Therefore, studying the correlation between TMD characteristics and measures of white matter integrity can provide insight into whether chronic pain drives changes in white matter microstructure.

With regard to the second route by which the CNS may contribute to the development and/or maintenance of chronic pain, pre-existing abnormalities may be a factor for the development of
TMD. These structural abnormalities in the CNS could be related to personality traits that are related to chronic pain, such as neuroticism [315]. However, not all patients with chronic pain have high neuroticism scores, and not all persons with neuroticism have chronic pain [179]. Therefore, neuroticism alone is not sufficient to develop chronic pain. Rather, there may exist an abnormal relationship between neuroticism and brain structure, which may impair function in pain-modulatory brain regions, such as the orbitofrontal cortex (OFC) [954] or the medial prefrontal cortex (mPFC) [245,386] and this could facilitate or maintain chronic pain. However, the precise mechanism by which neuroticism can contribute to TMD development remains to be elucidated.

Given the poor understanding of the mechanisms underlying TMD, it is challenging to develop rational treatment approaches for this chronic pain condition. Moreover, it is becoming clearer that TMD may be mediated by central mechanisms. However, while there are some data demonstrating neuropsychological and cognitive, as well as psychosocial contributors to TMD, there are little data demonstrating whether there are underlying neuroanatomical changes that could support the notion that TMD is a centrally-mediated pain condition.

Therefore, the **main aims** of this thesis are:

1. To examine gray matter abnormalities in patients with idiopathic TMD and to determine the contribution of pain-related characteristics and neuroticism;

2. To determine whether the cumulative effect of idiopathic TMD interacts with the normal aging process in focal cortical regions associated with nociceptive processes;

3. To evaluate white matter brain and trigeminal nerve (cranial nerve five: CNV) abnormalities in patients with idiopathic TMD and to determine the contribution of pain-related characteristics.
Chapter 2
Literature Review

2.1. What is pain?

The current definition of pain, established by International Association for the Study of Pain (IASP) in 1986, defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of tissue damage, or both.” It is important to recognize that pain and nociception are not synonymous. Nociception, based on the 2011 IASP taxonomy [110], is “the neural process of encoding noxious stimuli” and is initiated by the activation of peripheral receptors that preferentially respond to stimuli in the noxious range, referred to as nociceptors. It includes the neural activity in the peripheral nervous systems (PNS) and CNS, elicited by a noxious stimulus. In contrast to nociception, pain requires a conscious perception.

Pain is a complex, multidimensional experience involving sensory, affective, cognitive, attentional, motivational, and motor components [593]. The sensorial dimension of pain relates to the discriminability of pain qualities, location, intensity and duration of pain. Pain is inherently salient. Based on the context, our psychological and affective state, we initiate a response to noxious stimulus. Therefore, different people respond to pain differently.

Pain can be categorized based on its temporal profile: short-term pain is known as acute pain, and lasts from seconds to days. Acute pain plays an essential role in signaling actual or potential tissue damage [442]. However, chronic pain persists more than three to six months, and is not necessarily related with tissue damage [791]. The aetiology of chronic pain is not as evident as it is in acute pain. Often, chronic pain persists beyond the time necessary for the tissue to heal normally following injury and, in these cases, it is believed that nociceptive input and/or the CNS maintain pain [576]. The IASP has devised a classification scheme for chronic pain [600], based on five axes: (1) the region of the pain, (2) the system affected, (3) temporal characteristics of the pain, (4) time since onset, and (5) the aetiology of the pain.
2.1.1. Historical perspectives and theories of pain

A number of theories have been postulated to describe mechanisms underlying pain perception. These theories date back several centuries, and even millennia [483,729]. This thesis will primarily focus on theories that were postulated since the 17th century.

2.1.1.1. Specificity Theory

Specificity theory refers to the presence of dedicated pathways for each somatosensory modality. The fundamental tenet of the specificity theory is that each modality has a specific receptor and associated sensory fibre (primary afferent) that is sensitive to one specific stimulus [273]. For instance, the model proposes that non-noxious mechanical stimuli are encoded by low-threshold mechanoreceptors (LTM), which are associated with dedicated primary afferents that project to mechanoreceptive second-order neurones in the spinal cord or brainstem (depending on the source of the input). These second-order neurones project to higher mechanoreceptive areas in the brain. Similarly, noxious stimuli would activate a nociceptor, which would project to higher pain centres through a pain fibre. These ideas have been emerging over several millennia, but were experimentally tested, and formally postulated as a theory in the nineteenth century by physiologists in Western Europe. This thesis will briefly review some of the key ideas that have led to the development of specificity theory.

René Descartes was one of the first western philosophers to describe a detailed pain pathway in humans. Descartes’ manuscript Treatise of Man (originally written in French) was illustrated, edited and published posthumously, first in Latin in 1662 [241], and then in French in 1664 [242]. In Treatise of Man, based on the French edition by Louis La Forge (who was also one of the illustrators), Descartes describes pain as a perception that exists in the brain and makes the distinction between the neural phenomenon of sensory transduction (today known as nociception) and the perceptual experience of pain. What is essential to the development of Descartes’ theory is his description of nerves, which he perceived as hollow tubules that convey both sensory and motor information. This understanding of neural function was by no means novel. In the 3rd century BCE, Herophilus demonstrated the existence of sensory and motor nerves, and Erasistratus demonstrated that the brain influenced motor activity [729]. Half a millennium later, Galen demonstrated that sectioning the spinal cord caused sensory and motor deficits [651]. Within the spirit of scientific enquiry that resurfaced in the renaissance,
anatomical studies by Vesalius published in 1543 reiterated and confirmed Galen’s findings [651]. In relation to this, Galen had postulated that three conditions be met for perception: (1) an organ must to be able to receive the stimulus, (2) a connection from the organ to the brain, and (3) a processing centre that converts the sensation to a conscious perception [729]. Descartes contributed to Galen’s model by postulating that a gate existed between the brain and the tubular structures (the connections), which was opened by a sensory cue [242]. A sensory cue would “tug” on the tube, which would then open a gate between the tube and the brain. The opening of this gate would then allow “Animal Spirits” (an extension of the Greek pneuma¹) to flow through these tubes and within the muscles to move them. Although this sensory system was not specific to pain, La Forge’s drawing (based on Descartes’ concept) of a foot near a flame is one of the most ubiquitous figures in neuroscience (see Figure 2-1). This example describes the pathway for promptly moving one’s foot away from a hot flame. In the figure (and its description in the text) the heat of the flame near the foot activates a fibril (or fibre) within the nerve tubule that traverses up the leg, to the spinal cord and finally to the brain. Descartes compared this fibre to a cord attached to a bell – by pulling on the other end of cord, the bell will ring. The proverbial bells in this case are the pores that line the ventricles in the brain. Once these pores open in response to the sensory input, the Animal Spirits were thought to flow through the tubule and elicit a motor response. This motor response included turning the head and the eyes to see the flame, and raising the hands and folding the body away from the flame for protection. Descartes conceived that there are many of these fibrils and that their movements elicit the sensations. For example, the perception of pain would be felt in the brain when there is a significant “tug” on the fibre, which caused it to sever. In contrast, a tug of the same magnitude that doesn’t cause the fibre to break would evoke a tickling (or tingling – Descartes uses the French word chatouillement) perception. Although Descartes’ figure of the boy and the flame suggests that there is a dedicated pain pathway, a closer read of the text indicates that Descartes believed that the pattern and rate of firing (intensity of tugging) of a fibre provided the adequate information to the brain about the stimulus intensity and quality.

¹In ancient Greek medicine, the concept of pneuma represents air, breath, or motion. It was also called the breath of life and the spirit of man. [87] Bernard C, Magendie F. Leçon d’ouverture du Cours de Médecine du Collège de France. Paris: Bailliére, 1856.
**Figure 2-1:** Line drawing of the pain system by Florentius Schuyl (left) and Louis La Forge (right), based on Descartes description in *Treatise of Man*: The fire (A) is close to the foot (B). Particles from the fire move and press the skin, and tug on the fibril (C), which opens the pore (d, e) where the fibril terminates. Opening the pore is akin to tugging on a rope attached to a bell, thus ringing the bell. The open pore allows the “animal spirits” to flow from the cavity (F) into the fibril and, part of them activate the muscles to move the foot away from the fire, and part of them activate the muscles to turn the eyes and the head toward the fire to look at it, and part of them are used to bring forth the hands and fold the body to protect it. (Image on the left is reproduced from and the image on the right and text is reproduced from [242], out of copyright; translated by Massieh Moayedi).
The concept of a dedicated pain pathway (also known as specificity theory) was developed by Charles Bell in his landmark essay *Idea of a new anatomy of the brain; submitted for the observation of his friends*, first published as a conference proceeding in 1811, and later reproduced in a journal [79]. In this essay, Bell provided an alternative perspective about the organization of the nervous system. First he suggested that the brain is not “common sensorium” as suggested by Descartes, which was the accepted model of the brain at the time. Instead, he provided anatomical evidence that the brain was a heterogeneous structure, a theory first postulated by Willis in the seventeenth century [729]. He then suggested that nerves were bundles of heterogeneous neurones that have specialized functions, and that their bundling was only for ease of distribution. Thus, Bell spoke of different sensory neurones for different types of stimuli, motor neurones, and so-called vital neurones that are wired to the mind, rather than the brain. He did, however, maintain that perception of stimulus (such as vision and nociception) is different than the perceptual experience (e.g., colour and pain, respectively). Importantly for specificity theory, Bell states:

…that while each organ of sense is provided with a capacity of receiving certain changes; to be played upon it, as it were, yet each is utterly incapable of receiving the impressions destined for another organ of sensation. It is also very remarkable that an impression made on two different nerves of sense, though with the same instrument, will produce two distinct sensations; and the ideas resulting will only have relation to the organ affected [79].

This is the fundamental tenet of specificity theory, which postulates that there is a dedicated fibre that leads up a dedicated pain pathway to the sensory modality’s region of the brain. This model, therefore, suggests that a pathway specific to pain exists.

François Magendie was a French physician considered by some as the father of experimental physiology [87,773,827]. Magendie made substantial contributions to neurophysiology, including reiterating Bell’s findings regarding the existence of both motor and sensory nerves, and that these have separate paths to and from the spinal cord (the ventral and dorsal roots, respectively) [827]. This differentiation of spinal nerves is known as the Bell-Magendie Law, which is a fundamental aspect of the organization of the nervous system.
Concurrently in Germany, Johannes Müller published a *Manual of Physiology*, which echoed Charles Bonnet’s manual published a century earlier [729]. Müller’s manual, published in 1840, sought to summarize and synthesize findings in physiology. The purpose of this synthesis was to understand how different stimuli were so clearly sensed and how the brain could distinguish them from one another. He, like Bonnet, concluded that specific receptors have must have specific energy of stimulation, and that there were infinite numbers and types of fibres, each to a specific sensory stimulus; e.g., there is a specific fibre for the smell of bananas, and another for the scent of an apple, and yet another for the scent of an orange. Furthermore, because of a sense organ’s specific energy, the sensory neurone will only encode a single perceptual quality. For example, if a warm fibre is artificially stimulated by electric current, the brain will perceive warmth. In line with these findings, Erasmus Darwin (Charles Darwin’s grandfather) provided the first evidence for a set of specific nerves for the perception of heat [204].

The discovery of specific cutaneous touch receptors such as Pacinian corpuscles [148], Meissner’s corpuscles [149], Merkel’s discs [394], and Ruffini’s end-organs [393] in the latter half of the nineteenth century provided further evidence that specific sensory qualia were encoded by dedicated nerve fibres (see Section 2.3.1). However, there remained a debate about the nature of pain as part of the five senses. An end-organ specific to pain stimuli (nociceptor) had not yet been discovered. In contrast to the idea of a dedicated pain pathway, it was argued that pain was different than the other senses in that it is inherently unpleasant [106,199]. These ideas persisted from Plato and Aristotle’s writings of pain as an emotion [767]. This inherently makes pain the antithesis of pleasure, and, because pleasure is a characteristic of the mind (*i.e.*, an emotion), it was inferred that pain was also a characteristic of the mind, and not a percept of the body.

Further evidence for the specificity theory came from Schiff’s and Woroschiloff’s findings of a pain pathway in the spinal cord in a series of experiments between 1854 and 1859. These findings built upon Charles-Édouard Brown-Séquard’s observations that sensory fibres decussate in the spinal cord [18,199]. Schiff and Woroschiloff established the presence of two pathways through observations of the effect of incisions at different levels of the spinal cord: the anterolateral pathway for pain and temperature, and the posterior bundles for tactile sensibility [199,729]. However, Schiff and Woroschiloff noted that the tactile pathway did not decussate at the level of the spinal cord. These findings were supported by a case study reported by William
Richard Gowers, a physician in London. In this report, Gowers reported that a patient with a bullet wound to the gray matter of the spinal cord lost the sense of pain and temperature, but not touch [729]. He concluded that there were specific pathways for pain and temperature, separate from that of touch. However, those who held onto the Aristotelian dogma argued strenuously against specificity theory. They insisted that pain is a quality of all senses, a percept of the mind. Only when Blix and Goldscheider published their findings of sensory spots on the skin independently did specificity theory gain momentum, and did pain become a recognized sense [199]. Sensory spots were defined as tiny areas of the skin that elicit a specific sensation when touched. These sensory spots were specific to warmth, cold, pressure or pain. However, both Blix and Goldscheider moved away from specificity theory some years later, and moved towards the intensity theory of pain, a concurrent theory (see section 2.1.1.2).

Between 1894 and 1896, Max von Frey’s carried out experiments that advanced the specificity theory. Von Frey indicated that there were four somatosensory modalities: cold, heat, pain and touch and that all of the other skin senses were derivatives of these four modalities. To test this idea, he developed his now well known von Frey hairs (termed an aesthesiometer) that consisted of a hair, usually from a human, but sometimes he used a horsehair or a hog bristle, attached to a wooden stick [682]. By measuring the hair’s diameter, length and the maximal weight precisely it could support (maximal tension) without breaking off of the stick, it was possible to measure the force applied to a very specific spot. Today, von Frey hairs are made of fine nylon filaments of varying thicknesses (and hence stiffness) to deliver different forces and pressures upon bending. Using these hairs, he could carefully determine the pressure required to elicit a sensation at each of the skin spots identified by Blix and Goldscheider. Further, his experimental setup allowed him to determine which spots responded to innocuous pressure, and which ones responded noxious pressure. Von Frey demonstrated that there were distinct spots for innocuous pressure and for noxious pressure. He presented a model of the skin that comprised of a “mosaic of distinct tactile, cold, warm, and pain spots distributed across the skin with distinctive regional variation” [683]. Von Frey related the distribution of the pressure points to the distribution of Meissner’s corpuscles (see section 2.3.1), whereas pain points were related to the distribution of free nerve endings in the skin (see section 2.3.3.1). Despite these remarkable findings, the specificity theory made a number of assumptions about the anatomical basis of somesthesis. For instance, when von Frey postulated the theory, pain receptors had yet to be identified, nor were
the peripheral pathways and brain centers specific to pain sensation established, as well as other factors (for a review, see [199,729]).

Charles Scott Sherrington addressed some of the assumptions of the specificity theory in his proposed framework of nociception. He applied a Darwinian (i.e., evolutionary) approach to describe the specificity of neurones, which included the four basic modalities recognized by von Frey. He stated that “the main function of the receptor is […] to lower the excitability threshold of the arc for one kind of stimulus and heighten it for all others” [796,797]. This approach resolved the divide between the intensity theory (see below) and specificity theory [729]. He also coined the term “nocicipient” [795] to describe the specificity of the cutaneous end-organ for pain, later termed nociceptor [796]. Sherrington developed a framework that advanced the specificity theory of pain even further. However, the nociceptor had yet to be identified definitively.

The discovery of myelinated primary afferent fibres that respond only to mechanical noxious stimuli occurred much later – in 1967 [124]. Soon thereafter, Bessou and Perl [89] discovered nociceptive unmyelinated afferent fibres: polymodal nociceptors and high-threshold mechanoreceptors (HTM). These findings revolutionized the field of pain research, and helped advance and develop a number of theories of pain. Since Sherrington’s endorsement of the specificity theory of pain, this became the dominant theory at the time. However, its popularity waned with the postulation of the Gate Control Theory of pain (see Section 2.1.1.4) postulated by Melzack and Wall [595].

### 2.1.1.2. Intensity theory of Pain

The intensive (or summation) theory of pain (now referred to as the intensity theory) has been postulated at several different times throughout history. First postulated in the 4\textsuperscript{th} century BCE by Plato in his œuvre *Timaeus* [698], the theory defines pain not as a unique sensory experience, but rather as an emotion that occurs when a stimulus is stronger than usual. Centuries later, Erasmus Darwin reiterated this concept in *Zoonomia* [204]. One hundred years after Darwin, Wilhelm Erb also suggested that pain occurred in any sensory system when sufficient intensity was reached, rather than being a stimulus modality in its own right [199]. Arthur Goldscheider further advanced the intensity theory, based on an experiment performed by Bernhard Naunyn in 1859 (cited in: [199]). These experiments showed that repeated tactile stimulation (below the
threshold for tactile perception) produced pain in patients with syphilis who had degenerating dorsal columns. When this stimulus was presented to patients 60-600 times per second, they rapidly developed what they described as unbearable pain. Naunyn reproduced these results in a series of experiments with different types of stimuli, including electrical stimuli. It was concluded that there must be some form of summation that occurs, in order for the subthreshold stimuli to become unbearably painful. Goldscheider’s suggested a neurophysiological model to describe this summation effect: repeated subthreshold stimulation or suprathreshold hyper-intensive stimulation could cause pain. Goldscheider suggested that the increased sensory input would converge and summate in the gray matter of the spinal cord. This theory competed with the specificity theory of pain, which was championed by von Frey. However, the intensity theory lost support with Sherrington’s evolutionary framework for specificity theory; and postulated the existence of sensory receptors that are specialized to respond to noxious stimuli, for which he coined the term “nociceptor”.

2.1.1.3. Pattern Theory

In an attempt to overhaul theories of somaesthesis (including pain), J. P. Nafe postulated a “quantitative theory of feeling” [636]. This theory ignored findings of specialized nerve endings and many of the observations supporting the specificity and/or intensive theory of pain. The pattern theory stated that any somaesthetic sensation occurred by a specific and particular pattern of neural firing, and that the spatial and temporal profile of firing of the peripheral nerves encoded the stimulus type and intensity. Lele, Sinclair and Weddell [531] championed this theory, and added that cutaneous sensory nerve fibres, with the exception of those innervating hair cells, are the same. To support this claim, they cited work that had shown that distorting a nerve fibre would cause action potentials to discharge in any nerve fibre, whether encapsulated or not. Furthermore, intense stimulation of any of these nerve fibres would cause the percept of pain [807,924].

2.1.1.4. Gate Control Theory of Pain

In 1965, Ronald Melzack and Patrick (Pat) David Wall proposed a theory that would revolutionize pain research: the Gate Control theory of pain. The Gate Control theory recognized the experimental evidence that supported specificity theory and pattern theory, and provided a model that could explain these seemingly opposed findings. In a landmark paper, Melzack and
Wall [595] carefully discussed the shortcomings of the specificity and pattern theories – the two dominant theories of the era. The Gate Control theory attempted to bridge the gap between intensity and specificity theory with a framework based on the aspects of each theory that had been corroborated by physiological data. Specifically, Melzack and Wall accepted that there are nociceptors (pain fibres) and touch fibres, and proposed that these fibres synapse in two different locations within dorsal horn of the spinal cord: the substantia gelatinosa and the “transmission” cells. The model (see Figure 2-2) proposed that signals produced in primary afferents from stimulation of the skin were transmitted to three regions within the spinal cord: (1) the substantia gelatinosa, (2) the dorsal column, and (3) a group of cells that they called “transmission” cells. They proposed that the gate in the spinal cord is the substantia gelatinosa in the dorsal horn, which modulates the transmission of sensory information from the primary afferent neurones to “transmission cells” in the spinal cord. This gating mechanism is controlled by the activity in the large and small fibers. Large fiber activity inhibits (or closes) the gate, whereas small fiber activity facilitates (or opens) the gate. Activity from descending fibres from supraspinal regions to the dorsal horn could also modulate this gate. When nociceptive information reaches a threshold that exceeds the inhibition elicited, it “opens the gate” and activates pathways that lead to the experience of pain and its related behaviours. Therefore, the Gate control theory of pain provided a neural basis for the findings that supported and in fact helped to reconcile the apparent differences between the pattern and specificity theories of pain.
**Figure 2-2:** Schematic of the Gate Control Theory of Pain mechanisms. L – large diameter fibres; S – small diameter fibres. The fibres project to the substantia gelatinosa (SG) and first central transmission (T) cells. The inhibitory effect exerted by SG on the afferent fibre terminals is increased by activity in L fibres and decreased by activity in S fibres. The central control trigger is represented by a line running from the large-fibre system to the central control mechanisms; these mechanisms, in turn, project back to the gate control system. The T cells project to the entry cells of the action system. +, Excitation; –, inhibition. (Figure and legend are reproduced with permission from: [595]).
2.1.2. Multidimensional aspects of pain

Melzack and Casey [593] described pain as being multidimensional and complex, with sensory-discriminative, affective-motivational and a cognitive-evaluative components. Additionally, recent work has shown that pain can also affect and interact with motor systems [23]. The concept of pain as a multidimensional experience has been described in ancient texts, dating as far back as the Syriac Empire (circa 200 BCE). In the Book of Medicines [122], it is suggested that pain is the product of bile and phlegm mingled with cold and heat. These simple combinations occur in the brain, and, according to Syriac medicine, pain is a product of the brain (a concept which has passed the test of time, and that we still hold true today). Different types of pains would thus arise from differential combinations of these substances affecting the type of pain. It is noteworthy that the concepts of bile and phlegm, and even those of cold and hot were understood in a different paradigm of philosophical thought – these are not the simple compounds we know today, but are rather used as a classification of the world. For instance, certain foods make the body “cool”, whereas others make the body “warm.” These concepts are not unique to the Syrians, since they follow a long tradition of ancient medicine passed down from the Egyptians (who were the first to record medical texts, e.g., The Papyrus Ebers [120]), to the Greeks (e.g., most-famously, Hippocrates and Galen), to the Babylonians and to the Assyrians.

The contemporary definition used by the IASP is based on the divisional (multidimensional) definition proposed by Melzack and Casey, in 1968 [593]. These dimensions include the sensory-discriminative (intensity, location, quality, and duration), the affective-motivational (unpleasantness and the subsequent flight response) and the cognitive-evaluative (appraisal, cultural values, context and cognitive state) dimensions of pain. These three dimensions are not independent, but rather, interact with one another. They are, however, partially dissociable: the cognitive state of a person can modulate one or both of these dimensions of pain perception. In general, the more intense a noxious stimulus is, the more unpleasant it will be [279]. However, there are exceptions to this rule: hypnosis has been shown to modulate pain unpleasantness without affecting intensity – that is, the person felt the pain, but was not as bothered by the sensation [500,590,714-716,937]. This is an example of how the cognitive state can modulate the percept of the affective-motivational component of pain and can be referred to as cognitive
modulation. Cognitive modulation of pain is reflected in the effects of placebo, nocebo [171,172,295,908], cognitive behavioural therapy [752,753,793] and other treatments for chronic pain.

Theories about chronic pain have continued to evolve as knowledge accumulates concerning the structure and function of pathways underlying pain perception and pain modulation. Recent advances in neuroimaging and cellular and molecular medicine have vastly expanded our understanding of pain, and some of these key findings relevant to this thesis will be discussed further when anatomical pathways and brain regions implicated in pain are described (see Sections 2.3).

2.1.3. Chronification of pain

An important issue that is not fully understood is the way in which acute pain becomes chronic. For example, how does pain change from being a beneficial signal of a potential threat that engages protective mechanisms, to become a state where pain itself becomes a threat, dysfunction, despair, and much suffering.

2.1.3.1. Sensitization, Hyperalgesia and Allodynia

When the skin is injured and pain is evoked, the tissue can in some situations become tender and inflamed. The tenderness can be associated with two processes: allodynia and hyperalgesia. *Allodynia* is defined as a “pain due to a stimulus that does not normally provoke pain” [110]. The definition further specifies that allodynia is not related to any specific mechanism; rather it specifies an unexpected behavioural response (pain) to a normally innocuous stimulus. Furthermore, to demonstrate allodynic responses, it is preferred that responses be tested in unaffected body areas to show normal responses, where possible. Because the normative response to the stimulus is not painful, and the allodynic response is painful, “allodynia involves a change in the quality of the sensation” [110]. *Hyperalgesia* is defined as a “increased pain from a stimulus that normally provokes pain” [110]. There are two types of hyperalgesia: primary (at the injury site) and secondary (outside the site of injury). These types of hyperalgesia can be described with the following example. When glabrous skin (non-hairy skin, like the palm of the hand) is burned, the burn spot (primary zone) becomes more sensitive to heat and mechanical stimuli – a stimulus that was mildly painful before the burn is subsequently perceived as being
much more painful [609]. In normal cases, it is believed that allodynia and hyperalgesia provide warning signs, and are likely related to nocifensive behaviours – that is, behaviours that are meant to protect the damaged limb, prevent further damage, and ultimately promote healing.

The perceptual states of hyperalgesia and allodynia are related to the physiological process known as sensitization, which is defined as “increased responsiveness of nociceptive neurones to their normal input, and/or recruitment of a response to normally subthreshold inputs” [110]. There are different mechanisms of sensitization, depending on the type of tissue and/or injury (chemical, mechanical or heat) [610]. This sensitization process can result from threshold related changes in nociceptors (i.e., lower firing threshold to sensory stimuli and/or an increased firing rate to suprathreshold stimuli, spontaneous firing and/or an expansion of the receptive field). Evidence suggests that primary hyperalgesia occurs as a result of nociceptors becoming sensitized [609,853]. Similarly, evidence supports that allodynia in the primary zone is also related to sensitization. Peripheral sensitization occurs as a result of the prolonged activation of peripheral nociceptors, or exposure to the algesic chemicals, which leads to the release of a number of chemical messengers that reduce nociceptive neurones’ threshold (for a comprehensive review, see: [515,951]). This process of sensitization causes amplification of the response to a noxious stimulus as well as to non-noxious thermal and mechanical stimuli. In the example of the burn described above, the region around the burn (secondary zone) is more sensitive to mechanical stimuli, but not to heat stimuli [718]. In fact, the secondary zone is less sensitive to heat stimuli than normal skin. Peripheral sensitization does not fully explain secondary hyperalgesia and there is now much evidence that secondary hyperalgesia is largely mediated by central mechanisms [512], although there may also be some peripheral contribution. Specifically, neurones in the CNS show enhanced responses to mechanical and thermal stimuli in the secondary zone [806], i.e. a central sensitization. The IASP defines central sensitization as an “increased responsiveness of nociceptive neurones in the central nervous system to their normal or subthreshold afferent input.” These abnormal neuronal states are thought to be, in part, the neural basis for hyperalgesia and allodynia. This state of hyperalgesia is likely related to dysfunctional endogenous pain-modulatory mechanisms (see section 2.3.6).
2.1.3.2. The role of glia in sensitization

There are many more glial cells in the nervous system than there are neurones. Classically, it was believed that glia only served as support or structural cells. However, recent evidence has suggested that these cells play a much more significant role in both the PNS and CNS processing. In the context of pain, recent evidence suggests that glia are important to the pathogenesis of pain [159]. Specifically, satellite glial cells in dorsal root ganglia and trigeminal ganglia are implicated in nociceptive conduction and transmission, and microglia and astroglia in the CNS are implicated in sensitization and chronification of pain [159]. However, microglia and astroglia have no effect on normal nociceptive processing, they are only implicated in pathological states [614,921]. Specific mechanistic details of the role of glia in sensitization are outside of the scope of this thesis (for a comprehensive review, see: [159]). Microglia are also activated after inflammation, and are implicated in CNS inflammatory processes [159].

Taken together, it is possible that prolonged nociceptive input may induce central sensitization, a process that is mediated by neurones and glia. Over time, this sensitization can lead to changes in the function and structure of the nervous system (*i.e.*, plasticity – see section 2.6). Normally, there is a delicate balance between nociceptive and antinociceptive signaling in the central nervous system. For instance, persistent nociceptive input is accompanied by an increase in activity in pain-modulatory pathways. This functional change is considered a form of beneficial plasticity, or change in the nervous system (see Section 2.6). However, in some cases plasticity can be harmful, or maladaptive. For instance, it is believed that in persons who develop chronic pain, the supraspinal modulatory pathways become dysfunctional or ineffective. This maladaptive plasticity can contribute to the development and/or maintenance of chronic pain, even in the absence of a noxious stimulus [576] (see Section 2.6.2). Furthermore, pain sensitivity and a reduction in the ability to modulate pain may be related to pre-existing abnormalities in the CNS.

2.1.4. Functional pain syndromes

Pain without a known physiological or “organic” cause is diagnosed as functional pain disorders. Functional pain syndromes include the most prevalent of all pain conditions, which can be divided into two categories: somatic or visceral pain syndromes. Somatic pain syndromes include TMD, vulvodynia, low-back pain and fibromyalgia (FMS). Visceral pain syndromes include
irritable bowel syndrome (IBS), chronic cardiac pain syndrome, and internal cystitis (or painful bladder syndrome). Functional pain syndromes are prevalent in 15-20% of adults worldwide [562]. Relevant to this thesis is the prevalence of TMD, which is the most prevalent of these functional pain syndromes (discussed in more detail in Section 2.2.3).

2.1.4.1. Central abnormalities in functional pain syndromes

Functional pain disorders are more prevalent in women than in men [581,582]. Furthermore, some functional pain disorders are more likely to have increased co-morbidity in women than in men [582]. Functional pain syndromes, such as TMD, are diagnosed as such because of a lack of a clear physiological cause for the pain. This means that there are no consistent findings for the source of the pain, and there are no clear peripheral abnormalities. Functional pain syndromes do, however, share some common pathophysiological features, including changes to neurobiological, physiological and anatomical aspects of the CNS. A number of behavioural studies have also shown that patients with functional pains are hypersensitive to experimental pain [581]. These patients also have dysfunctional pain-modulatory systems (e.g., [491,693]), and, in some cases, have cognitive deficits (e.g., [365,412,455,489,666]). There is also a greater degree of comorbidity with mood and anxiety disorders in patients suffering from functional pain disorders [637]. With the lack of peripheral aetiologica factors, these behavioural abnormalities have led researchers to suspect that central mechanisms contribute to and mediate functional pain syndromes. Findings specific to idiopathic TMD are described in further detail in section 2.2.3, and support the hypothesis that central sensitization may be the underlying pathophysiological processes underlying functional pain syndromes. These hypotheses have been corroborated by a number of functional and structural neuroimaging studies, and are described in further detail in Section 2.5.2.

2.1.5. Pain-cognition interactions

There is a two-way interaction between cognitive processes and pain in that cognitive processes can interfere with and modulate pain, and pain can interfere with and modulate cognitive processes (e.g., [123,193,290,529,776,778,781,893,935]). The interaction is best illustrated by a common example: that of a football player who injures his ankle, but doesn’t feel the pain until after the game. This suggests that pain can be attenuated when a person is distracted or engaged in a cognitively-demanding task [851]. Conversely, pain can hamper cognitive performance (e.g.
reduced task accuracy or slower task reaction times) [713]. The cognitive interference of pain may be due to the possibility that pain has a cognitive load, and that there could be limited cognitive resources in the brain. Therefore, the brain must prioritize between competing tasks, such as pain and a cognitive task. More recent work has demonstrated that cognition-pain interactions are the product of complex interactions between two modes of attention selections: bottom-up and top-down [529]. The bottom-up attention selection is related to the salience of pain in that pain can be attention demanding because it is behaviourally relevant. Interestingly, the salience of pain is partly relevant to its novelty [530,632,886]. This process of habituation is likely related to top-down attention selection processes. These processes allow the redirection of attention to other behaviourally relevant stimuli, and thus reduce the attention-grabbing (or salience) effect of pain [529,892].

Several seminal studies in the 1990s demonstrated the interaction of pain and cognitive performance in healthy subjects, showing that their task performance decreased (i.e., longer reaction times) when concurrently presented with a noxious stimulus [192-194]. These studies indicate that pain can interrupt or detract from the ability to perform a cognitive task, and thus suggests that it is difficult to disengage from the painful stimulus. Later studies showed that difficult tasks, which required presumably more cognitive resources, could briefly reduce subjective pain ratings. Therefore, pain can affect cognitive performance, and cognitive performance can modulate pain ratings (for a comprehensive review see [778]). These processes have been summarized in the neurocognitive model of attention to pain [529]. This model describes a “bottom-up capture of attention by pain”, whereby pain grabs one’s attention to signal a relevant and threatening stimulus. However, this bottom-up process can be modulated by top-down factors, such as cognitive load, emotional state and motivation. Inherent to this form of modulation is the context of the noxious stimulus. If a subject is in an emotional state he/she may report heightened pain, as he/she is more focused on stimuli with a negative valence. Alternatively, a subject may report a heat stimulus as less painful if the stimulus is perceived as beneficial, such as a heating pad, or a hot tub in a therapeutic setting.

In the context of chronic pain, a number of studies have shown that chronic pain negatively impacts cognitive abilities, including concentration, learning, memory, and attentional focus [26,250,275,365,369,455,489,666,928]. Eccleston and colleagues [289] showed that chronic pain patients could briefly be disengaged from pain during attentional switches. They further showed
that pain has a cognitive load, and that only when there is heavy use of cognitive resources, do pain ratings decrease. Taken together, these findings suggest that the brain has a limited cognitive resource, where a more salient task can overcome pain – however, only for a brief period of time. Over time, patients report getting tired, and not being able to focus on the task, and reducing the subject’s ability to block out pain from consciousness. In a later study, Eccleston and colleagues [292] showed that the cognitive interference effect of chronic pain was related to levels of negative affect and somatic awareness. In fact, it has been shown that negative affect can enhance perceived pain [936].

A study by Harman and Ruyak [409] reported that people with persistent low-level pain have cognitive deficits, indicative of the interference of pain. There is evidence of functional and structural plasticity that occurs in chronic pain (see Section 2.5.1 and 2.5.2, respectively and 2.6.2): over time, pain can change the connectivity of brain regions, which can lead to cognitive impairment. Another possible mechanism is the aforementioned limited resource model – the cognitive load of pain reduces resources to perform a task. These mechanisms are not mutually exclusive, as prolonged nociceptive input can alter the connectivity of cognitive regions, and further reduce resources available for cognitive processing.

In line with the limited resource model, is the concept of cognitive branching – the ability to put a pending task “on hold” to execute a more salient (or rewarding) task [497]. Therefore, the cognitive branching model provides a functional basis for the limited resource model, and provides a model that explains reductions in pain ratings when engaged in a cognitive task, or reduced cognitive performance whilst receiving prolonged nociceptive input. Specifically, if the cognitive branching system is limited, as is suggested by Koechlin and Hyafil [497], then the system can be overloaded, and affect task performance. This explains findings that tasks that require little cognitive engagement do not modulate pain ratings, and that low noxious stimulus intensities do not have a significant effect on task performance [776]. Conversely, as cognitive processes increase in difficulty, pain ratings can be modulated; or if nociceptive input increases, cognitive performance can be negatively affected. For example, Weissman-Fogel and colleagues [928] found no group differences in task performance between controls and patients with TMD in a neutral Stroop task (no interference). However, when presented with a more difficult interference counting Stroop task, patients showed sluggish reaction times – their cognitive branching system was either overwhelmed, or prioritized pain over the Stroop task. Finally, this
effect was modulated by an emotional counting Stroop task. Subjects were asked to report the number of TMD-pain related words visually presented. Specifically, these words were: ache, tight, annoying, pain, stress, hurt, clench, anxious, throb, unpleasant, pressure, and tiring. The patient group reported that the TMD pain words affected their pain intensity and unpleasantness during the Stroop task, whereas controls did not. The increase of the patients’ pain rating affected the TMD group’s reaction times for the Stroop task.

In sum, pain and cognition have a complex interaction that can be modulated by affective state, context and motivation. Pain is inherently salient, but its behavioural relevance can be up- or downregulated based on these factors. Furthermore, chronic pain can alter brain structure, connectivity and function, which may further impact cognitive abilities.

2.1.6. Salience and pain

Pain plays an essential role in survival – it signals a potential or actual injury. Because of the behavioural relevance of pain, noxious stimuli are inherently salient. Furthermore, studies have shown that consciousness is required to perceive pain. Specifically, these studies have investigated the neural correlates of nociception in subjects in a persistent vegetative state using neuroimaging techniques (these techniques are discussed in detail in Section 2.5). We must clearly indicate that these studies were not investigating pain (recall that the definition of pain requires that a person report pain – it is a subjective perceptual experience), and so, these studies were comparing neural correlates of normally noxious stimuli in persistent vegetative state patients to pain perception in the control group. One study found that the regions along the ascending nociceptive pathways, involved in the sensory-discriminative dimension (midbrain, thalamus and primary somatosensory cortex (S1)), showed activation, but the other regions that showed activation in the control group (including the secondary somatosensory cortex (S2), insula, cingulate, prefrontal cortex (PFC) were not activated [516]. Conversely, Kassubek and colleagues [479] found that the patients did have activation in brain regions involved in nociception and pain modulation. However, it is worth noting that recent studies have shown that some, but not all, patients in a vegetative state show brain activity in response to stimuli in the environment [517,659]. Therefore, it is possible that in a subset of these patients, where consciousness may persist, pain perception and modulation occurs. However, in patients that
have lost consciousness, nociception occurs [768], but there is no evidence that they feel pain, as
regions underlying different dimensions of pain do not show activation.

2.1.7. Pain-motor interactions

Pain-motor interactions are a hallmark of pain-related behaviours, the most obvious example
being nocifensive behaviours. Descartes portrayed nocifensive behaviour in the *Treatise of Man*
[242] in his famous depiction of the boy burned by the fire, and Charles Darwin describes
nocifensive behaviours in his text *Expression of Emotion in Man and Animal* [203]. There is no
doubt that the sensory and motor system interact, a concept supported by the many sensorimotor
reflexes observed in animals and humans. However, the interactions of pain and motor functions
are not as clear. Furthermore, the effects of prolonged (or chronic) pain on motor output are
equally ambiguous. A number of experimental pain paradigms have attempted to shed light onto
these topics. For instance, it has been shown that noxious input inhibits the motor cortex
excitability [3,157,301,302,638,856]. Similarly, motor cortex stimulation has been shown to
modulate pain [103,117,118,301,331,342,344,345,535,548,657,689]. Furthermore, a study by
Boudreau and colleagues [109] reported that motor cortex plasticity is inhibited by noxious
stimulation of the intraoral cavity. Therefore, it seems that motor learning is modulated, in this
case inhibited, by a tonic noxious stimulus.

A number of theories have been postulated to explain the relationship between pain and motor
output. The two dominant theories are the vicious cycle theory, first postulated by Travell [866],
and the pain adaptation model, postulated by Lund and colleagues [556]. The vicious cycle
theory suggests that the presence of a reciprocal relationship between pain and dysfunction: pain
causes muscular dysfunction, which increases pain, creating a vicious cycle. This theory states
that, over time, the muscle hyperactivity contributes to and/or maintains pain. Specifically,
tonically hyperactive muscles become ischaemic, which in turn leads to an increase in algesic
chemicals within the musculature. This has been supported by findings of increased motor
activity in experimental models of muscle pain [431]. However, some studies of orofacial pain
have failed to demonstrate that pain leads to muscular hyperactivity [634,675]. Therefore, Lund
and colleagues [556] postulated the pain adaptation model, which suggests that there is both
muscle hyper- and hypo-activity. Specifically, the model states that there is a decrease in activity
of muscles that are painful or that hurt with movement, and that antagonist muscles have
increased activity. However, more recent experimental findings have not supported the concept of uniform inhibition of a painful muscle. Instead, they have shown variable patterns of change in activity both spatially and temporally (for a review, see: [431]). To address these inconsistencies, Hodges and colleagues propose a “new theory of motor adaptation to pain” [431], which maintains the basic premise that pain modifies muscular activity as a nocifensive mechanism. This theory proposes that the body adapts to muscular pain is several ways. When there is pain or threat of pain, there is a redistribution of activity within a muscle, and between muscles, so that force is maintained. This redistribution occurs upon the recruitment of new motor units, and changes the mechanical behaviour of the muscle including increased stiffness and altered load distribution. In the short term, this reduces movement of the muscle and decreases the load on the muscle, which is beneficial and promotes healing. In the long term, however, these changes can lead to increased load, decreased movement and decreased variability, which can increase the risk of injury.

2.1.8. Neuroticism and pain

Psychological factors have been associated with sensitivity to pain, and the development and maintenance of chronic pain. Inherent personality-related factors reduce the brain’s capacity to effectively modulate nociceptive input and pain perception, likely affecting the cognitive modulation system (discussed in Section 2.1.2 and Section 2.3.6). Of particular interest to this thesis is the personality dimension of neuroticism [178]. Neuroticism has been defined as a stable personality trait associated with heightened sensitivity and/or processing of negative affective stimuli [178,179,907]. Therefore, it is not surprising that patients with high neuroticism scores have a lower pain threshold and pain tolerance, and are more distressed by pain in experimental settings and chronic pain [408]. Specifically, neuroticism has been associated with pain-related suffering [35,408], pain sensitivity [177,373,907], nerve injury outcomes and the development of neuropathic pain [848], and TMD [315]. However, not all chronic pain patients have high neuroticism scores, and not all persons with neuroticism have chronic pain [179]. Therefore, neuroticism alone is not sufficient to develop chronic pain; however it can contribute to the severity of the pain reported by chronic pain patients.
2.2. Temporomandibular disorders

2.2.1. Historical perspectives

Reports of TMD-like symptoms date back 1500 years when Hippocrates described a method for reducing dislocated mandibles [423]. These treatments often led to TMD-like disorders, including arthritis and infection of the TMJ. The first documented surgeries for disc displacements in the TMJ occurred in the nineteenth century, and shortly thereafter, artricular disc removal was used to manage TMD symptoms [587]. In the late 1800s and early 1900s, dentists primarily attributed TMD symptoms to problems with the muscles of mastication and their contribution to malocclusion. By 1934, malocclusion was formally considered to be the cause of TMD symptomology [181]. Costen’s range of symptoms, that far exceeds the recognized pathophysiological understanding of TMD today, led to the development of numerous treatments and devices to treat TMD and other symptoms believed to be related to malocclusion (for a review of these, see [587]). However, this range of symptoms was criticized for being too broad [587]. More recent, randomized control research studies have refuted the use of occlusal devices in the treatment of TMD, suggesting that the positive effects of these sorts of treatments are related to the patients’ expectation of the treatment [174,882]. Therefore, it has been suggested that less invasive and less expensive methods of treatment may be as efficacious in pain management as occlusal splints [872]. Despite an exceptional historical presence and extensive research, little is known of TMD pathophysiology, and few treatments are effective. Today, TMD continues to be a prevalent pain syndrome that poses a significant financial burden on healthcare and society.

2.2.2. Prevalence and social costs

TMD are estimated to affect between 3-20 percent of the United States’ adult population [269,539,540,562]. Of these, only a small proportion of persons suffering from TMD pain seek treatment and end up at tertiary and quaternary medical facilities such as pain clinics [350].

TMD are 1.5-9 times more prevalent in women [113,201,284,285,312,721]; a study found that 84% of persons with TMD were women in a nationwide epidemiological study in the United States [284]. It has been estimated that 5.3 million people in the United States seek medical attention for a TMD-related problem per year [349]. Furthermore, TMD physical symptoms (e.g., pain) and psychological symptoms (e.g., depression) have been reported as more severe in
women than in men. The predominance of TMD symptoms in women has been attributed to differences in sex hormones, such as oestrogen, as well as the use of oral contraceptives. Older (55-74 years old) and younger (< 20 years old) subjects report fewer TMD-like symptoms than subjects in reproductive years (20-55 years old). The annual incidence of TMD is 2-4%, with 0.1% going on to develop chronic pain. There are different disease outcomes for different patients, with 31% of cases persisting over 5 years, 33% whose pain resolves, and 36% who have recurring TMD. The direct healthcare costs for treatment of TMD in the United States have been estimated at $2 billion. However, TMD is often co-morbid with other disorders, such as FMS, IBS, anxiety, and depression, and may have greater economic impact based on indirect costs (such as missing work, unemployment, disability, limitations, etc.). The estimated total of both direct and indirect costs in the United States exceeds $4 billion dollars per annum [350]. Taken together, then, it is clear that TMD are a prevalent disorder with a significant economic burden on society.

2.2.3. Clinical aspects

TMD comprise of a cluster of clinical problems involving the structures of, and around, the TMJ, the masticatory musculature, or both [1,418]. TMD are primarily characterized by spontaneous pain and/or pain associated with jaw function, which can affect mandibular range of motion and produce TMJ sounds (e.g., crepitus, clicking) during jaw function. TMD pain can also spread to the muscles of the neck. The most common diagnostic criteria (and classification scheme) for TMD is the TMD-Research Diagnostic Criteria (RDC) established by Dworkin and LeResche in 1992 [285]. The American Association of Orofacial Pain (AAOP) has adopted this classification scheme. Based on the TMD-RDC, there are two primary types of painful TMD: myogenous (pain generated by the masticatory muscles) and arthrogenous (pain generated by the TMJ) [285]. Myogenous TMD, often called masticatory muscle pain, is diagnosed on the basis of patient history and clinical examination. Arthrogenous TMD conditions are usually caused by displacement or derangement of the TMJ disc, but also by inflammation within or around the joint. Hence, the pain in arthrogenic TMD can be attributed to inflammatory processes that occur as a result of intra-articular events or anatomical factors including disk displacement – although extra-articular events can also cause TMJ inflammation [646]. Additionally, TMD can be classified as idiopathic or as post-traumatic. Idiopathic TMD have no clear aetiology for pain onset and/or maintenance, although, as discussed above, there are a large number of factors that
have been considered in the past to play an important aetiological role. However, over time, and with increasing research, these factors have either been put into question or even eliminated. In fact, this is why it has been suggested that TMD is generally an idiopathic condition. In contrast, post-traumatic TMD are caused by traumatic events in the craniofacial region. These traumatic events can be as serious as head injury, hyperextension-flexion injury (e.g., following a motor vehicle accident), or as minor as a dental treatment (e.g., a long dental procedure) [81].

2.2.3.1. Sensory abnormalities

Sensory function in TMD has been assessed with a variety of psychophysical measures and quantitative sensory testing (QST) approaches to determine detection and pain thresholds to experimental stimuli across thermal, mechanical, electrical and chemical modalities. The details of these studies are provided in Table 2-1, and a summary of the findings is provided in Table 2-2. In general, these studies demonstrate that patients with TMD can have sensory abnormalities such as hyperalgesia, reduced pain tolerance and reduced DNIC.

QST studies have demonstrated that patients with TMD have hyperalgesic responses\(^2\) to acute experimental pain in the trigeminal region as well as in extra-trigeminal regions, as measured by pain thresholds. Specifically, studies investigating pressure pain thresholds in TMD have reported that patients with TMD have reduced pain thresholds to noxious pressure stimuli in the trigeminal region, and outside the trigeminal region [42,139,305,565,841]. Interestingly, one of these studies did not find significant group differences in pressure pain thresholds between controls and patients with TMD, but did so when they exposed study participants to a psychosocial stressor [139]. Most studies investigating heat thresholds in the trigeminal region and outside the trigeminal region reported that patients with TMD have lower heat pain thresholds than controls [313,491,563-565,841]. However, two studies failed to find differences in heat pain thresholds in the arm between the TMD group and controls [113,313]. Also, five studies have investigated pain thresholds to experimental ischaemic pain in the arm of patients with TMD versus controls. Three of these studies reported reduced pain thresholds in patients with TMD versus controls [313,563,564], whereas two studies [113,180] did not identify any differences between patients with TMD and controls. Patients with TMD also have lower

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\(^2\) Whether decreased pain thresholds are related to hyperalgesia or allodynia, or both remains a contentious issue.
pinprick pain thresholds in the orofacial region [42]. Four studies have investigated electrical pain thresholds in the orofacial region, and one study examined these thresholds outside the orofacial region [42,211,391,792]. Of these, only one identified lower pain thresholds to electric stimuli in the orofacial region [42]. Together, these studies demonstrate that, in most cases, patients with TMD have reduced sensory thresholds to noxious stimuli across different modalities. Furthermore, patients with TMD show prolonged pain and increased peak pain in response to hypertonic saline injected into muscles in the trigeminal and extra-trigeminal regions [841]. These hyperalgesic responses may be related to either peripheral sensitization or central sensitization, or both (see Section 2.1.2.1).

In addition to pain thresholds, some studies have investigated pain tolerance in TMD. It is believed that pain tolerance reflects a more integral view of the pain system, and its various dimensions. Because pain tolerance reflects “how much” pain a subject can withstand, this measure not only captures the sensory-discriminative dimension of pain, but also cognitive-evaluative and affective-motivational dimensions. Pain tolerance data are variable so it is not clear what mechanistic factors underlie this attribute seen in patients with pain. Specifically, two studies [42,841] have reported reduced pressure pain tolerance in trigeminal and extra-trigeminal regions of TMD patients compared to controls. Four studies have investigated ischemic pain tolerance, and two studies [563,564] reported lower pain tolerance in patients with TMD compared to controls, and two studies failed to find any group differences [90,313,563,564]. Five studies investigated heat pain tolerance in TMD, and four of these identified lower pain tolerance in patients with TMD compared to controls, compared to controls, whereas one of these studies failed to identify any group differences. Three studies tested pain tolerance to experimental electrical stimulation in trigeminal and extra-trigeminal regions, and found no differences between patients with TMD and controls [211,391,792]. In sum, there are variable findings with regard to pain tolerance in patients with TMD.

Taken together, there seems to be variability in the findings of quantitative sensory testing studies. This may be due to the heterogeneity of TMD, which was the motivation for the development of the TMD-RDC. Therefore, it is difficult to compare studies conducted prior to the establishment of the RDC, or that do not use the RDC to select patients to studies that have abided by the RDC.
Temporal summation is the production of a gradual and progressive increase in pain from the repeated painful stimuli. A typical protocol to test for temporal summation involved delivering painful stimuli at 3s inter-stimulus interval [711] in a short timeframe [31]. It is believed that temporal summation is related to “wind-up” – a process of increased nociceptive firing of dorsal horn neurones to repeated noxious stimulation, related to central sensitization [915]. Studies have demonstrated that the degree of temporal summation is related to central hyperexcitability: if the subjects have central sensitization, they have increased temporal summation. Two studies have reported augmented temporal summation in patients with TMD, compared to pain-free controls [42,565]. One study by Sarlani and colleagues reported that women with TMD had greater temporal summation than men with TMD, and healthy controls [755]. Therefore, these studies support the hypothesis that patients with TMD may have central sensitization.

In support of central sensitization in TMD, King and colleagues [491] reported that patients with TMD had dysfunctional endogenous pain modulation systems. The authors used an experimental method that modulates pain via descending inhibition: a counter-stimulation method. Under normal circumstances, when a conditioning noxious stimulus is administered to a remote body part, it activates the descending modulation system. This activation of the modulation pathway attenuates pain responses to a second focal stimulus, a process called DNIC [519,521]. In King et al’s [491] study, patients with TMD did not report less pain to a second focal noxious stimulus, but rather reported higher pain ratings than when the second stimulus was administered without the conditioning stimulus. Similarly, Maixner and colleagues [563] reported that patients with TMD could not effectively engage anti-nociceptive mechanisms when given a remote noxious stimulus, which would be expected to dampen their spontaneous TMD pain. In this regard, it has been shown that in concert with findings showing that patients with TMD have increased pain sensitivity outside the orofacial region, they are also unable to modulate pain perception when a second noxious stimulus is presented.

In sum, there is evidence that patients with TMD have both peripheral and/or central sensitization, as well as dysfunctional anti-nociceptive systems.
**Table 2-1:** Summary of quantitative sensory testing studies in TMD

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>TMD subtype*</th>
<th>Stimulus laterality</th>
<th>Body site</th>
<th>n patients (# females)</th>
<th>n controls (# females)</th>
<th>Pain threshold</th>
<th>Pain tolerance</th>
<th>Temporal summation</th>
<th>Other</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td>myofascial</td>
<td>Bilateral</td>
<td>V\textsubscript{1}\textsuperscript{a}, V\textsubscript{3}\textsuperscript{b} and wrist</td>
<td>20 (20)</td>
<td>20 (20)</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td>[305]</td>
</tr>
<tr>
<td>Ischaemia</td>
<td>combined</td>
<td>Left\textsuperscript{c}</td>
<td>arm</td>
<td>36 (N/A)</td>
<td>80 (N/A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[313]</td>
</tr>
<tr>
<td>Heat</td>
<td>myofascial</td>
<td>Left\textsuperscript{d}</td>
<td>forearm, palm</td>
<td>14 (14)</td>
<td>28 (28)</td>
<td>↓</td>
<td></td>
<td>↓ DNIC</td>
<td></td>
<td>[491]</td>
</tr>
<tr>
<td>Heat</td>
<td>myofascial</td>
<td>Left\textsuperscript{d}</td>
<td>forearm, palm</td>
<td>23 (23)</td>
<td>24 (24)</td>
<td>↓</td>
<td></td>
<td>↑</td>
<td></td>
<td>[565]</td>
</tr>
<tr>
<td>Pressure</td>
<td>myofascial</td>
<td>Bilateral</td>
<td>face and forearm</td>
<td>64 (64)</td>
<td>23 (23)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>[564]</td>
</tr>
<tr>
<td>Heat</td>
<td>myofascial</td>
<td>Left\textsuperscript{d}</td>
<td>forearm</td>
<td>52 (44)</td>
<td>23 (19)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>[563]</td>
</tr>
<tr>
<td>Heat</td>
<td>myofascial</td>
<td>Bilateral</td>
<td>masseter and anterior tibialis</td>
<td>22 (16)</td>
<td>21 (15)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑ peak pain</td>
<td>[841]</td>
</tr>
<tr>
<td>Heat</td>
<td>myofascial</td>
<td>Bilateral</td>
<td>masseter and anterior tibialis</td>
<td>22 (16)</td>
<td>21 (15)</td>
<td>↓</td>
<td>↓</td>
<td>↑ pain duration</td>
<td></td>
<td>[841]</td>
</tr>
<tr>
<td>Heat</td>
<td>myofascial</td>
<td>Left\textsuperscript{d}</td>
<td>forearm</td>
<td>20 (20)</td>
<td>42 (20)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>[113]</td>
</tr>
</tbody>
</table>

\textsuperscript{a}V\textsubscript{1}: Trigeminal nerve, \textsuperscript{b}V\textsubscript{3}: Mandibular nerve, \textsuperscript{c}Left\textsuperscript{c}: Lateral left, \textsuperscript{d}Left\textsuperscript{d}: Lateral left.
<table>
<thead>
<tr>
<th>Stimulus</th>
<th>TMD subtype*</th>
<th>Stimulus laterality</th>
<th>Body site</th>
<th>$n$ patients (# females)</th>
<th>$n$ controls (# females)</th>
<th>Pain threshold</th>
<th>Pain tolerance</th>
<th>Temporal summation</th>
<th>Other</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischaemia combined</td>
<td>Non-dominant arm</td>
<td></td>
<td></td>
<td>28 (10)</td>
<td>20 (20)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>[180]</td>
</tr>
<tr>
<td>Pressure myofascial</td>
<td>Bilateral</td>
<td>masseter and temporalis muscles and finger</td>
<td></td>
<td>35 (33)</td>
<td>35 (33)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure</td>
<td>Bilateral</td>
<td>TMJ, non-dominant finger</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tactile f</td>
<td>Bilateral</td>
<td>TMJ and face</td>
<td></td>
<td>43 (19)</td>
<td>20 (17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric</td>
<td>Ipsilateral to pain in TMD, random in controls</td>
<td>TMJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric myofascial</td>
<td>N/A</td>
<td>incisor tooth pulp</td>
<td></td>
<td>12 (11)</td>
<td>12 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric combined</td>
<td>Bilateral</td>
<td>masseter</td>
<td></td>
<td>30 (19)</td>
<td>31 (18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric myofascial</td>
<td>Bilateral</td>
<td>masseter and forearm</td>
<td></td>
<td>12 (10)</td>
<td>12 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a - Frontalis muscle, which receives afferent input from the ophthalmic branch of the trigeminal nerve (V1)
b - Masseter muscle, which receives afferent input from the ophthalmic branch of the trigeminal nerve (V1)
c - No information was provided about pain laterality or handedness.
d - The study investigated thermal heat pain thresholds with and without a cold water immersion conditioning stimulus

e - Group differences only found when a psychosocial stressor was presented

f - Tactile stimuli were two von Frey filaments; 5.16g and 84.96g for tactile and pin-prick stimuli, respectively

* "combined" refers to patients with both myofascial and articular (joint) pain or a mixed population of myofascial TMD or joint TMD.

**Abbreviations:** DNIC – diffuse noxious inhibitory controls; TMJ – temporomandibular joint; V₁ – ophthalmic branch of the trigeminal nerve; V₃ – mandibular branch of the trigeminal nerve; - no difference; ↓ – decrease
**Table 2-2:** Number of quantitative sensory testing studies testing sensory thresholds and tolerance in TMD

<table>
<thead>
<tr>
<th>Stimulus (n studies)</th>
<th>Trigeminal Region</th>
<th>Extra-trigeminal Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Threshold</td>
<td>Tolerance</td>
</tr>
<tr>
<td></td>
<td>↓ no change</td>
<td>↓ no change</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric (4)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ischaemia (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure (5)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Thermal heat (7)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pin-prick (1)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are based on studies presented in Table 2-1. ↓ indicates a decrease in TMD compared to controls. Blank cells indicate that the measure was not tested or reported.
2.2.3.2. Motor abnormalities

Some studies have shown that patients with TMD have motor abnormalities in the orofacial region, although there is much discordance across studies. For instance, three studies reported that patients with TMD have hyperactivity in their muscles of mastication, whereas other studies did not find any differences when comparing patients to controls (for a review, see [197,832]). Furthermore, some studies have shown that there are individual differences in muscle reactivity to pain [634,675]. One study that tested the excitability of central motor controls of masticatory pathways in patients with TMD, and found that central motor pathways were not hyperexcitable [195]. These variable findings between the interaction of TMD-related pain and orofacial motor function may be related to a unique experience of pain, reflective of the heterogeneity of TMD. In addition, some patients with TMD have limited unassisted jaw opening (with or without pain) [539], whether they were symptomatic or asymptomatic [286]. Therefore, in the context of TMD, it seems that the revised pain adaptation model (see section 2.1.6) best explains the observed variability in motor abnormalities. This model accounts for individual differences in changes in motor output to maintain homeostasis – the muscle, or region maintains contraction force, and promotes nocifensive behaviour. Based on these findings, Svensson and Graven-Nielsen [839] proposed that TMD pain interferes with sensorimotor integration and inhibits motor output to the craniofacial region. They also suggested that differential patterns of excitation of masticatory muscles occur in a phase-dependent manner to dampen jaw movements. Specifically, there can be increased excitation of antagonistic muscles during contraction of the agonistic (painful) muscle. Additionally, there is increased activity in the antagonistic phases of muscle movement and decreased excitation during agonist muscle contraction, presumably to reduce agonistic muscle activity, and thus pain [374]. In contrast to this proposed model, Cairns, Sessle and Hu [133] reported that the injection of noxious chemicals into the TMJ increased both agonist and antagonist muscle activity.

2.2.3.3. Cognitive abnormalities

There is extensive literature about the cognitive dimension of pain, and interactions between cognition and pain in general (see section 2.1.4). A few studies have examined cognitive performance in TMD and reported various cognitive deficits. For instance, Goldberg and colleagues [370] showed that post-traumatic TMD patients performed poorly on simple and
complex reaction time tasks, as well as neuropsychological tests testing interference effects, compared to patients with idiopathic TMD. In two follow-up studies, Grossi et al. [379,380] determined that a patient’s performance on neuropsychological test, including he simple and multiple-choice reaction-time tests, California Verbal Learning Tests, the Brown-Peterson Consonant Trigram Auditory Memory Test, Sleep Assessment Questionnaire, and Beck Depression Inventory, as well as fatigue and energy level assessments (100-mm visual analog scale), predicted their treatment outcome. Weissman-Fogel and colleagues [928] used a cognitive interference task, the counting Stroop task, and found that patients with idiopathic (non-traumatic) TMD had sluggish reaction times compared to controls. Together, these studies suggest that patients with TMD may have cognitive deficits. However, although there is evidence that patients with TMD have a variety of cognitive deficits, it is not clear whether these deficits existed prior to the onset of TMD pain or are caused by TMD pain.

2.3. Anatomy and physiology of orofacial pain and sensation

In order to understand concepts related to the possibly neurological and pathophysiological aspects of orofacial pain and TMD in particular, it is important to describe the ascending sensory and nociceptive pathways from sensory transduction in peripheral receptors, to transmission to the brainstem, and supraspinal levels. The emphasis will be on the trigeminal system but some differences with the spinal (i.e., in the trunk, neck and limbs) system are also noted.

Sensory information, such as touch, temperature, proprioception, kinaesthesia, itch and pain are processed by the somatosensory system. In general, peripheral receptors transduce stimulus energy and encode them as neural signals. These neural signals are propagated from the periphery to the CNS via primary afferent neurones. The cell bodies of these afferents are generally located in the periphery – with the notable exception of the mesencephalic nucleus of the CNV (see below). These peripheral neurones project to central neurones. There are two main routes for orofacial sensory information to travel from the brainstem to the brain: the trigeminal lemniscus, and the trigeminothalamic tract (TTT) for craniofacial information.

2.3.1. Peripheral receptors and sensory afferents

The nervous system monitors the state of the internal and external milieus. There are many types of specialized peripheral receptors, each with specific properties to encode different stimuli
Receptors in muscles, deep tissues, joints and tendons provide sensory information about movement and limb position [573]. These include Golgi tendon organs and muscle spindles. Other aspects of the somatosensation, such as touch, flutter, vibration and skin indentation are encoded by encapsulated nerve endings that are either fast adapting (Meissner corpuscles for flutter, Pacinian corpuscles for vibration) or slowly adapting (Merkel discs for touch, Ruffini endings for stretch – both are related to skin indentation) [573]. Sensations of pain and temperature in the skin and muscles are encoded by free-nerve endings [941]. There are also receptors specific to hair follicles, which are mechanically-sensitive (mechanoreceptors) [346]. These nerve fibres are inside the hair follicle and wrap around the sheath. These rapidly adapting fibres encode movements of the hair. Information regarding position of a limb is provided by proprioceptors. In relation to this investigation, the TMJ is innervated by proprioceptors that provide information about jaw position and the force being applied by masticatory muscles [843].

Each receptor is the peripheral ending of a primary afferent fibre. The peripheral somatosensory primary afferents can be classified based on their conduction velocity, a classification system developed by Erlanger and Gasser [297]. The free nerve endings have unmyelinated small-diameter C-fibres with slow conduction velocities (~< 2 m/s) [612], and Aδ fibres, which have a conduction velocity of ~2-30 m/s [612]. The Merkel discs and encapsulated endings are generally associated with large-diameter myelinated fibres (Aβ) with conduction velocities of ~30-70 m/s [449]. These axons transmit information pertaining to touch and proprioception from the periphery to the CNS. Muscle spindles and Golgi spindle afferents that carry proprioceptive and movement information are large myelinated fibres (Aα), which have a conduction velocity of 70-120 m/s. Receptors that respond preferentially to noxious stimuli are called nociceptors [796] and lack a receptive structure or corpuscle, but rather are free nerve endings [826]. Primary afferents with free nerve endings respond to mechanical, thermal and/or chemical stimuli [941]. In cutaneous tissues, LTM and thermoreceptors (receptors that detect changes in temperature) also have free nerve endings [941]. Similarly, LTM in skeletal muscles have free nerve endings [598]. Free nerve endings have small-myelinated Aδ fibre or unmyelinated C-fibre axons fibres. For instance, a subpopulation of unmyelinated fibres (C) detects stimuli that warm the skin [462] and a subpopulation of small-myelinated fibres (Aδ) detects stimuli that cool the skin [202].
There are also some reports that a small number of Aβ afferents may have nociceptive functions [255].

Nociceptors can respond to a single modality (mechanical, thermal, chemical) or to multiple modalities (e.g., mechano-heat, polymodal). Mechanical nociceptors respond to suprathreshold mechanical stimuli [681], and thermal nociceptors respond to noxious temperatures [202]. Polymodal nociceptors respond to various modalities of noxious stimuli, including mechanical, thermal and chemical stimuli [612]. Furthermore, nociceptors can be classified based on the type of afferent fibre to which they are associated, Aδ or C fibres. There are also C fibre mechano-heat sensitive afferents (CMH) and Aδ-mechano-heat sensitive afferents (AMH). It is believed that these different types of nociceptors encode the different qualia of pain, such as burning, aching, pricking, prickle [612]. The CMH and AMH are considered polymodal nociceptors, and some respond to noxious chemical stimuli [224].

There are other classification schemes for primary afferents, including nociceptors, but they are outside the scope of this thesis.

2.3.2. Orofacial nervous system: the trigeminal nerve

The CNV is a mixed nerve with both sensory and motor components. However, the sensory segment is much larger than the motor portion [884]. The CNV has three main branches: the ophthalmic branch (V₁), the maxillary branch (V₂) and the mandibular branch (V₃).

V₁ and V₂ are entirely sensory nerves, while V₃ is comprised of both sensory and motor fibres [843]. The V₁ innervates the scalp, forehead, upper eyelid, the cornea and the eye’s conjunctive tissue, the nose, the nasal mucosa, the frontal sinuses and the dura and blood vessels of the meninges. The V₂ innervates the lower eyelid, the cheek, the nostrils and nasal mucosa, the upper lip, the upper teeth and gingiva, the palate, the roof of the pharynx, the maxillary, ethmoid and sphenoid sinuses and some of the meninges. The third branch of CNV, V₃, innervates the lower lip, the lower teeth and gingiva, the chin and parts of the jaw, parts of the external ear, and some of the meninges. Somatosensory information from the floor of the oral cavity is also encoded by V₃. CNV is not the only nerve that encodes signals from the orofacial region. In relation to this, somatosensory information from certain parts of the oral cavity, ear and the meninges is encoded by the facial nerve (cranial nerve VII; CNVII), the glossopharyngeal nerve (cranial nerve IX;
CNIX) and the vagus nerve (cranial nerve X; CNX). Sensory fibres from CNVII, CNIX and CNX enter the brainstem and synapse onto the same nuclei as CNV (see below).

The sensory aspect of the nerve receives input from receptors that encode touch, nociceptive, proprioceptive and temperature stimuli from the face, facial and masticatory muscles and the oral cavity [784]. Aδ and C fibres in the orofacial region, especially in the TMJ and muscles of mastication are very similar to those present in the spinal nociceptive system [784]. There are some notable tissues that are innervated by the trigeminal nerve, such as the tooth pulp, the cornea and the dura regions that are only (or predominantly) innervated by nociceptors, but do not have other somatosensory receptors. For instance, Davis and Dostrovsky [216] have demonstrated that stimulation of the middle meningeal artery and the sagittal sinus activates central nociceptive neurones in the trigeminal system.

The sensory fibres of the three branches of the CNV converge at the trigeminal ganglion, where the cell bodies of afferent fibres are located. The trigeminal ganglion is similar to the dorsal root ganglion in the spinal somatosensory system in terms of markers associated with nociception, and nociception-related receptors [9,33,82,487,569]. From the ganglion, a single sensory root emerges and enters the CNS at the level of the pons. Primary afferent fibres terminate in the principal sensory nucleus (or main sensory nucleus; MSN) and the spinal trigeminal nucleus (STN). There is another specialized trigeminal nucleus in the brainstem: the mesencephalic nucleus of the CNV (MeT) (see below). Together, the MSN, the STN and the MeT form the trigeminal brainstem sensory nuclear complex (VBSNC). Unlike the dorsal horn of the spinal cord, the VBSNC is comprised of several nuclei with unique cytoarchitectonic and organizational features [82].

The MSN is similar to the dorsal column (gracile and cuneate) nuclei where large-diameter myelinated primary afferents that encode touch and position information from the body synapse onto second-order fibres. Like these nuclei, the MSN maintains a somatotopic map of the regions from which it receives touch and position information, namely the orofacial region [411]. The second-order fibres from the MSN bifurcate, and a larger branch of these second-order fibres forms the trigeminal lemniscus, which decussates and runs parallel to the medial lemniscus. The medial lemniscus, which carries touch and position from the body, terminates at the
ventroposterior lateral (VPL) nucleus of the thalamus. The trigeminal lemniscus terminates in the ventroposterior medial (VPM) nucleus of the thalamus.

The STN is a long nucleus that is located between the pons and upper cervical spinal cord (around C2) and is comprised of three subnuclei, organized rostro-caudally: oralis, interpolaris and caudalis (V_o, V_i and V_c, respectively) [656]. V_c is cytoarchitectonically similar to the spinal dorsal horn [656], and has been termed the medullary dorsal horn [366]. A-β fibres bifurcate and terminate in the MSN and along the STN, and Aδ and unmyelinated C fibres terminate predominantly in the STN at the level of V_c and to the upper cervical spinal cord dorsal horn. Interestingly, some Aδ primary afferents carrying pain and temperature information synapse onto second-order fibres in V_o and V_i, but this does not occur with C-fibres [947]. There are several somatotopic maps along the VBSNC. Specifically, somatotopy is maintained in the dorsoventral plane of each nucleus: the mandibular region is encoded in the dorsal segment, then the maxillary region, and, ventrally, the ophthalmic region. In V_c, the somatotopic organization has been shifted, and has an “onion-skin” arrangement, where oral regions are represented rostrally, and lateral regions of the face are represented more caudally. There is a second somatotopic representation along the mediolateral axis of V_c where the head is inverted [453,801]. Second-order fibres from the STN form the TTT and, as implied by the name, project to the thalamus, while maintaining a somatotopic organization. These pathways are described in greater detail in Section 2.3.3.1.

A unique feature of the trigeminal system is that the cell bodies of primary afferents that encode proprioceptive information from the masticatory muscles and periodontal ligaments are located within the central nervous system in the MeT [167,176,549]: this is the only site where soma of afferent neurones occur within the CNS. The MeT fibres bifurcate in the brainstem. One branch of these fibres forms a reflex loop: it synapses on to the trigeminal motor nucleus, which has efferent fibres that projects back to the periphery. These loops form the basis of orofacial reflex arcs (such as the jaw jerk reflex) [399-401,411,557]. The other branch of these fibres projects to other brainstem targets via the tract of Probst [138]. For instance, some of these axons project to the MSN where they synapse onto second-order neurones [918].
2.3.3. White matter pathways

This section will provide a brief overview of the major white matter tracts in the brain, especially those related to brain regions implicated to nociception and pain processing and modulation will be described, particularly in terms of the functional neuroanatomy of these tracts (although there has actually been little work on the functional anatomy of white matter in humans).

White matter tracts are classically divided into three groupings: (1) projection fibres, that connect the cortex with subcortical, brainstem and other regions; (2) commissural fibres, that connect cortical regions from one hemisphere to the other hemisphere; and (3) association fibres that connect cortical regions within a hemisphere [147] (See Figure 2-3).

Today, it is recognized that there are several types of white matter tracts in the brain, including long association fibre pathways, striatal pathways, commissural fibres, and projection fibres [237,760] (see Figure 2-3). There are also short “U” association fibres that connect neighbouring gyri [237,311].

The corona radiata contain ascending and descending projection fibres that include motor tracts that emerge from the primary motor cortex (M1), supplementary motor area (SMA) and premotor cortex (PMC) and project to the basal ganglia, the brainstem motor nuclei (corticobulbar tracts) and the spinal cord (corticospinal tract; CST) [147,644]. There are also corticobulbar tracts that emerge from various PFC regions that are involved in various homeostatic functions. Of particular interest are tracts from the PFC that project to the periaqueductal gray (PAG), which is implicated in pain modulation. The corona radiata also includes ascending tracts that project from the thalamus to the cortex, forming the thalamocortical tracts (TCT) [644]. Furthermore, there are corticothalamic tracts that run parallel to the TCT.

Somatosensory (including nociceptive) input from the orofacial region is mainly transported to the CNS via the CNV. The primary afferents project to the VBSNC. Second-order neurones form the TTT and the trigemino-bulbar tracts, which project to the thalamus and the various brainstem nuclei (see above). Third-order neurones from the various thalamic nuclei project to cortical regions, forming the TCT. For instance, tracts from VPM project to S1 and S2. These tracts ascend to these cortical targets through the corona radiata, via the posterior limb of the internal
capsule (plIC) [237]. The MD thalamus projects to the cingulate cortex and the insular cortex via the anterior corona radiata (ACR), which course through the anterior limb of the internal capsule (ICAL) [237]. Similarly, there are corticothalamic tracts, which run parallel to the TCT.

Association tracts are intrahemispherical and project between distant cortical regions. For example, the cingulum connects the entire cingulate cortex, and is connected to every lobe of the brain: the medial frontal, insular, parietal, occipital, temporal lobes [147,644]. The superior longitudinal fasciculus is comprised of three major tracts (I, II, III). It runs antero-posteriorly, and connects frontal regions along the sylvian fissure, and the insula to the parietal and temporal lobes [147], and form most of the external and extreme capsules (EC/ExC). Another association fibre is the uncinate fasciculus, which connects the OFC to the temporal lobe, including the hippocampus and amygdala [146].

Animal studies have demonstrated that the trigemino-bulbar (as well as the spinobulbar) tracts project to supraspinal regions via the hypothalamus [258], thereby bypassing the canonical thalamocortical projections. Therefore, it is likely that these fibres provide input to the circuit of Papèz [664], the limbic circuit of the brain. This circuit includes the hippocampus, the mammillary bodies, the anterior thalamic nucleus, and the cingulate gyrus [644]. These gray matter regions are connected through several white matter tracts: the fornix, the mammillothalamic tract, the thalamocingulate tract, and the cingulum. The hippocampus projects to the hypothalamus via collaterals of the fornix [644]. The mammillary bodies receive input from brainstem nuclei, including the parabrachial nuclei, and further project to the anterior nucleus of the thalamus via the mammillothalamic tract. The thalamus projects to the cingulate gyrus via the thalamocingulate tract. The cingulum bundle courses the entire length of the cingulate gyrus, and projects posteriorly to the hippocampus [644,664]. Anteriorly, there are reciprocal projections with the PFC [644]. Therefore, the circuit of Papèz may be implicated in processing the affective-motivational dimension of pain. Furthermore, key parts of the circuit of Papèz, especially the cingulate cortex, are connected to other brain regions implicated in pain [21,23].

Commissural tracts comprise of interhemispherical tracts. The corpus callosum contains fibres that project from one hemisphere to the other, including the frontal, parietal, occipital and temporal lobes, and so is thought to transmit information between the hemispheres. Little is
known about the role of the corpus callosum in the context of pain, but one study has demonstrated abnormalities in the anterior body of the corpus callosum of patients with CRPS [356]. The bilateral amygdalae and the ventromedial temporo-occipital cortices are connected by the anterior commissure.

**Figure 2-3:** Diffusion tensor imaging reconstruction of Meynert’s classification of white matter tracts in the brain. The tracts are superimposed on medial and lateral images of the brain. Reproduced with permission from: [147].
2.3.4. Nociceptive pathways and mechanisms

The ascending nociceptive pathways can broadly be divided into three components: primary sensory afferents that transmit nociceptive information from the periphery to the CNS; second-order neurones that transmit nociceptive information to the thalamus; and third-order neurones that transmit nociceptive information from the thalamus to cortical brain regions. There are also collateral projections from trigeminal brainstem nuclei to other brainstem nuclei, including the reticular formation (RF) and other brainstem nuclei. Here, the peripheral nociceptive mechanisms and both the spinal nociceptive pathways and the trigeminal nociceptive pathways will be described.

2.3.4.1. Peripheral nociceptive mechanisms

Free nerve endings that respond preferentially to noxious stimuli are called nociceptors [89,124,796]. The transductive properties of nociceptors are based on the receptor molecules and/or ion channels on the free nerve endings.

Some nociceptors are specialized and have specific response properties (unimodal), whereas other types of nociceptors (polymodal) respond to several noxious stimuli. Unimodal nociceptors are the basis of different pain qualia, such as burning, stinging, and aching. Polymodal nociceptors, for example, can either be C or Aδ (and some Aβ) afferents that respond to intense mechanical and heat stimuli (CMH, AMH), and can also respond to chemical stimuli [224,944]. Furthermore, AMHs can be classified as type I and type II [870]. Type I AMH cells (Aβ or Aδ fibres) have low mechanical thresholds and a high heat threshold. Type II AMH cells (Aδ fibres) have high mechanical thresholds and moderate heat thresholds. Another class of nociceptive afferents is C and Aδ fibres that do not respond to mechanical stimulation, or have very high mechanical thresholds, and are thus called mechanically insensitive afferents or silent nociceptors [611]. There are several types of C fibre nociceptors: CMH cells, mechanically-insensitive C fibres nociceptors and polymodal nociceptors.

Another feature of the different fibres is the quality of pain they encode. Type I AMH fibres are present in both hairy and glabrous skin, and respond to sustained high-intensity heat stimuli (i.e., they are slowly adapting to the noxious thermal stimulus). Type II AMH fibres are rapidly adapting fibres and are also present in hairy and glabrous skin [444,863]. CMH cells respond to
noxious heat stimuli near the pain threshold, and the neural response increases with the stimulus temperature. Because of the different conduction velocities of the fibres, the percepts from the different fibres are felt at different times: myelinated Aδ neural responses reach the CNS before neural responses from unmyelinated C fibres. For instance, when a noxious heat stimulus is presented to glabrous skin, Aδ (type II AMH) fibres give rise to first-pain, which is a sensation of pricking, sharp and aching pain. This sensation is followed by second pain, which is a sensation of dull or burning pain elicited by C fibres [136,543,710,711,867,870]. It is also noteworthy that stimulation of glabrous skin does not elicit a dual percept of pain; the absence of type II AMH fibres eliminated the possibility of a first-pain sensation [612].

Muscles and joints also have nociceptors. It is believed that the primary cause of muscular pain is ischaemia (reduced blood flow and oxygenation), which activates nociceptors [572]. Furthermore, the gradual accumulation of metabolites formed by muscular exercise can also sensitize nociceptors and induce alldynia [375,376,432]. The percept of muscular pain is usually mediated by Aδ and C fibres, which are HTM [81], which can be sensitized by algesic chemicals [941]. There are also mechanically insensitive nociceptors that respond to algesic chemicals (chemonociceptors) [480]. The TMJ is also innervated by nociceptors. Specifically, the TMJ and its periarticular connective tissues are innervated by nociceptors. Furthermore, evidence suggests that the articular disk contains low-threshold mechanoreceptors [34].

Another distinction in pain qualities is based on the spread of the pain. Specifically, cutaneous pain is often localized, whereas muscle pain is can be diffuse [482,805,946]. This is caused by the size of the receptive field of a single nociceptor and less dense innervation of muscles. The receptive field of cutaneous nociceptors is in the mm² range [89,483], whereas that of muscle nociceptors is in the cm² range [504,599]. Further, muscular and cutaneous pains have several different characteristics. For instance, deep muscular pain is reported as more unpleasant [838] and it has a different quality than cutaneous pain. These differences can be attributed to different neurophysiological phenomena in the peripheral innervation of muscles and skin, as well as differential central pathways encoding nociceptive information from these tissues.

2.3.4.2. Spinal nociceptive pathways and mechanisms

Primary afferents encoding innocuous and nociceptive information from the skin, the viscera, muscles and joints enter the central nervous system at the dorsal horn of the spinal cord. These
tracts join Lissauer’s tract, posterolateral to the spinal cord, and send collaterals into the dorsal horn at various levels of the spinal cord [941]. Thermoreceptive and nociceptive primary afferents synapse onto second order neurones in the dorsal horn of the spinal cord. Rexed [727,728] identified several layers in the spinal cord based on cytoarchitectonic features. He identified six layers in the dorsal horn, three layers in the intermediate region and ventral horn and one layer surrounding the central canal. The dorsal horn layers can further be classified as the superficial layers (laminae I, or the marginal zone, and II, or the substantia gelatinosa) and the deep layers (laminae III-V, or the nucleus proprius, and lamina VI) [410,720,941]. Cutaneous Aδ fibres terminate in laminae I, II, and V, and cutaneous C-fibres synapse onto laminae I and II. Deep tissue, including muscles and joints, and visceral afferents synapse onto various layers. There are two paths for HTM afferents: one set terminates exclusively onto lamina I, and the other set terminates onto laminae I, IV, and V [857]. Unmyelinated nociceptive afferents from the deep tissues project to lamina I and deeper laminae, but not lamina II. There are two types of nociceptive second-order neurones: nociceptive-specific (NS) neurones and wide-dynamic range (WDR) neurones, which predominate in laminae I/II and V/VI. A small proportion of nociceptors also synapses onto second-order cells in the other laminae (III, IV and VI). Fibres that encode tactile information other than pain and temperature bifurcate upon their entry to the spinal dorsal horn, and form an ascending branch which forms the dorsal column and a descending branch that synapses to fibres in laminae IV and V [857].

There are two types of second-order neurones that receive input from primary nociceptive afferent fibres: NS and WDR cells. NS neurones respond exclusively to noxious stimuli [160]. WDR neurones respond to innocuous stimuli but have greater responses noxious stimuli. The NS cells include the polymodal heat-pinch-cold (HPC) cells [397]. Second-order neurones form the STT. The second-order fibres originating in the marginal zone are mostly modality-specific, and include thermoreceptive (which respond to cooling), HPC, and NS cells [20,397,964,967]. These fibres form 50% of the fibres in STT [185]. The substantia gelatinosa (lamina II) receives nociceptive input and is mostly comprised of interneurones which project to laminae I and V and to adjacent spinal segments, and thus these cells do not contribute to the STT. STT neurones originating from deeper laminae (the border of IV/V, the lateral aspect of VI, and a smaller proportion from VII and VIII) [24] of the spinal cord are mostly WDR cells [943]. There are two STT pathways: the lateral and the medial STT [545]. The lateral STT is a monosynaptic tract that
encodes sensory-discriminative aspects of pain, which project directly to the ventrolateral thalamus in a somatotopically organized fashion. The lateral STT receives input mostly from the superficial layers of the dorsal horn [545]. The medial STT is comprised of monosynaptic and polysynaptic tracts that encode affective-motivational aspects of pain, and receives its input from deeper laminae of the dorsal horn. The medial STT projects to the medial thalamus as well as several brainstem nuclei (see below). The receptive fields of the medial STT neurones are typically large, unspecific and not related to stimulus intensity [363], and there is no somatotopic organization [102]. Unlike the neurones in lamina I, lamina V cells do not maintain somatotopic organization [913,941,942,944]. Furthermore, lamina I cells have small receptive fields compared to lamina V cells, which have more extensive receptive fields. Therefore, it is likely that lamina I cells are more involved in the stimulus localization, whereas lamina V cells, which have a graded response to innocuous and noxious stimuli, are likely more involved in intensity coding.

As mentioned above, lamina II neurones receive input from primary nociceptive afferents, but rather than ascend along the STT, these cells play a largely modulatory role in the spinal cord. Specifically, lamina II cells comprise mostly of interneurones that modulate second-order neurones originating in lamina I and V. These cells may, in fact, represent the gate discussed in the Gate Control theory of pain (see Section 2.1.1.4). In addition to the STT, there are other spinal nociceptive pathways, but these are outside the scope of this thesis.

About 85% of the STT decussates and 15% of the fibres remain ipsilateral to the input [258,545]. Both pathways ascend the spinal cord to project to several thalamic nuclei. For instance, there are STT projections to ventroposterior region of the thalamus, including VPL and the inferior ventroposterior thalamus (VPI) [258]. STT fibres originating from lamina I also project to the ventromedial aspect of the posterior (vmPO) nucleus and the ventrocaudal aspect of the medial dorsal (MDvc) nucleus of the thalamus [184]. Lamina V and deep laminae neurones also project to the centromedian (CM) and centrolateral (CL) nucleus of the thalamus [258]. Canonically, these formed the lateral and medial pain systems. The lateral system included projections to VPI and VPL and vmPO and their cortical projections. The medial pain system includes MDvc and CL thalamus [869].
As mentioned above, the medial STT has collaterals that project to several brainstem nuclei, which form the spinobulbar tract [184]. There are four main regions where this tract projects to: (1) the catecholamine cell groups (A1-A7), the parabrachial nuclei, the PAG and the brainstem RF. The role of these regions is pain is discussed in further detail in Section 2.3.6. There are other spinal nociceptive pathways, including the spinothalamalic pathway, the postsynaptic dorsal column system, and the spinocervicothalamic systems [258], but specific details about these pathways are outside the scope of this thesis.

### 2.3.4.3. Trigeminal nociceptive pathways and mechanisms

The trigeminal nociceptive system includes primary afferents from peripheral nociceptors. Nociceptors are specific receptors in the skin and muscles and joints that are free nerve endings. Different nociceptors encode different types of noxious stimuli, such as mechanical, thermal (heat and cold) and chemical stimuli, and some encode more than one of these stimuli (polymodal nociceptors) (see Section 2.3.3.1).

Small myelinated Aδ and unmyelinated C primary nociceptive afferent fibres have their cell bodies in the trigeminal ganglion, and project to the VBSNC [784]. Nociceptive afferents synapse all along the VBSNC, including the three subnuclei of the STN and MSN [214,216,784]. Specifically, these project mostly to Vc, although small-myelinated primary nociceptive afferents (Aδ) fibres have been shown to synapse (in lesser density) along the entire VBSNC, including Vo and Vi, and MSN [453]. The laminar organization of the Vc is similar to that of the spinal dorsal horn. Cutaneous nociceptors from the orofacial region project to the outermost lamina (lamina I).

Dense connections within and between the STN nuclei, termed deep fibre bundles of the VBSNC [367,452,639], link different nuclei within the complex that have a common somatotopic representation [82]. Although the role of these connections has yet to be established, evidence suggests that the connections from Vc have a facilitatory effect on the other nuclei. Evidence for the facilitatory role of these interneurones comes from studies reporting that inhibiting or lesioning Vc reduced or abolished the excitability of rostral portions of the STN to noxious stimuli in the trigeminal region, and the rostral nuclei showed increased responses to innocuous stimuli in the periphery [82,83,452]. There is also evidence of ascending tracts in the deep fibre bundles [82,215,450,452,501] that have a modulatory effect of rostral regions of the STN onto Vc [425].
Similar to the spinal dorsal horn, primary afferents have specific targets within the \( V_c \) \( (i.e., \ V_c) \) \[784\]. For instance, LTM project to the deeper layers of \( V_c \) (laminae III – VI) \[453\]. Small-diameter nociceptive afferents synapse onto laminae I, III, V and VI of \( V_c \). Many of the aforementioned local circuit neurones, which modulate rostral regions of the STN emerge from lamina II \[784\]. This lamina, akin to the spinal \textit{substantia gelatinosa} modulates other neurones in the \( V_c \). Second-order neurones in the STN can be categorized like those in the spinal dorsal horn: NS neurones, which receive input from nociceptive A\( \delta \)- and C-fibres, and WDR neurones, which receive convergent input from neurones which encode both innocuous and nociceptive somatosensory information \[784\]. Both cell types show graded responses to noxious stimulus intensity, or as more of the receptive field is stimulated \[784\]. NS and WDR neurones predominate in laminae I/II and V/VI \[783-785\]. NS cells have ipsilateral, localized receptive fields in the skin, whereas WDR neurones have larger, more-complex receptive fields, with tactile and, often, pinch-sensitive areas \[784\]. There are also NS and WDR in more rostral portions of VBSNC, including \( V_o \), \( V_i \) and nuclei outside the VBSNC, such as interstitial and paratrigeminal islands \[784\]. However, as these neurones show a graded response to stimuli in the oral cavity and perioral regions, they are outside of the scope of this thesis.

Additionally, most NS and WDR neurones in \( V_c \) receive converging input from both cutaneous afferents and from afferents supplying other structures in the craniofacial region, including dura, cerebrovasculature, the cornea, the tooth pulp and, relevant to this thesis, the muscles of mastication and TMJ \[17,141,214,586,597,645,708,787,788\]. Furthermore, some of these neurones also receive afferent input from other cranial nerves and upper cervical cord neurones \[784\]. Together, these converging afferent inputs provide a neural substrate for the poor localization, spread and referral of pain in painful conditions of the TMJ and deep craniofacial tissues \[784\].

As in the STT, the TTT projects to the thalamus, as implied by its name. Sensory fibres from the VBSNC synapse onto third-order neurones in the VPM, the posterior nucleus (PO), the parafascicular nuclei (Pf), and the submedial nucleus (SM) of the thalamus \[257\] (for further discussion, see 2.3.5). Other tracts emerging from second-order neurones in the \( V_c \) project to brainstem and brain regions involved in nociception and pain modulation (see Section 2.3.5 and 2.3.6). These include projections to the brainstem RF, the PAG, the hypothalamus, brainstem
motor nuclei and brainstem autonomic nuclei [784]. The brainstem targets likely form the neural basis for sensorimotor and autonomic reflex loops.

2.3.4.4. Trigeminal motor pathways

Abnormalities in motor output in the trigeminal system have been associated with parafunctional habits, including bruxism (grinding) and clenching of the jaw [628]. These habits have been associated with pain in the orofacial region, and with TMD [840]. Furthermore, patients with TMD show abnormal motor function in the orofacial region (see Section 2.1.6), and so an understanding of the interaction of sensory and motor pathways is essential to this thesis.

The sensory and motor systems interact and show connections throughout the CNS [361]. These interactions allow for rhythmic motor activity (such as walking and chewing), reflexes (such as withdrawal from a noxious stimulus) and smooth execution of a voluntary task. These interactions are the neural basis of feedback loops. Feed-forward loops allow for the use of sensory information and cues in the external environment to prepare for motor output. These loops are used for rapid actions, such as catching a ball [361]. The sensory visual information about the trajectory of a ball provides necessary information for the motor system to properly anticipate where the ball can be caught. Conversely, feedback loops allow for sensory information to modulate or correct motor output to attain a desired output. The sensory signal is compared to a reference, and if these do not match, error signals are produced to correct the proper trajectory. These loops are used for slow movements, e.g., such as reaching for an object [361].

The motor aspect of the CNV supplies the muscles of mastication, including the masseter muscles, the temporalis muscles, the anterior digastrics, the medial mylohyoids, the medial pterygoids, and the lateral pterygoids [912]. The motor fibres originate in the trigeminal motor nucleus in the brainstem, and receive input from supraspinal motor centres and sensory information from afferents in the brainstem [555]. The trigeminal motor nucleus is located in the lateral pons, medial to the MSN. Motor efferents emerge from the trigeminal motor nucleus and leave the pons at the ventral surface, forming the motor root of the CNV. The motor root is much smaller than and medial to the sensory root [256]. Other cranial nerves also innervate the masticatory muscles. For instance, CNVII innervates the posterior digastric muscle (as well as
other facial muscles), CNX innervates the palatoglossus part of the tongue, and the rest of the
tongue is innervated by CNXII [644].

There are three levels of parallel and hierarchical motor control: the cortex, the brainstem and the
spinal cord (or in the case of the trigeminal system, the sensory and motor nuclei of the CNV)
[361]. At the most basic level, the trigeminal sensory and motor nuclei mediate simple reflexes,
such as the jaw-jerk reflex. This monosynaptic reflex occurs when there is pressure on the
mandibular teeth. Proprioceptive information of the mandible is relayed to the trigeminal MeT
nucleus in the midbrain via primary afferents in the third branch of the sensory root of the CNV
[176,644]. These neurones’ cell bodies are located in the MeT nucleus, and some collaterals
synapse directly onto the trigeminal motor nucleus. The trigeminal motor nucleus projects
efferent fibres to the masseter muscle, the internal pterygoid, and the temporalis muscle, which
contract and depress the mandible.

The brainstem motor nuclei are also responsible for repetitive (or rhythmic) motor behaviour,
such as mastication (or chewing) [644]. In the case of mastication, it is believed that a central
pattern generator maintains the rhythmic behaviour. The controlled and patterned contraction of
muscles to produce rhythmic mandibular motion can be broken down into three different stages
[555]: the preparatory, reduction and pre-swallowing stages. The first step is breaking food into
chewable pieces. In some cases, such as a steak, this is done before the food reaches the mouth.
In other cases, such as biting into an apple or a sandwich, this step occurs at the mouth. In this
stage, the digastric muscles (jaw-opening muscles) are very active, and the masseter and the
temporalis muscle show little activity, but are involved as they are responsible for fast-closing.
The chewing (or reduction) cycle is characterized by three phases: opening and fast-closing and
slow-closing. The slow-closing phase occurs when teeth come in contact with food, and muscles
move the jaw laterally to grind food between the molars. The final stage of chewing – pre-
swallowing – consists of fast-closing, slow-closing, and three sequential opening phases. These
coordinated movements rely heavily on sensory feedback – location of food in the oral cavity,
state of the food, position of the jaw, lips, and tongue, etcetera. These sensory cues modulate the
rhythmicity of masticatory muscles while chewing. Taken together, there is evidence that a
central pattern generator at the level of the trigeminal motor and MeT nuclei exists, and
coordinates muscular activity during chewing [555,644]. The central pattern generator in the
brainstem can be modulated by corticobulbar tract (motor efferent tracts from motor cortical
regions) [555]. Further modulation can come from RF of the brainstem, which receives convergent input from the cortex, and the VBSNC. It is important to distinguish that reflexes and pattern generators do not require input from upper motor neurones (corticobulbar tracts), but cannot function without this input [555].

The next level of motor control comes from the brainstem – namely, the RF [361]. As described above, the RF modulates the central pattern generator based on sensory cues and efferents from cortical regions and other brain regions such as the amygdala and the hypothalamus. Other brainstem motor regions that project to the trigeminal motor nucleus include the red nucleus [368], and the vestibular nucleus [256]. These findings suggest that motor output to the orofacial muscles can be modulated by a number of different sources. The cortical regions represent the highest level of motor control: voluntary motor movement [361,644]. These movements are initiated in the brain. Specifically, M1 (Brodmann’s Area 4 (BA4)) is where voluntary motor output is initiated. M1 receives dense input from the S1 (BA 1, 2, 3a, and 3b) [674,706,747,748], and two regions involved in motor planning: the SMA, and the PMC (both regions are designated as BA6) [644]. It has been suggested that somatosensory input into M1 may be related to motor learning. In line with these findings, it has been shown that acute nociceptive input inhibits motor learning, and that patients with TMD have abnormal motor output (see Section 2.2.1.2).

M1, SMA and PMC have motor efferents that project to the trigeminal motor nucleus either directly, via corticobulbar tracts, or indirectly, via the RF [644]. In primates, M1 is somatotopically organized: the map is organized ventrolateral-dorsomedially, with the toes along the medial wall, and the hand region in the middle of gyrus, and the orofacial region is represented on the ventrolateral surface of the gyrus [644]. The corticobulbar tracts originate at the lateral aspect of the M1 (or SMA, or PMC), descend along the genu of the internal capsule (IC), and course through the cerebral peduncles in the brainstem [644]. There is both ipsilateral and contralateral contribution (contralateral being dominant) to the corticobulbar tracts, which decussate at the level of the cerebral peduncles in the midbrain, and synapse onto lower motor neurones in the trigeminal motor nucleus. Therefore, the sensory and motor systems are highly interconnected, and sensory information modulates motor output at several levels within the CNS [556,644].
2.3.4.5. Supraspinal nociceptive and pain regions

Peripheral and brainstem nociceptive pathways project to the thalamus and other brain regions implicated in pain. Herein, the subcortical and cortical brain targets of second-order neurones of the TTT and its collaterals that bypass the thalamus will be described. Additionally, this thesis will describe experimental findings from animal studies and electrophysiological studies implicating these regions in nociception and the putative role of these regions in the experience of pain. Neuroimaging studies describing neural correlates of acute and/or chronic pain will be discussed in Section 2.5.

It is important to recognize that it has not always been clear that the cerebral cortex is involved in pain perception. It has been proposed that the brain is where perceptions occur (c.f., sensations) – even Aristotle recognized that perception was different from sensation. Sensation means to encode stimuli, and perception is the interpretation of this sensation. Aristotle posited that sensations were a property of the physical being, whereas he relegated percepts to the soul (or the mind) [810]. Nonetheless, until the mid-twentieth century, there was little evidence for the cortex to be involved in pain perception. For instance, Hitzig [426] stated that pain is a subcortical phenomenon. Head and Holmes [417] found little evidence that the cortex is involved in pain – a patient with extensive cortical damage still felt pain, and pain unpleasantness. In fact, they suggested that, in the case of central pain – a condition where a stroke affects the thalamus – pain arises because the thalamus is released from cortical control [514]. Cushing could not produce a painful response in patients with electrical stimulation of the cortex [196], and so thought that pain was a percept of the thalamus. Penfield and Boldrey echoed these findings in their paper in 1934 [677], stating that electrical stimulation of the brain did not elicit painful responses. However, in a later study, they found that stimulating the postcentral gyrus (S1) did elicit painful responses, but that these responses were rare – 11 of 800 stimulations produced a “painful” experience, none of which were intense enough for the patient to object [679]. Additionally, they suggested that the removal of cortex does not relieve pain [678]. From these findings, they concluded that pain has little cortical representation. In support of this hypothesis, Adrian [6] could not identify any impulses reaching the cerebral cortex from noxious or thermal stimulation in the periphery in several animals.
Conversely, to treat a patient with thalamic syndrome (or central pain), procaine was injected into the somatosensory cortex of a patient, who reported pain relief for a period of 2 months [845]. Further, Russel and Horsley [746] excised a portion of S1 (in the area of the arm), and the subject reported a loss of sensation and analgesia in the affected limb, however not all sensory qualities were abolished. Similarly, a number of studies reported that patients who suffered cortical lesions, especially in the region of S1, had altered pain sensations – in the absence of thalamic damage (reviewed by [571]). Electrical stimulation of the cortex provided variable results, as some groups found that stimulating S1 caused the patient to experience pain, whereas several other studies S1 stimulation did not elicit pain. In one of these cases, stimulation of S1 reproduced the patients’ pain, and resection of S1 alleviated their pain [542,571]. Furthermore, Dusser de Barenne and McCulloch showed that the function of VPL and S1 were interdependent, and when deeper layers of S1 or VPL were ablated, the spontaneous electrical activity in the other was diminished [281].

Although other theories have postulated that the percept of pain occurs in the brain, none had incorporated a specific role for it in their model. However, no study has shown that pain is solely due to a single area in the brain. As Marshall [571] suggested, S1 (and likely other cortical regions) are involved in pain, but may not be the “endpoint” of pain – that S1 receives and processes nociceptive input (likely sensory-discriminative information); however, this is not the region where the nociceptive input is perceived as pain. Rather, this information is processed and “sent” to other brain regions where the percept of pain occurs. To address this point, Melzack suggested that a network of brain regions working together is required for the percept of pain [592-594]. This pain “neuromatrix”, as he called it, is supported by the multidimensional experience of pain. This theory required the concerted activation of several brain areas to produce the experience of pain, each set underlying a dimension of pain. Therefore, it was proposed that to have a conscious experience of pain, many brain regions, each related to a dimension of pain (cognitive, modulatory, sensory-discriminative, affective, motor) are required. This theory provides an explanation for the finding that stimulating a single brain region does not consistently produce pain. However, as will be described, to some extent in this section, and in further detail in Section 2.5, few (if any) studies have been able to show that these regions of the brain are, effectively, functionally connected – a fundamental tenet of a “pain matrix”. In fact,
the concept of the so-called “pain matrix” idea has now fallen out of vogue, and other theories about cortical brain regions have been postulated, and will be discussed herein.

**Thalamus**

The STT (and the TTT) project to the thalamus. Interestingly, the TTT ascends bilaterally, albeit the contralateral thalamus receives a greater density of projections [784]. These tracts project to the ventroposterior nuclear group (ventrobasal complex in subprimates) in a somatotopic fashion. In fact, the lateral STT projects to the lateral portion (called the VPL) [351] and the TTT projects to the medial portion (called the VPM) [340]. These projections maintain the somatotopic organization of the VBSNC. The ventral STT projects to other thalamic nuclei, including MD, Pf and CL [869]. Gaze and Gordon [351] identified NS neurones in the thalamus of cats and monkeys for the first time. Similarly, Perl and Whitlock [684] identified NS neurones in the ventrobasal thalamus of cats and monkeys. These neurones showed responses only to noxious stimuli, and had a specific (c.f. large or diffuse) receptive field in the contralateral body. Similarly, Applebaum and colleagues [29] confirmed these findings with antidromic mapping of VPL neurones in the upper lumbar region of the spinal cord. There are also WDR neurones in the thalamus of humans and animals [142,527]. Electrophysiological recordings and stimulation studies in humans undergoing neurosurgery have confirmed that the human thalamus receives nociceptive input, and these inputs are topographically organized in the ventroposterior nucleus [223,396,534]. The somatotopic organization of STT and the ventroposterior thalamus suggests that these nuclei may be related to the sensory-discriminative aspect of nociception, *i.e.*, the location of the stimulus, and the graded response to nociceptive stimulus suggests that this region is implicated in magnitude encoding, *i.e.*, the intensity of a stimulus. These concepts were supported by findings that stimulating VPL results in localized pain in humans [415]. Additionally, some STT neurones projecting to VPL have collateral projections that synapse onto CL [30,362,589].

Furthermore, Perl and Whitlock [684] demonstrated that the STT also projects to CM and intralaminar nuclei, which only receive nociceptive input and have bilateral receptive fields, and the posterior nuclear group of the thalamus, which has diffuse receptive fields. These findings were confirmed by several tracing studies [25,102,129,485,488,603,701]. Additionally, connections from the STT to the VPI were also found [25,85,701].
The STT and TTT also have topographical input to MDvc: the STT projects to the anterior portion, and the TTT projects more caudally [186,341]. There is also evidence of a pain- and temperature-specific nucleus – the VMpo [188]. Neurones that responded to cold perception, in the vicinity and had some of the same properties attributed to VMpo were located in the human thalamus [223]. Blomqvist et al. [101] found a site in the human thalamus with STT projections in the vicinity of VMpo.

Projections of the TTT are similar to those of the STT. As mentioned above, these ascending tracts project to different nuclei within the ventroposterior thalamus: the VPM and VPL, respectively. The VPM receives input from all levels of the VBSNC, however, only dorsal MSN neurones project to the ipsilateral VPM, whereas ventral MSN and STN project to the contralateral VPM [126,127,140,574,618,719,811,812,831,861,948,957]. Furthermore, there are also projections to the PO and MDvc [127,341], and projections to CL and CM have also been identified [948].

Finally, the ventrolateral nucleus of the thalamus (VL) receives input from deep dorsal horn, via the STT [85]. These projections overlap with cerebellar projections to VL. Third-order neurones project from VL to the motor cortex, and so it has been suggested that these inputs are related to sensorimotor integration, or the motor dimension of pain [794].

In summary, the thalamus receives thermal and nociceptive information from the STT and TTT. The tracts project to several nuclei within the thalamus, which have various cortical targets. These various projections suggest that the different targets contribute to various dimensions of pain. These concepts are discussed in further detail herein.

**Primary somatosensory cortex**

In primates, S1 is located along the postcentral gyrus, and is comprised of four cytoarchitectonic rostrocaudally organized regions: BA 3a, 3b, 1, and 2. These regions have functional differences: areas 3b and 1 receive cutaneous tactile input, and areas 3a and 2 respond to proprioceptive input. Furthermore, each of the four regions in the somatosensory cortex has a complete body (homuncular) map: the foot is located medially, and the face is located on the ventrolateral bank [196,652,679].
S1 receives input from the somatosensory thalamus. Specifically, in primates, there is evidence of projections from the VP thalamus to each of the cytoarchitectonic regions of S1 [644]. As information is processed from BA 3b to BA 1, the size of the neuronal receptive field increases, and the neurones have more complex response properties, which is an indication of integration and modulation of sensory input within S1 [644]. Similarly, BA 3a projects to M1, BA 2 and S2. The receptive fields in BA 2 and S2 are larger and the response properties are more complex than in BA 3a. Furthermore, there is evidence of projections from S1 to other cortical regions, including reciprocal connections between 3b, 1, and 2 and the posterior parietal cortex (PPC, BA 5 and 7), and contralateral somatosensory cortex. Area 3a projects to M1. There are also projection to distant cortical regions, including the insula, the cingulate cortex and the parietal lobule and operculum [644]. There is also evidence for descending projections from S1 to subcortical regions [644].

As discussed above, the role of the S1 in pain processing is contentious. Some of the earliest documented evidence comes from a study by Dusser de Barenne in 1916 where he reported that cats showed an allodynic response (which he called hyperalgesic) to noxious stimuli in the periphery by applying strychnine, an alkaloid that blocks inhibitory signaling [825], to S1 cortex [280]. In humans, electrical stimulation of S1 only sometimes elicited a feeling of pain in the patient (e.g., [679]). Similarly, in some but not all cases, phantom limb pain was resolved after S1 ablation [293,383,542,833] (reviewed in: [296,571,845]). Furthermore, resection of S1 was ineffective in relieving pain in other pain disorders. Conversely, patients with Rolandic seizures (epilepsy in the region of S1 and M1) reported pain [961], and lesions to parts of S1 caused a disorder similar to thalamic syndrome [941].

Studies in non-human primates support the aforementioned findings in humans undergoing neurosurgery – that S1 has a role in nociception. For instance, Peele showed that ablating specific BA in S1 or PPC in monkeys led to a loss of nociception for nine months, and a permanent loss of the ability to locate a noxious stimulus [676]. Furthermore, electrophysiological studies in S1 in non-human primates have demonstrated that nociceptive regions of the thalamus project to BA 1 and 2 [485,631]. There is also evidence of somatotopic organization [270,484]. Kenshalo and Isensee [486] identified neurones in areas 3b and 1 that responded maximally to pinching in the receptive field, and a proportion of these showed a
graded response to noxious heat stimuli in the receptive field. Furthermore, there is evidence that area 3a neurones contribute to alldynic and hyperalgesic phenomena [929].

Specific to the trigeminal system – and of particular interest to this thesis – Biedenbach and colleagues electrically stimulated the tooth pulp in non-human primates, and reported graded responses in the face area of S1, mainly in area 3a [91]. Similarly, Chudler and colleagues found cells that in the somatotopic region of the face in BA 1 and 2, which also showed a graded response to nociceptive input [161]. Interestingly, Biedenbach and colleagues suggested that S1 cortex is likely essential for localizing noxious stimuli on the body, but also observed responses in neurones outside S1, and so suggested that S1 contributes to pain perception, but this role is not exclusive to S1.

The parasylvian cortex: S2 and insula

The parasylvian cortex includes several regions: S2, insular cortex, and the ventrolateral prefrontal cortex (vlPFC, which will be discussed later). S2 is comprised of two adjacent cytoarchitectonic regions: BA 40 and 43, which are in the ventrolateral bank of the postcentral gyrus, and within the parietal operculum [644]. The insula is a very large, heterogeneous region. It is comprised of two long sulci in the posterior portion, and three short sulci in the anterior portion [39, 40]. The insula can be divided into three subregions: the anterior, middle and posterior insula, based on functional and anatomical features.

S2 receives dense connections from S1, and some input from the VP thalamus. S2 also has a somatotopic organization, but much less detailed than that of S1 [735, 737, 930, 941, 952]. S2 maintains this somatotopic organization in its projections to M1 [859]. Evidence from non-human primate studies has shown that, in addition to performing integrative aspects of somatosensory processing [294, 644], neurones within S2 respond to noxious stimulation [65, 736, 930], and that these neurones show somatotopic organization and graded response to nociceptive laser-evoked potentials [65]. Electrophysiological studies in humans have shown that S2 responds to noxious stimulation [335, 338, 583, 734, 871, 890, 953]. Frot and colleagues have demonstrated that S2 shows graded responses to increasing intensity of non-noxious and noxious somatosensory stimuli [335]. Taken together, it would seem that S2 is implicated in processing the sensory-discriminative dimension of pain. However, the receptive fields in S2 are larger and more complex than those of S1, and so it is possible that S2 plays a more integrative role and
outputs this information to motivational and motor regions to respond to a noxious stimulus accordingly.

The insula is implicated in a variety of functions. Evidence for this comes from anatomical studies that have revealed that the insula receives input from primary sensory, limbic, motor, and other brain areas, and projects to a number of brain regions [40]. The insula is considered to be a visceral sensory and visceromotor area [40]. Further evidence for the role of the insula in several systems comes from neuroimaging studies – the insula is one of the most commonly activated brain regions in various experimental paradigms [956]. Craig has suggested that the insula is the homeostatic/interoceptive centre of the brain [187 10572,190]. It has also been suggested that the insula is a node in a general salience network [263,265,616]. In terms of pain perception, the insula is considered as a part of the medial pain system [869]– albeit it has also been suggested that it may be part of the lateral pain system (i.e., its role in sensory-discriminative dimensions of pain are under debate). The dorsal posterior subregion of the insula receives direct input from VMpo, a nucleus in the thalamus that exclusively receives thermal and nociceptive input [188]. Evidence for nociceptive input to the insula comes from studies of electrical stimulation and recordings of the insular cortex [7,337,583,658,688]. Further evidence comes from lesion studies, where patients suffered sensory loss after a lesion of the insula (Obrador, 1957, cited in: [343,377,532]) and surrounding areas [92]. Another lesion study found that the patient showed hypalgesic responses to noxious stimuli after an ischaemic stroke affected the parasylvian cortex (insula, parietal operculum and S1) [233]. Greenspan and Winfield [378] reported that a patient with a tumour that was compressing the posterior insula and the parietal operculum had increased pain thresholds to heat, cold, and mechanical noxious stimuli. Conversely, Starr and colleagues [829] found that two patients with insular lesions showed increased heat pain intensity ratings. Greenspan and colleagues [377] showed that patients with lesions to the parietal operculum, where the insula was spared, had decreased discrimination of noxious stimuli, whereas in patients where the insula was damaged, there was decreased motivation to escape the noxious stimuli (it was less aversive).

Because of the diverse input into the insula, a number theories about its role in pain perception have been postulated. For instance, it has been postulated that the insula is a multidimensional integration site for pain [116]. This suggests that the many dimensions of pain are integrated and/or interoceptive region [183]. Alternatively, Coghill [169] suggested that because of the
behavioural relevance of pain, and its essential role is survival, there are several “backup” pain intensity coders, including S1 and the insula. In line with these findings, it has recently been suggested that the insula is a central magnitude estimator for the brain [48], and that there is also pain-specific magnitude estimator in the insula. These estimators have differential connections and are hubs of cortical and subcortical networks, respectively. An alternative perspective is that the insula may be a salience detector in the brain (for a more comprehensive discussion of these ideas, see [616], and Section 2.5.1.1)

**The cingulate cortex**

The cingulate cortex surrounds the corpus callosum, anterior to the genu, dorsal to the body and posterior to the splenium. In 1937, Papèz [664] included the cingulate cortex as part of the limbic circuit he identified, the brain’s emotional circuit. Although this remains true today, many other functions have been ascribed to the cingulate cortex, including cognitive, motor, limbic nociceptive, pain-modulatory, visuospatial and memory [899]. However, there are several subregions within the cingulate that are specialized for these functions, although, like many other associative brain regions, there is much integration and overlap of function between adjacent subregions [899].

There are many classification systems used to differentiate subregions within the cingulate cortex, which add to the complexity of comparing studies that use different species or different nomenclatures. In this thesis the nomenclature developed by Vogt and colleagues [905] will be used, whenever possible. It is noteworthy that the region previously referred to as the anterior cingulate cortex (ACC) has now been divided into two regions: the mid-cingulate cortex (MCC) and the ACC. This nomenclature was developed based on a multimodal approach, including electrophysiological properties of cells, cytoarchitectonic studies, lesion studies and neuroimaging studies [900,905]. The subdivisions of the cingulate include the ACC, MCC and the posterior cingulate cortex, and the following description of these regions comes from: [899]. The ACC can be further subdivided into the subgenual ACC (sgACC; BA 25) and the perigenual ACC (pgACC; BA 32, 24 a, b, c). The MCC can be further subdivided into the anterior MCC (aMCC; BA 33’, 32' and 24 a', b', c'), which includes the rostral cingulate motor area (rCMA) and the posterior MCC (pMCC; BA 32' and 24 a', b', c'), which includes the caudal cingulate motor area (cCMA). The PCC is also divided into dorsal (dPCC; BA 31, 23) and ventral (vPCC) areas.
There is additionally the rostral caudal zone (RCZ; BA 29, 30), which is ventral and anterior to the dPCC [900]. This thesis will focus on the ACC and MCC, whereas the PCC and RCZ are outside the scope of this thesis.

It is worth prefacing the specific descriptions of the roles of the ACC and the MCC with the observation that these regions are among the most often activated in brain imaging studies, across various paradigms [956]. Therefore, descriptions of specific findings may be related to broader functions, such as salience, rather than specific functions.

Tracing studies in non-human primates have elucidated thalamocingulate connections. The ACC receives the ventral anterior, CL, Pf, and other midline and intralaminar nuclei of the thalamus [798]. There are some, albeit a small proportion, projections from the MD nucleus. Specifically, the anterior thalamus projects to the BA 32 region of pgACC and sgACC. MD thalamus projects to both subregions of the ACC (all three BAs). The ACC has been implicated in modulating emotional, mood state and visceromotor functions in humans. In monkeys, the ACC projects to subcortical and brainstem structures involved in these functions, including the hippocampus and bed nucleus of the stria terminalis, hypothalamus and the PAG and parabrachial nucleus. There are also extensive projections from area 24 to the MD thalamus [803]. The sgACC is interconnected to several frontal brain regions, including the pgACC, the OFC, vlPFC, and mPFC. The pgACC receives input from the sgACC, MCC, PCC, the insula, the frontal pole, the OFC, mPFC, the frontal operculum, and the dorsolateral prefrontal cortex (dLPFC).

The MCC includes the CMA, which has direct projections to motor neurones in the ventral horn of the spinal cord [623,691]. This subregion is involved in the planning and execution of volitional movement. The MCC receives input from VL, VPL, CL, CM-Pf, and MD [798]. Corticothalamic projections from the MCC synapse terminate onto ventroanterior nucleus (VA) and MD. Tracing studies in non-human primates have also elucidated anatomical connections between the MCC and other cortical regions, including the insula, the temporal, parietal, frontal and limbic association areas. The MCC connects to the sgACC, pgACC, the OFC, pre-supplementary motor areas, mPFC, PCC, PMC, frontal operculum, the vlPFC and the dLPFC [686]. Additionally, the CMA receives input from M1 and more ventral regions of the MCC [416].
Of relevance to this thesis are the intrinsic connections (via the cingulum bundle) within the cingulate cortex, which modulate and coordinate attention, cognitive processes and response behaviour [622,901]. Additionally, these intrinsic connections are modulated by input from and output to the dIPFC – a region involved in selecting information about the sensory environment (attention, self-monitoring, personality, working memory) and decision-making. Therefore, the connection between the dIPFC and the cingulate cortex is of particular interest in the context of pain processing (heightened awareness of pain) and pain modulation. Another important connection is between the OFC and the cingulate [150,686]. The OFC is involved in autonomic and emotional processes, and goal-directed behaviour [742]. It is believed that the OFC and dIPFC modulate sgACC output (potentially via the MCC’s intrinsic connections) to subcortical and brainstem nuclei that are involved in pain modulation (see Section 2.3.6).

Lesion and electrophysiological studies in humans have implicated the MCC in pain processing. Specifically, patients who had large regions of their cingulate cortex lesioned to relieve intractable pain (a procedure called “cingulotomy” [50]) showed attenuated emotional responses to pain – they still perceived pain, but it was no longer distressing [327,326]. These findings suggest that the cingulate cortex is implicated in the affective dimension of pain processing. Later studies reported equivocal post-cingulotomy outcomes for pain relief [50,51,414,696,938,939]: from 23% [439] to 75% [327]. This variability is likely due to the location and size of the lesion within the MCC [733]. In addition to changes in the affective dimension of pain post-cingulotomy, Davis and colleagues [217] report that a patient with a relatively small MCC lesion showed hyperpathic intensity and unpleasantness responses to noxious thermal stimuli, suggesting that the cingulate may also have a role in the sensory-discriminative (or intensity coding) dimension of pain. Furthermore, Dierssen and colleagues elicited a painful response in the contralateral body and face of a patient with phantom limb pain when they stimulated the MCC (case V.G.D., point no. 8 in [254]).

In line with these findings, Hutchison and colleagues [440] identified neurones in the human MCC that respond to painful cutaneous stimuli, and Lenz and colleagues [533] reported laser-evoked potentials in the MCC as measured by microelectrode recordings to noxious laser stimuli in the periphery. Iwata and colleagues [451] identified neurones in the CMA that are nocireponsive, which are involved in attention to pain, and nocifensive behaviour. Similarly, in a reward and pain-avoidance cued task, Koyama and colleagues [499] found that cells in the
MCC (likely the CMA) were implicated in stimulus prediction and anticipation, and response selection. Taken together, these findings suggest that the cingulate cortex is implicated in the affective-motivational dimension of pain: as pain becomes more bothersome or aversive, there is greater motivation to escape it, and these processes are encoded within the MCC.

However, it is essential to note that the MCC is involved in a number of complex tasks, and therefore, may serve a more general role in the brain: salience detection and response selection. Neurones in the MCC are activated during tasks that have a cognitive and/or emotional load and that are attention demanding, such as mental arithmetic, word generation, and Stroop tasks [218,229]. Furthermore, cingulotomy increased errors in motor output in a monetary reward task [940]. Therefore, the MCC may, in fact, be a generalized system for orienting the self to a more salient stimulus, and choosing an appropriate response, whether it is to avoid a noxious stimulus, or to increase rewarding behaviour.

**Prefrontal cortex**

The PFC is a large, heterogeneous region of the brain comprised of a number of subregions involved in various higher cognitive and limbic functions. Broadly defined, it is believed that the PFC is central to the cognitive-evaluative dimension of pain. Specifically, various regions of the PFC have been implicated in cognitive modulation of pain; however, most of these data come from neuroimaging studies, and so will be discussed in Section 2.5.1 and 2.5.2. Non-imaging evidence to support a role for the PFC in pain perception and/or modulation is paltry. One line of evidence comes from an extreme surgical procedure: frontal lobotomies, which were used to relieve intractable pain, amongst other conditions [154,288,330,880,923]. However, the extent and specificity of these surgeries is unclear, and regions such as the cingulate cortex – which is known to be involved in pain perception and modulation – could have been destroyed.

Furthermore, a tracing study that injected murine VBSNC neurones with a neuroanatomical tracer, horseradish peroxidase (a retrograde tracer), did not identify any cortical targets in the PFC [570]. Additionally, a later study injected a viral tracer (an anterograde, trans-synaptic neural tracer) into the murine tooth pulp, and failed to identify any infected cells in the PFC. Additionally, Dum and colleagues [277] injected the STT with a viral tracer, and also failed to identify any infected cells in the PFC. However, tracing studies identified corticofugal tracts from the PFC to brainstem regions involved in pain modulation in the rats [70,406,536], cats
and monkeys [405,520], and stimulation of the PFC in rats modulates nociceptive input [403,404]. Further, anaesthetization of the PFC of rats reduced pain modulation, as measured by the jump reflex threshold [175]. In a recent histological study of a rat model of spared-nerve injury, Metz and colleagues [608] reported that there are morphological and functional changes in pyramidal neurones in the mPFC. Specifically, they found increased dendritic branching, increased spine density in the nerve injury group, compared to the sham group. Additionally, these structural changes were associated to functional changes in synaptic currents, which correlated with tactile thresholds in the injured limb. More recently, Ji and Neugebauer [460] have attempted to elucidate the neural underpinnings of observed abnormal mPFC activity in neuroimaging studies of pain [47,356] (see Section 2.5.1). They found that cells in the mPFC were effectively inhibited in a murine chronic pain model, and may be related to cognitive deficits in chronic pain [461]. Additionally, there is evidence that transcranial stimulation of the dlPFC modulates pain in experimental pain paradigms [310] and in chronic pain [114,649,754,802]. Taken together, it seems that the PFC does not play a role in pain perception, but rather may be involved in cognitive appraisal and evaluation of pain, and its subsequent modulation.

Motor regions

Pain and motor interactions have been described in Section 2.1.6, and the anatomical pathways pertinent to this thesis are described in 2.3.2. This section will describe tracing and electrophysiological studies in animals and humans that have implicated suprabulbar motor regions in pain. In general, nociceptive input into motor regions is believed to serve two purposes: (1) orient the body toward the threat (noxious stimulus) and (2) initiate nocifensive behaviour (e.g., avoid the stimulus). Several lines of evidence have implicated suprabulbar motor regions in pain. The first line of evidence comes from a study that investigated dorsal column stimulation in monkeys [53]. This study found that the stimulation of the dorsal column modulated nociceptive input into the Pf thalamic nucleus, which indicated that modulation of nociceptive input occurred at the level of the thalamus, rather than in (or in addition to) the spinal cord.

Further evidence for supraspinal motor-pain interaction comes from unexpected findings that M1 stimulation (motor cortex stimulation; MCS) reduced spontaneous hyperactive firing in a model
of central pain in the cat [874]. Interestingly, stimulation of S1 did not have an effect on hyperactivity in the thalamus. Furthermore, studies have demonstrated that MCS in cats suppresses nociceptive activity in the spinal and medullary dorsal horns [182,789]. MCS has become a prevalent therapy for intractable neuropathic pain; however, the mechanism of action remains unclear. It is believed that MCS recruits distant antinociceptive brain regions through corticocortical connections [643]. Similarly, there is ample evidence that transcranial magnetic stimulation (TMS) – a less invasive method to stimulate the cortex – of the M1 is effective tool to modulate neuropathic pain (e.g., [8,331,463,643,821]). Additionally, there is likely activation of CST that can modulate nociceptive input (as postulated in the Gate Control theory of pain, see Section 2.1.1.4).

Several studies have investigated the interaction of pain and motor learning in the brain. For instance, one study found that a novel tongue-protrusion task is associated with acute and reversible neuroplastic changes in the face region of M1, as measured by TMS [41,842]. However, noxious stimulation of the oral cavity disrupts learning the motor task, and related neuroplasticity in M1 [109].

Another line of evidence for motor-pain interactions in the brain comes from viral tracing studies of the STT and that have identified infected neurones in subcortical and cortical motor regions. For instance, Dum and colleagues [277] found that, in addition to S1, S2, and the posterior insula, M1 (BA 4), the ventral premotor cortex (PMv; BA 6) and the CMA (BA 32) receive input from thalamic targets of the STT.

The basal ganglia are a group of subcortical nuclei that have classically been associated to motor behaviour [13,239,640,945]. However, anatomical, functional and behavioural studies have elucidated several roles of the basal ganglia. The basal ganglia are organized in a number of parallel and segregated anatomical circuits forming functional loops [665] and the role of these loops is integrated to mediating goal-directed behaviours [388]. In the context of nociception and pain, there are a number of findings suggesting that the basal ganglia have a complex, and yet undetermined role. For instance, Dum and colleagues [277] reproduced (but did not present) findings that subcortical structures, including the basal ganglia receive direct input from the anterolateral tract of the spinal cord. Previous studies demonstrated that nocireponsive neurones project directly to the globus pallidus and the putamen [642], and that neurones in the globus
pallidus respond to nociceptive input [88,164]. Furthermore, the caudate nucleus of the rat and the cat has neurones that respond to noxious mechanical stimuli in the periphery and that are somatotopically organized [164,544,732,769]. Also, there is evidence that striatum is densely populated with opiate receptors in rats [38] and humans [97]. In line with these findings, stimulation of the caudate nucleus has been shown to produce analgesic effects in the monkey [550].

Further support for the role of the basal ganglia in pain comes from evidence that patients with movement disorders related to the dysfunction of the basal ganglia, such as Parkinson’s disorder, often report pain [162,385,398,624,635,707,759,965]. Furthermore, depletion of dopamine in the substantia nigra (the region affected in Parkinson’s disorder) in mice leads to hyperalgesic responses to noxious stimuli in the periphery [163]. Stimulation of the striatum also modulates orofacial pain: stimulating dopaminergic neurones has been shown to modulate the nociceptive jaw-opening reflex in rat [55,78].

In a recent study of patients with lesions in the putamen, Starr and colleagues [828] found that patients showed lower cold pain detection thresholds (i.e., less sensitive), lower pain intensity ratings to suprathreshold heat pain and decreased pain intensity ratings for prolonged noxious heat stimuli on the affected side (contralateral to the lesion), compared to the affected side and to controls. However, the patients’ tactile thresholds remained unchanged. Therefore, it seems that damage to the putamen causes decreased pain sensitivity, but not tactile thresholds, suggesting a role in the putamen in the sensory-discriminative dimension of pain.

The nucleus accumbens, integral to the reward circuit in the mammalian brain [388], is another region within the basal ganglia that has recently been implicated in pain processing. The concept of the reward pathway encoding aversive stimuli is reminiscent of the age-old dichotomy of pleasure and pain, and can be dated back to Platonic and Aristotelian ideas [199]. It has been reported that, in rats, the nucleus accumbens is pronociceptive, and that inhibiting the nucleus’ function with opioids induces antinociception [352].

It is tempting to conclude that the basal ganglia are involved in determining a behavioural response to pain - the motivational and nocifensive dimensions of pain. However, the substantial input of nociceptive information from different regions of the brain and the extensive connectivity of the basal ganglia suggest that these subcortical nuclei may have a more
prominent role in pain perception. For instance, it has been suggested that the basal ganglia may be the site of convergence of the different dimensions of pain (i.e., pain integration) [828]; however, more research is required to substantiate this theory. It is noteworthy that sensory convergence has been demonstrated in several nuclei within the basal ganglia [162,165].

Finally, there is evidence that changes to sensory input to the brain, including prolonged nociceptive input, can modulate M1 excitability and output. Specifically, when a peripheral nerve is transected in mice, neuroplasticity occurs in M1 [2,4,328,329]. However, it is possible that these changes in M1 reflect changes in S1 cortex, which has dense projections to M1 [41]. Furthermore, evidence in humans suggests that acute pain in the periphery can decrease M1 excitability [3,157,301,302,744] and acute intraoral pain can prevent learning a novel tongue-protrusion task [109].

In sum, motor regions in the brain receive nociceptive input, have nociresponsive neurones and, in some cases, are modulated by nociceptive input. The most obvious role for motor regions in the context of pain is to initiate a motor response to pain (nocifensive behaviour), however, evidence suggests that they may have a more prominent role in pain integration, and pain modulation.

2.3.5. Descending modulation

The perception of pain is modulated by several psychological factors, including attention, arousal and expectation, as well as the context in which the stimulus is presented [309]. This can be illustrated by two examples: athletes injured during a sporting event and a soldier wounded in combat. In both cases, the injured person often reports less pain than persons sustaining the same injury in another context [153]. This variability in the perception of pain has been associated to endogenous pain-modulatory systems, which are the neural basis by which attention, arousal, expectation and cognitive processes alter the perception of pain. It has been suggested that these networks modulate the transmission of nociceptive information to suppress or enhance nocifensive responses to increase the chance of survival [309]. In the example of a soldier in combat, suppressing pain reduces pain-related distraction, and facilitates escape from combat. Conversely, the pain-modulatory systems can enhance sensitivity to pain, and enhance nocifensive behaviour, which promote healing [309].
It has long been recognized that pain-modulatory mechanisms exist. In 1911, Head and Holmes [417] proposed that the thalamus was the site of pain perception, and corticothalamic connections modulated the nociceptive processing in the thalamus. Further, they suggested that such inhibitory mechanisms were present at lower levels in the sensory pathway. Evidence suggests that both sensory input and motor output can be modulated by supraspinal regions. For instance, in 1915, Sherrington and Sowton demonstrated that, in a decerebrated animal, spinal transection reduced stretch reflexes and enhanced flexor reflexes. In 1954, Hagbarth and Kerr [389] demonstrated that supraspinal mechanisms do influence afferent (sensory) input from the periphery to the spinal cord in the cat. Specifically, stimulation of M1, S1, S2, ACC, RF and ventral anterior part of the cerebellar vermis depressed the dorsal root reflex, suggesting modulatory effects of brain regions onto the spinal cord. It was also shown that cortical stimulation could inhibit cutaneous sensory input to the VBSNC [e.g., 421]. In 1964, Kuypers demonstrated that there are several tracts emerging from the brainstem that terminate onto the dorsal horn [507]. However, the Gate Control theory of pain [595] (See Section 2.1.1.4) was the first to propose that there were supraspinal regions that modulated nociceptive input from the periphery at the level of the spinal cord.

Animal studies have demonstrated that there are, indeed, specific nuclei in the brainstem that have a modulatory effect on dorsal horn nocireponsive neurones (for a comprehensive review, see: [420,521,580,726]. The first study to demonstrate this was by Reynolds [730], in 1969. In this study, electrical stimulation of the mesencephalic central gray matter in rats provided an analgesic effect. Specifically, during an invasive surgical procedure, a laparotomy, the rats did not show aversive behaviour, without paralysis. The potent analgesic effect of stimulating the brainstem, specifically the periaqueductal gray (PAG), has been confirmed in the rat, cat, non-human primate and in humans [580] [437]. This stimulation-produced analgesia is selective, in that animals remain alert and respond normally to environmental stimuli, with the exception of nociceptive stimuli – these responses are abolished [309]. Furthermore, if a noxious stimulus is presented outside the region of analgesia, the animal responds with typical pain-related responses [580]. Studies have demonstrated that stimulation-produced analgesia of the PAG is opiate-based. Specifically, studies have found that injecting morphine, and other opiates induces modulation [252,307,309,613]. Furthermore, naloxone, an opioid antagonist, abolishes the analgesic effect when injected into the PAG [5,873,959] or the third ventricle [958]. The
analgesic effect of the stimulation of the PAG is mediated through a descending pathway from the brainstem (via the rostral ventromedial medulla (RVM)) to the dorsal horn, and has been demonstrated to inhibit nociceptive reflexes (e.g., the tail flick in rats), and nociceptive neurones in the spinal and medullary dorsal horns [260,354,786,789].

It is noteworthy that descending pathways not only inhibit somatosensory input and pain transmission, but can also play a facilitatory role at the spinal level [353,613,725]. It is believed that this facilitatory role may be related to the chronification of pain [724].

### 2.3.5.1. Descending modulation pathways

There are several descending pathways that can modulate nociceptive input and pain transmission. In general, these pathways are modulated by brain regions through corticobulbar tracts and other tracts originating from subcortical nuclei that terminate in the brainstem [95,309,420]. The best characterized of these pathways involves a circuit connecting the PAG to nuclei within the RVM, including the nucleus raphe magnus (NRM), and the spinal dorsal horn, via the dorsolateral funiculus [58]. Another modulatory region is the dorsolateral pontomesencephalic tegmentum (DLPT), which connects to the cuneiform nucleus in the midbrain, and the locus coeruleus/subcoeruleus (LC/SC), and other noradrenergic nuclei.

The PAG receives input from the ascending fibres from spinal lamina I, via the spinomesencephalic tract [43,441,481,596] and the VBSNC [77,567,931], from the cerebral cortex, the hypothalamus and other brainstem nuclei, such as the RVM, including the NRM, and the nucleus cuneiformis [77]. There are direct projections from the PFC (with the exception of the medial OFC), ACC and the insula to the PAG [406,405,413]. There are also some corticobulbar connections to the PAG from M1 and the PMC (BA 4 and 6, respectively) [405,619]. Furthermore, the amygdala has dense projections to the PAG. The amygdala, in turn, is modulated by several cortical and subcortical regions. Furthermore, the PAG receives dense input from the hypothalamus, and stimulation and opioid injection to the hypothalamus have analgesic effects [433,566,731]. There are also cortical projections to the PAG via the anterior pretectum [158,960]. In addition to its descending projections, and the afferent input from brain regions to the PAG, the PAG has ascending efferents to MD thalamus and the OFC [135,168].
The RVM is another brainstem region essential for descending pain modulation. The RVM is a group of nuclei that are the primary relay site for projections from the PAG to the spinal (or medullary) dorsal horn [420]. The most important nucleus in the RVM in the context of pain modulation is the NRM. For instance, inhibiting NRM neurones abolishes the analgesic effect of PAG stimulation [10,71,355], whereas stimulation of the NRM produces analgesic effects by inhibiting nociceptive neurones in the spinal dorsal horn [253,308,469]. The RVM projects to the spinal dorsal horn via the DLF [58]. Therefore, the RVM is essential for the analgesic effect of the PAG to occur. Therefore, the PAG is an important antinociceptive region, which receives convergent input from ascending tracts, cortical regions and other brainstem regions. The PAG projects to the RVM, which directly modulates nociceptive neurones in the STN and the spinal dorsal horn.

Recent studies have also found that descending projections from the PAG to the spinal dorsal horn and Vc, via the RVM, can also be facilitatory, i.e., these projections have a pronociceptive effect, rather than an antinociceptive effect [654,835]. It has been suggested that these mechanisms may contribute to the chronification of pain [704].

In addition to the PAG-RVM descending pathway, a parallel pathway exists in the brainstem: the DLPT pathway [272,309,354]. The components of the DLPT include the nucleus cuneiformis, which is adjacent to the PAG and is similar in function to the PAG in terms of pain modulation, and pontine noradrenergic nuclear groups, including the LC/SC. It is believed that this pathway modulates deeper laminae in dorsal horn, as it has moderate projections to these laminae, compared to few projections to the superficial dorsal horn [166]. The LC/SC projects directly to the dorsal horn via the ventrolateral funiculus, and electrical stimulation of these nuclei has an inhibitory effect on nociceptive cells [726]. This pathway is separate from that of the PAG-RVM pathway because inhibition of the RVM does not disrupt the analgesic effects of the LC/SC [468].

2.3.5.2. Diffuse noxious inhibitory controls

It has long been known that a stimulus perceived as painful can diminish or mask a pain arising from elsewhere in the body. For instance, Hippocrates stated in his *Aphorisms*: “of two pains occurring together, not in the same part of the body, the stronger weakens the other” [424]. This effect has been described as counter-irritation analgesia, and only occurs when “distant” body
parts are affected, *i.e.*, extrasegmental body parts. In 1979, Le Bars and colleagues [520] proposed the DNIC hypothesis. This hypothesis states that nociceptive signals can be inhibited by nociceptive input from another body part, that projects to a different segment of the spinal cord. To test this hypothesis, the sural nerve RIII reflex was recorded in subjects who were presented with a painful “conditioning” stimulus (a hot water bath) [521]. Normally, when the sural nerve is electrically stimulated, a painful pinprick sensation is felt in the dermatome of the nerve and a knee flexor muscle nociceptive (RIII) reflex is evoked. It has been proposed that DNIC is a spinal cord/STN mediated process [251]. However, when a noxious stimulus was applied to the hand, the reflex and the perceived pain intensity of the sural nerve stimulation was decreased. DNIC has also been demonstrated in animal models. For example, in the anaesthetised rat, the C fibre reflex which is strongly inhibited by heterotopic noxious stimulation [521]. It is believed that DNIC is a spinally-mediated process [521], and WDR neurones, mainly in lamina V of the spinal cord, appear to be key for the effect of DNIC [519]. However, DNIC does not occur in animal models where the spinal cord is sectioned, or in tetraplegic human subjects [739]. This suggests that supraspinal regions contribute to DNIC. Specifically, there is evidence that the dorsal reticular nucleus in the caudal medulla contribute to DNIC [519,896]. This nucleus receives afferent nociceptive input from the spinal cord, as well as convergent input from PAG, RVM, RF, the hypothalamus, the amygdala, M1, S1, OFC, and the insula [309]. The dorsal reticular nucleus projects to the thalamus, suggesting a role in both nociceptive-signal transmission as well as antinociception [621]. Similarly to the descending modulation pathways, it has been shown that DNIC may also have a facilitatory role for nociceptive-signal transmission, but this is outside the scope of this thesis. For a review of these concepts, see [546,895]. DNIC has also been demonstrated in the orofacial region [917]. Furthermore, a remote noxious stimulus can affect jaw muscle activity [131,132,894].

In sum, there are both negative and positive feedback loops that are consistently modulating nociceptive-signal transmission to the brain. These loops are comprised of a network of brainstem nuclei that are modulated by cortical regions. This delicate balance of inhibition and facilitation can be very useful, based on the context of the nociceptive stimulus. Cortical regions can upregulate the inhibitory effects in order to block out excess or unwanted stimuli. Conversely, in a dangerous or threatening environment, when a subject is hypervigilant, the facilitatory mechanisms can be upregulated as a protective mechanism.
2.4. Structural brain imaging

With the advent of functional neuroimaging, there has been a large increase of studies investigating the function of the brain in health and disease. These studies have vastly improved our understanding of brain function, and its relationship to perception, cognition and behaviour. However, brain function is intimately related to brain structure, and so, studying neural structure can provide a fundamental framework for understanding brain function. Aristotle, often considered the father of comparative anatomy [145], described the basic tenet of physiology as the structure-function relationship [100]. For Aristotle, function is what is of importance, structure is secondary, and thus form and function are not separate disciplines, but are complementary [100]. In the context of the brain, functional MRI (fMRI) has provided a wealth of functional information, but the study of brain structure supporting brain function is only just burgeoning [145]. Scientists have used the structure-function relationship to understand normal function and abnormalities in disease. In a recent editorial, Catani [145] criticizes this approach as reverse logic. He argues that form begets function, or, in evolutionary terms, the form comes from the necessity for a function. Therefore, modification of form causes dysfunction. Only by studying “form” (or anatomy) can we understand function, and dysfunction.

MRI technologies can be used to examine both gray and white matter structure in humans. These techniques will be discussed below.

2.4.1. MRI

2.4.1.1. What is magnetic resonance?

A brief overview of the concepts related to MRI signal production, analysis and applications to structural brain imaging will now be presented. For a comprehensive review of the basic concepts of magnetic resonance (MR) and MRI, see [119,438] – the information reviewed below was derived from these sources.

The concept of MR is based on the interaction of an atomic nucleus that possesses a spin and an applied magnetic field ($B$). Nuclear spin angular momentum is an intrinsic property of an atom. Most atoms have a nuclear spin, meaning that they are spinning at a constant velocity around an axis. Nuclear spin angular momentum is a vector quantity, and it is conserved for different elements. The human body is primarily comprised of water and fat, which contain hydrogen
atoms and so the $^1$H nucleus is an ideal choice for MRI. The $^1$H nucleus is a single proton (termed the protium, and will be referred to as a proton herein), and has a spin of $\frac{1}{2}$, and is the most abundant hydrogen isotope (c.f. deuterium) for hydrogen. Also, its response to an applied magnetic field is one of the most robust observed in nature.

A proton has a magnetic moment, which is parallel to the axis of rotation. The orientation of the spin and the changes induced to it by a magnetic field provide the basis for the MR signal. However, in MR, it is the population, ensemble or collection of spins (e.g., in a tissue) that is key. The net magnetic moment of a population of spins (i.e., the vector sum of the proton moments) is zero, and so no net magnetization is observed in a tissue. However, when the tissue is placed into a magnetic field ($B_0$), the protons begin to precess about the magnetic field. The proton is slightly tilted away from the axis of $B_0$, but the axis of rotation is parallel to $B_0$. This precession is caused by the interaction of $B_0$ and the spinning proton. The rate of precession ($\omega_0$) is proportional to the strength of the magnetic field and can be summarized by the Larmor equation:

$$\omega_0 = \gamma B_0$$

where $\omega_0$ has units of Rad/s, $B_0$ has units in Tesla (T; 1T is roughly five orders of magnitude greater than the magnetic field of the Earth, and three orders of magnitude greater than that of a refrigerator magnet), and $\gamma$ is the gyromagnetic ratio, which is a constant for each nucleus. In the case of protons, $\gamma = 267.52 \times 10^6$ Rad/s/T ($\approx 42.577$ MHz/T).

Another feature of spins is that they have two possible states when they are in a magnetic field: “spin-up” and “spin-down”. These are the two quantum-allowed states, although they can make transitions between these two states, there are no intermediate states. These two states have different energies associated with them (potential energy). The spin-up configuration is associated with a greater potential energy than the spin-down configuration. Transitions between the spin states require a specific energy, produced by molecular motion and collision. The energy required for the transition to occur must be quantized – they must have the correct amplitude to induce the “flip”. In an ideal system and at rest, there are an equal number of spin-up and spin-down positions, and the net magnetization is zero. However, when the ensemble is placed in an external magnetic field, the spins return to their lower energy level configuration due to interactions with surroundings. The spin-down configuration is of lower energy, and is parallel to
$B_0$, whereas the spin-up configuration is of higher energy, and is anti-parallel to $B_0$. This effect is called the Zeeman effect. Therefore, a small excess of the spins will try to flip to the lower energy state. This process requires time because collisions are required to produce the quanta of energy for the flip. Over time, the ensemble will reach an equilibrium state with the applied magnetic field, and the transition probability of the spins becomes balanced because a small excess of the spins have flipped to the lower energy state. This number of protons at each energy level is governed by the Boltzmann distribution:

$$\frac{N_{\text{up}}}{N_{\text{down}}} = e^{\Delta E/kT}$$

where $N_{\text{up}}$ is the number of protons in the spin-up configuration, $N_{\text{down}}$ is the number of protons in the spin-down configuration, and $k$ is the Boltzmann constant, which is $1.381 \times 10^{-23}$ J/K.

When a vector sum of spins in the transverse plane (perpendicular to $B_0$) is performed, the net magnetization is zero because of the random distribution of the position of the perpendicular component of the protons’ precession. However, along the longitudinal axis (parallel to $B_0$), there is a net magnetization parallel to the field. This means that the tissue is polarized and magnetized when placed in a field $B_0$, and the level of net magnetization is $M$, which is parallel to $B_0$. This induced magnetization, $M$, is the basis of the MR signal.

A rotating frame is defined as a Cartesian plane rotating about an axis, which remains stationary. If we assume that $B_0$ is parallel to the $z$-axis, and that the $xy$-plane is rotating at the Larmor frequency with the proton, then we observe what looks like a stationary spin. If we sum these vectors, the resultant vector resembles a vertical magnetization. A time-varying magnetic field creates an electric field. The created electrical signal is what we measure as the MR signal.

However, in the summated vector of $M$ is not time-varying – recall that the transverse vectors cancel each other, and the longitudinal vectors are stationary. To induce a time-varying magnetic field, a new magnetic field ($B_1$) is introduced at an axis perpendicular to $B_0$ and $M$. $B_1$ rotates at the Larmor rate. $M$ will rotate about the axis of $B_1$ (and therefore perpendicular to $B_1$,) at a given frequency ($\omega_1 = \gamma B_1$). We can control the angle of rotation by setting the duration and amplitude of $B_1$. This rotation of the spins produces a time-varying magnetic field. For instance, if we rotate the spins 90°, then the spins are now precessing about $B_0$ in the transverse plane. Given that a rotating magnetic field induces a voltage in a loop of wire, if a loop of wire is placed around the ensemble of the rotating spins, and attached to an amplifier, signal within the frequency range of
radio signals (3 kHz to 300 GHz) can be detected. The recorded signal is the summated magnetization of the difference of the up and down spins of the entire population.

2.4.1.2. MR signals

The second magnetic field that is introduced to rotate $M$ is a short pulse of radiofrequency (rf), also called an excitation pulse. The excitation pulse is comprised of several frequencies within a narrow range, or bandwidth. During the pulse, the protons in the sample absorb the energy of a given frequency ($\omega_0$), and after a given time, depending on the T1, reemit this energy at the same frequency. When the pulse is turned off, the protons begin to realign themselves to their stable state, and they reemit the energy they absorbed in order to realign. As more protons undergo relaxation and reemit their absorbed energy, the transverse magnetization of the protons creating the signal is lost, and the signal decays.

Relaxation is a fundamental feature of MR, and relaxation time is measured for an ensemble, rather than individual protons. For example, relaxation times for cerebrospinal fluid (CSF), gray matter and white matter within the brain are different, and we can measure the relaxation time for the entire tissue, rather than individual water, protein or fat cells. There are two relaxation times that we can measure: T1 and T2.

Magnetization time (T1)

After the spins have been rotated, symbolized by $M$, the protons have a natural tendency to return to their stable state – relaxation. The relaxation time “T1” represents the time required for $M$ to return to 63% of its original value ($M_0$) before the rf pulse, in the z-axis (along $B_0$). This relaxation follows an exponential recovery function:

$$M(t) = M_0(1 - e^{-t/T1})$$

where TR is the time between the rf pulse (repetition time), and T1 is a constant and is specific to the tissue. T1 represents the time it takes for tissues to relax after magnetization. This relaxation is named a “spin lattice” relaxation. The motion of the surrounding spins provides a fluctuating magnetic field that enables spins to be flipped back to their stable state.

The mobility of atoms varies based on the size of the molecule it is part of, and its interactions with the immediate environment. The consequence of this is that different tissues magnetize at
different rates. For instance, smaller molecules, such as water (the main component of CSF), have a very long T1 (up to several seconds), whereas larger molecules, such as fat (which is the main component of myelin, and thus white matter), have a very short T1 (less than a second). Grey matter has an intermediate T1, between that of CSF and white matter. These differences in T1 provide a useful contrast mechanism in MRI.

**Signal decay time (T2)**

The relaxation time “T2” refers to the time required for the transverse component of $M$ to return to 37% of its initial value. When $M$ is rotated into the xy-plane, the spins of the protons are in phase. However, over time, the signal decays because the spins begin to dephase. This loss of coherence occurs because of molecular interactions with the protons, and because of the transfer of energy from one spin to another. The signal decay can be summarized by the equation:

$$S(t) = M_{xy}(t)$$

$$= M_0 e^{-TE/T2}$$

Where “TE” is time from excitation (or echo time), and is an adjustable parameter that represents the time after the rf pulse during which the signal data are collected. T2 is also tissue specific, and is slower in small molecules (the signal from CSF, which is mostly water, decays over several seconds), and faster in larger molecules (e.g., gray matter and white matter, where the signal decays in less than a hundred milliseconds). Again, differences in T2 allow for a contrast useful to MRI. Further, by increasing TE, the contrast between tissues can be increased but less signal is actually received as it decays.

**Spatial Encoding**

The creation of an image requires a process that encodes signals in tissues in 3D space. The Larmor relation states that the signal’s frequency is related to the magnetic field affecting the protons. For instance, if we maintain a homogenous magnetic field, $B_0$, in the bore of an MRI, there would be a single frequency present in the MR signal. However, if we vary the field with a gradient coil, then frequency encodes location. To do so, we need to have different frequencies representing different points in space. Therefore, we introduce a gradient pulse that distorts the magnetic field, and produces a gradient in the magnetic field. The effect is that at one end of the
gradient there is slightly less magnetic field strength \((B < B_0)\), in the middle there is still a net magnetic field strength, which is equal to \(B_0\) \((B = B_0)\), and at the other end there is a slightly higher magnetic field strength \((B > B_0)\). In a simplified model, this will affect the frequency of the nuclear spins – they are precessing at a slower rate at the lower end, and precessing faster at the higher end, compared to the middle, where \(B = B_0\). This is done in three orthogonal planes to localize signals in all three dimensions. The relationship between frequency and \(B\), allows us to encode spatial position as a function of precession frequency.

**Contrast**

Given that the relaxation times, T1 and T2, are specific to tissues, and that there are parameters that are specific to each relaxation time’s contrast (TR and TE, respectively), we can vary these parameters to adjust the contrast in MR images. The signal we obtain be summarized by:

\[
S = k\rho M_0 \left(1 - e^{-\frac{TR}{T1}}\right) e^{-\frac{TE}{T2}}
\]

where \(\rho\) is proton density, \(k\) is an instrument specific calibration factor.

We can experimentally adjust TR, which affects the T1 contrast. Alternatively, we can adjust TE, which affects the T2 contrast. If we set low values for the TR and the TE, then the contrast of our image will be T1-weighted. However, if we have a long TR and TE, the image will be T2-weighted. Water molecules that interact with small molecules have relatively low signal in a T1-weighted image, and relatively high signal in a T2-weighted image. Conversely, water molecules that interact with larger molecules have relatively high signal in a T1-weighted scan, and a relatively low signal in a T2-weighted scan. Therefore, in a T1-weighted scan white matter is bright, and CSF is dark. The opposite occurs in a T2-weighted scan: white matter is dark and CSF is bright.

In summary, rf pulses convert nuclear magnetization to signal. Rf pulses add energy by displacing longitudinal equilibrium. The contribution of intrinsic tissue properties, T1 and T2, can be manipulated by adjusting the timing of TR and TE, respectively.
2.4.2. Gray matter imaging

Structural studies of the human brain used to be limited to post-mortem investigation and sometimes biopsies (e.g., [844]). However, for the past few decades, it has been possible to use MRI to examine brain structure in living humans non-invasively. Structural MRI can be used to measure many structural features including the thickness of the cortical mantle, the gyrification index, and other measures of interest, at a very high spatial resolution: the millimeter scale. As newer technologies emerge, even greater spatial resolution can be achieved, but these are outside the scope of this thesis (for a review, see: [282]).

Structural MRI analysis of gray matter is commonly performed on T1-weighted images, as these acquisitions can be optimized to increase the contrast between gray matter and white matter. In a T1-weighted image, the CSF is dark (almost black, due to the long T1 relaxation time), the white matter is bright (due to the myelin sheath surrounding axons) and the intensity of gray matter is between that of CSF and white matter. Many automated and semi-automated techniques have been developed to characterize gray matter differences within a population and between populations. These measures include the shape of gray matter structures, gray matter density (the relative amount of gray matter within a voxel) and cortical thickness. Brain imaging software packages typically include toolboxes that use algorithms to segment the three tissue classes in the brain (gray matter, white matter and CSF), and perform statistical analyses. Currently, the two most common methods are voxel-based morphometry (VBM) and cortical thickness analysis (CTA). Specific information about the methods and the relative advantages and limitations of each are discussed below.

2.4.2.1. Voxel-based morphometry

One of the first automated toolboxes to analyze gray matter was Christian Gaser’s VBM toolbox, implemented in the Statistical Parametric Mapping (SPM). A VBM analysis of brains from a group of subjects includes the following stages: 1) segment high-resolution brain images into gray matter, white matter and CSF; 2) spatially normalize the brains to a common standard stereotactic space; 3) apply a spatial filter to “smooth” the gray matter segments (this effectively blurs the gray matter to improve alignment) [36,37]; and 4) perform voxelwise parametric or non-parametric statistics. Using either statistical method, the results are corrected for multiple comparisons. More recently, Jacobian modulation has been added to the processing pipeline
prior to the smoothing step so that findings can be considered as volumes, rather than relative gray matter density [36,371]. To do so, for each brain the software calculates the deformation for normalization to the standard space (stereotactic) template – a Jacobian determinant, and then modulates each voxel in standard space to account for the expansion that occurs. VBM is a widely used tool in neuroimaging, and its popularity can be attributed to its relative ease of use, and the little processing power required. However, this technique has been criticized because it is sensitive to misalignment, especially in the cortex [104,210]. Therefore, results in cortical regions should be interpreted with caution. Nonetheless, the alignment of subcortical gray matter regions is robust, and thus more reliable.

2.4.2.2. Cortical thickness

It has been suggested that cortical thickness (rather than gray matter volume) may provide a better measure to examine changes or variability of the cortical mantle and so a technique called CTA was developed. CTA measures the thickness of the cortex in millimeters, which is a well-defined physical measure. However, CTA is limited to the cortex, as implied by its name, and thus we must use it in conjunction with VBM to get a full picture of gray matter in the brain. Nonetheless, CTA is purportedly superior to VBM because of improved alignment of cortical gray matter, especially gyral folding patterns. This method inflates the cortical gray matter sheet into a sphere, and aligns the gyri and sulci based on landmarks. Several software packages offer a CTA toolbox, and the most common one is FreeSurfer. The analysis pipeline relies on some of the basic principles of VBM, but with a few differences. First, high-resolution T1-weighted brain images are aligned to a different standard stereotactic space than the one used in VBM, which uses the Montreal Neurological Institute’s standard template of 152 brains (MNI152). FreeSurfer uses the Talairach and Tournoux [844] brain as a template for normalization. Then, the brains undergo intensity normalization to remove coil-related inhomogeneities. The image is then skull-stripped, and the subcortical structures are identified and labeled, which aids in the segmentation of white matter from gray matter. The pial surface is then defined, and the hemispheres are separated. The software then measures the distance at every point between the edge of the white matter surface and the edge of the pial surface, which represents the thickness of the cortex. This cortical sheet is then parcellated and the individual gyri and sulci are identified and labeled using an atlas that is based on manual parcellations [243,318,321]. Parametric univariate statistics can be used to perform group-level analyses on cortical thickness. To do so, the brains must first be
aligned to one another. This is done by inflating the cortical surface into a sphere, and iterative alignment algorithms, which use landmarks and the individual parcellation to align specific sulci and gyri [319,320]. These steps allow for better alignment of the cortex, and thus overcome the limitations of VBM.

2.4.3. White matter imaging

DWI with MRI can be used to non-invasively evaluate human white matter. The study of white matter paths is called brain hodology, and comes from the Greek *hodos*, which means path. Brain hodology in humans has mainly been on post-mortem studies of white matter, which date back to antiquity. Notably, in the seventeenth century, Steno stated:

"To say that the white matter is but a uniform substance like wax in which there is no hidden contrivance, would be too low an opinion of nature’s finest masterpiece. We are assured that wherever in the body there are fibres, they everywhere adopt a certain arrangement among themselves, created more or less according to the function for which they are intended [translated by 760,830]."

Neuroscientists in the nineteenth century then attempted to elucidate structure-function relationship [145,306]. However, these scientists recognized that local structure-function relationships could not fully explain dysfunction in the brain; they understood that distant brain regions interacted to produce behaviour. This is exemplified by Wernicke’s statement that sensory and motor regions must work together through connections (*i.e.*, white matter tracts) in the brain [145]. Déjérine was a French neurologist, medical historian, and neuroanatomist, whose investigation of white matter in the brain is the most comprehensive of the time – and some would argue until today [607]. He agreed with Wernicke, proposing that the key to understanding brain function relied on understanding the connections of association fibres within the brain [145,237]. More recently, the concept of studying the anatomic basis of behaviour, and functional relevance in anatomic circuits has reemerged [147,760], notably by Geschwind [359,360] and Kuypers (*e.g.*, [387,663]). Geschwind proposed that “brain function is the result of effective communication between structures geographically distributed around the nervous system”[761]. Recently, Mesulam [607] suggested that the function of a neurone is defined by its connections, and that understanding the connection pattern of the cortex is the only way to understand how the brain functions. Therefore, in addition to functional and gray matter studies of the brain, elucidating the connectional anatomy, and the integrity of these tracts in the brain
will provide essential insight into the connections (or disconnections) of patients with chronic pain. The advents of DWI and diffusion tensor imaging (DTI) have provided a method to essentially perform in vivo dissections of the white matter in humans [60], and to elucidate abnormal white matter structure related to clinical correlates in disorders, such as chronic pain. Furthermore, of particular relevance to this thesis, is that DTI (see below) can also be used to examine peripheral nerves [429], such as the CNV in trigeminal neuralgia [525] (discussed below and in Chapter 7).

2.4.3.1. Diffusion tensor imaging

DTI is a mathematical model that is fit to DWI data. DWI is a brain imaging technique that can be used to visualize white matter. Specifically, DWI is an MR modality that is sensitive to the diffusion of water molecules [63]. The following description of DWI and DTI are based on information from: [52,60,62,63,67,522,524,626,660]. Diffusion is the process of molecules moving, and is the result of Brownian motion. All molecules, when suspended in a liquid or gas, show random translation movements (i.e., Brownian motion), resulting from thermal energy of the molecules. The diffusion of water molecules can be summarized by Einstein’s equation:

\[ X^2 = 2DT_d \]

where \( X^2 \) is the average mean-squared diffusion distance (in m\(^2\)) along one direction, \( T_d \) is the diffusion time (in s), and \( D \) (in m\(^2\)/s) is the diffusion coefficient. DWI’s ability to discern microstructural features of a tissue is based on the notion that water molecules sample the microscopic environment at a very high resolution (much higher than the average MRI resolution), roughly 10 µm per 50 ms [524]. This sampling includes collisions, interactions and crossing of water molecules with cell membranes, fibres and other macrostructural features, such as macromolecules. A DWI provides a summary of the bulk motion of a diffusion molecule within a voxel, which can reveal structural and geometric organization of a tissue [60,523]. The molecule of choice in human DWI is the water molecule, because 90% of the protons in the human body are located in water molecules. Diffusion occurs in all three dimensions of space, and when unhindered, will diffuse equally in all directions (the expected vector-sum of displacement is zero), a process known as isotropic diffusion. When diffusion is hindered, e.g., by a tissue, we observe anisotropic diffusion. Anisotropic diffusion of water has been observed in muscle, spinal cord and cerebral white matter [524]. Einstein’s equation does not apply to
anisotropic diffusion, and we have to use a symmetric tensor, \( D \), to describe diffusion in this scenario:

\[
D = \begin{bmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{xy} & D_{yy} & D_{yz} \\
D_{xz} & D_{yz} & D_{zz}
\end{bmatrix}
\]

It is noteworthy that in the scenario of anisotropic diffusion, we assume that diffusion is Gaussian in all directions.

Anisotropic diffusion in brain white matter has generated much interest in understanding the connectional anatomy of the brain. Specifically, water molecules within or between white matter tracts have anisotropic diffusion, as the bulk diffusion is parallel to the main axis of the fibre tract. This anisotropy comes from the geometrical structure of the axon – it is tubular in shape, and many axons form a fibre tract. Specifically the diffusion of water is restricted by cellular barriers, such as myelin, cell membranes, and macromolecules (such as microtubules and neurofilaments), and the molecules tend to diffuse along the primary axis of the tract, whereas diffusion perpendicular to this axis is impeded. MRI acquisition parameters can be adjusted to acquire images that provide information about the diffusion of water, and thus, the connectivity, integrity and orientation of white matter in the brain (see below). This thesis will briefly review the basic principles of DWI signal generation and image acquisition (for a comprehensive review, see: [522,524,626]).

MRI can be used to acquire several different types of image contrasts (T1, T2; see section 2.4.1.2). As described above, the MR signal equation can be summarized as:

\[
S = k\rho M_0 (1-e^{-TR/T1}) e^{TE/T2}
\]

where \( S \) is the acquired signal, TR is the repetition time, TE is the echo time, \( \rho \) is proton density and T1 and T2 represent relaxation times specific to tissues. This equation can be modified to include a diffusion term, bD:

\[
S = k\rho M_0 (1-e^{-TR/T1}) e^{TE/T2} e^{-bD}
\]
where the b, or b-factor, summarizes all of the gradient effects, and D is the diffusion tensor. To measure the diffusion of water molecules, we must acquire two different b-values. Therefore, for $S_b$ we obtain:

$$S_b = k\rho M_0 (1 - e^{-\frac{TR}{T1}}) e^{-\frac{TE}{T2}} e^{-bD}$$

and for $S_{b0}$, we obtain:

$$S_{a} = k\rho M_{a} (1 - e^{-\frac{TR}{T1}}) e^{-\frac{TE}{T2}} e^{-b_{0}D}$$

We can then solve the equation for $D$:

$$D = \frac{-\ln\left(\frac{S_b}{S_{b0}}\right)}{b - b_{0}}$$

where $S_b$ is a signal acquired from the scanner with a given value for b and $S_{b0}$ is the signal acquired with a b-factor equal to zero. The signal acquired from b = 0 is not sensitive to diffusion, and is referred to as a non-diffusion-weighted image. All of the other terms in the signal equation are kept constant, and so cancel out.

The signal (S) that we acquire is generated. To do so, we apply a phase encoding gradient pulse along a particular direction, using different combinations of the x, y, z gradients. After the rf pulse is applied (for a given duration), the protons which are precessing about the z-axis begin to dephase. We then apply a second re-focusing pulse, which is the same as the first pulse, but in the opposite gradient direction. This paired-pulse sequence sensitizes the signal to diffusion. Specifically, the refocusing pulse can only rephase the protons if they have not moved. If the water molecules diffuse (i.e., change location between the first and second rf pulse), then this motion will disrupt the phase-gradient. This imperfect rephasing is measured as signal loss. As previously mentioned, by using two different b-values we can acquire two different signals, which allows us to solve for D. The b-value is related to $\gamma$, the strength of the gradient pulse (G_i), the length of time we apply the gradient ($\delta$), and the time between the application of gradients $\Delta$). These relationships can be summarized by the following equation:
which can be simplified to:

\[ b = \gamma^2 \int_0^\infty \left[ \int_0^t G(t') dt' \right]^2 dt \]

This equation demonstrates that the simplest way to modify the b-value is by manipulating the time between the two gradient pulses.

In a DTI experiment, a symmetric b-factor tensor (b-matrices) is calculated for each DWI acquired with a different b-value (b ≠ 0 and b = 0). Usually the b ≠ 0 is acquired at a b-value equal to 1000 s/mm². We then solve for D and the result is all 6 components of tensor model, which can be summarized in an image of the apparent diffusion coefficient (ADC). Therefore, the signal ratio equation becomes:

\[ \ln \left( \frac{S_b}{S_{b0}} \right) = -b(ADC) \]

The intensity of a voxel in the ADC image represents the direction of the bulk diffusion of water molecules with regard to the direction of the applied gradient. In the ADC image, the brighter the voxel intensity, the more diffusion has occurred. However, complex features in microstructure, such as crossing fibres, can produce erroneous measures of diffusion. Thus, to properly determine the principal diffusion direction within a voxel, an enormous number of directions should be sampled. However, this is not a feasible approach, as MRI scanning is costly, the hardware is limited, and the subject cannot undergo such long scan durations. Therefore, to overcome these limitations, a tensor model is used to calculate the bulk direction of water diffusion within a voxel. In a tensor model, diffusion only needs to be acquired along six directions; however, the more directions acquired, the better the signal-to-noise ratio of the image, which will improve the precision of the DTI estimation, and hence the angular resolution. Some imaging studies acquire up to 120 directions, although 60 directions has now become standard practice. The tensor (D) is a positive, definite tensor, and describes a three-dimensional ellipsoid in displacement space, called the diffusion-ellipsoid. The three diagonal terms of the tensor model (Tx, Dy and Dz) represent the three axes of the ellipsoid. These relative
diffusivities of water along these axes can be expressed by three eigenvalues \((\lambda_1, \lambda_2, \lambda_3)\), and their orientation is represented by three corresponding eigenvectors \((v_1, v_2, v_3)\) (see Figure 2-4). For a comprehensive review of the tensor model, see: [60,61]. These values allow us to calculate several useful measures (or contrasts), such as mean diffusivity \((MD)\), fractional anisotropy \((FA)\), and radial diffusivity \((RD)\) [62,494,524,626] (for the biological basis of these, see 2.6.4), and their respective equations are presented in Figure 2.4.

\[
\begin{align*}
\text{MD} &= (\lambda_1 + \lambda_2 + \lambda_3)/3 \\
\text{FA} &= (3/2)^{1/2} \times \{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2\}^{1/2}/(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)^{1/2}
\end{align*}
\]

\[
\text{RD} = (\lambda_2 + \lambda_3)/2
\]

\textbf{Figure 2-4}: Schematic of diffusion tensor imaging, and summary of eigenvectors and metrics. Lambda \((\lambda)\) represents each of the eigenvalues that make up the tensor model.

In DTI analysis, FA is the most commonly used measure, as it is scaled between 0 and 1, where 0 represents complete isotropic diffusion, and 1 represents complete anisotropic diffusion. Assuming that these measures represent white matter integrity along a tract (see below), then we can evaluate white matter integrity within a population, or compare between populations (see next section). By combining FA as a threshold and the primary eigenvector, \(v_1\), then we can determine the orientation of white matter tracts, at each voxel.

Despite the great advances that DTI has afforded us, there are a number of inherent limitations and challenges that must be overcome in data acquisition and analysis. For instance, as DTI
measures the relative displacement of water molecules, it is very sensitive to head motion and physiological motion. To minimize head motion, the subject’s head is padded, and to minimize physiological motion, some investigators measure respiration rate and cardiac rhythm during the scan, and control for these during analysis. Furthermore, to correct for relatively small amounts of motion during a scan, some scanners collect several non-diffusion-weighted images at several points during the acquisition, interspersed between the diffusion-encoding runs (usually at a ratio of 5:1 for diffusion-encoding directions: non-diffusion-weighted images). These non-diffusion encoding directions are aligned to one another, and the transformation matrix is applied to the DWIs between these images. Other motion correction algorithms also exist (for a review of these, and other denoising methods, see: [694]). Another source of noise is the production of eddy-currents artifacts, which occur due to residual gradient effects and the rapid switching of gradient pulses. Most motion-correction toolboxes also correct for these artifacts.

There are some notable limitations with the diffusion tensor model. For instance, some voxels may appear to have low FA values despite their location in white matter. We would expect voxels in gray matter and CSF to have low FA, as there are few barriers and little geometric organization that restrict the diffusion of water. Regions of complex white matter organization, such as crossing fibres or fibre dispersion, cause these relative hypointensities of FA in white matter [14]. Another limitation of DTI is that it cannot resolve the direction of the fibre, but only its orientation in three-dimensional space [14]. Therefore, one cannot make any directional interpretations about whether a tract is connecting to or coming from a gray matter region.

2.4.3.2. Between Group Comparisons of White Matter: Tract-based spatial statistics

One of the major challenges in applying DTI to study clinical populations has been the development of an approach to compare white matter metrics between subjects, given the intersubject variability, but now, several approaches have been developed. For instance, some groups use an average value (or summary measure) of FA, MD and RD across the whole brain [112,152]. Yet, summary measures of white matter integrity are not very informative, as there is no information about the location of the white matter abnormality. This method is useful when investigating a diffuse disease, such as multiple sclerosis [722]. Other groups have used a VBM-style approach (see Section 2.4.1.2) to delineate regions of white matter abnormality between subject cohorts, or to identify regions where white matter correlates with a behavioural measure
within a group of subjects [36,37]. However, VBM-style analyses have been heavily criticized for their lack of reproducibility (due to the parameters that can be selected) and its lack of accuracy (due to misalignment) [151,210,813]. In the former case, one example is smoothing. Two studies have shown that results vary largely based on the selection of the size of the smoothing kernel [466,667]. Another limitation of VBM-style white matter analysis arises when aligning white matter tracts for comparison between subjects. To obtain a reliable measure, it is essential to compare the same tracts between subjects, and to compare the same part of the tract (as FA is not the same at the edge of a tract, compared to the centre of the tract), the so-called correspondence issue [813,815]. These issues have been encountered in several studies (e.g., [80,804,822]. Another white matter analysis approach has been to confine the analysis to specific white matter regions-of-interest (ROI). ROI analyses of white matter can be beneficial, in that they are very sensitive to changes in measures of white matter integrity, so long as the ROI is specific. Furthermore, this approach is not very computationally intensive. However, delineation of an ROI requires expert knowledge of the anatomy, in order to identify appropriate landmarks to draw reliable and reproducible ROI [151].

To address some of the issues of these white matter analysis methods, Smith and colleagues developed tract-based spatial statistics (TBSS), a toolbox implemented in the Oxford Centre for Functional MRI of the Brain’s (FMRIB) software library (FSL) [813,815,818]. TBSS is a fully-automated program that has a similar pipeline to that of VBM, but it has been optimized for white matter analysis. TBSS uses FA to align subjects to a common template using a non-linear algorithm. A standard FA template (FMRIB58_FA) with 58 subjects in the MNI152 standard space was developed for this purpose. Alternatively, FSL allows the creation of a study-specific template for populations where a standard-space template is not suitable, such as a pediatric population, or in the case of severe pathology. TBSS then calculates a group mean FA map, and “thins” this map to create a skeletonized mean FA image (see Figure 2-5). This skeletonized FA map represents the centre of the white matter tracts common to all subjects. The highest FA value of each subject’s tract, deemed to be the centre of the tract, is projected onto the skeleton at every voxel, in order to overcome any residual misalignments. Voxelwise univariate statistical tests can then be performed on the skeletonized FA map. Furthermore, once alignment is achieved, other indices of white matter integrity, or shape can be projected onto the skeleton for analysis. Therefore, TBSS effectively addresses the limitations of the other methods described.
However, restricting the analysis to the peak FA value may reduce sensitivity to changes outside the centre of the tract.

![White matter skeleton from tract-based spatial statistics. The skeleton is shown in green, and is overlaid onto the MNI152 standard brain in FSL.](image)

**Figure 2-5:** White matter skeleton from tract-based spatial statistics. The skeleton is shown in green, and is overlaid onto the MNI152 standard brain in FSL.

### 2.4.3.3. Tractography

Another method to evaluate white matter in the brain with DTI is tractography. In general, this method allows us to perform *in vivo* “dissections” of white matter tracts (*i.e.*, visualize the tract), and to delineate the anatomical connectivity of the brain. The concept of tractography is based on the signal information collected in a DWI scan and the tensor model utilized in DTI. Briefly, when we apply the tensor model to a DWI data set, we obtain eigenvalues and eigenvectors for each voxel in the image, which represent the orientation and magnitude of water diffusion within the voxel. When diffusion is restricted, by a biological tissue for instance, then the diffusion is anisotropic. We can use the orientation information within each voxel to reconstruct white matter pathways in the brain, called tractography. The fundamental assumption of white matter tractography is that the primary eigenvector ($v_1$) is parallel to the primary axis of the white matter tract. It is, however, noteworthy that this assumption is often violated in regions of complex white matter (*e.g.*, crossing fibres and fibre dispersions). More complex algorithms have been developed to address these issues, and to increase the sensitivity and reliability of DTI. Two of the most commonly used methods, deterministic and probabilistic tractography, are described in further detail below. Although there are other, more complex methods being developed, these are outside the scope of this thesis.
Streamline (deterministic) tractography

Deterministic tractography algorithms use the orientation information within voxels to track the path of white matter tracts [14]. The resultant image is a tractogram, which is based on the primary (or major) eigenvector within each voxel. The tractogram is initiated from a seed location (a single voxel, or a group of voxels of interest). Because the algorithms use a threshold for FA to distinguish between gray and white matter, the seed is usually located within the white matter [14]. If the seed were in the gray matter, or in a voxel with FA below the threshold, then tracts could not be generated. Several restraints can be placed on the algorithm to remove spurious connections, and to ensure that the tracts are consistent with the predicted pattern of connectivity [14].

There are several deterministic tractography algorithms that can produce tractograms. Most of these methods utilize a streamline approach. This approach calculates the stepwise progression of the tract (or the path), beginning from a seed. The progression of the path is based on the direction of the primary eigenvector. The termination of a tract is based on an arbitrarily selected parameter [465]. For instance, an FA value is selected where the primary eigenvalue is well defined, e.g., FA \( \geq 0.15 \). Another parameter used to terminate a tract is the curvature angle, which is also arbitrarily chosen. The specific aspects of deterministic algorithms are outside the scope of this thesis, but for a detailed review of these methods see: [14].

There are both advantages and disadvantages to deterministic tractography. The most significant advantage is that fewer diffusion-encoding directions are required to produce tractograms, compared to other algorithms, such as probabilistic tractography, or diffusion spectrum imaging (see below). Furthermore, the analysis is not as computationally intensive as some of the other methods (including probabilistic tractography). However, deterministic tractography can only be used as a qualitative means of visualizing tracts in the brain, although the images can serve as masks to perform quantitative analysis, e.g., evaluate measures of white matter microstructure, such as FA. The images produced with deterministic tractography are visually appealing, but this can instill a false sense that the visualized tracts are accurate. However, there are many potential sources of errors in deterministic tractography. As previously discussed, DTI is very sensitive to motion and other sources of noise. Even small perturbations in the image can lead to tracking spurious connections. The largest source of error in tractography is tract dispersion, or the
variance of trajectories, which increases from the seed because there are an increasing number of potential paths [14]. Another source of error comes from tract deviation, or errors in tract position. These are related to the step size, especially in regions where the curvature of the tract is high. In general, a larger step size will increase the chance of error [518]. Another source of error in deterministic tractography is that of crossing fibres, as discussed above. Crossing fibres can lead to false-negatives in the tractograms [145]. For instance, most deterministic algorithms are not able to resolve tracts within the CST emerging from the lateral motor cortex and projecting to the pyramids in the brainstem [14]. This is the result of the crossing fibres in the centrum semiovale, which contains association fibres, callosal fibres and short U-shaped fibres, which project in different directions. As a result of the multiple fibre directions in voxels within this region, the primary eigenvalue is diminished, and the tractography algorithms fail to track the rest of the tract. Therefore, experiments that seek to resolve tracts using deterministic algorithms should have strong hypotheses and predictions based on known anatomical pathways, from animal studies and human post-mortem studies.

**Probabilistic tractography**

Probabilistic (or stochastic) tractography is a method that attempts to address some of the limitations of deterministic tractography. Specifically, probabilistic tractography acknowledges that there are inherent errors that occur in tract-tracing algorithms, and accounts for this variability. Therefore, the inherent variability in the data is included in probability estimates of a tract [668]. To do so, algorithms use probability density functions (PDF), which considers the expected distribution of the possible fibre orientations, at the level of the voxel [671]. The PDF are used instead of discrete measures of fibre orientation. An advantage of probabilistic tractography, compared to deterministic tractography, is that the PDF allow tractography to continue in a region where deterministic tractography would normally stop. Another advantage of probabilistic tractography is that we can quantitatively compare the connectivity of tracts based on the probability of connections.

The PDF is comprised of three orientation density functions (ODFs): the diffusion ODF (dODF), the fibre ODF (fODF) and the uncertainty ODF (uODF) [72]. The dODF and the fODF are biophysical properties of the tissue that is being measured. The fODF describes the proportion of the fibres in each direction. For instance, as in the example above, we have two orthogonal fibres
in a voxel, the resultant fODF will appear as a cross, in the direction of the fibres [862]. The fODF is useful in that it contains a proportion, which can be used to estimate the connectivity of a tract connecting A to B, providing a quantitative method that is both useful and biophysically meaningful [72]. However, DWI measures the diffusion of water within each voxel in the brain, and not the proportion of fibres, and so the measure we obtain is dODF, a measure of the orientation of diffusion within a voxel [879]. If water molecules diffused exactly along the axis of a tract, then the dODF would be exactly equal to the fODF. However, in reality, water molecules diffuse primarily along the axis of the tract, but there is also diffusion in other directions. Therefore, the dODF is necessarily broader than the fODF. Whereas the dODF and fODF are properties of the tissue which can be measured, the uODF is a measure of the error that can be predicted, or as Behrens puts it: “it [uODF] is a function that describes our belief” [72]. Specifically, the uODF represents the uncertainty that is included in the data because of the noise inherent to it.

These variables have very important implications in tractography. It can no longer be assumed that the primary eigenvector is the path of the tract, as there are an infinite number of paths within each voxel, each associated to a probability of being the correct orientation. To calculate the probability of connection of two regions, every possible path and its probability must be considered, and then the probabilities associated with each path connecting the two regions is summed [75]. Rather than attempt to solve this equation, more reasonable approaches have been developed that allow us to sample the probability distributions [75,392,465,669-671]. Briefly, these algorithms sample many paths from a seed at sub-voxel to sub-voxel steps (or for lower resolution, or larger step size, at a voxel to voxel steps). Each step considers the distribution of possibilities, resulting in many possible paths, each with a probability. These results are summarized within the voxel as a proportion of the number of samples that pass through the voxel [465]. Whereas deterministic algorithms rely on arbitrary FA values or curvature angles to stop tractography, probabilistic algorithms do not rely on these parameters for termination. In contrast to deterministic tractography, this allows the algorithm to propagate even when a seed is in gray matter. The criterion for termination of a tract is usually the angular deviation between successive steps (c.f. FA values in deterministic tractography), which prevents a tract from looping back onto itself, or if a streamline enters the same voxel more than once [75,465]. The
specific differences between probabilistic tractography algorithms are outside the scope of this thesis. For a comprehensive review, see: [72,671].

The results of probabilistic tractography should be interpreted with care. These results have a very specific meaning: a calculated probability is the probability that a connection from a given seed to a target through the diffusion data exists within the context of the model for the diffusion signal and the model of connections. These tracts do not provide anatomical evidence for the existence of a path within the brain. However, if a hypothesis is based on known anatomical pathways from post-mortem studies in humans (and homologues from tracing studies in monkeys), then we can infer that the tract we have identified is “real”. Otherwise, we must interpret the presence or absence of a tract with caution.

Crossing fibres are still a consideration in probabilistic tractography; in fact, it is a shortcoming of the tensor model. Some groups have developed alternate, non-parametric models to replace the tensor model, that can better resolve crossing fibres, such as diffusion spectrum imaging [878,925], q-ball imaging [877,879], spherical deconvolution [862], persistent angular structure MRI [456], and diffusion orientation transform [661]. However, these are outside the scope of this thesis (for a review, see: [15]). Some groups have tried to resolve crossing fibres within the tensor model. The most common approach is to model more than one tensor per voxel: the multi-tensor model [73]. The number of “fibres” that can be modeled per voxel depends on the signal-to-noise ratio, and the number of diffusion-encoding directions.

In sum, there are several advantages and limitations to tractography. Nonetheless, when care is taken to acquire, check and de-noise the data, and seeds are selected carefully, based on known landmarks, and results correspond to known tracts within the brain, then tractography can be a very useful and informative tool. Additionally, tractography can be qualitative (visualizing tracts) and quantitative (calculating the probability of a connection). The latter allows group comparisons of connectional anatomy.

2.5. Brain imaging of pain

2.5.1. Functional neuroimaging of pain

Functional neuroimaging has confirmed and advanced our understanding of brain regions implicated in nociception and pain modulation – especially the role of the PFC. With the
exception of the PFC, the pain-related brain regions identified with functional neuroimaging are similar to the brain regions previously identified in animal models and human electrophysiological studies. These regions, however, are implicated in sensory, motor, attention, salience, cognitive processes, decision-making, and other functions. Therefore, further research is required to understand the role of these regions in pain perception and modulation, and further to determine how pain is integrated.

Functional brain imaging encompasses a number of modalities, including positron emission topography (PET), single-photon emission computed tomography (SPECT), fMRI, and magnetoencephalography (MEG). Also, electroencephalogram (EEG) is sometimes considered as a brain imaging technique when it is coupled with visualization of the brain areas activated. Each of these modalities has its benefits and shortcomings because of spatial and temporal resolution, fundamental principles (e.g., indirect hemodynamic measures, direct electrical measures, etc.), invasiveness, availability, cost, and other scientific and practical issues. Because this thesis focuses on structural abnormalities in the brains of patients with TMD, findings from functional neuroimaging will only be briefly described.

2.5.1.1. Imaging acute pain

The first two brain imaging studies to investigate brain regions involved in nociception and pain perception and modulation in healthy subjects were performed using PET [464,846]. One study reported that heat pain, compared to an innocuous warm stimulus, evoked an increase in regional cerebral blood flow (rCBF) in the contralateral thalamus, lentiform nucleus and ACC (MCC, with the current nomenclature) [464]. The other study reported increased rCBF in S1, S2 and the MCC [846]. The first fMRI study to investigate the neural correlates of pain perception found that painful electrical stimulation increased the blood-oxygenation-level-dependent (BOLD) signal in S1 and MCC [231]. Many studies have since investigated pain-related brain activation, and some have specifically attempted to identify the neural correlates of the different dimensions of pain. Several reviews and meta-analyses have summarized these findings [23,95,143,212,274,304,690,709,714,869]. In general, brain imaging studies of pain have reported the activation of S1, S2, MCC, insula, prefrontal and motor regions. Relevant to this thesis, two studies have examined BOLD correlates of pain in the orofacial region. One study by Ettlin and colleagues [300] reported that electrical stimulation of the tooth in healthy subjects
was related to BOLD activity in the cerebellum, superior temporal gyrus, anterior insula, MCC, vIPFC and dIPFC. Furthermore, they reported that BOLD activity in the posterior parietal cortex, anterior insula, MCC, cerebellum, and caudate nucleus were correlated to pain intensity ratings. Another study of the neural correlates of acute orofacial pain reported that acute electrical stimulation of the tooth activated nuclei believed to be related to trigeminal nociceptive processing (within the VBSNC) and brainstem nuclei implicated in pain modulation, such as the RVM [926]. Furthermore, they found significant clusters of activation in S1, S2, anterior insula, MCC, PMC, M1, SMA and dIPFC. Therefore, it seems that acute pain in the orofacial region activates the same set of brain regions as acute experimental pain outside the orofacial region.

The putative roles of these regions in pain-related processing as determined by neuroimaging studies are described below.

**S1**

It is well known from classic brain stimulation studies in humans and animal electrophysiology studies that S1 is somatotopically organized (see Section 2.3.5). This S1 homunculus has been largely confirmed in human brain imaging studies (for a review, see: [23]), generally showing an inverted organization with the face represented ventrolaterally, the upper limb more dorsolateral, and the lower limb represented within the medial wall. Of relevance to this thesis, several studies have demonstrated that the dermatomes of the three branches of the CNV are somatotopically represented on the ventrolateral surface of S1: V1 is more superior and V3 is more inferior, although there is some overlap [205,446,630]. Of these studies, one has explicitly tested the somatotopy of the branches of the CNV with a noxious stimulus, compared to an innocuous stimulus. Specifically, DaSilva and colleagues [205] found that the representation of noxious and innocuous stimuli in the receptive fields of V1 and V2 were the same, whereas noxious stimuli in the receptive field of V3 seemed to have a broader representation than innocuous stimuli in the same receptive field. This broader cortical representation of V3 overlapped with the representation of V1 and V2.

Similar to the electrophysiological data presented in Section 2.3.5, neuroimaging data has not provided a clear role for S1 in pain processing [130]. It is unclear whether S1 is implicated in pain perception. It has been proposed that the activation of S1 is due to the tactile component of noxious stimuli, e.g., when a heat probe is used to stimulate the arm, the probe places some
pressure on the arm, and fibres that encode innocuous warm temperatures or touch may also be firing. Furthermore, imaging studies have found vastly different findings in S1 activation in pain paradigms. Specifically, Talbot et al. [846] reported significant contralateral S1 activation to the stimulation of the arm, whereas Jones et al. [464] did not find S1 activation. In a later study, Apkarian et al. [28] found that S1 actually showed deactivation in response to noxious thermal stimuli. However, in a meta-analysis, Apkarian and colleagues [23] reported that 69% of PET, 76% of fMRI, 10% of EEG and 70% of MEG studies reported an S1 activation in acute pain paradigms. More recently, MEG [699,855], EEG [885] and diffuse optical tomography [68] studies have reported S1 responses evoked by painful stimuli. Also, a meta-analysis carried out by Duerden and Albanese [274] identified S1 as a region that is activated across 140 neuroimaging studies of acute experimental heat and cold pain. Several imaging studies in humans have found that S1 (in addition to other cortical areas) shows graded activation in response to the increased intensity of experimental pain stimuli [169,240,629,705]. Also, Rainville and colleagues [715] reported that when subjects’ perceived pain intensity and unpleasantness were manipulated under hypnosis, S1 and possibly S2 responses correlated with perceived pain intensity, whereas the ACC response correlated with the perceived pain unpleasantness. In sum, neuroimaging studies have provided inconsistent findings with regards to the role of S1 in nociception and pain processing. However, more recent and sensitive methods have clearly demonstrated that S1 may, indeed, be implicated in pain perception. Therefore, neuroimaging studies have replicated many of the previous findings from electrophysiological and brain stimulation of the role of S1 in nociception and pain perception. Specifically, neuroimaging studies have confirmed the somatotopic organization of S1, and have shown that the haemodynamic response in S1 is graded to noxious stimulus intensity. Together, these findings suggest that S1 is implicated in the sensory-discriminative dimension of pain, encoding the location and intensity of a noxious stimulus.

**S2/Parietal operculum**

Although many imaging studies report that S2 is activated during painful stimuli, the findings have not clarified the role of S2 in pain processing. Apkarian et al. [23] reported that 68% of PET, 81% of fMRI, 60% of EEG and 95% of MEG studies reported S2 activation in studies investigating the neural correlates of acute pain in healthy controls. Also, Duerden and Albanese’s meta-analysis [274] identified S2 as one of the regions that is most commonly
activated in neuroimaging studies of experimental pain. However, it is not clear how S2 activations reflect various aspects of the pain experience. It is also unclear whether S2 receives nociceptive information from the thalamus via a relay through S1 (i.e., serial processing) [16, 407], or directly in parallel to information being sent to S1 [699, 702]. Furthermore, evidence from EEG studies of laser-evoked potentials (LEP) have demonstrated that the early electrophysiological nociceptive response (N2) is caused by activity in S2/posterior insular cortex (e.g., [335]), which suggests that there is parallel processing in S1 and S2, although recent findings have opposed a parallel processing model with regard to the sensory-discriminative dimension of pain [885].

One study investigating painful LEP with subdural electrodes has demonstrated somatotopic organization along the anterior-posterior axis, with the foot anterior to the face, within S2 [897]. This organization is different from that of tactile input, and it has been suggested that there may be a pain somatotopic map independent of the tactile somatotopic map [23].

**Insula**

The insula also has several somatotopic maps for different noxious stimuli, and within different subregions [64]. In this somatotopic map, the face is anterior to the foot. The insula was activated in many neuroimaging studies [187], that investigated many paradigms, including gustation, emotion, olfaction, empathy, interoception, motor output, attention, language, memory, speech, and pain [506]. Apkarian et al. [23] reported that 88% of PET, 100% of fMRI, 30% of EEG and 20% of MEG studies reported insular activation in imaging studies of acute pain in healthy volunteers. Duerden and Albanese’s [274] meta-analysis of 140 experimental pain neuroimaging studies also identified the insula as one of the most likely activated regions. In a meta-analysis of the function of the insula, Kurth and colleagues [506] investigated 79 studies that showed insular activation in imaging studies of acute pain stimulation paradigms in healthy controls, and found that all of the subregions of the insula were activated. It is, however, noteworthy that this same study identified that different subregions of the insula showed responses to paradigms that tested the neural correlates of processes related to pain, such as somatosensation, motor output, attention, interoception, and emotion, and that these activations generally overlapped with that of pain.
A number of groups have postulated a role for the insula in terms of pain perception. For instance, evidence that the insula is involved in intensity encoding comes from a study by Coghill and colleagues [169] who reported that the insula responds in a graded fashion to increasing intensity of noxious stimuli. Brooks and Tracey [116] suggested that the insula is a multidimensional integration site for pain. An alternative hypothesis about the role of the insula proposed by Craig [183] suggested that the mid-posterior insula was a multimodal homeostatic or interoceptive area. The former hypothesis maintained pain as a separate modality, whereas Craig’s hypothesis established pain as a subset of homeostatic function. Baliki and colleagues [48] have posited another hypothesis suggesting that, in addition to integrating the dimensions of pain perception, the insula is a central, multimodal magnitude estimator and a nociceptive-specific magnitude estimator. However, the insula has been implicated in a multimodal sensory stimulus salience detection network, where salience stimuli are encoded and processed to appropriately orient attention [263-265,445,530,632,633].

In sum, because the insula receives multimodal input, it is an ideal site for integrating information from the various dimensions of pain – it is the site where nociception becomes pain. However, it is activated in many other paradigms, and across different modalities [265,956]. Therefore, the insula may best be considered as a region that encodes behaviourally-salient stimuli, including pain.

**Cingulate cortex**

The cingulate cortex is a large, heterogeneous brain region that can be subdivided into several subregions (see Section 2.3.5) [169]. Apkarian et al. [23] reported that 94% of PET, 81% of fMRI, 100% of EEG and 25% of MEG studies reported cingulate activation in imaging studies of acute pain in healthy volunteers. Furthermore, Coghill and colleagues demonstrated that the cingulate, specifically the MCC, showed graded responses to increasing intensity of noxious stimuli [169]. The different subregions of the cingulate cortex have been implicated in different dimensions of pain, including the affective, cognitive, modulatory and motor dimensions [128,860,902,903]. The aMCC/pgACC is a complex, multimodal region that has been implicated in a number of functions [69]. For instance, this region has been identified as a node in the salience network, and has been implicated in multimodal salience detection [213,229,262,263,774,849,927], including detection of noxious stimuli
The cingulate has also been implicated in the cognitive and affective processing of pain [218,230,529,716,777,934,936], and the MCC, which includes the CMA, is involved in action selection and modulation of motor output in response to aversive stimuli [790,898,904]. The sgACC has been implicated in pain modulation [95]. Furthermore, the pgACC and sgACC have been implicated in emotional processing and depression [299,579,790] (See section 2.3.6).

**Prefrontal cortex**

The PFC is a large, heterogeneous region, in the frontal lobe anterior to the motor cortex. There is no evidence suggesting that the PFC receives nociceptive information directly from the thalamus (see Section 2.3.5). The PFC does, however, receive input from a number of nociceptive brain regions, such as the cingulate cortex, the insula, and the somatosensory cortex [644]. Apkarian et al. [23] reported that 39% of PET, 70% of fMRI studies reported PFC activation in imaging studies of acute pain in healthy volunteers. Interestingly, no EEG or MEG studies reported PFC activation. The PFC has been implicated in the cognitive-motivational dimension of pain. Evidence for this role comes from a number of studies that have investigated the interaction of pain and cognition of the brain. For instance, the OFC is activated when subjects are distracted from pain, which suggests that this region is involved in pain modulation [888]. Furthermore, some studies have found that the dlPFC is implicated in pain modulation [552], although other studies have not found this. Conversely, neuroimaging studies of persistent pain, and experimental models of hyperalgesia and allodynia have shown that increased sensitization is related to increased activity in the dlPFC [56,443,551,775]. Furthermore, one study has demonstrated that regulation strategies can modulate pain via the vlPFC, which is involved in top-down cognitive modulation of pain [932]. However, another regulation study did not find that the vlPFC was implicated in pain modulation [753]. Alternatively, it has been suggested that the vlPFC is implicated in pain anticipation, rather than modulation [752,908]. The medial PFC has been implicated in the integration of cognitive and motivational dimensions of pain [935]. Therefore, although the PFC does not receive nociceptive input from the thalamus, neuroimaging has revealed that the PFC plays an extensive role in the cognitive dimension of pain, and, through it connections to antinociceptive brainstem regions (see Section 2.3.6), pain modulation.
Cortical and subcortical motor regions

Motor regions are activated in acute pain paradigms in healthy controls, but less reliably than the aforementioned brain regions [23]. As previously discussed (Section 2.3.5), in the context of pain, it is believed that motor regions serve two purposes: to orient the body toward the source of pain and to initiate nocifensive behaviour (e.g., avoid the stimulus). Similarly, another study reported that hypertonic saline injection into the masseter muscle, an experimental acute pain model, was related to deactivation in the face region of M1, as measured by fMRI [638]. It is, however, noteworthy that some studies have not observed activation in M1 during noxious stimulation [395,744].

The basal ganglia are a set of subcortical nuclei that have been associated with motor function. However, it is noteworthy that the basal ganglia do receive input from many cortical and subcortical regions, and form several functional loops [644]. It has been proposed that this extensive wiring to the brain suggests that the basal ganglia may be implicated in more than just motor functions [388]. For instance, the basal ganglia are often activated in neuroimaging studies of experimental pain [107]. It has been suggested that the cortico-thalamo-basal ganglia-cortical loops may provide a unique anatomical substrate for the integration of the various dimensions of pain [107]. However, more work is required to further investigate this possibility.

Other motor regions are also commonly activated in brain imaging studies of experimental pain in healthy volunteers, including the PMC and SMA [23,274]. The PMC has been implicated in cognitive modulation of motor output, and the SMA has been implicated in motor planning, and the temporal organization of movements [692]. Therefore, their activation during experimental pain is likely related to the cognitive and motivational dimensions of pain, with regard to nocifensive behaviours.

2.5.1.2. Imaging salience and pain

Salience and pain are closely related. Pain is inherently salient and therefore can engage brain regions that serve as “salience-detectors”. Specifically, salient stimuli have been shown to activate a right-lateralized brain network, including regions in the frontal, parietal, insular and cingulate cortex [364,604-606,647]. In an fMRI study, Downar et al. [262-264] identified a multimodal salience network in humans. In the context of pain, a network of regions, comprised
of the MCC, temporoparietal junction, PFC, and the putamen encode stimulus salience. Similarly, an fMRI study investigating the neural basis of perceptual decision-making in the context of pain as a perceived threat and increased salience stimulus, reported that the insula integrated the perceived threat to form a decision about the stimulus [933]. It is thus conceivable that these regions form a system that appraises, encodes and modulates a salient (and potentially threatening) stimulus based on the context in which it is presented – or its relative salience. Another study demonstrated that brain regions that are commonly activated by noxious stimuli are also activated by equally salient, non-noxious stimuli [530]. Therefore, results from experimental pain paradigms attempting to investigate the roles of brain regions in pain must be interpreted with caution, as the aforementioned studies have demonstrated that they are non-specific to pain, and may be implicated in encoding any behaviourally-relevant stimulus.

2.5.1.3. Chronic pain

Many studies have used functional neuroimaging to investigate the central abnormalities in chronic pain patients. One of the differences between BOLD-fMRI and PET is that BOLD-fMRI requires a stimulus, as it relies on differences in activation, whereas PET can assess baseline function. In terms of imaging chronic pain disorders, fMRI cannot capture brain activity related to spontaneous pain, or other clinical features. PET can capture these features, but is invasive as it requires a radioactive tracer. Therefore, some groups use percept-related fMRI, where subjects are continuously rating a quality of a painful stimulus throughout an fMRI scan [213, 226, 509]. In a meta-analysis of all functional neuroimaging studies of pain up to 2005, Apkarian and colleagues [23] compared noxious stimulus-evoked brain activation in healthy, pain-free subjects and chronic pain patients. They reported that, in general, chronic pain patients showed less noxious stimulus-activation of S1, S2, insula, and the ACC/MCC compared to pain-free subjects. They reported no difference between the groups for the thalamus (although studies have reported thalamic findings), and that the PFC showed significantly greater activation in chronic pain patients, compared to normal subjects. However, not all chronic pain disorders have the same symptomatology. Therefore, it is more likely that different types of chronic pains have different “brain signatures” or “fingerprints” – the pattern of abnormally functioning brain regions is specific to the symptom profile of the pain disorders. A challenge for neuroimaging of pain is to disentangle which abnormally-functioning brain region is specific to a pain disorder, and which is common to all pain disorders.
Another method of analyzing functional brain imaging data is to identify regions that may be functionally connected [333]. Conceptually, this method is based on the idea that brain regions do not process information in isolation, but rather process information in different schemes, either hierarchical, or in parallel. Therefore, these regions are functionally connected during a task, and in task-free paradigms (or at rest) [213]. Recent evidence has shown that some of these functionally connected brain regions may be disrupted in chronic pain (e.g., [49,560]).

Few studies have investigated functional brain abnormalities in TMD. The first study to do so, investigated neural responses to tactile-flutter stimuli on the index finger [641] and found that the patients reported the stimuli as more intense, but not painful, than the control subjects. Further, compared to the control subjects, the tactile stimuli evoked less activation in S1, S2 and insula, and greater activation in the thalamus, MCC, the amygdala, and another region of S1 in the patients with TMD. This study reported that patients with TMD have both increased and decreased activation across several regions, including S1, S2 and the insula to a vibratory stimulus applied outside the orofacial region, compared to controls. Patients with TMD show increased activation in the MCC, in the contralateral insula and thalamus. Therefore, patients with TMD show abnormal processing of an innocuous tactile stimulus outside the orofacial region. It would have been more informative to have also applied a vibratory stimulus to the orofacial region, to test whether tactile thresholds were different in this region, and the neural correlates of these differences. However, a vibratory stimulus on the face in an MRI magnet would likely be a source of noise and therefore difficult to test.

Our laboratory has conducted an fMRI study to investigate brain responses to cognitive and emotional attention-demanding tasks in patients with TMD [928]. In this study, a group of patients with idiopathic TMD and a group of age- and sex-matched pain-free controls underwent fMRI scanning while performing three counting Stroop tasks (neutral, conflict numbers, emotional). We found that behaviourally, the patients with TMD had sluggish reaction times, compared to controls, in all three Stroop tasks. The fMRI data indicated that patients had greater deactivation in the mPFC, OFC, medial temporal gyrus and dIPFC during the neutral Stroop task, compared to controls. Furthermore, patients showed increased activation in the dIPFC, vIPFC, insula, ACC, SMA and amygdala during the high conflict Stroop task, compared to controls. During the emotional Stroop task, patients showed greater activation than controls in the sgACC, MCC, SMA, PCC, insula, vIPFC, and the inferior parietal lobule. Finally, patients showed less
functional connectivity than controls between the dIPFC and the aMCC, and the pgACC and the amygdala during the high conflict task. During the emotional Stroop task, patients showed increased connectivity between the dIPFC and the MCC. In sum, these findings suggest that patients with idiopathic TMD have cognitive deficits related to poorer task performance, compared to controls. These deficits may be related to abnormal brain function in cognitive/affective and attention-related brain regions.

Resting state networks reflect the intrinsic connectivity of brain regions when subjects are not actively engaged in a task. It has been posited that these networks reflect the anatomical connectivity, as well as the “history” of prior co-activation during task [235,236]. In the context of TMD, a recent study investigated the connectivity of the insular and cingulate cortices in myofascial patients with TMD during rest, and during experimental pressure pain in the orofacial region [448]. These studies reported that during resting state, in patients with TMD compared to controls, the left anterior insula was more functionally connected to the right pgACC and the left parahippocampal gyrus, and the right anterior insula was more connected to the right thalamus. During noxious stimulation, both groups showed greater functional connectivity between the left anterior insula and the left S2, cerebellum, the left posterior insula showed greater connectivity to the right dIPFC. Finally, the study reported a group-by-pressure interaction in the functional connectivity between the left anterior insula and the left pgACC, bilateral frontal polar cortex, and the right anterior insula with the right pgACC. Therefore, these findings suggest that in TMD there is increased functional connectivity between sensory and pain-modulatory regions at rest, and increased connectivity between cognitive and modulatory regions during experimental pressure pain.

In sum, few studies have used functional neuroimaging in TMD. These studies have found abnormal function in sensory, motor, cognitive/modulatory and salience-related brain regions.

2.5.2. Structural imaging of pain

2.5.2.1. Acute Pain

To date, only two studies have investigated structural gray matter correlates of acute pain. Teutsch and colleagues [852] demonstrated that eight days of noxious heat pain stimulation induced increased gray matter volume in MCC and S1. Furthermore, as subjects habituated to the
noxious stimulus over eight days, these gray matter increases resolved (i.e., the gray matter volume returned to baseline). In a study by Erpelding et al. [298], cortical thickness in S1 correlated with individual subjects’ heat and cold pain sensitivity, and cortical thickness of the MCC correlated with heat pain sensitivity. To date, the relationship between white matter microstructure and acute pain thresholds or tolerance has not been studied in healthy subjects.

2.5.2.2. Chronic Pain

Gray matter abnormalities

There are several approaches to investigating gray matter structure in chronic pain patients. For instance, studies can either investigate the whole brain, or focal areas (ROIs) that have been hypothesized to show abnormal gray matter structure, or investigate a specific ROI. Furthermore, the question posed can also vary: (1) do the patients have abnormal structure, compared to a control group?; and (2) Are there structural neural correlates of behavioural measures, such as pain characteristics and personality traits? Furthermore, as discussed in Section 2.4.2, there are various MRI-based analysis methods to measure gray matter. It is noteworthy that studies that only used CTA to evaluate differences in gray matter structure did not study subcortical structures.

A summary of all studies examining gray matter structure in chronic pain populations is provided in Table 2.3. It includes chronic back pain [27,121,764,782], FMS [125,502,558,712,738,766,949], IBS [98,225,779], migraine [207,490,740,762,889], trigeminal neuropathic pain [206,382], trigeminal neuralgia [382], TMD [358,963], phantom-limb pain [268], provoked vestibulodynia [772], complex regional pain syndrome (CRPS) [356], cluster headache [578], chronic tension-type headache [765], medication-overuse headache [765], hypnic headache [434], chronic pelvic pain [303], chronic posttraumatic headache [650], persistent idiopathic facial pain (previously atypical facial pain) [763], hip osteoarthritis [741], rheumatoid arthritis [919] and pain disorder [887] (a diagnosis based on the DSM-IV, defined as persistent and chronic pain at one or more sites that cannot be fully explained by a physiological process or physical disorder). Overall, the most common brain regions showing gray matter abnormalities were the PFC, insula, the ACC and the MCC. Other regions that were abnormal in chronic pain included the thalamus, basal ganglia, S1, S2, and the brainstem. Additionally, some studies demonstrated abnormalities in the temporal lobe and the PCC. In every region, with the
notable exception of the basal ganglia and the brainstem, most studies showed a decrease in gray matter in chronic pain.

Of particular interest to this thesis, three studies used VBM to investigate gray matter abnormalities in TMD. One study by Gustin and colleagues [382] reported no gray matter abnormalities in the brain of patients with TMD. Gustin and colleagues did not provide the diagnostic criteria used to select patients with TMD. Another study, by Younger and colleagues [963], tested for gray matter abnormalities in myofascial patients with TMD and reported four main findings: 1) compared to controls, patients with TMD had increased gray matter in the thalamus, the basal ganglia, insula, the vIPFC, and the brainstem; 2) a negative correlation between jaw pain and gray matter volume in the dorsal posterior cingulate, the MCC, SMA/preSMA, and the superior temporal gyrus; 3) a negative correlation between experimental pressure pain thresholds (as measured by pressure algometry) and brainstem gray matter volume; and 4) a positive correlation between TMD duration and gray matter volume in the posterior cingulate, the hippocampus, and the brainstem. Another study by Gerstner and colleagues [358] investigated myofascial patients with TMD and found that patients with TMD had decreased gray matter in the vIPFC, MCC, PCC, SMA, insula and the regions within temporal lobe. These findings seem to conflict with the Younger et al. [962] study, but could be attributed to methodological differences between the studies. For instance, the age range and the range of durations of TMD in the Younger patient sample were larger than those of the Gerstner patient sample. These factors could contribute to changes in gray matter, and they cannot be adequately controlled with the statistical methods used to evaluate group difference in structural gray matter analyses. For a complete discussion of these points see: [615].

Interestingly, recent evidence suggests that some gray matter abnormalities in some chronic pain disorders are caused by the pain, rather than pre-existing abnormalities. For instance, Rodriguez-Raecke and colleagues [741] showed gray matter abnormalities in several brain region of patients with primary hip osteoarthritis. These patients underwent surgery, and for the most part, the pain was resolved. The post-operative scan of a subgroup of these patients revealed that some, but not all, of the gray matter abnormalities had resolved (i.e., there were no differences between controls and patients). Four other studies have since demonstrated similar effects – partial reversal of gray matter changes in brain once patients’ pain has been resolved, in the same [384] and other chronic pain disorders, including chronic low back pain [782]. However, it remains
unclear whether these changes in gray matter are due to the resolution of pain, or are secondary to the resolution of pain. For instance, it is possible that a person with chronic pain in the hip will be more sedentary, compared to when they are no longer in pain. Furthermore, pain has been associated with changes in mood, including increased incidence of depression and anxiety, which could resolve once the pain is gone. Furthermore, it has recently been shown that medications can alter the structure of the brain [916,962]. Therefore, when patients are no longer taking pain-killers, the changes in the brain may resolve.

Furthermore, it is noteworthy that not all of the observed gray matter abnormalities were abolished with the cessation of pain. This may be related to lasting effects of medications, such as opiates, which have been shown to produce long-term changes in the pgACC, MCC, amygdala, the vIPFC, hypothalamus, brainstem, and the hippocampus of patients with chronic low-back pain [962]. These changes did not show reversal after the patients had ceased managing their pain with morphine for an average period of 4.7 months. Therefore, there may still be pre-existing abnormalities that contribute to the development of chronic pain.

Additionally, it is noteworthy that functional pain syndromes, such as IBS, FMS and TMD, are defined as disorders that disturb normal function without any obvious structural or biochemical abnormality [246]. Therefore, it is possible that mechanisms other than increased nociceptive drive from the periphery may be driving gray matter abnormalities in the brain. An alternative possibility is that gray matter abnormalities pre-date the onset of chronic pain. In this scenario, gray matter abnormalities are pre-existing vulnerabilities that may contribute to the development of chronic pain [213]. Evidence for this proposition comes from studies that have identified regions of gray matter abnormalities related to stable personality traits, such as neuroticism or pain catastrophizing [98,772], which are related to heightened pain sensitivity (see Section 2.1.7).

In sum, there are both pre-existing and pain-driven gray matter abnormalities in the brain of chronic pain patients. In general, there appears to be increases in sensory/nociceptive brain regions, indicative of increased nociceptive drive; decreases in pain modulation brain regions, indicative of potentially dysfunctional pain-modulatory systems.
White matter abnormalities

There have only been eight studies investigating white matter abnormalities in the brain of chronic pain patients. Of these, two have used VBM to evaluate white matter volume from T1-weighted images, and four have used DTI to delineate abnormalities in white matter microstructure, or gray matter microstructure. The two studies that have used DTI to investigate gray matter [334,558] and the white matter VBM studies [358,553] are omitted from this thesis because of flaws in the experimental design and methodologies. Of the four remaining studies that used the approach of DTI, one did not find any group differences between patients with chronic pelvic pain and controls [303]. The other three that did identify abnormalities are described below.

The first study to test for abnormalities in white matter microstructure associated with chronic pain, by DaSilva and colleagues [208], reported decreased FA along tracts between the brainstem and the thalamus and the thalamus and S1 cortex of patients with migraine. The authors concluded that there are abnormalities along the ascending nociceptive pathways in migraine patients.

Geha and colleagues [356] reported white matter abnormalities in patients with CRPS that consisted of lower FA in the cingulum bundle and the adjacent corpus callosum. This was the first study to investigate the abnormalities in white matter tracts by elucidating the connectivity of the region. They reported that abnormal white matter region had fewer connections per unit of distance in CRPS patients compared to controls.

A study by Chen et al. [156], from our laboratory, investigated white matter abnormalities in patients with IBS, and reported that patients had increased FA in the fornix and the external/extreme capsule, adjacent to the insula. It was also found that FA in the anterior insula and the VPL thalamus were significantly correlated to pain severity. Further, FA in the left insula correlated with pain unpleasantness and duration. Finally, in patients with IBS, FA in the cingulum bundle was negatively correlated to the PCS, and a positive correlation between FA in the MD thalamus and neuroticism. These findings suggest that IBS patients have abnormal connections in white matter tracts related to nociception and/or cognitive/limbic function.
In addition to the aforementioned studies that have investigated white matter abnormalities in the brains of chronic pain patients, two DTI studies have investigated abnormalities along the CNV in patients with trigeminal neuralgia. One study by Fujiwara et al. [339] did not find any significant abnormalities in the FA, $MD$, or the cross-sectional area of the patients’ CNV. This study reported that no significant group differences in the ratio of FA of the affected to the unaffected nerve. However, the study reported significantly more variance in the FA values of patients’ CNV, compared to controls, and this value was positively correlated to the affected:unaffected ratio of the cross-sectional area of the CNV. The other study, by Leal et al. [525], reported that the affected CNV had significantly increased $MD$, and decreased FA, nerve volume, and cross-sectional area. Furthermore, the decrease in FA was positively correlated to the decrease in volume and cross-sectional area of the CNV. Decreased FA and increased $MD$ indicate that there is increased diffusion, or less organization. Therefore, it is possible that the CNV has a larger diameter, is inflamed, or damaged. However, the interpretation is limited as other measures of white matter integrity (RD and $\lambda_1$) were not provided. These studies indicate that there are structural abnormalities along the CNV in patients with trigeminal neuralgia, and suggest that DTI can be used to investigate the CNV for abnormalities in TMD.
Table 2-3. Summary of regions with gray matter abnormalities in chronic pain

<table>
<thead>
<tr>
<th>Chronic pain disorder</th>
<th>Method</th>
<th>n patients (# females)</th>
<th>n controls (# females)</th>
<th>Thal</th>
<th>BG</th>
<th>S1</th>
<th>S2</th>
<th>IC</th>
<th>ACC</th>
<th>MCC</th>
<th>M1</th>
<th>PFC</th>
<th>Bstem</th>
<th>Other?</th>
<th>Threshold</th>
<th>Multiple comparisons correction</th>
<th>Contributing factors</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBP</td>
<td>VBM</td>
<td>26 (16)</td>
<td>26 (16)</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ dl</td>
<td>p&lt;0.05</td>
<td>Permutation</td>
<td>D</td>
<td>[27]</td>
<td></td>
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**Note:** The table represents data comparing patients with chronic pain disorders to control subjects, showing changes in brain activity or structure. The columns indicate the method used, the number of patients and controls, and the changes observed in various brain regions.
a. the p-value was reduced for some findings, but the lower threshold is not provided
b. Between patient groups analysis: Chronic vs. Episodic
c. Comparing temporary post-traumatic headache to chronic post-traumatic headache (temporary > chronic)
d. Longitudinal study comparing time 1 scans with subjects after the pain has resolved
e. Decrease GM on the right, increase GM on the left
f. Longitudinal study comparing time 1 scans with post-operative scans, when pain is resolved
g. Pain disorder is a diagnosis based on the DSM-IV, defined as persistent and chronic pain at one or more sites that cannot be fully explained by a physiological process or physical disorder."

**Abbreviations:** Amyg — amygdala; ACC — anterior cingulate cortex; BG — basal ganglia; Cb — cerebellum; CH — cluster headache; CPP — chronic pelvic pain; CPTH — chronic posttraumatic headache; CRPS — complex regional pain syndrome; CTA — cortical thickness analysis; DBM — deformation-based morphometry; dlPFC — dorsolateral prefrontal cortex; dmPFC — dorsomedial prefrontal cortex; FDR — false discovery rate; FWE — family-wise error; GMV — gray matter volume; GP — globus pallidus; HC — hippocampus; Hip OA — hip osteoarthritis; HT — hypothalamus; HypH — hypnic headache; IBS — irritable bowel syndrome; IC — insular cortex; iTL — inferior temporal lobe; M1 — primary motor cortex; MCC — midcingulate cortex; MOH — medication-overuse headache; mPFC — medial prefrontal cortex; MTL — medial temporal lobe; NC — caudate nucleus; OFC — orbitofrontal cortex; PCC — posterior cingulate cortex; PFC — prefrontal cortex; PHG — parahippocampal gyrus; PIFP — persistent idiopathic facial pain; PMC — premotor cortex; PPC — posterior parietal cortex; pTL — posterior temporal lobe; Put — putamen;
PVD — provoked vestibulodynia; RFT — random field theory; Rheum Arth — rheumatoid arthritis; ROI — region of interest; S1 — primary somatosensory cortex; S2 — secondary somatosensory cortex; SMA — supplementary motor area; SN — substantia nigra; STG — superior temporal gyrus; SVC — small-volume correction; Thal — thalamus; TMD — temporomandibular disorder; TN — trigeminal neuralgia; TNP — trigeminal neuropathic pain; TL — temporal lobe; V1 — primary visual cortex; vlPFC — ventrolateral prefrontal cortex; vmPFC — ventromedial prefrontal cortex; vSTR — ventral striatum
2.6. Neuroplasticity

It is clear that brain structure is defined by genetics and experience [577]. In fact, it has been suggested that neuroplasticity is “the normal ongoing state of the nervous system throughout the life span” [672], in order to better adapt to the environment [969]. This concept of brain neuroplasticity was recognized early in the twentieth century. For instance, Cajal described the concept:

The labor of a pianist [...] is inaccessible for the un-educated man as the acquisition of new skill requires many years of mental and physical practice. In order to fully understand this complex phenomenon it becomes necessary to admit, in addition to the reinforcement of pre-established organic pathways, the formation of new pathways through ramification and progressive growth of the dendritic arborization and the nervous terminals. [134] (cited and translated in [672])

The term plasticity was first used by Ernesto Lugaro in 1906 [554] to describe changes in the nervous system during maturation, compensatory changes after lesions, and changes related to learning and memory. He suggested the specific chemicals, today known as neurotrophins, that are responsible for the development and organization of the nervous system, continue to reorganize the neuronal connections in the brain. This property of the nervous system, which he called plastic, occurred through chemical signaling. Lugaro recognized two processes specific to neurones: conduction of an impulse, and transduction of a neural signal between neurones [86]. In line with Hebbian doctrine [419], Lugaro postulated that transduction would lead to modifications of the neural circuit: increased firing would enhance a connection by modifying the synaptic distance [86]. Despite Lugaro’s postulation that the brain remains plastic throughout life, scientific dogma maintained that only the developing nervous system could undergo changes. The concept of plasticity was not established until pioneering studies were published in the second half of the twentieth century. These studies used animal models to demonstrate that plasticity and “learning” occur at several levels of the nervous established systems, from the periphery, and the spinal cord, to the brain (see [477]). Today, it is established that plasticity occurs in the mature nervous system. It is also now known that plasticity can occur rapidly at several levels of the nervous system. These neuroplastic events can either be beneficial, as in learning a novel task, or maladaptive, as is the case in chronic pain (discussed below).
One of the first studies [914] to report plasticity in the somatosensory system found that after destruction of the dorsal column neurones in the VPL region of the thalamus that normally responded to tactile stimulation in the foot changed their receptive fields. The representation of the arm in VPL expanded to the region that previously represented the foot. Later studies have confirmed that plasticity occurs throughout the adult nervous system, including in the somatosensory system (for a complete review, see: [347,475]).

2.6.1. Use-dependent neuroplasticity

One type of beneficial plasticity occurs during learning. This concept was first described anecdotally by Hebb [419]: rats that were exposed to enriched environments performed better on tasks requiring complex behaviour, compared to rats raised in cages. In humans, a study by Pascual-Leone and colleagues [673] reported that learning a novel motor task is associated with motor cortex plasticity, as measured by TMS. Interestingly, these changes returned to baseline once the task had been learned, and became part of implicit knowledge. An fMRI study [478] investigating motor learning (the learning of sequential finger movements) found increased brain activation in M1 in subjects that have learned a new task, but, in contrast to Pascual-Leone et al.’s study, these changes persisted for several months. Another example of beneficial plasticity is a study by Nudo and colleagues [648], which showed that after an experimentally-induced ischaemic stroke in the hand region of M1 of non-human primates, retraining of the affected hand preserved the cortical tissue adjacent to the lesion. This preserved tissue underwent plasticity and contributed to the functional recovery of the hand. These studies demonstrate the concept of use-dependent plasticity. Motor learning, or retraining, can change the structure and function of the brain – in these cases, M1.

Longitudinal studies of use-dependent plasticity have found that behavioural changes are related to both functional and structural gray and white matter changes in the brain [111,266,267,271,771]. Animal studies in non-human primates have demonstrated that the nervous system undergoes functional reorganization (changes in cortical representation fields) in response to massive changes in the environment, such as sensory loss due to deafferentation or brain lesions [454,458,703]. Furthermore, studies in non-human primates have demonstrated massive white matter plasticity in response to learning a novel task [422] and brain lesions [200].
Other studies have shown that experience-dependent (or use-dependent) plasticity is related to cellular changes, specifically in dendritic spine density [436,493,865].

Plasticity in the nervous system can include changes to a receptive field (e.g., with increased use of a limb, its representation in the brain can be expanded), changes in neuronal responses or firing thresholds, and structural changes in neuronal connections.

However, not all use-dependent neuroplasticity is beneficial [576]. For instance, Rosenkranz and colleagues [745] compared healthy musicians to a group of patients with musician’s dystonia, and a group of controls. The authors reported that musicians showed functional plasticity in the motor cortex. Patients with dystonia, however, had even more changes, which disrupted normal motor output. This study demonstrates that neuroplasticity can actually interfere with normal function. Similar mechanisms may occur in patients that develop chronic pain: increased or prolonged nociceptive input can induce “excess” plasticity, which can itself become the aetiology for pain (see below).

2.6.2. Neuroplasticity and pain

Normally, pain is a behaviourally-relevant signal that can promote survival by signaling injury or potential injury. However, in some cases, pain persists long after its usefulness, even after the injury has healed. This occurs due to a process called the chronification of pain (see Section 2.1.2). In cases of chronic pain, pain is no longer a useful protective signal which is promoting survival, but rather the pain becomes a disease in itself. This chronification occurs due to functional and structural changes at several levels of the nervous system, or maladaptive plasticity.

One level of plasticity is in the periphery at the nociceptor. When injury occurs, pain experience is heightened by increasing the sensitivity of nociceptors to thermal and mechanical stimuli (i.e., peripheral sensitization) [470]. This occurs, in part, because of chemical mediators that are released at the site of injury, which modify the properties of the nociceptors such as their threshold. There are also changes in the neurotransmitters produced by these neurones. The dorsal horn of the spinal cord and the VBSNC also undergo plasticity [159,784,950,951]. For instance, in phantom limb pain, where the peripheral end of the nerve undergoes changes that cause pain, blocking conduction along the peripheral nerve at the stump does not eliminate
ongoing pain in all patients [96,323]. Furthermore, when a nerve is severed, there is a cascade of events that occur in the CNS. For instance, microglia are activated, and chemical mediators (including inflammatory molecules) are released [568]. Interestingly, when these chemical mediators are inhibited in the spinal cord and VBSNC [159], patients with neuropathic pain report that their pain is resolved [568]. These factors can initiate or maintain pain after peripheral nerve injury by sensitizing or disinhibiting second-order neurones. Importantly, immune cells are not implicated in acute pain conditions, but rather in persistent and chronic pain states (see [159,568]). Further neuroplastic events occur in suprabulbar regions, in the thalamus and the cortex. For instance, after peripheral nerve injury (including amputation) in non-human primates, spinal, brainstem, thalamic and cortical neurones can undergo large-scale reorganization [324,325,467,472-474,703]. Further evidence for pain-related neuroplasticity comes from a study that showed that thalamic stimulation in phantom limb patients could elicit their phantom limb pain [219]. This suggests that neuronal representation of the phantom limb remains in the thalamus, even after the loss of the limb.

Neuroimaging studies have contributed to our understanding of brain plasticity in response to repeated noxious stimulation. For instance, healthy subjects who received 20 minutes of noxious stimulation over eight days habituated behaviourally (i.e., they reported the same stimulus as less painful) [93,94,852] and showed decreased pain-evoked activation in the thalamus, S2, insula and the putamen, and increased activation in the sgACC [94]. Structural imaging in these subjects also revealed increased gray matter volume in the PMC, MCC, S1, inferior parietal lobule, and the medial temporal gyrus after the eight days of stimulation, compared to baseline [852]. Although not explicitly tested, it is inferred that these structural and functional changes observed were related to the observed behavioural changes. Although the functional and structural changes were located in different regions, the findings nonetheless provide evidence that repeated noxious stimulation induces functional and structural brain plasticity. Further support for the concept that increased nociceptive input alters the structure of the brain comes from studies that have demonstrated that gray matter abnormalities are reversible upon the successful treatment of the chronic pain disorder [384,741,782]. However, not all gray matter abnormalities in chronic pain conditions are reversible. It is possible that chronic pain patients may not have effective pain-modulatory systems. In TMD, evidence suggests that some pain-modulatory systems, such as DNIC [113,491,756], are dysfunctional. Also, most studies
investigating the structure of the brain in chronic pain have reported decreased gray matter in the pain-modulatory regions [575]. Additionally, it is possible that, to some extent, hypersensitivity to nociceptive stimuli is related to changes that have occurred in brain structure. In line with this concept, studies have demonstrated that some patients with TMD show allodynic and hyperalgesic responses to experimental stimuli, consistent with the concept of central sensitization (See section 2.2.3). It is possible that the combination of increased barrage of nociceptive processing, an increase in the activity of pain-facilitatory pathways and decreased activity in pain-inhibitory pathways induce central sensitization. This central sensitization can lead to further functional and structural changes that can maintain painful states.

Based on the concept of that pain may drive CNS plasticity, structural abnormalities in chronic pain patients would correlate with pain characteristics pain, such as duration, intensity and unpleasantness. For instance, if we expect increased nociceptive activity, then we would expect a positive correlation between measures of brain structure (volume or thickness in gray matter; FA in white matter) in nociceptive brain regions and pain duration, intensity and unpleasantness. Conversely, we would expect a negative correlation between structural measures and pain characteristics in antinociceptive regions. Alternatively, if there are pre-existing abnormalities in the brain that predispose subjects to the development of pain, then we would not expect correlations between structural measures in pain-related brain regions and pain characteristics. However, in the latter scenario, we can expect that brain structure would be related to personality factors that predispose persons to developing chronic pain, such as neuroticism.

2.6.3. Age-related neuroplasticity

It has long been recognized that, over time, the brain undergoes atrophy. These age-related changes have been observed in post-mortem studies [115,191,232,261,427] as well as in brain imaging studies [322,749-751,911,968]. These studies have found that, in general, the brain underwent atrophy with age, although some regions were spared, and others showed an increase with age. For instance, Ziegler and colleagues [968] showed that the MCC and sgACC were thicker in older adults than in younger adults. Some disease states have been shown to modulate this aging process. For instance, Alzheimer’s disease has been shown to increase the rate of gray matter atrophy. Furthermore, two studies have shown that patients with chronic pain, specifically FMS [502] and chronic back pain [27], also had accelerated gray matter atrophy. It is not known
whether this form of plasticity is related to duration of pain, or other factors that may contribute to accelerated gray matter atrophy.

### 2.6.4. Microstructural basis of neuroplasticity

MRI-detectable changes occur in the brain as part of the natural aging process, with increased use (experience, learning and memory), in response to peripheral nerve injury, and amputation. However, the neural basis of these changes is not well understood. Pakkenberg and Gundersen [662] reported that whole brain age-related atrophy in humans is due to neuronal loss and reduced cell packing density; whereas Peters et al. [685] reported that age-related atrophy in macaques is related to a decrease in the number of neuropils, not neurones. There are also other proposed hypotheses to explain mechanisms of gray matter change. For instance, rather than neural loss, there may be glial death [575]. Recent evidence suggests that gray matter loss may be related to the density of small dendritic spines [278,608], and the remodeling of neuronal processes [537]. Alternatively, reversible gray matter changes in chronic pain may be caused by neuroinflammation [238,381,920], and induce MRI-detectable increases in gray matter. This mechanism could explain both abnormal age-related increases and maintenance of gray matter volume/thickness. For the observed gray matter losses, however, we cannot rule out cell death as a factor in age-related gray matter loss – healthy populations lose neurones as they age, and persons with neurodegenerative diseases suffer increased rates of atrophy related to cell death.

Changes in measures of white matter integrity, as indicated by FA, $MD$, RD and $\lambda_1$, each reflect a different aspect of white matter microstructure. The most commonly computed and assessed measure of white matter integrity is FA, because it is scaled between 0 and 1, where 0 represents complete isotropic diffusion, and 1 represents complete anisotropic diffusion, and studies have demonstrated that a threshold value of 0.2 for FA value can suitably delineate white matter from gray matter [820]; however, this needs to be checked for specific populations. There could be macrostructural factors that contribute to reduced FA, such as increased branching, crossing fibres or larger tracts (more axons) and/or microstructural changes such as cell swelling (œdema), changes to protein filaments (neurofilament phosphorylation), disruptions to the cell membranes, and, to a certain extent, decreased myelin [66,67]. However, as FA is a summary measure, it is not specific to any microstructural features of white matter tracts [695]. Histological studies have demonstrated that RD and $\lambda_1$ are related to alterations in specific
structures [67]. FA can be increased by any decrease in RD, and increases in $\lambda_1$, and thus RD and $\lambda_1$ provide greater insight into the cellular properties underlying the observed changes in FA, and presumably, white matter tracts. Specifically, RD is related to membrane integrity, and, to some extent, myelination; whereas $\lambda_1$ is related to factors that may disrupt the axon or neurofilament phosphorylation [67].

Recent studies have suggested that chronic pain is associated with neuroinflammation in the brain [238,381,920], which can lead to changes in both gray matter and white matter that can be detected by MRI. Therefore, changes in the measures of white matter microstructure may, in fact, be related to neuroinflammation rather than increases or decreases in the number or size of cells and/or axons present in the fields being studied. In this scenario, we would expect that changes in FA are associated to changes in $MD$ and RD, which are markers of inflammation and/or oedema [63,66].
Chapter 3
Rationale, Specific Aims and Hypotheses

The general aim of this thesis is to determine whether patients with TMD have structural abnormalities in the brain or CNV, compared to healthy, pain-free controls. To this end, three MRI-based studies have been conducted to assess gray and white matter abnormalities. The rationale, specific aims and hypotheses for each of these studies are provided below.

3.1. Study I. Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in TMD patients

TMD can be idiopathic in that there may not be any clear peripheral etiological factors identifiable [283,286,653] (see Chapter 2). In this scenario, it is thought that the CNS may initiate and/or maintain the pain [757]. Previous structural MRI studies of chronic pain populations have found both increases and decreases in brain gray matter (See Section 2.5.2). There are two main routes by which the CNS may contribute to the development and/or maintenance of chronic pains such as TMD. One possibility is that persistent or long-term nociceptive input into the brain induces maladaptive brain plasticity that can play a role in maintaining pain [12,950]. This maladaptive plasticity could hamper a patient’s ability to habituate to sustained nociceptive activity, possibly related to a reduced capacity of the brain to dampen pain by descending (top-down) controls [95]. Indeed, many structural MRI studies have found that chronic pain-related characteristics (intensity, unpleasantness, or duration) can in part account for gray matter abnormalities in chronic pain populations (See Section 2.5.2, and Table 2-3). Alternatively, inherent personality-related factors that reduce the brain's capacity to modulate nociceptive activity may contribute to gray matter abnormalities, and represents a vulnerability to develop chronic pain. For example, there is evidence that neuroticism may be associated with pain-related suffering (See Section 2.1.7), nerve injury outcomes and neuropathic pain [848] and inhibition of negative thoughts [178]. However, not all chronic pain patients have high neuroticism scores, and not all persons with neuroticism have chronic pain [179]. Therefore, neuroticism alone is not sufficient to develop chronic pain. Rather, the normal relationship between neuroticism and brain structure and function may be disrupted within regions involved in pain modulation, and this could facilitate or maintain chronic pain.
Therefore, the main aim of Study I is to examine gray matter abnormalities in patients with idiopathic TMD and to determine the contribution of pain-related characteristics and neuroticism.

**Specific aims:**

1. To determine whether patients with TMD have increased gray matter in regions of the brain implicated in pain perception, compared to healthy, pain-free controls.

2. To determine whether patients with TMD have reduced gray matter in regions of the brain implicated in pain modulation and motor function, compared to healthy, pain-free controls.

3. To determine the relative contribution of TMD pain characteristics (duration, intensity and unpleasantness) to gray matter abnormalities in the brains of patients with TMD.

4. To determine the relative contribution of neuroticism to gray matter abnormalities in the brain of patients with TMD.

**Specific hypotheses:**

Compared to healthy, pain-free controls, patients with TMD will have:

1. increased gray matter in brain areas associated with pain perception;

2. decreased gray matter in areas associated with pain modulation and motor function;

3. a positive correlation between gray matter in pain perception regions and pain intensity, unpleasantness and TMD duration; but a negative correlation between gray matter in regions implicated in pain inhibition and pain intensity, unpleasantness and TMD duration;

4. a positive correlation between gray matter in regions implicated in the affective dimension of pain and neuroticism, but a negative correlation between gray matter in regions implicated in pain inhibition and neuroticism.
3.2. Study II. Age-related gray matter abnormalities in patients with TMD

The brain undergoes structural changes due to normal conditions such as aging. For example, normal aging is characterized by cortical gray matter atrophy [84,99,372,585,627,824], although gains have also been reported in some brain areas [322,749]. Brain plasticity also occurs with dysfunction, injury, in specific diseases, and with chronic pain (see Section 2.5.2). The cumulative effect of chronic pain has been shown to interact with normal aging processes. For example, accelerated age-related whole brain gray matter atrophy has been reported in FMS [502] and chronic back pain [27]. Most aging studies in chronic pain have assessed global gray matter but little is known about the interaction between chronic pain and age in specific brain areas.

Although the functional significance of gray matter changes is not understood, MRI and histological studies indicate that changes can be stimulus-dependent. For instance, studies have demonstrated gray matter changes in response to learning [267], training [266], and repetitive noxious stimulation [852]. These data suggest that abnormal gray matter progression in chronic pain may be related to pain duration. While it is plausible that age-related changes specific to chronic pain are the product of cumulative pain exposure, this hypothesis has not been tested empirically in TMD.

Therefore, the main aim of study II is to determine whether the cumulative effect of idiopathic TMD interacts with the normal aging process in focal cortical regions associated with nociceptive processes.

Specific aims:

1) To determine whether patients with TMD exhibit greater rates of whole brain gray matter atrophy, compared to healthy, pain-free controls.

2) To determine whether TMD is associated with abnormal gray matter aging in focal cortical regions associated with pain processes.

3) The degree to which the cumulative effects of pain (i.e. TMD duration) contributes to the observed age effects.
Specific hypotheses:

1) Patients with TMD will have increased rates of whole brain gray matter atrophy, compared to healthy, pain-free controls.

2) Age-related gray matter changes will be increased in brain regions implicated in pain perception of patients with TMD,

3) Abnormal gray matter changes in patients with TMD will be driven by TMD duration, rather than age.

3.3. Study III. White matter brain abnormalities in temporomandibular disorder

Abnormal CNS function is thought to initiate or maintain TMD pain [757] based on TMD symptomology such as persistent pain, allodynia, and hyperalgesia (sometimes extending to regions distant from the face) [305,314,390,565,756,883], enhanced temporal summation of pain to repetitive noxious heat stimuli [563], and dysfunctional DNIC [113,491,756]. Abnormalities of these centrally-mediated processes suggest that ascending nociceptive pathways and/or descending pain-modulatory pathways [515] are affected in TMD. Additionally, patients with TMD can exhibit cognitive [370,379,380] and motor dysfunction [837] possibly related to abnormalities in brain regions associated with these functions [799,800,839,928].

Studies (including this thesis) indicate that patients with TMD have gray matter abnormalities in sensory, motor and cognitive/limbic regions (see Section 2.5.2 and Chapter 5). Our finding of a correlation of thalamic gray matter with TMD duration [617] supports the concept of long-term peripherally-induced central plasticity. Therefore, although clinical observations have not clearly identified gross peripheral abnormalities in TMD, the CNV may indeed undergo changes due to abnormal persistent activity resulting in microstructural abnormalities.

Previous studies examining white matter in other clinical conditions with sensory abnormalities and/or chronic pain [156,356,558,847] found white matter abnormalities in brain areas involved in sensory, modulatory and cognitive functions, and some of these white matter abnormalities correlated with clinical findings. Therefore, studying the correlation between TMD
characteristics and measures of white matter integrity can provide insight into whether chronic pain drives changes in white matter microstructure.

Therefore, the main aim study III is to evaluate white matter brain and CNV abnormalities in patients with idiopathic TMD and to determine the contribution of pain-related characteristics.

**Specific aims:**

1) To determine whether there are white matter abnormalities along the CNV of patients with TMD compared to pain-free controls.

2) To determine whether there are abnormalities in white matter tracts in the brain associated with sensory, cognitive or motor functions, compared to healthy controls.

3) To determine the connectivity of main regions of white matter disruption.

4) To determine whether there is a link between white matter microstructure and clinical characteristics of TMD.

**Specific hypotheses:**

Patients with TMD will have:

1) Abnormal white matter along the CNV;

2) Abnormal white matter in sensory, cognitive and motor tracts;

3) Abnormal connectivity of white matter regions with abnormal microstructure;

4) Abnormalities in white matter will be correlated to pain characteristics (TMD duration, pain intensity and pain unpleasantness).
Chapter 4
General Methods

This project was conducted in a cohort of female patients with TMD and an age-matched group of control subjects (see below).

4.1. Overview of Project

All study participants underwent two experimental sessions:

- **Questionnaires and task training session** – This session consisted of handedness and personality questionnaires. All subjects were also trained in an fMRI task that is not part of this thesis. Furthermore, patients underwent an interview to characterize their pain, and completed pain questionnaires.
- **Brain imaging session** – This session included acquisition of structural MRI sequences to assess gray and white matter structure. This session also included acquisition of Stroop task fMRI [928] and resting state fMRI that are not part of this thesis.

4.2. Subject Recruitment

Patients were recruited by dentists working in the Facial Pain Program at the Mount Sinai Hospital Dental Clinic. Patients were diagnosed with non-traumatic TMD based on the Mount Sinai Hospital Dental Clinics’ TMD diagnostic criteria (that are based on the TMD-RDC).

Specifically, inclusion criteria included: 1) Self-report of TMD pain in the masticatory muscles of greater or equal to 4/10 (with ‘0’ being equivalent to no pain at all and ‘10’ representing maximal pain) for at least 3 months or pain that is aggravated by mandibular function; and 2) moderate pain to palpation and/or persisting pain after examination in at least 3 masticatory muscle sites and/or moderate pain to palpation of the TMJ region and/or limitation in mandibular movement (opening less than 40 mm). Exclusion criteria included: 1) serious metabolic, rheumatoid or vascular disorders and other diseases; 2) other craniofacial pain disorders, previously diagnosed psychiatric disorders (e.g., depression, schizophrenia and attention-deficit hyperactivity disorder) or self-reported history of an abnormal neurological examination; 3) any contraindication to MRI scanning (e.g., claustrophobia, pregnancy and metal); and 4) use of psychotropic drugs. All participants were female because of the increased prevalence of TMD in females. If patients were eligible to participate in the study, the study was outlined to them, and
any of their questions were answered. If there were interested in participating in the study, they were provided with a letter outlining the general goals and methods of the study, and inviting the patients to participate (see Appendix I: Recruitment letter). If patients chose to participate, contact information was collected, so that they had a week to reconsider whether to participate in the study. Nineteen patients with TMD were initially included in the study, of which two were later excluded because they did not meet inclusion criteria.

The final study cohort included 17 patients with TMD (age ± SD: 33.1 ± 11.9 years; range: 18 – 59 years). Additionally, a group of 17 age-and sex-matched healthy controls (age ± SD: 32.2 ± 10.1 years; range: 20 – 50 years) was recruited from the University and Hospital environments. To recruit control subjects, the study was advertised by postings within the hospital. Control subjects would contact us by telephone or email, and undergo a basic screening over the telephone. The exclusion criteria for the healthy control cohort were the same as those mentioned for the patient cohort with the addition of a history of past chronic pain.

All study participants provided informed written consent to procedures approved by the University Health Network and Mount Sinai Hospital Research Ethics Boards (see Appendix II: Consent Forms).

One additional control subject was recruited and consented to REB approved procedures to replace one subject subsequently removed from the subcortical analysis (see below) because of poor segmentation in the pre-processing pipeline.

4.3. Questionnaires

4.3.1. Edinburgh Handedness Inventory

Handedness was determined using the Edinburgh handedness inventory [655] (see Appendix III). This questionnaire consists of ten questions about which hand is used to perform everyday manual activities. Subjects report whether they use their right or left hand, and whether they could use the opposite hand if they so desired. To tabulate, a laterality quotient is calculated with the following equation:
where $X(i,R)$ is the number of right-handed responses for each item, and $X(i,L)$ is the number of left-handed responses for each item. If a subject reports that the other hand cannot be used for a given task, then their response is weighted as 2 responses. The range of possible scores is between -100 and +100. If subjects scores between $<-40$ he or she is categorized as left-handed; if the subject scores between -40 and +40, then he or she is considered ambidextrous; and if the subject scores $>+40$, he or she is deemed right-handed.

### 4.3.2. NEO-Five Factor Inventory-Short Form

All subjects completed the NEO (Neuroticism Extraversion Openness) Five Factor Inventory (NEO-FFI; Psychological Assessment Resources, Lutz, Florida, USA) [178]. Participants were asked to indicate the degree to which they agree with a statement on a five-point Likert scale (strongly disagree, disagree, neutral, agree and strongly agree), each of which is coded to a number (0–4). A total of 15 of 60 questions probe for aspects of neuroticism in this questionnaire. Neuroticism was the only personality dimension that was assessed.

### 4.3.3. Pain Catastrophizing Scale

The Pain Catastrophizing Scale [836] (see Appendix IV) was also collected for all participants of this study, but they were not included in the analysis within this thesis.

### 4.3.4. Patient Interview

Patients were interviewed to characterize their TMD-related pain (Appendix V). Patients were also asked to provide a numerical pain score for their current pain intensity and pain unpleasantness verbally and were asked to report their average pain intensity and unpleasantness over the last month before scanning. They were specifically asked the following questions: “Please rate your current/average pain/unpleasantness rating over the last month on a scale of 0 to 10 (0 = no pain, 10 = worst pain imaginable)”. The duration of TMD was also documented for each patient.
Furthermore, patients completed the McGill Pain Questionnaire [591] (see Appendix VI). The McGill Pain questionnaire was used to assess the different dimensions of pain, and thus to specify the qualia of pain. The questionnaire comprised 102 words classified into three classes and sixteen subclasses. Different pain disorders have been related to a specific set of words from the questionnaire. For the current thesis, the McGill Pain Questionnaire was only collected as a qualitative tool, and was not further assessed. Results are presented in Appendix VII.

4.4. MR Imaging

4.4.1. Study design

All subjects participated in a 1-hour imaging session that included: 1) a low-resolution anatomical scan for co-registration of fMRI scans, 2) three “task” fMRI scans (not included in this thesis), 3) a high-resolution T1-weighted whole brain anatomical scan, 4) a resting state fMRI scan (not included in this thesis), and 5) two runs of DWI covering the whole head. After brain scanning, subjects were asked to complete a questionnaire related to the task fMRI scans. Of note, the task fMRI required subjects to wear special goggles to view images, and to use a response button box. These devices can increase the inhomogeneity during structural MRI acquisition and so the goggles and the response button-box were removed from the MRI prior to structural imaging.

4.4.2. Imaging parameters

Brain imaging data were acquired using a 3.0 T GE Signa HDx MRI system (General Electric, Milwaukee, WI) fitted with an eight-channel phased-array head coil. Subjects were placed supine on the MRI table and each subject's head was padded to reduce movement. Each subject was given a “panic button” which they could use if they felt uncomfortable and wanted to stop the scan.

For gray matter analysis, a whole brain three dimensional (3D) high-resolution anatomical scan was acquired with a T1-weighted 3D IR-FSPGR sequence: 128 axial slices, $0.94 \times 0.94 \times 1.5$ mm$^3$ voxels, $256 \times 256$ matrix size, field of view $= 24 \times 24$ cm, one signal average, flip angle=$20^\circ$, TE =5 ms, TR =12 ms, inversion time=300 ms.
For white matter analysis, two DWI scans were performed \((TR = 14,500 \text{ ms}, \text{field of view: } 24 \text{ cm x } 24 \text{ cm}, 128 \times 128 \text{ matrix, } 1.875\text{mm x } 1.875 \text{ mm in-plane resolution, } 3 \text{ mm thick axial slices, with Array Spatial Sensitivity Encoding Technique (ASSET) with a factor of } 2, \text{ maximum gradient strength } = 40 \text{ mT/m, maximum slew rate } = 150 \text{ T/m/s}) \text{ acquired along } 23 \text{ non-collinear, isotropic directions (}b = 1,000 \text{ s/mm}^2\). Additionally, 2 non-diffusion-weighted scans \((b = 0 \text{ s/mm}^2; b0)\) were acquired at the beginning of each run. The scans covered from the top of the head to the second cervical spinal process (C2).

### 4.4.3. Gray Matter Analysis

Two analysis methods were used to assess gray matter: CTA for cortical analysis \([316,538,559]\), and VBM for subcortical analysis \([36]\). We used masks of specific brain regions \((i.e., \text{ROI analysis})\) to test our specific \textit{a priori} hypotheses; this focus also reduced the statistical burden associated with the need to adjust the \(\alpha\) level in multiple comparisons to regions of no interest (masks specific to the different studies are described in Chapters 5 and 6).

#### 4.4.3.1. Cortical Thickness Analysis

FreeSurfer software v 4.5 (http://surfer.nmr.mgh.harvard.edu) was used to assess cortical thickness \([198,316,317,319,320]\). There are two automated pipelines embedded into the FreeSurfer analysis pipeline: the cortical (surface) stream and the subcortical (volumetric) stream. The subcortical stream is out of the scope of this thesis, and so it will not be discussed further. The cortical stream is described in \([198]\). The pipeline is as follows: (1) T1-weighted scans were normalized to Talairach stereotaxic space \([844]\). To do so, transformation parameters are computed “by using gradient descent at various scales to maximize the correlation between an individual volume and an average volume composed of a large number of previously aligned brains”\([198]\). The resultant transformation matrix (from individual space to Talairach space) is stored for later steps. (2) Anatomical T1-weighted images have MRI-susceptibility artifacts and RF-field inhomogeneities which cause varying intensities and contrast across the volume. These need to be corrected for proper segmentation. To do so, white matter is used to correct inhomogeneities and artifacts, because it is more spatially extensive than gray matter, and thus has minimal partial voluming issues, and allows for low spatial frequency inhomogeneities to be estimated. Inhomogeneities are corrected by adjusting for the variability in peak white matter signal between different slices. This process is called “estimating a B1 bias field” \([198]\). (3) The
skull is removed from the brain image. To do so, the inner surface of the skull is modeled using a deformation-based tessellated ellipsoid template [198] (see Figure 4-1).

**Figure 4-1:** Template deformation process for skull-stripping in FreeSurfer. Reproduced with permission from [198].

(4) Intensity values are used to classify white matter voxels as “white matter” or “not white matter”. (5) Planes based on the Talairach location of the corpus callosum and the pons are applied to separate the two hemispheres, remove the cerebellum and the brainstem (see Figure 4-2).

**Figure 4-2:** Cutting planes overlaid on sections through white matter-labeled volume. Reproduced with permission from [198].

(6) The surface that is the border of white matter and gray matter (*i.e.*, the outer surface of the white matter) is modeled as an initial surface for each hemisphere (see Figure 4.5). The modeled surface (called “white surface”; see Figure 4-3 and 4-5) undergoes smoothing based on local MRI intensity values to better reflect the gray/white border. (7) The white matter surface is then inflated to the gray/CSF surface (the “pial surface”; see Figure 4-4 and 4-5). Cortical thickness is defined as the distance between the white surface and the pial surface.
Figure 4-3: White surface overlaid on original T1 scan. Reproduced with permission from [198].

Figure 4-4: Pial surface overlaid on original T1 scan. Reproduced with permission from [198].

Figure 4-5: Original (left), gray/white boundary (middle), and pial surface (right) reconstructions of a left hemisphere. Reproduced with permission from [198].

Each subject’s homologous gyri and sulci were aligned to the standard average brain provided in FreeSurfer. A Gaussian spatial smoothing kernel of 6 mm full-width half-maximum (FWHM)
was applied to compensate for topographical heterogeneity prior to statistical analysis. For this thesis, the final sampling distance between adjacent points on the cortex (or two vertices) was 0.71 mm.

### 4.4.3.2. Voxel-Based Morphometry

A VBM analysis was used to measure the gray matter volume of subcortical structures.

Preprocessing of T1-weighted images and statistical analyses were performed in the SPM v5 software package ([http://www.fil.ion.ucl.ac.uk/spm/software/spm5/](http://www.fil.ion.ucl.ac.uk/spm/software/spm5/)) running under Matlab (The Mathworks, Inc, Natick, MA). We used the VBM 5.1 toolbox ([http://dbm.neuro.uni-jena.de/vbm](http://dbm.neuro.uni-jena.de/vbm)) implemented in SPM 5 to perform VBM. The methods are described in [36]. The pipeline included the following steps: DICOM file format images were converted to the NIfTI-2 file format using the DICOM import tool in SPM. Next, images were normalized to a standard template (International Consortium for Brain Mapping (ICBM) template), using a 12-parameter affine transformation. Next, the images were intensity normalized, and segmented based on tissue types using a Markov random field model and prior probability maps. The segmented gray matter posterior probability maps, underwent Jacobian modulation. The images were then spatially smoothed with a 10 mm FWHM Gaussian kernel. An absolute threshold mask of 0.10 (*i.e.*, the posterior probability of being gray matter must exceed 0.1) was set to restrict the results to gray matter. All coordinates were converted from Montreal Neurological Institute Stereotaxic Space (MNI; also the ICBM Standard Template) to Talairach space [844] using Yale BioImage Suite non-linear MNI to Talairach Coordinate Converter [510] ([http://www.bioimagesuite.org/Mni2Tal/index.html](http://www.bioimagesuite.org/Mni2Tal/index.html)) for results in Chapter 5, and the Lancaster transform [513] implemented in GingerALE v.2.0.4 ([http://www.brainmap.org/ale](http://www.brainmap.org/ale)) in Chapter 6.

### 4.4.4. White matter analysis

Diffusion-weighted images were converted from DICOM image file format to the NIfTI-1 file format (for FSL) using the dcm2nii application, as part of Chris Rorden’s MRIcon software package ([http://www.mccauslandcenter.sc.edu/micro/mricron/dcm2nii.html](http://www.mccauslandcenter.sc.edu/micro/mricron/dcm2nii.html)). The NIfTI-1 diffusion-weighted images were imported into FSL v.4.1.8 ([http://www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)) for quality control [816]. Pre-processing included eddy current and motion artifact correction using the FSL diffusion toolbox (FDT) [459]. This was achieved by aligning each 3D volume (each
DWI direction forms a 3D image; the image with all of the directions is called a 4D image) to the b0 (non-diffusion-weighted image) collected at the beginning of each scan. In this thesis, two b0 images were acquired for each run, and the 4D dataset was aligned to the second b0 image. Next, each subject’s two DWI runs were averaged to a single volume to achieve a greater signal-to-noise ratio. Then, individual brain masks were created using the Brain Extraction Tool (BET) [814] to cover the entire brain and exclude the skull, the eyes and other non-brain structures. The parameters were adjusted for each individual subject to obtain maximal brain coverage, while excluding as much of the non-brain tissue. These images were then processed through two different pipelines for (1) voxelwise analysis using TBSS and (2) probabilistic tractography.

4.4.4.1. Tract-based spatial statistics

TBSS v.1.2 [815,817] was used to compare FA between the TMD and healthy control cohorts. FA maps were created using DTIFIT tool in FDT. For TBSS, FA maps underwent non-linear registration to a 1 x 1 x 1 mm FA map in standard space (FMRIB58_FA, available in FSL), a mean image derived from all the subjects was created and thinned to represent the centre of major white matter tracts common to all subjects, forming a white matter skeleton. Each subject’s peak FA value perpendicular to the skeleton was then projected onto the skeleton. One skeleton was created for analysis of group differences that included all study participants, and a second skeleton based on the patients’ FA maps was created for correlation analyses of TMD pain characteristics (see below). The mean skeleton images were set at a threshold of FA > 0.2 to include FA values that are related to white matter [815]. Other DTI metrics were also evaluated to characterize FA findings (see Figure 7-1). Additionally, a separate patient group analysis was performed to assess which areas of the skeleton correlate with TMD characteristics (pain intensity, unpleasantness, duration).

4.4.4.2. TBSS with other DTI metrics

To gain more insight into FA findings, other DTI metrics were assessed in the clusters with significant group difference. \( \lambda_1 \) is thought to reflect diffusion along a tract, and reductions in this value suggest disruptions along the tract diffusivity. RD is believed to reflect changes in neural membrane permeability and, to some extent, myelination [66,67]. RD is calculated by averaging the two radial vectors of the tensor model (\( \lambda_2 \) and \( \lambda_3 \)). Finally, \( MD \) was measured because it is associated to oedema and inflammation. Figure 7-1 provides a schematic of these DTI metrics.
DTI metrics (FA, \(MD\), \(\lambda_1\), \(\lambda_2\) and \(\lambda_3\)) are calculated using DTIFIT in the FDT toolbox. The TBSS skeleton was created based on FA, and peak FA values are projected onto the skeleton. For statistical analysis of the other DTI metrics, \(MD\), RD and \(\lambda_1\) values from the same voxels are projected onto the skeleton. To test for group differences, we performed a multivariate analysis of variance (MANOVA) with each of the DTI metrics as dependent variables, and group as an independent variable.

4.4.4.3. Probabilistic tractography

We sought to better characterize the corpus callosum cluster identified using cluster-mass correction (see section 7.3.3) by elucidating its connectivity. Therefore, we performed multi-fibre probabilistic tractography (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html) [73,74] to determine pathways passing through this cluster, and to elucidate its connectivity. To do so, the preprocessed diffusion-weighted images were downsampled in order to create isotropic voxels (3 x 3 x 3 mm). The images were then processed in FDT. Probability density functions (PDF) on up to 2 principal fibre directions were estimated at each voxel in the brain. Multi-fibre tractography was used and 5000 samples were drawn from each seed voxel along the PDFs. Significant clusters from the TBSS group difference analysis were binarised and used as seeds for tractography. Together, each seed voxel’s streamlines provide an estimate of its connectivity. When a streamline reaches a voxel with more than one fibre direction, the streamline follows the direction closest to the direction at which it arrives at the voxel. The pathways generated by the algorithm represent the number of samples that have passed through a voxel. The pathways generated in each subject were thresholded at 10 samples (of the 5000 generated from each seed voxel; \(i.e., \, 0.2\%\)) to eliminate spurious connections. These thresholds provided consistent tracts between subjects. For visualization, each of the subject’s tracts were binarised and overlaid on a standard brain to produce a probabilistic map of the pathways for controls and patients. The values in these maps represent the number of subjects who share a common pathway.
Chapter 5
STUDY I: Contributions of chronic pain and neuroticism to abnormal forebrain gray matter in TMD

This study was published in *Neuroimage*:


5.1. Introduction

TMD represent the most common orofacial chronic pain disorder, and are more prevalent in women than in men [721]. TMD can be idiopathic in that there may not be any clear peripheral etiological factors identifiable [283,286,287,653]. Thus, central mechanisms have been proposed to account for TMD pain [757].

Although a clear pattern of change has yet to be determined, previous structural MRI studies of chronic pain populations have found both increases and decreases in gray matter. For instance, some studies of headache and chronic facial pain populations have found that patients with chronic pain had gray matter increases in regions likely associated with pain perception [207,575,650,963]. Additionally, most studies of chronic pain patients have found reduced gray matter in cortical regions likely associated with antinociception and limbic function [98,356,575]. Interestingly, some studies have also reported gray matter loss in cortical and subcortical motor areas [22,575,763]. However, the increases are not limited to regions thought by some to be associated with pain perception, and the decreases are not limited to pain-modulatory regions. Although the role of motor regions in pain is not fully established, there is evidence suggesting these areas play a role in pain modulation [3,117,189,342,345,547]. In support of this concept are the motor abnormalities that can accompany chronic pain [471,492,839,928], possibly related to nocifensive behaviour [634].

There are two main routes by which the central mechanisms may contribute to the development and/or maintenance of chronic pains such as TMD. One possibility is that long-term nociceptive input into the brain induces maladaptive brain plasticity, which may play a role in maintaining pain [12,950]. For example, a recent study demonstrated that experimental pain that increased
gray matter in nociceptive regions [852], induced pain habituation over time that was accompanied by decreased activity within nociceptive areas and increased activity within the antinociceptive system [94]. Chronic pain patients, however, may not be able to adapt in this way to nociceptive activity. For example, neuroimaging studies of chronic pain have shown hyperactivity in nociceptive regions, and hypoactivity in antinociceptive regions [23,541]. Chronic pain patients’ inability to habituate to increased nociceptive activity may be related to a reduced capacity of the brain to dampen pain by descending (top-down) controls [95]. Indeed, many structural MRI studies have found gray matter differences in chronic pain populations associated with pain-related characteristics (intensity, unpleasantness or duration) [27,98,575,741,963].

The second route by which the CNS may contribute to the development and/or maintenance of chronic pain relates to inherent personality-related factors that reduce the brain’s capacity to modulate nociceptive input. This poor pain control represents a vulnerability to develop chronic pain. For example, there is evidence that neuroticism may be associated with pain-related suffering [408], pain sensitivity [177,373,907], nerve injury outcomes and neuropathic pain [848] and inhibition of negative thoughts [178]. However, not all chronic pain patients have high neuroticism scores, and not all persons with neuroticism have chronic pain [179]. Therefore, neuroticism alone is not sufficient to develop chronic pain. Rather, the normal relationship between neuroticism and brain structure and function may be disrupted within regions involved in pain modulation, such as the OFC [954] or the mPFC [245,386] and this could facilitate or maintain chronic pain.

Thus, in the current study we examined gray matter abnormalities in patients with idiopathic TMD and focused our investigation on the contribution of pain-related characteristics and neuroticism. Towards this goal, we measured gray matter in patients who had suffered from TMD over a range of pain intensities, unpleasantness and for varying durations, and neuroticism scores. Based on the aforementioned behavioural and neuroimaging studies, we specifically tested the hypotheses that patients with TMD will have: 1) increased gray matter in areas associated with pain perception; 2) reduced gray matter in areas associated with pain modulation and motor function; 3) gray matter positively correlates with pain intensity, unpleasantness and TMD duration within areas associated with pain perception areas and negatively correlates with gray matter in areas associated with antinociception; 4) negative correlation between neuroticism
and gray matter in regions implicated in pain modulation, and positive correlation in regions implicated in the affective dimension of pain, because of the interaction between affective processing and pain modulation in TMD [881].

5.2. Methods

A detailed description of methods is provided in Chapter 4.

5.2.1. Subjects

A group of 17 females with idiopathic TMD (mean age ± SD: 33.1 ± 11.9 years) and 17 healthy females (mean age ± SD: 32.2 ± 10.1 years) provided informed written consent to procedures approved by the University Health Network and Mount Sinai Hospital Research Ethics Boards. All subjects were right-handed. Patients with TMD were screened with the Mount Sinai Hospital Dental Clinic TMD diagnostic criteria, based on the TMD-RDC [285]. Inclusion criteria included: 1) TMD pain masticatory muscle region greater or equal to 4/10 for at least 3 months or pain that is aggravated by mandibular function; 2) moderate pain to palpation and/or persisting pain after examination in at least 3 muscle sites and/or moderate pain to palpation of the TMJ region and/or limitation in the mandibular movement (opening less than 40mm). Patients were asked to remain analgesic-free for 24 hours prior to scanning, as functional data was also being collected during the scanning session. For both patients and control subjects, exclusion criteria included: 1) serious metabolic, rheumatoid or vascular disorders and other diseases; 2) other craniofacial pain disorders, previously diagnosed psychiatric disorders (e.g., depression, schizophrenia, ADHD) or self-reported history of an abnormal neurological examination; 3) any contraindication to MRI scanning (e.g., claustrophobia, metal); 4) use of psychotropic drugs. In addition, healthy controls were not eligible for the study if they had a history of chronic pain.

5.2.2. Questionnaires

Each participant completed the NEO-FFI [178]. The NEO-FFI is a self-report questionnaire comprising of 60 statements. Participants were asked to indicate the degree to which they agree with a statement on a five-point scale (strongly disagree, disagree, neutral, agree, strongly agree), each of which is coded to a number (0-4). A total of 15 of 60 questions probe for aspects of neuroticism in this questionnaire.
Patients were also asked to rate their current pain and unpleasantness and their average pain intensity and unpleasantness over the last month before scanning with the following instructions: “Please rate you current/average pain/unpleasantness rating over the last month on a scale of 0 to 10 (0 = no pain, 10 = worst pain imaginable)”. The duration of the patients’ TMD was also recorded for each patient.

5.2.3. Imaging parameters

Brain imaging data were acquired using a 3T GE MRI system fitted with an eight-channel phased array head coil. Subjects were placed supine on the MRI table and each subject’s head was padded to reduce movement. A whole brain three dimensional (3D) high-resolution anatomical scan was acquired with a T1-weighted 3D IR-FSPGR sequence: 128 axial slices, 0.94 x 0.94 x 1.5 mm³ voxels, 256 x 256 matrix size, field of view = 24 x 24 cm, one signal average, flip angle = 45°, TE = 5ms, TR = 12ms, TI = 300ms.

5.2.4. Structural brain imaging analysis

Because we were interested in both cortical and subcortical structures, we used two analysis approaches to measure gray matter from high-resolution MRI images. To evaluate differences in cortical thickness (measured in mm) we used CTA [316,538,559], and VBM [36] was used to measure subcortical gray matter density. We used masks of specific brain regions (i.e., ROI analysis) to test our specific a priori hypotheses; this focus also reduced multiple comparisons to regions of no interest (see below).

5.2.4.1. Cortical thickness analysis

CTA was carried out using the Freesurfer software (http://surfer.nmr.mgh.harvard.edu); methods are described in full detail elsewhere (see:[198,316,317,319,320]. Briefly, pre-processing included intensity normalization, skull stripping, separation of the hemispheres, and gray matter segmentation. The white matter/gray matter border (i.e., white surface) and gray matter/CSF border (i.e., pial surface) were identified and modeled as surfaces. The software then calculated the distance between the two borders at every point on the cortex, for each hemisphere. The gray matter surface was then warped such that homologous gyri and sulci were aligned across all subjects. Each individual subject’s cortex underwent automatic anatomical parcellation, and each sulcus and gyrus was labeled during pre-processing [321]. To restrict our search to regions
hypothesized to be involved in TMD chronic pain, we used the cortical parcellations map implemented into FreeSurfer (aparcs2005) on a standard subject (fsaverage). Two masks were created: (1) a sensorimotor/pain mask which included S1, S2, M1, and MCC (see Figure 1 a); and (2) a cognitive/modulatory mask of the frontal lobe that included the OFC, PFC, insula and the ACC/MCC (see Figure 1 b). The MCC has been implicated in both nociception and pain modulation [23] and so this region was included in both masks. To compensate for topographical heterogeneity amongst the different subjects, a 6 mm FWHM Gaussian spatial smoothing kernel was applied to the data prior to statistical analysis. A vertex represents a point on a two-dimensional sheet, and, in this study, the distance between two vertices is 0.71 mm.

5.2.4.2. Subcortical analysis with VBM

Image processing and statistical analyses were performed in the SPM5 software package (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) running under Matlab (Mathworks). The VBM analysis used Gaser’s VBM 5.1 toolbox (http://dbm.neuro.uni-jena.de/vbm) within SPM 5; detailed methodology is described in elsewhere [36]. Briefly, the preprocessing included setting the origin of the image at the anterior commissure of each subject, spatial normalization to the ICBM-152 template, gray matter segmentation, Jacobian modulation to adjust for the effects of spatial normalization, and spatial smoothing with a 10 mm FWHM Gaussian kernel. Total intracranial volume (TIV) was calculated for each subject from the volume estimates output during segmentation. An absolute threshold mask of 0.10 was applied to restrict the results to gray matter only. We constructed a single mask for our subcortical VBM analysis (See figure 1 c). We used prefabricated anatomical masks in WFU Pickatlas (http://www.nitrc.org/projects/wfu_pickatlas). The subcortical mask was used to investigate regions that cannot be investigated using CTA, and comprised the basal ganglia, amygdala, brainstem and thalamus.

5.2.5. Statistical analyses

5.2.5.1. Demographics

Group differences between characteristics of the patients and controls were evaluated using a multivariate analysis of variance in SPSS 18.0 (http://www.spss.com/). Age and neuroticism scores were compared between the groups. The significance threshold was set at p < 0.05.
5.2.5.2. Gray matter group differences

CTA

To restrict our search to regions hypothesized to be involved in TMD chronic pain, we used the cortical parcellations map implemented into FreeSurfer (aparcs2005) on a standard subject (fsaverage). Two masks were created: (1) a sensorimotor/pain mask which included S1, S2, M1, and the MCC (see Figure 5-1 a); and (2) a cognitive/modulatory mask of the frontal lobe that included the OFC, PFC, insula and the ACC and MCC (see Figure 5-1 b). The MCC has been implicated in both nociception and pain modulation [23] and so this region was included in both masks.

To test our first hypothesis that patients have thicker cortex in perception areas, we ran a general linear model (GLM) testing for group differences in cortical thickness at every vertex within the sensorimotor/pain mask (see Figure 5-1a). Age was included as a regressor of no interest. To test our second hypothesis that patients have thinner cortex in modulatory/cognitive areas, we ran a second GLM testing for group differences at every vertex within the cognitive/modulatory mask (see Figure 5-1b). Percent change was calculated using the following formula: ((Mean of patients’ thickness - mean of controls’ thickness)/mean of controls’ thickness) × 100.

All data were thresholded at an experiment-wide Bonferroni corrected p < 0.05. To do so, we calculated the corresponding cluster-wide p-value to reach significance and so the experiment-wide correction was based on conducting 6 tests: (1) thicker cortex in pain perception regions, (2) thinner cortex in cognitive/modulatory regions, (3) the contribution of pain intensity, (4) the contribution of pain unpleasantness, (5) the contribution of TMD duration to cortical abnormalities, and (6) group by neuroticism interactions in cortex. Thus the correction for the threshold was 0.05/6 = 0.008. Therefore, to achieve a Bonferroni-corrected threshold of p < 0.05, CTA data were thresholded at image-wide threshold of p < 0.008. Our two masks (see Figure 5-1) were of different sizes; thus, we used Monte Carlo permutation to calculate an image-wide threshold for each. For the sensorimotor/pain mask, this was derived from an uncorrected voxelwise p < 0.01 and 190 contiguous vertices (379 voxels), and for the cognitive/modulatory mask, this was derived from an uncorrected voxelwise p < 0.01 and 220 contiguous vertices (436 voxels). Monte Carlo simulations were run with 1000 iterations using AlphaSim (http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim), as we have used previously [847].
We constructed a single mask for our subcortical VBM analysis (See figure 5-1c). We used prefabricated anatomical masks in WFU Pickatlas (http://www.nitrc.org/projects/wfu_pickatlas). The subcortical mask was used to investigate regions that cannot be investigated using CTA, and comprised the basal ganglia, amygdala, brainstem and thalamus. To test both our first and second hypotheses, we used student’s t-test to test for group differences in each voxel within the subcortical mask (see Figure 5-1c). The general linear model included age and TIV as covariates of no interest. All data were thresholded at a voxelwise corrected p < 0.05 using false discovery rate (FDR), as implemented in SPM 5.

5.2.5.3. Clinical correlates

To assess pain-related plasticity, we correlated pain intensity, pain unpleasantness and TMD duration to gray matter. For all the correlations, age was included as a regressor of no interest. However, for the correlation of gray matter to TMD duration we could not include age as a covariate, as these two explanatory variables (age and TMD duration) are correlated (r = 0.536, p = 0.027). For the CTA analysis, pain intensity, pain unpleasantness and TMD duration correlations were performed within the sensorimotor/pain mask and the cognitive/modulatory mask. For the VBM analysis, we ran correlations in each voxel within our subcortical mask, as previously described. TIV was included as a covariate of no interest in every model.

5.2.5.4. Neuroticism effects

To determine whether neuroticism may be affected by chronic pain in TMD, we performed a Pearson correlation between TMD duration and neuroticism scores in the patient group.

To investigate whether neuroticism has an abnormal relationship to gray matter, and thus contribute to TMD onset, we performed an interaction analysis. Specifically, in CTA, we performed a group by neuroticism interaction using the FreeSurfer software on a vertex-wise basis within the cognitive/modulatory mask. In the VBM analysis, to test this hypothesis, we performed a group by neuroticism interaction in each voxel within our subcortical mask.
5.3. Results

5.3.1. Patient demographics

The characteristics of individual patients are shown in Table 5-1. Patients and controls did not differ in age (patients: mean age ± SD: 33.1 ± 11.9 years; controls: 32.2 ± 10.1 years; p = 0.94) or in neuroticism scores (patients: mean age ± SD: 19.35 ± 6.84; controls: 18.35 ± 7.95; p = 0.70). Of importance for this study is that there was variability in the durations of TMD, intensity and unpleasantness of pain that facilitated assessment of these factors in correlation analyses. The duration of TMD ranged from 0.75 – 30 years (mean ± SD: 9.8 ± 8.2 years), while pain intensity ranged from 2 – 7 (mean ± SD: 4.3 ± 1.8), and unpleasantness ranged from 1 – 8 (mean ± SD: 5.4 ± 2.1). Interestingly, TMD pain intensity and pain unpleasantness scores were not significantly correlated (r = 0.40, p = 0.12), unlike the tight correlation of these dimensions in healthy subjects in acute pain paradigms [717]. Patients reported that their TMD pain was confined to the TMJ (n = 6), the muscles of mastication (n = 3), or both the joint and muscles (n = 8). Most patients reported bilateral pain (n = 13), but some reported unilateral pain (three right-sided, one left-sided). The duration of TMD significantly correlated with the patients’ age (r = 0.536, p = 0.027).

Patient neuroticism scores showed no significant relationship to TMD duration (r = 0.33, p = 0.21).

5.3.2. Group differences: S1 and frontal thickening in TMD

There were prominent group differences (p < 0.05, corrected) in gray matter thickness in the patients with TMD compared to controls (see Table 5-2, Figure 5-2). In line with our first hypothesis, the ventrolateral S1 was 22% thicker in the TMD group compared to controls. This region of thickening was located in a region consistent with the somatosensory representation of the face [205,630]. However, contrary to our second hypothesis, we did not identify statistically significant cortical thinning in any pain modulation or motor region. Unexpectedly though, we identified regions in the left frontal pole and vIPFC that were thicker in the patient group compared to controls by 17% and 15% respectively (see Table 5-2 and Figure 5-2). The VBM analysis did not reveal any significant subcortical differences between patients and controls.
5.3.3. Effect of chronic pain intensity and unpleasantness

We also examined the relation between pain intensity and pain unpleasantness in gray matter within our cortical masks. Cortical thinning that correlated with the degree of TMD pain intensity or unpleasantness was found within three brain regions (see Figure 5-3 and Table 5-3). A significant negative correlation was found between pain intensity and gray matter thickness in the aMCC (BA32; r = -0.83), and in the ventrolateral aspect of M1 in a region consistent with the representation of the face [952] (r = -0.83). Our test of the impact of pain unpleasantness on cortical thickness within our two masks revealed a significant effect only in left OFC. This region showed a negative correlation between gray matter thickness and unpleasantness (BA 47; r = -0.74). Interestingly, in these three brain areas, the patients reporting intense pain had gray matter that was thinner than the healthy controls, but the individuals with only mild pain had gray matter either slightly thicker than the controls or within the normal range. Of note is that we did not identify any significant gray matter subcortical correlations with pain intensity or unpleasantness.

5.3.4. Effect of TMD chronicity

The subcortical VBM analysis revealed that gray matter in the sensory thalamus was positively correlated to the length of time that patients had been suffering with TMD (r = 0.91; see Table 5-4 and Figure 5-4 for details). This region of gray matter increase included the left PO, the left VL, and bilateral VPM of the thalamus. Interestingly, the patients who had TMD for only a few years showed gray matter within the normal range (see Figure 5-4), but patients who had TMD for 7 or more years showed a stronger relationship between duration and thalamic gray matter. We did not find any significant correlations between TMD duration and gray matter thickness in the a priori hypothesized cortical ROI.

5.3.5. Effect of neuroticism

Because of the expectation that neuroticism manifests differently in patients than healthy controls, we tested whether there was neuroticism by group interaction in the cognitive/modulation mask. The controls showed a negative correlation (r = -0.32) between gray matter thickness in the left OFC (BA11; Talairach coordinates: -18, 22, -18) and neuroticism scores, whereas patients show a positive relationship (r = 0.61) in this region. The area of the interaction in this ROI was 517 mm² (peak vertex t-score = -2.87; p < 0.05, experiment-wide
Bonferroni corrected; see Figure 5-5). We did not identify any significant neuroticism by group interactions in the subcortical VBM analysis.
Table 5-1: Patient demographics.

<table>
<thead>
<tr>
<th>#</th>
<th>Age</th>
<th>TMD Dur (yrs)</th>
<th>Pain Intensity</th>
<th>Pain Unpl</th>
<th>Pain Sites</th>
<th>TMD Laterality</th>
<th>Other pains</th>
<th>Meds</th>
<th>Oral contraceptive</th>
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<tr>
<td>1</td>
<td>22</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>J</td>
<td>Bilateral</td>
<td>Knee pain</td>
<td>NSAID</td>
<td>Yes</td>
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<tr>
<td>2</td>
<td>20</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>M, J</td>
<td>Bilateral</td>
<td></td>
<td>A*, cyc*</td>
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</tr>
<tr>
<td>3</td>
<td>24</td>
<td>7</td>
<td>4</td>
<td>8</td>
<td>J</td>
<td>Right</td>
<td>Headache</td>
<td>N, P</td>
<td>Yes</td>
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<tr>
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<td>38</td>
<td>20</td>
<td>7</td>
<td>6</td>
<td>J</td>
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<td>NSAID</td>
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<td>5</td>
<td>42</td>
<td>0.75</td>
<td>2</td>
<td>3</td>
<td>M</td>
<td>Bilateral</td>
<td>F*</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>M, J</td>
<td>Bilateral</td>
<td>Shoulder, Neck</td>
<td>A*, F*</td>
<td>No</td>
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<tr>
<td>7</td>
<td>28</td>
<td>17</td>
<td>3</td>
<td>5</td>
<td>M, J</td>
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<td></td>
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<td></td>
<td>Hy*</td>
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<td>10</td>
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<td>6</td>
<td>M</td>
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<td>A, Ch, Di</td>
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<td></td>
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<td>59</td>
<td>13</td>
<td>7</td>
<td>7</td>
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<td>(neck and shoulder), Gastric Distress</td>
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<tr>
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<td>34</td>
<td>15</td>
<td>2</td>
<td>3</td>
<td>M</td>
<td>Right</td>
<td>Neck, Sciatica</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>52</td>
<td>30</td>
<td>4</td>
<td>5</td>
<td>M, J</td>
<td>Bilateral</td>
<td>A</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>31</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>M, J</td>
<td>Right</td>
<td>Ovarian cyst</td>
<td>N</td>
<td>No</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>17</td>
<td>5</td>
<td>8</td>
<td>M, J</td>
<td>Bilateral</td>
<td>A, F, NSAID</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>22</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>J</td>
<td>Bilateral</td>
<td></td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>23</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>J</td>
<td>Bilateral</td>
<td></td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: M= masticatory muscles; J=TMJ; NSAID: Non-steroidal anti-inflammatory, over the counter, as needed; A: Arthrotec; cyc: cyclobenzapine; Ch: Champix; Di: Dixarit (Clonidine); F: Flexoril; Hy: Hydromorphone; Meds: Medications; N: Naproxen; P: Prevacid; TMD Dur: TMD duration; Unpl: unpleasantness; yrs: years. The asterisk (*) denotes that subjects discontinued the use of the drug prior to our study. A and N are also NSAIDs, but were
patients were prescribed to take these medications daily- rather than when needed. Gastric distress was described as “burning in the stomach” by the patient.
Table 5-2: Group differences in cortical thickness.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Region</th>
<th>BA</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Area (mm²)</th>
<th>Peak T-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients &gt; Controls</td>
<td>R S1</td>
<td>2</td>
<td>50</td>
<td>-18</td>
<td>37</td>
<td>1132</td>
<td>5.18</td>
</tr>
<tr>
<td></td>
<td>L FP</td>
<td>10</td>
<td>-30</td>
<td>45</td>
<td>6</td>
<td>569</td>
<td>4.87</td>
</tr>
<tr>
<td></td>
<td>L vlPFC</td>
<td>9/10</td>
<td>-38</td>
<td>35</td>
<td>4</td>
<td>531</td>
<td>3.41</td>
</tr>
</tbody>
</table>

Peak vertex Talairach coordinates are reported. All results are significant at p < 0.05, corrected for multiple comparisons. Abbreviations: R: right; L: left; BA: Brodmann’s Area; FP: frontal polar cortex; S1: primary somatosensory cortex; vlPFC: ventrolateral prefrontal cortex.
Table 5-3: Cortical thickness negatively correlates with TMD pain intensity or unpleasantness.

<table>
<thead>
<tr>
<th>TMD attribute</th>
<th>Region</th>
<th>BA</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Area (mm$^2$)</th>
<th>T-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain Intensity</td>
<td>L aMCC</td>
<td>32</td>
<td>-4</td>
<td>18</td>
<td>19</td>
<td>718</td>
<td>-3.94</td>
</tr>
<tr>
<td></td>
<td>L M1</td>
<td>4</td>
<td>-57</td>
<td>-3</td>
<td>13</td>
<td>674</td>
<td>-4.15</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>L OFC</td>
<td>11/47</td>
<td>-27</td>
<td>16</td>
<td>-19</td>
<td>472</td>
<td>-4.22</td>
</tr>
</tbody>
</table>

Peak vertex Talairach coordinates (TAL) are reported. All results are significant at $p < 0.05$, corrected for multiple comparisons. Abbreviations: L: left; aMCC: anterior mid-cingulate cortex; M1: primary motor cortex; OFC: orbitofrontal cortex.
Figure 5-1: Masks used to restrict analyses. Three masks were used to restrict the analyses to regions of interest: a) the sensorimotor/pain mask included cortical sensorimotor regions (primary and secondary somatosensory cortices (S1, S2), the primary motor cortex (M1), and the mid-cingulate cortex (MCC), and b) the cognitive/modulatory mask which included the insula, the prefrontal cortex (PFC), the orbitofrontal cortex (OFC), and the cingulate cortex (ACC/MCC), c) a subcortical mask used for voxel-based morphometry, included the basal ganglia, thalamus, amygdala and the brainstem. CTA results are overlaid onto the fsaverage standard brain in FreeSurfer.
**Figure 5-2:** Cortical thickening in TMD. The CTA GLM group analyses within the cortical masks identified three cortical regions (shown in blue on the brain images) that showed significant group differences at a corrected $p < 0.05$. The top panel shows that the TMD patient group had thicker right primary somatosensory cortex (S1) (peak $T$-score = 5.18) compared to controls, with the effect of age regressed out. The bottom panel shows that the TMD patient group has thicker ventrolateral (peak $T$-score = 3.41) and frontal polar cortices (peak $T$-score = 4.87). All labeled regions are significant at a corrected $p < 0.05$. The graphs indicate the mean cortical thickness ± SE (age not regressed out). CTA results are overlaid onto the fsaverage standard brain in FreeSurfer.
**Figure 5-3:** TMD pain intensity and unpleasantness are negatively correlated to modulatory regions. The CTA regressions within the cortical masks in the TMD group identified three cortical regions (shown in blue on the brain images) that were significantly correlated with individual pain scores at a corrected $p < 0.05$. The top panel shows that pain intensity has a negative correlation to anterior mid-cingulate cortex (aMCC) ($r = -0.83$, peak $T$-score $= -3.94$).
The middle panel shows that pain intensity is negatively correlated to cortical thickness in the left primary motor cortex (M1) \(r = -0.83\), peak \(T\)-score = -4.15). The bottom panel shows that pain unpleasantness negatively correlated to cortical thickness in the left orbitofrontal cortex (OFC; \(r = -0.75\), peak \(T\)-score = -4.22), with the effect of age regressed out). All labeled regions are significant at a corrected \(p < 0.05\). The graphs indicate the mean cortical thickness \(\pm SE\) for each subject versus pain intensity (age not regressed out). CTA results are overlaid onto the fsaverage standard brain in FreeSurfer.
Figure 5-4: TMD duration is related to plasticity in the thalamus. The TMD patient group showed a positive correlation between TMD duration (measured in years) and gray matter in a cluster in the sensory nuclei in the left thalamus (posterior (PO), ventral lateral (VL), ventral posterior medial (VPM) (2312 voxels, peak T-score = 7.84, r = 0.91). The finding is significant at a false-discovery rate corrected p < 0.013. The graph indicates each TMD patient’s gray matter volume versus her individual TMD duration (in years). The dashed lines represent the mean gray matter volume of controls ± SE. VBM results are displayed on the single subject standard brain in SPM.
Figure 5-5: Patients with TMD showed a positive relationship between OFC thickness and neuroticism, which is considered to be abnormal. Neuroticism was correlated with changes in the left orbitofrontal cortex (OFC; peak T-score = 3.97), with the effect of age regressed out. For all correlations, mean cortical thickness ± SE (age not regressed out) versus the raw neuroticism score was graphed. CTA results were overlaid onto the fsaverage standard brain in FreeSurfer.
5.4. Discussion

This structural imaging study identified striking abnormalities in patients with chronic idiopathic TMD pain and highlights the contribution of both TMD-related and neuroticism-related factors. Our key findings were that, compared to controls, patients with TMD had 1) cortical thickening of the S1, frontal pole and vlPFC, 2) pain intensity-dependent cortical thinning in the aMCC and M1 and pain unpleasantness-dependent cortical thinning in the OFC, 3) TMD duration-dependent gray matter increase in the sensory thalamus, and 4) a positive correlation between cortical thickness in the OFC and neuroticism, in contrast to a normally negative correlation.

5.4.1. S1 and thalamic gray matter increases in TMD

Our finding of S1 thickening in TMD is consistent with other gray matter studies of chronic pain in the trigeminal system [207]. However, trigeminal chronic pains are heterogeneous, and so may produce different patterns of gray matter changes specific to the particular symptomology or pathology. Nonetheless, the convergence of increased S1 thickness suggests that there may be prolonged or repeated barrage of nociceptive input to the cortex from the thalamus. Evidence for this hypothesis comes from a study by Teutsch and colleagues [852] that showed persistent noxious stimulation in healthy subjects can induce increased gray matter in S1. Since we also found that in patients with TMD there was a positive correlation between thalamic gray matter and TMD duration, it is possible that sustained trigeminothalamic nociceptive activity over time leads to increased gray matter in sensory thalamus. The patients included in our study have a larger range of TMD durations (0.75-30 years) compared to the study by Younger et al. [962] (0-11 years). Thalamic changes were more apparent in patients with longer reports of TMD duration, which may explain why our findings are different than those reported by Younger and colleagues [963]. Increased firing to the thalamus may lead to increased thalamocortical activity to S1 and plasticity [950]. The cellular basis of cortical thickening (plasticity) is not yet established but is thought to include increases in synaptic boutons, dendritic branching, glial cells, and/or neurones [575,608].

5.4.2. Cortical thickening in cognitive and modulatory regions in TMD

We found that patients with TMD had cortical thickening in the frontal pole. These findings diverge from those of Younger et al. [963] study. A factor that may have contributed to the
different findings is that their study examined patients with myofascial TMD, whereas our TMD group consisted of a somewhat more clinically representative group of mixed muscular and/or joint TMD.

Activation of the frontal pole has been reported during spontaneous pain in patients with chronic pain [47]. The frontal pole has also been implicated in a number of complex executive cognitive functions such as learning behavioural routines [457,496,834], cognitive branching (the ability to put a pending task on hold to execute an ongoing one) [497], behavioural flexibility/adaptability [105] and post-hoc monitoring or evaluating decisions based on feedback [875]. Therefore, the frontal pole may process the cognitive dimension of pain, which suggests that pain has a cognitive load. We propose that the cognitive load of pain may require constant engagement of the frontal polar cortex for cognitive branching. That is, chronic pain may need to be put “on hold” in order for a patient to engage in another competing cognitive load. However, if the cognitive branching system is limited, a chronic pain load may impact the ability to properly complete tasks [529]. In support of this, we have recently shown that patients with TMD have sluggish reaction times to low and high conflict cognitive interference tasks, but not to a simple sensorimotor task [928]. Further evidence for cognitive branching of pain comes from studies showing that an increase in cognitive load modulates acute pain perception in healthy controls [935]. Therefore, one possibility for the thickening of the frontal polar cortex is that chronic TMD pain bears a cognitive load that the brain needs to ‘put on hold’ in order to address more immediate environmental demands.

The other frontal area we found to be thicker in TMD than controls was the vlPFC. This region has been implicated in pain anticipation [752], pain modulation [909], and behavioural inhibition [32]. There is evidence that patients with TMD are hypervigilant [435,808], which is related to increased pain anticipation. Therefore, vlPFC thickening may be related to patients’ hypervigilance and increased anticipation to pain.

5.4.3. Relationship between chronic pain intensity and cortical thickness in M1 and aMCC

We found that gray matter in the orofacial region of M1 showed a negative correlation to pain intensity. Several studies have reported abnormal stimulus-evoked fMRI activity in motor regions of chronic pain patients, although the implication of these abnormalities is not often
discussed (see: [23]). One interpretation of our finding is that there is an adaptive mechanism that responds to sustained, intense nociceptive activity by dampening motor output to prevent further damage to the affected region. Evidence for this concept comes from studies of both acute and chronic orofacial pains. For instance, TMD pain inhibits craniofacial motor function [839], and dampens motor neurone output [556]. Similarly, acute pain stimuli applied to the orofacial region can decrease M1 excitability [3,109]. Interestingly, Kirveskari et al. [492] reported that patients with CRPS had weaker M1 reactivity with increased spontaneous pain ratings, that is to say that there is a negative correlation between pain intensity and M1 activation. Another possibility is that patients with TMD make fewer jaw movements to avoid eliciting pain. This reduced jaw activity may lead to atrophy of the orofacial region of the motor cortex. Conversely, there is evidence to suggest that M1 may play a role in central processing of pain [11,244,476,785] and pain modulation [189]. For instance, motor cortex stimulation has been shown to have some, albeit limited, analgesic effect in chronic pain [342,689]. Therefore, another interpretation of our finding is that M1 may be, in part, involved in descending modulation of pain.

The other gray matter region that we identified that was negatively correlated to pain intensity is the aMCC. This finding had also been identified in a previous study of gray matter in TMD [963]. Experimental acute pain in healthy subjects is associated with activity in the MCC (caudal BA 24) [690] and NS neurones have been identified in caudal BA 24 [440]. The top-down descending antinociceptive system is thought to arise from the aMCC (possibly triggered by activity in cingulate nociceptive neurones) [95]. The aMCC also shows increased rCBF during analgesic thalamic stimulation [228]. Furthermore, this region shows opioid-related pain modulation [342].

Therefore, both the M1 and the aMCC have been implicated in descending pain modulation [689]. We found that patients with thicker cortex in both the aMCC and M1 report lower pain intensity, whereas patients with thinner cortex in these regions report higher pain intensity. Therefore, thicker cortex in these regions may provide a greater capacity to modulate TMD pain, whereas thinner cortex may be related to an impairment of this descending modulation system.
5.4.4. Interaction between neuroticism and group in prefrontal gray matter

We investigated two factors that have not been previously examined in imaging studies of TMD: neuroticism and TMD pain unpleasantness. Neuroticism is described as a personality trait associated with heightened sensitivity and/or processing of negative affective stimuli [178,179,907]. We found that in patients with TMD there is a positive correlation between neuroticism and cortical thickness in the left ventromedial PFC (vmPFC; part of the OFC); an area that normally shows a negative correlation between gray matter and neuroticism [954]. Neuroticism is considered to be a stable trait across the lifespan [178]. Therefore, our observation highlights the contribution of neuroticism to abnormal gray matter in the OFC of patients with TMD and, perhaps, to the development of TMD. An alternative interpretation is that chronic pain may have modified the relationship between neuroticism and OFC thickness. However, the lack of correlation of TMD duration and neuroticism scores in our patients with TMD does not support this hypothesis.

We also found that unpleasantness is negatively correlated to gray matter in an adjacent region of the OFC. The OFC has been implicated in cognitive reappraisal and emotional regulation [723] related to interoception and somatoviscero stimuli, for directing our behaviour appropriately [57,935]. The OFC is also thought to play a role in mental flexibility and adaptability (for a critical review, see: [770]. Therefore, our finding suggests that patients with TMD may have abnormal emotional regulation and reappraisal, which may lead to pain behaviours and exaggerated affective responses to pain.

5.4.5. Study limitations

A few limitations in study design and results should be considered in interpretation of our data. First, it is not known whether medications used by some of the patients have an effect on gray matter. Second, some of our patients had co-morbid chronic pains (see Table 5-1), and these pains (mostly related to TMD) may have contributed to our findings. Third, we cannot disassociate an age effect from a pure TMD duration effect because of the strong correlation between age and TMD duration (see Chapter 6). Furthermore, duration (and intensity) may not fully describe the effect of pain on gray matter structure. Due to the nature of TMD pain, other factors that we did not collect, such as the number of days of pain in a month may have more
explanatory variance. Our patients did not self-report any previous diagnosis of depression or anxiety, although these exist in some patients with TMD [283,808,850]. Thus we cannot exclude the contribution of these factors in white matter abnormalities in TMD in general. Finally, we cannot totally rule out a contribution of ventricular volume to the VBM analysis of subcortical structures. Despite these limitations, our study does provide considerable evidence in line with previous findings in the literature about gray matter abnormalities in chronic pain. Further study limitations are discussed in Section 8.6.

5.5. Conclusions

This study provides evidence for gray matter abnormalities in both the ascending pain and descending antinociceptive systems as well as motor and cognitive areas in TMD. Further, we have shown that the personality trait neuroticism and TMD intensity and duration can affect gray matter, suggesting the presence of both personality-based and chronic pain-related abnormalities. This structural imaging study identified striking abnormalities in patients with chronic idiopathic TMD pain and highlights the contribution of both TMD-related and neuroticism-related factors. Our key findings were that, compared to controls, patients with TMD had 1) cortical thickening of the S1, frontal pole and vIPFC, 2) pain intensity-dependent cortical thinning in the aMCC and M1 and pain unpleasantness-dependent cortical thinning in the OFC, 3) TMD duration-dependent gray matter increase in the sensory thalamus, and 4) a positive correlation between cortical thickness in the OFC and neuroticism, in contrast to a normally negative correlation.
Chapter 6
STUDY II: Age-related gray matter abnormalities in temporomandibular disorder

This study has been published in *Brain Research*:


6.1. Introduction

There is no doubt that the brain undergoes structural changes as part of a normal aging process. For example, normal aging is characterized by cortical gray matter atrophy [84,99,372,585,627,824], although gains have also been reported in some brain areas [322,749]. Brain plasticity also occurs with dysfunction, injury, or specific disease. Chronic pain in particular is associated with brain plasticity, including reduced gray matter in regions associated with pain modulation and limbic function, such as the ACC, insula, dIPFC [98,225,575,577,617,779,780,963], and gray matter increases in regions associated with pain perception, such as S1 [209,577,617]. Additionally, some studies of chronic pain populations have identified gray matter decreases in the basal ganglia [741,764-766,772,963] and pain-related gray matter decreases in other motor regions, such as M1 (see Chapter 5). We have previously reported that thalamic gray matter volume in patients with TMD is correlated to the duration of their pain [617] (see Chapter 5). However, it is unclear whether these changes are caused by the cumulative effect of TMD pain over time, or whether age contributes to the observed abnormalities.

Chronic diseases such as pain may interact with normal aging processes. For example, accelerated age-related whole brain gray matter atrophy has been reported in FMS [502] and chronic back pain [27]. However, most aging studies in chronic pain have assessed global gray matter and little is known about the interaction between chronic pain and age in gray matter volume/thickness of specific brain areas.

Although the functional significance of gray matter changes is not understood, MRI and histological studies indicate that changes can be stimulus-dependent, as has been demonstrated in
studies of learning [267], training [266], and repetitive noxious stimulation [852]. These data suggest that the aforementioned findings of abnormal gray matter progression in chronic pain may be related to pain duration. While it is plausible that age-related changes specific to chronic pain are the product of cumulative pain exposure, this hypothesis has not been tested empirically.

Therefore, the aims of this study were to determine 1) whether chronic pain in TMD is associated with abnormal gray matter aging in focal cortical regions associated with nociceptive processes, and 2) the degree to which the cumulative effects of pain contributes to age effects.

Prolonged nociceptive activity may disrupt or even reverse normal gray matter atrophy in nociceptive and motor regions, and increase rates of atrophy in pain-modulatory regions. Therefore, we hypothesized that normal age-related gray matter changes would be enhanced in brain regions implicated in pain perception (e.g., the thalamus, S1, posterior insula and MCC, and suppressed in motor regions (e.g., the M1, PMC, SMA and the basal ganglia) and in regions implicated in pain modulation (e.g., ACC and the anterior insula) of patients with chronic pain.

6.2. Methods

A detailed description of methods is provided in Chapter 4.

6.2.1. Subjects

Seventeen patients with non-traumatic TMD and 17 pain-free healthy subjects with no prior history of chronic pain were recruited and provided informed written consent to procedures approved by the local Research Ethics Boards. All subjects were right-handed. Structural MRI data in this cohort unrelated to age have been presented [617] (See Chapter 5). Patients with TMD were screened with the Mount Sinai Hospital Dental Clinic TMD diagnostic criteria, based on the TMD-RDC [285]. Patient demographics and specific screening criteria have been previously described elsewhere [617,928] (see Chapter 5 and Table 5-1). All patients reported the number of years of TMD symptoms, herein described as TMD duration. Exclusion criteria (for all study participants) included: a history of serious diseases (metabolic, rheumatoid, vascular), concurrent craniofacial pain disorders, or any contraindication to MRI scanning (e.g., claustrophobia, metal).
6.2.2. Imaging & Analysis

Each study participant was placed in a 3T GE MRI (Signa HDx) system fitted with an eight-channel phased array head coil, lying supine with foam padding around their head to reduce movement. The 3D brain scan was acquired with a T1-weighted scan (IR-prep 3D-FSPGR with 128 axial slices, 0.94 x 0.94 x 1.5 mm³ voxels, 256 x 256 matrix size, field of view = 24 x 24 cm, one signal average, flip angle = 20°, TI = 300 ms, TE = 5 ms, TR = 12 ms).

6.2.3. Global age effects

To assess group differences in global age effects, we estimated whole brain gray matter volume from tissue segmentation performed in the SPM5 software package (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) running under Matlab v.7.0.4 (Mathworks). We used a general linear model to test for age-by-group interactions for whole brain gray matter. All statistical analyses were performed in SPSS v.19.0 (www.spss.com).

6.2.4. Age-by-group effects in gray matter

CTA

FreeSurfer software (http://surfer.nmr.mgh.harvard.edu) was used to assess cortical thickness. Detailed methods are described elsewhere [198,316,317,319,320]. Briefly, T1-weighted scans underwent intensity normalization, skull stripping and segmentation of each hemisphere based on tissue type (gray matter, white matter, CSF). Within each hemisphere, the boundary between each tissue type was modeled as a surface so that the distance between each surface could be calculated at each point on the cortex. Each subject’s homologous gyri and sulci were aligned to the standard average brain provided in FreeSurfer. A Gaussian spatial smoothing kernel of 6 mm FWHM was applied to compensate for topographical heterogeneity prior to statistical analysis. For this study, the distance between two points on the cortex (or two vertices) was 0.71 mm.

To restrict our search to regions hypothesized to be involved in TMD chronic pain, a single mask using FreeSurfer’s cortical parcellations was constructed [321] that included the OFC, PFC, insula, M1, SMA, cingulate cortex, postcentral gyrus and sulcus (including S1 and S2) and the posterior parietal cortex [23]. Age-by-group interactions in cortical thickness were tested at each vertex on the cortex within the cortical mask. To correct CTA results for multiple comparisons, we used a Monte Carlo simulation with 1000 permutations with the AlphaSim software package.
VBM

VBM analysis was used to measure subcortical structures. One additional control subject was recruited and consented to REB approved procedures to replace one subject subsequently removed from the subcortical analysis because of poor segmentation in the pre-processing pipeline.

Preprocessing of T1-weighted images and statistical analyses were performed in the SPM5 software package (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) running under Matlab (Mathworks). We used the VBM 5.1 toolbox (http://dbm.neuro.uni-jena.de/vbm) implemented in SPM 5 to perform VBM (for details see: [36]). Briefly, images were normalized to a standard template (ICBM-152), tissue types were then segmented using a Markov random field model, and underwent Jacobian modulation, followed by spatial smoothing of gray matter with a 10 mm FWHM Gaussian kernel. We set an absolute threshold mask of 0.10 to restrict the results to gray matter. All coordinates were converted from MNI space to Talairach space [844] using the Lancaster transform [513] implemented in GingerALE v.2.0.4 (http://www.brainmap.org/ale).

A gray matter mask of subcortical regions was constructed using the WFU Pickatlas toolbox (http://www.nitrc.org/projects/wfu_pickatlas). The mask was constructed using the “Sub-lobar” label mask and the restricted by the “Gray Matter” label mask. The final mask included the bilateral basal ganglia, amygdala and thalamus. Age-by-group interactions in gray matter volume were tested for each voxel within the subcortical mask. All significant results are reported at a voxelwise false discovery rate (FDR) [357] corrected \( p < 0.05 \), as implemented in SPM 5.

6.2.5. Contribution of TMD duration to age-related gray matter abnormalities

We examined the degree to which any of the observed age effects are attributed to cumulative duration of chronic pain. To do this, we performed a forward model multiple linear regression, which measures the degree to which one independent variable correlates to the dependent variable. Additional independent variables are added to the equation and the degree to which the
independent variable predicts the dependent variable. Age and TMD duration were entered as explanatory (independent) variables and each of the significant findings as dependent variables.

6.3. Results

6.3.1. Patient characteristics

The mean age of subjects in the patient group (mean ± SD: 33 ± 12 years) was not significantly different than the control group (CTA analysis cohort: 33 ± 9.8 years, \( p = 0.94 \); VBM analysis cohort: 32 ± 10.1 years, \( p = 0.81 \)). The range of the controls ages was 20 to 50 years old, and the age range for patients was 18-59 years old. The distribution of controls’ and patients’ ages is presented in Table 6-1. Patient characteristic details are found in Table 5-1 [617,928]. Patients reported having TMD for durations of 0.75-30 years (mean ± SD: 9.8 ± 8.3 years). Of importance for this study is that there was variability in the durations of TMD pain that facilitated assessment of this factor, compared to age.

6.3.2. Global age effects

Both the control and TMD groups showed age-related whole brain gray matter atrophy, and there were no significant group differences in whole brain gray matter volume (\( p = 0.88 \), see Figure 6-1a). Specifically, there was a significant negative correlation between whole brain gray matter and age for the controls (\( r = -0.55, p = 0.024 \), slope (m) = -3.46 cm³/year) and for the patients (\( r = -0.72; p = 0.001 \), m = -5.11 cm³/year). However, there was an accelerated overall whole brain aging effect in the patients with a significant group interaction of the slopes of the gray matter/age curves (i.e., rate of change of gray matter with age) (\( p = 0.0002 \); see Figure 6-1b).

There was a significant correlation between patients’ duration of TMD and their age (\( r = 0.54, p = 0.026 \); see Figure 6-1c), however TMD duration was not significantly correlated to gray matter volume in the patient group (\( r = -0.37, p = 0.14 \), see Figure 6-1d).

6.3.3. Focal age effects

A significant age-by-group interaction (\( p < 0.05 \), corrected for multiple comparisons) was localized to two focal regions within the right cortex. In one region, on the border of aMCC and pgACC (BA32), patients had cortical thinning with age (\( r = -0.54, m = -0.022 \) mm/year), whereas controls had age-related cortical thickening (\( r = 0.76, m = 0.033 \) mm/year). In another
region, the PMC, controls had age-related cortical thinning ($r = -0.87, m = -0.035$ mm/year), whereas patients did not have normal atrophy, but rather had a very modest age-related cortical thickening ($r = 0.11, m = 0.002$ mm/year) (see Table 6-2 and Figure 6-2 for details).

Three subcortical clusters were identified that had a significant age-by-group interaction ($p < 0.05$, FDR corrected; see Figure 6-3): the left PO thalamus and the right and left dorsal striatum. The cluster in the right dorsal striatum extends to VPL, VPM and VL thalamus. Interestingly, Figure 6-3 illustrates the strong positive correlation between gray matter volume with age in the TMD group ($r = 0.65$) in contrast to the weak correlation in the control group in the thalamus ($r = -0.27$). Figure 6-3 also illustrates the group differences in the dorsal striatum where the control group showed a clear age-related gray matter loss (left: $r = -0.78$; right: $r = -0.80$), in contrast to the age effect observed in the TMD group (left: $r = 0.42$, right: $r = 0.33$) (see Table 6-2, Figure 6-3).

### 6.3.4. The contribution of TMD duration to gray matter age effects

A schematic of the focal aging effects in control and TMD groups is shown in Figure 6-4a (also see discussion). The contribution of age and duration to the observed age-by-group interactions was examined because of the observed correlation between age and duration. We performed a multiple regression analysis with age and duration as independent variables to parse out the relative contributions of these two factors. To describe our results, we used the standard annotation for partial correlations, e.g., age • duration, such that age is being correlated to the dependent variable while regressing out the variance related to duration. The outcomes of these analyses are shown schematically in Figure 6-4b. In the right thalamus, the partial correlation coefficient between age and gray matter volume was no longer significant when controlling for duration ($r_{age} = 0.65, p = 0.005$; $r_{age \cdot duration} = 0.45, p = 0.082$), whereas duration remained significant when age was included in the model ($r_{duration} = 0.70, p = 0.002$; $r_{duration \cdot age} = 0.56, p = 0.026$). In the aMCC/pgACC, the age-by-group interaction was driven by the shared variance between age and duration. When the age was regressed out of the correlation between duration and cortical thickness, the relationship remained insignificant ($r_{duration} = -0.45, p = 0.073$ to $r_{duration \cdot age} = -0.22, p = 0.409$). Further, when duration was regressed out of the correlation between age and cortical thickness in the aMCC/pgACC, the relationship was no longer significant ($r_{age} = -0.54, p = 0.027$ and $r_{age \cdot duration} = -0.39, p = 0.133$) (see Table 6-3).
In some cases, duration did not contribute to the observed age-by-group interaction. For instance, in the bilateral dorsal striatum, the partial correlations between age and gray matter in these regions did not show much change when duration was regressed out. Similarly, when age was regressed out of the correlation between duration and gray matter, the partial correlations were not significant (see Table 6-3). In the case of the PMC, we found a suppressive effect [170], \textit{i.e.}, when duration was included in the regression model, age became a better predictor of the interaction as the correlation became more significant. That is, the partial correlation coefficient of age and thickness in the PMC, controlling for duration, was larger than the zero-order correlation (zero-order correlation: 0.11, partial correlation: 0.38). Similarly, when correlating thickness to TMD duration and controlling for the effect of age, the correlation coefficient for the PMC decreased from -0.36 to -0.50 (see Table 6-3).
**Table 6-1:** Distribution of subject ages

<table>
<thead>
<tr>
<th>Age range</th>
<th>18-20</th>
<th>21-25</th>
<th>26-30</th>
<th>31-35</th>
<th>36-40</th>
<th>41-45</th>
<th>46-50</th>
<th>51-55</th>
<th>56-60</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Con</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>#Pat</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>17</td>
</tr>
</tbody>
</table>

Abbreviations: #Con – Number of control subjects; #Pat – Number of patients with TMD
Table 6-2: Age-related group differences in cortical thickness and subcortical gray matter volume. Shown are the statistically significant (p<0.05) group differences in correlation coefficients of gray matter against age. Peak vertex/voxel Talairach coordinates (TAL) are reported.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Region</th>
<th># Voxels</th>
<th>Correlation (age vs. thickness)</th>
<th>Peak TAL</th>
<th>Peak t-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical</td>
<td>C &gt; P</td>
<td>R aMCC/pgACC</td>
<td>451</td>
<td>-0.54 0.76</td>
<td>14 30 20</td>
</tr>
<tr>
<td>(CTA)</td>
<td>C &lt; P</td>
<td>R PMC</td>
<td>423</td>
<td>0.11 -0.87</td>
<td>8 12 54</td>
</tr>
<tr>
<td>Subcortical</td>
<td>C &lt; P</td>
<td>L dorsal Striatum</td>
<td>1537</td>
<td>0.42 -0.78</td>
<td>-18 10 10</td>
</tr>
<tr>
<td>(VBM)</td>
<td></td>
<td>R dorsal Striatum</td>
<td>4415</td>
<td>0.33 -0.80</td>
<td>11 -3 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L Thalamus</td>
<td>264</td>
<td>0.65 -0.27</td>
<td>-21 -23 14</td>
</tr>
</tbody>
</table>

Abbreviations: aMCC – anterior mid-cingulate cortex; pgACC – perigenual anterior cingulate cortex; CTA – Cortical thickness analysis; PMC – premotor cortex; VBM – voxel-based morphometry.
**Table 6-3:** Contributions of TMD duration to the age-gray matter relationships. Zero (0) order correlations and partial correlations are presented for age and TMD duration versus gray matter thickness/volume. The p-values in the age and duration columns represent the significance of the correlations.

<table>
<thead>
<tr>
<th>Significant group</th>
<th>Age</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r_{age \times duration}</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r_{duration \times age}</td>
<td>p</td>
</tr>
<tr>
<td>R PMC</td>
<td>0.11</td>
<td>0.677</td>
<td>0.38</td>
<td>0.145</td>
<td>-0.36</td>
<td>0.159</td>
<td>-0.50</td>
<td>0.051</td>
</tr>
<tr>
<td>R aMCC/ pgACC</td>
<td>-0.54</td>
<td>0.027</td>
<td>-0.39</td>
<td>0.133</td>
<td>-0.45</td>
<td>0.073</td>
<td>-0.22</td>
<td>0.409</td>
</tr>
<tr>
<td>L Dorsal Striatum</td>
<td>0.42</td>
<td>0.096</td>
<td>0.42</td>
<td>0.109</td>
<td>0.13</td>
<td>0.639</td>
<td>-0.12</td>
<td>0.650</td>
</tr>
<tr>
<td>R Dorsal Striatum</td>
<td>0.33</td>
<td>0.204</td>
<td>0.33</td>
<td>0.216</td>
<td>0.09</td>
<td>0.742</td>
<td>-0.10</td>
<td>0.706</td>
</tr>
<tr>
<td>R Thalamus</td>
<td>0.65</td>
<td>0.005</td>
<td>0.45</td>
<td>0.082</td>
<td>0.70</td>
<td>0.002</td>
<td>0.56</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Abbreviations: aMCC – anterior mid-cingulate cortex; pgACC – perigenual anterior cingulate cortex; PMC – premotor cortex; S1 – primary somatosensory cortex
Figure 6-1: Total gray matter volume and correlations with age and duration: (a) Total gray matter volume was not significantly different in the TMD group versus control group (p = 0.88). (b) Significant gray matter volume decreases with age for both controls (3.46 cm³/year) and TMD
group (5.11 cm$^3$/year) with a significant age-by-group interaction between the groups’ slopes (p = 0.0002). (c) TMD duration significantly correlated with patients’ age (r = 0.54, p = 0.026), (d) but not to total gray matter volume (r = -0.37, p = 0.139). n.s. = not statistically significant (p > 0.05); asterisks (*) = p < 0.05; m = slope (rate of gray matter change in cm$^3$/year).
Figure 6-2: Age-by-group interactions in cortical thickness. Patients with TMD had age-related thinning (0.022 mm/year) in the anterior mid-cingulate cortex/perigenual anterior cingulate cortex (aMCC/pACC), whereas controls show age-related thickening (0.033 mm/year) in this region. In the premotor cortex (PMC), only the controls had age-related thinning (0.035 mm/year).
**Figure 6-3:** Group differences in age effects within subcortical gray matter volume. Age-by-group interactions in the thalamus and dorsal striatum (p < 0.05, FDR). The graphs indicate gray matter volume (corrected for total intracranial volume; TIV) versus age for each subject by group. Colour bar shows t-statistic values.
**Figure 6-4:** Summary of age-related gray matter abnormalities (a) Schematic diagram of gray matter regions with normal aging in healthy controls and abnormal aging in TMD. (b) Schematic diagram summarizing the relative contribution of age and TMD duration to the regions of gray matter with abnormal aging. The line thickness of each arrow depicts the relative contribution of age and duration and the plus (+) and minus (-) signs depicts the direction of the relationship. Abbreviations: aMCC/pACC – anterior mid-cingulate cortex/perigenual anterior cingulate cortex; PMC – premotor cortex
6.4. Discussion

This study is the first to show that chronic pain associated with TMD in females is associated with abnormal gray matter aging in focal cortical regions associated with pain and motor processes. We found that patients with TMD have accelerated whole brain gray matter atrophy, compared to pain-free controls. We also identified three types of aberrant patterns of gray matter aging in five focal brain regions (Figure 6-4): 1) in the thalamus, patients with TMD gray matter volume increased with age, whereas gray matter in controls was relatively sustained; 2) in the aMCC/pgACC, patients with TMD had a progressive loss of cortical thickness with age, whereas the controls had age-related cortical thickening; 3) in the dorsal striatum and PMC, there was little change with age in the patients with TMD, whereas the controls had age-related atrophy. TMD duration added to the age effects in the thalamus and the aMCC/pgACC, suppressed the age effect in the PMC, but did not contribute to age effects in the dorsal striatum. Abnormal gray matter aging in TMD may thus be due to the progressive impact of TMD-related factors as well as inherent factors in individuals with this chronic pain.

6.4.1. Whole brain gray matter atrophy

Our finding of accelerated gray matter atrophy with age in TMD is consistent with previous studies of gray matter in FMS [502] and chronic back pain [27]. These previous studies attributed increased rate of gray matter loss to excitotoxicity and inflammatory molecules. Specifically, they suggest that, because chronic pain is inherently harmful to the body and is associated with negative affect and increased stress, the inflammatory response is upregulated centrally, inducing cell death. These processes have been implicated in chronic age-related diseases (Mattson, 2003), and therefore, it is plausible that they are implicated in age-related gray matter loss in chronic pain states.

6.4.2. Use-dependent plasticity

We have demonstrated that TMD duration added to the age effects in the thalamus and cingulate cortex. This form of plasticity is in line with the concept of use-dependent plasticity which comprises structural and functional changes in the brain in response to increased or decreased neuronal input [348,758]. For instance, studies in healthy human subjects have found that training [266] and learning [267] can increase gray matter in the brain. Conversely, limb
amputation, and other forms of sensory loss can induce reorganization of the cortical map that represents the affected limb \[601,602\] and gray matter loss \[268,847\]. Of particular interest are studies that report reversible gray matter changes in nociceptive and antinociceptive regions of the brain in response to repeated noxious stimulation in healthy subjects \[93,852\]. Furthermore, patients with chronic pain show age-independent gray matter changes in both nociceptive and pain-modulatory regions \[98,356,558,575,617,741,772,963\], some of which can be reversible \[384,741\]. Our finding of progressive thinning in the cingulate cortex and increasing thalamic gray matter are consistent with the concept of use-dependent plasticity. That is to say that increased input, activity or use will increase gray matter, and decreased use or activity is associated with decreased brain gray matter. Therefore, the observed age-by-group interaction in the thalamus and aMCC/pgACC may, in part, be driven by prolonged nociceptive activity, which could suppress the normal pattern of aging. These findings are consistent with our previous report that gray matter in the thalamus is positively correlated with TMD duration, and may be due to increased nociceptive activity, as previously discussed (see \[617\]).

In the aMCC/pgACC, we found that the shared variance of both age and TMD duration contributes to progressive atrophy in the patients. This region receives orofacial nociceptive input from thalamic nuclei that are part of the STT and TTT \[189,276\]. The aMCC/pgACC is a complex, multimodal region that has been implicated in a number of functions \[69\]. For instance, this region has been identified as a node in the salience network, and has been implicated in aspects of salience \[213,229,262,263,774,849,927\], and pain \[220,230,231,259,265,440,508,528,632,933\]. The cingulate cortex has also been implicated in the cognitive and affective processing of pain \[218,230,529,716,776,777,934,936\], and the MCC is involved in action selection and modulation of motor output in response to aversive stimuli \[790,898,904\]. Therefore, it is possible that the age-related thinning in the MCC is related to abnormalities in TMD with regard to cognitive and attentional processes related to prolonged TMD pain \[928\].

The suppressive relationship identified in the PMC is of particular interest. The PMC is a region that is often activated in neuroimaging studies of experimental pain \[304,511\]. In the current study, age and TMD duration uniquely and non-redundantly predicted variance in gray matter. When we included duration in the model, both variables (age and duration) better predicted the progression of cortical thickness over time. Furthermore, age and duration had differential effects
on the PMC. Specifically, patients had sustained cortical thickness with age, whereas TMD duration was related to cortical thinning in the PMC. The PMC receives nociceptive input from the ventral caudal portion of the MD thalamus [276]. Therefore, we would expect that a barrage of nociceptive input from the thalamus over an extended period of time could induce gray matter plasticity in the PMC, as we have observed in the thalamus, rather than the observed normalization. These paradoxical findings warrant further study to better understand the relationship of the PMC and aging in the context of chronic pain. It is therefore apparent that there is abnormal gray matter aging in TMD, independent of how long patients have had TMD.

The findings reported here are in contrast to previous findings that in some chronic pain conditions central gray matter changes are related to pathology where there exists a peripheral aetiology, such as osteoarthritis. It has been reported that once the peripheral cause of the pain has been resolved, gray matter changes resolve [384,741]. There is evidence suggesting that both central and peripheral mechanisms contribute to pain in TMD [561,757]. Therefore the source of age-related variance may derive from multiple mechanisms in addition to (dis)use-dependent plasticity. Interestingly, recent studies have reported that development of some brain regions is tightly regulated by genes, rather than the environment [680,854]. There is also evidence suggesting that there is a genetic predisposition to functional chronic pain syndromes [247-249,809]. It is therefore feasible that there is a genetic contribution to the observed abnormal aging effects. These findings provide a genetic source of plasticity [137,858] that may co-exist with other forms of plasticity.

6.4.3. Cellular basis of changes in gray matter

The cellular and molecular basis of MRI-detectable gray matter changes remains to be explained. However, several hypothetical mechanisms have been postulated, such as neuronal and/or glial death [575], but recent evidence suggests that, to some extent, gray matter losses are likely related to density of small dendritic spines [278,608], and remodeling of neuronal processes [537]. Alternatively, reversible gray matter changes in chronic pain may be caused by neuroinflammation [238,381,920], and induce MRI-detectable increases in gray matter. This mechanism could explain both abnormal age-related increases and maintenance of gray matter volume/thickness. For the observed gray matter losses, however, we cannot rule out that cell death does not occurring in age-related gray matter losses – healthy populations lose neurones as
they age, and persons with neurodegenerative diseases suffer increased rates of atrophy related to cell death.

6.4.4. Study limitations

One limitation of this study is that it is not possible to control for the impact of long-term use of medications in some of the patients on gray and white matter plasticity. Although the effect of such medications is not well understood, recent evidence suggests that medications commonly used to manage pain, such as anti-inflammatory medications and opiates, can impact brain structure [916,962]. Considering that thirteen of seventeen of the patients included in this study were taking NSAIDs, it is possible that the observed age-related findings (especially those of gray matter maintenance) may be related to the effect of these drugs. Only one subject was taking an opiate, hydromorphone, and so this is of less concern to this study.

Our patients did not self-report any previous diagnosis of depression or anxiety, although these exist in some patients with TMD [283,808,850]. Thus we cannot exclude the contribution of these factors in white matter abnormalities in TMD in general.

Another limitation of these findings is that it is a cross-sectional examination of structural abnormalities in TMD. Therefore, the interpretations of “age-driven” and “TMD duration-driven” abnormalities are limited to parsing out the relative variance that is accounted by each factor. Therefore, the results warrant further study, and should be interpreted with caution.

6.5. Conclusion

In sum, our findings provide novel evidence that chronic TMD pain patients have abnormal age-related gray matter changes in cognitive, motor and nociceptive brain regions. Our study highlights the importance of understanding the effects of age and TMD duration in structural studies of chronic pain, as progressive changes in gray matter may require differential therapeutic approaches.
Chapter 7

STUDY III: White matter brain and trigeminal abnormalities in TMD

The findings presented in this chapter are in press in *Pain*:

Moayedi M, Weissman-Fogel I, Salomons TV, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. White matter brain and trigeminal nerve abnormalities in temporomandibular disorder, *Pain* 2012; (in press).

7.1. Introduction

TMD comprise clinical problems involving the structures of and around the TMJ, the masticatory musculature, or both [1,418]. TMD represent the most common orofacial chronic pain disorder, prevalent in ~3-20% of the United States’ adult population [269,539,540,562], mostly in women [285,312,539,721]. In idiopathic TMD there is no clear aetiology [283,286,287,653]. Central mechanisms are thought to initiate or maintain TMD pain [757] based on TMD symptomology such as persistent pain, allodynia, and hyperalgesia, sometimes extending to regions distant from the face [305,314,390,565,756,883], enhanced temporal summation of pain to repetitive noxious heat stimuli [563], and dysfunctional DNIC [113,491,756]. Abnormalities of these centrally-mediated processes suggest that ascending nociceptive pathways and/or descending pain-modulatory pathways [515] are affected. Additionally, patients with TMD can exhibit cognitive [370,379,380] and motor dysfunction [837] possibly related to abnormalities in brain regions associated with these functions [799,800,839,928].

Structural brain imaging provides an opportunity to delineate anatomical substrates of CNS abnormalities in TMD. We reported that patients with TMD have increased cortical thickness in the orofacial region of the primary somatosensory cortex (S1), the ventrolateral prefrontal cortex (vLPFC) and the frontal pole [617]. Similarly, Younger and colleagues [963] reported increased gray matter volume in motor, limbic and sensory regions, including trigeminal brainstem nuclei, in myofascial TMD. These studies indicate that TMD patients have gray matter abnormalities in sensory, motor and cognitive/limbic regions. Our finding of a correlation of thalamic gray matter with TMD duration [617] supports the concept of long-term nociceptive-induced central
plasticity, possibly due to increased peripheral nociceptive activity, or dysfunctional antinociceptive systems. Therefore, the former possibility suggests that although clinical observations have not clearly identified gross peripheral abnormalities in TMD, the CNV may indeed undergo changes due to abnormal persistent activity resulting in microstructural abnormalities.

Previous studies examining white matter in other clinical conditions with sensory abnormalities and/or chronic pain [156,356,558,847] found white matter abnormalities in brain areas involved in sensory, modulatory and cognitive functions, and some of these white matter abnormalities correlated with clinical findings. Therefore, studying the correlation between TMD characteristics and measures of white matter integrity can provide insight into whether chronic pain drives changes in white matter microstructure.

The main aim of this study was to evaluate the white matter abnormalities in TMD. Specifically, we determined whether there are abnormalities along the CNVs or in white matter tracts in the brain associated with sensory, cognitive or motor functions, compared to healthy controls. Towards this goal, we used DTI, a brain imaging technique sensitive to the inherent diffusion of water molecules that allows us to assess white matter microstructure. FA is a DTI metric that reflects white matter tract integrity or structure [52,59,66,626]. We tested for abnormalities in FA along the CNV of patients with TMD. Further, we used two approaches to assess white matter in the CNS: First, we quantified brain FA abnormalities in TMD with TBSS [815], and second we used probabilistic tractography [74] to determine the connectivity of main regions of white matter disruption. Finally, we determined whether there is a link between white matter microstructure and clinical characteristics of TMD.

7.2. Methods

A detailed description of methods is provided in Chapter 4.

7.2.1. Subjects

The study consisted of a cohort of 17 right-handed females with idiopathic TMD (mean age ± SD: 33.1 ± 11.9 years) and 17 healthy right-handed females (mean age ± SD: 32.8 ± 9.8 years). Informed written consent was obtained from all study participants for procedures approved by the University Health Network and Mount Sinai Hospital Research Ethics Boards. Patients were
screened with the Mount Sinai Hospital Dental Clinic TMD diagnostic criteria, based on the TMD-RDC [285]. Inclusion criteria included: 1) pain in the muscles of mastication rated verbally as at least 4/10 for at least 3 months at the time of evaluation, or pain that is aggravated by mandibular function; 2) moderate (on a scale of no (0), low (1), moderate (2), or severe (3)) pain to palpation and/or pain persisting post-examination in at least 3 muscle sites and/or moderate pain to palpation of the TMJ and/or limited mandibular movement (opening < 40mm). Patients were analgesic-free for 24 hours prior to scanning. Exclusion criteria for all subjects included: 1) left-handedness; 2) self-report of metabolic, rheumatoid or vascular diseases or disorders, or any other serious diseases; 3) self-report of commonly co-morbid functional chronic pain disorders (IBS and FMS); 4) self-report of psychiatric disorders (e.g., depression, schizophrenia, attention deficit hyperactivity disorder); and 5) self-report history of an abnormal neurological examination; 6) contraindication to MRI scanning; 7) self-report of substance abuse. Additionally, healthy controls were excluded if they had a history of chronic pain and patients were excluded if they had a pain disorder other than TMD.

### 7.2.2. Questionnaires

Patients provided verbal ratings of their current pain and unpleasantness, as well as their average pain intensity and unpleasantness ratings over the last month before scanning on a scale of 0 to 10 (0 = no pain, 10 = worst pain imaginable). Specifically, patients provided a numerical pain score for pain intensity and pain unpleasantness by answering the following questions: 1) “Please rate the intensity of your average pain over the last month, 0 being no pain and 10 being the worst pain imaginable?” and 2) “Please rate the unpleasantness of your average pain over the last month, 0 being not unpleasant and 10 being the most unpleasant pain imaginable?”. The patients also reported the duration of their TMD symptoms.

### 7.2.3. Imaging parameters

All subjects underwent scanning in a 3-Tesla GE MRI system fitted with an eight-channel phased array head coil. Each subject’s head was padded to reduce movement. The DTI acquisition consisted of two runs of diffusion-weighted scans (TR = 14,500 ms, field of view: 24 cm x 24 cm, 128 x 128 matrix, 1.875mm x 1.875 mm in-plane resolution, 3 mm thick axial slices, with ASSET with a factor of 2, maximum gradient strength = 40 mT/m, maximum slew rate = 150 T/m/s) acquired along 23 non-collinear, isotropic directions (b = 1,000 s/mm²). Additionally, 2
non-diffusion weighted scans (b = 0 s/mm²; b0) were acquired at the beginning of each run. The scans covered from the top of the head to the second cervical spinal process (C2).

### 7.2.4. DTI pre-processing

The diffusion-weighted images were imported into FSL 4.1.8 (http://www.fmrib.ox.ac.uk/fsl) for quality control [816]. Pre-processing included eddy current and motion artifact correction using FDT [459]. Each subject’s two DTI runs were averaged to a single volume to achieve a greater signal-to-noise ratio. Then, individual brain masks were created using BET [814]. These images were then processed through two different pipelines for (1) voxelwise analysis and (2) tractography.

The preprocessed images were fit with a diffusion tensor model using DTIFIT in FDT v. 2.0. We then calculated voxelwise values of FA, and other DTI metrics (see below).

#### 7.2.4.1. Other DTI metrics

To gain more insight into FA findings, we also assessed other DTI metrics in the clusters with significant group difference. \( \lambda_1 \) is thought to reflect diffusion along a tract, and reductions in this value suggest disruptions along the tract diffusivity. RD is believed to reflect changes in membrane permeability and, to some extent, myelination [66,67]. RD is calculated by averaging the two radial vectors of the tensor model (\( \lambda_2 \) and \( \lambda_3 \)). Finally, \( MD \) was measured because it is associated to oedema and inflammation. Figure 7-1 provides a schematic of these DTI metrics. DTI metrics (FA, \( MD \), \( \lambda_1 \), \( \lambda_2 \) and \( \lambda_3 \)) are calculated using DTIFIT in the FDT toolbox. The TBSS skeleton is created based on FA, and peak FA values are projected onto the skeleton. For statistical analysis of the other DTI metrics, \( MD \), RD and \( \lambda_1 \) values from the same voxels are projected onto the skeleton. To test for group differences, we performed a multivariate analysis of variance (MANOVA) with each of the DTI metrics as dependent variables, and group as an independent variable.

### 7.2.5. Assessment of CNV

To assess the CNV, we manually drew a ROI (3.75 mm x 3.75 mm x 3 mm) in the axial plane on each subject’s CNV root in the cisternal space in native space (see Figure 7-1). The nerves were identified on color orientation maps of the primary eigenvector (\( \lambda_1 \)) image overlaid onto the FA
map. FA, MD, RD, $\lambda_1$ values (see Figure 7-1) were calculated for each of the DTI volumes and extracted from the ROI. Independent samples $t$-tests were used to assess group differences in FA for each CNV. Furthermore, a paired $t$-test was utilized to compare left and right CNV FA values within the patient group. Lastly, FA values derived from the each of patients’ nerve were correlated with TMD characteristics (pain intensity, unpleasantness and duration). Statistical significance was set at $p < 0.05$. All statistical tests were performed in SPSS v. 19.0 (http://www.spss.com/).

![Diagram of diffusion tensor imaging](image)

**Figure 7-1:** Schematic of diffusion tensor imaging, and summary of eigenvectors and metrics. Lambda ($\lambda$) represents each of the eigenvectors that make up the tensor model. The equations to calculate MD, fractional anisotropy (FA) and radial diffusivity (RD) from these eigenvectors are also provided.

\[
MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}
\]
\[
FA = (3/2)^{1/2} \times \frac{[(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2]^{1/2}}{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)^{1/2}}
\]
\[
RD = \frac{\lambda_2 + \lambda_3}{2}
\]

**7.2.6. Tract-based spatial statistics**

TBSS v.1.2 [815,817] was used to compare FA between the TMD and healthy control cohorts. Briefly, FA maps underwent non-linear registration to a $1 \times 1 \times 1$ mm FA map in standard space (FMRIB58_FA, available in FSL), a mean image derived from all the subjects was created and thinned to represent the centre of major white matter tracts common to all subjects, forming a white matter skeleton. Each subject’s peak FA value perpendicular to the thinned track was then projected onto the skeleton. One skeleton was created for analysis of group differences that
included all study participants, and a second patient only skeleton was created for correlation analyses of TMD pain characteristics (see below). The mean skeleton images were set at a threshold of 0.2 to include FA values that are related to white matter [815]. Other DTI metrics were also evaluated to characterize FA findings (see Figure 7-1, and below). Additionally, we performed a separate patient group analysis to assess which areas of the skeleton correlate with TMD characteristics (pain intensity, unpleasantness and duration).

7.2.7. Probabilistic tractography

Probabilistic tractography was used to assess the connectivity of findings from the TBSS analysis (http://www.fmrib.ox.ac.uk.fsl/fdt/index.html)[74,76]. First, we downsampled the preprocessed diffusion-weighted images to create isotropic voxels (3 x 3 x 3 mm). The images were then processed in FDT. Probability density functions (PDF) on up to two principal fibre directions were estimated at each voxel in the brain. We then used multi-fibre tractography and drew 5000 samples from each seed voxel along the PDFs. The seed voxels were binarised images of the significant clusters. Together, each seed voxel’s streamlines provide an estimate of its connectivity. When a streamline reaches a voxel with more than one fibre direction, the streamline follows the direction closest to the direction at which it arrives at the voxel. The pathways generated by the algorithm represent the number of samples that have passed through a voxel. To eliminate spurious connections, each computed pathway in each subject was thresholded at 10 samples (of the 5000 generated from each seed voxel) that passed through the voxels. These thresholded tracts were consistent between subjects. To visualize the findings, each of the subject’s tracts were binarised and overlaid on a standard brain to produce a probabilistic map of the pathways for controls and patients. The values in these maps reflect the number of subjects who share a pathway.

7.2.8. Statistical analyses

7.2.8.1. Whole brain white matter

We assessed group differences in global (whole brain) FA and the white matter skeleton FA. To do so, we extracted mean FA values across every voxel in the brain and every voxel in the skeleton for each subject and performed a two-tailed t-test.
7.2.8.2. Mask analysis

A mask was created to restrict the analysis to *a priori* white matter ROI, including the white matter regions containing the pathways subserving nociceptive, antinociceptive, motor and cognitive functions. Thus, the mask included the brainstem white matter (including the white matter that contains the TTT and the CST, TCT (including tracts to S1 and the anterior corona radiata), the IC containing the CST, the cingulum, the rostrum, genu and the body of the corpus callosum, the uncinate fasciculus, and the EC/ExC. The mask also included the thalamus, as white matter courses through this region. The ROI were identified with the Johns Hopkins University white matter Tractography Atlas, ICBM-DTI-81 white matter Atlas [625,910], the Harvard-Oxford Cortical Atlas and the Harvard-Oxford Subcortical Atlas, available within FSL (http://www.cma.mgh.harvard.edu/fsl_atlas.html). The final mask corresponded to voxels within the FA skeleton.

A between group voxel-wise *t*-test within the skeleton mask was performed to identify regions with significant group differences in FA, with age included as a covariate of no-interest. We used two complementary statistical thresholding methods with 5000 permutations testing in FSL’s randomise toolbox: (1) threshold-free cluster enhancement (TFCE; correct cluster *p* < 0.01) [819], which is sensitive to spatially extensive areas of significant difference; and (2) cluster-mass correction (*p* < 0.05), which requires clusters to meet a specific height threshold and, therefore, is only sensitive to *t*-values above the threshold, which we set at *t* > 2.3.

An additional set of post-hoc of analyses was run to characterize regions with significant group differences in FA. First, we determined whether the findings were related to specific characteristics of TMD. To do so, we extracted each subject’s mean FA values from significant group difference clusters. The patients’ FA values from each of these clusters then were correlated with the TMD pain intensity, unpleasantness and duration in SPSS v. 19.0 (http://www.spss.com/). Statistical significance was set at *p* < 0.05. Second, we extracted the mean values of mean diffusivity *MD*, RD and *λ*₁ for each of the clusters and assessed whether there were significant group differences between patients with TMD and controls using a multivariate analysis of variance (MANOVA) with each of the DTI metrics as dependent variables and group as an independent variable. Statistical significance was set at *p* < 0.05.
All significant TBSS results are thickened with the “tbss_fill” tool implemented in FSL for ease of visualization. This tool uses a 3mm Gaussian smoothing kernel to thicken significant results to the white matter tract (as defined by FA > 0.2).

7.2.8.3. Quantitative Tractography: connection probability

We identified that patients and controls had differential connectivity between the corpus callosum seed and the dIPFC, and between the corpus callosum seed and the frontal pole. However, these findings were only qualitative, and we therefore sought to quantify these group differences. To do so, we performed a second tractographic analysis with specified targets based on the observed differences. Specifically, we thresholded the tractography maps for each group at 5 of 17 subjects (~30%) (see Figure 7-7A). Subsequently, we binarised the resultant image for each group, and subtracted them one from another to determine the regions of difference between the groups. We extracted the tracts for each hemisphere and restricted them to gray matter of the target region. For example, the tract projecting to the frontal pole was restricted to the frontal polar cortex. This was done by multiplying the binarised subtraction image to a binarised mask of the frontal pole (from the Harvard-Oxford Cortical Structural Atlas; http://www.cma.mgh.harvard.edu/fsl_atlas.html). Similarly, the tracts projecting to the dIPFC were restricted to the middle frontal gyrus. We then performed tractography between the corpus callosum seed and these 4 targets (the left and right frontal pole, and the left and right dIPFC). Next, we extracted the number of samples that project to each target in each subject, and the number of voxels in the seed that project to each of the targets. Group differences were tested using two-tailed, independent samples t-tests. Significance was set at p < 0.05

7.3. Results

7.3.1. Patient characteristics

The ages of the patient and control groups were not significantly different (patients: mean age ± SD: 33.1 ± 11.9 years; controls: 32.8 ± 9.8 years; p = 0.94). Details of the individual patient demographics have been tabulated in our previous study of this cohort (see Table 5-1; and [617]). Of importance for this study is that there was variability in the durations of TMD, intensity and unpleasantness of pain that facilitated assessment of these factors in correlation analyses. The duration of TMD ranged from 0.75 – 30 years (mean ± SD: 9.8 ± 8.2 years), while pain intensity ranged from 2 – 7 (mean ± SD: 4.3 ± 1.8), and unpleasantness ranged from 1 – 8
Patients reported that their TMD pain was confined to the TMJ (n = 6), the muscles of mastication (n = 3), or both the joint and muscles (n = 8). Most patients reported bilateral pain (n = 13), but some reported unilateral pain (three right-sided, one left-sided). The duration of TMD significantly correlated with the patients’ age (r = 0.536, p = 0.027).

7.3.2. Trigeminal nerve FA

We tested the CNV roots, in the cisternal space (see Figure 7-2), for group differences in FA, MD, RD and \( \lambda_1 \). We found that patients with TMD had significantly lower FA in both CNVs (right and left: \( p < 0.001 \)) (see Figure 7-3A). FA in the right CNV was also negatively correlated with TMD duration (\( r = -0.53, p = 0.028 \)) (See Figure 7-3B). The left CNV was not significantly correlated with TMD characteristics. MD and RD were significantly higher in both CNVs (right: \( p < 0.01 \); left: \( p < 0.05 \)), compared to controls (See Figure 7-3C), but there were no significant group differences in \( \lambda_1 \).

7.3.3. Patients have lower white matter FA

To investigate global differences in white matter microstructure, we tested for group differences in FA across the whole brain and within the white matter skeleton. Compared to controls, the TMD patient group showed a 3.8% reduction in whole brain FA (mean ± SD: controls = 0.264 ± 0.012; patients = 0.254 ± 0.008; \( p = 0.003 \)) and 3.6% reduction in whole brain skeletonised FA (controls = 0.449 ± 0.018; patients = 0.433 ± 0.001; \( p = 0.006 \)) (see Figures 7-4 and 7-5). We also evaluated MD, RD and \( \lambda_1 \) across the white matter skeleton and found that there was a significant group difference (Pillai’s trace = 0.416; \( F(3,30) = 7.117; p<0.001 \)), and Bonferroni post-hoc tests revealed that all three DTI metrics were significantly higher in the patient group compared to the control group (all post-hoc tests: \( p < 0.05 \); see Table 7-1 for details).

The mask-wide TBSS analysis (\( p < 0.01 \); TFCE corrected) identified white matter clusters that had 6.6-16.8% lower FA in the patient cohort compared to controls (see Table 7-2 and Figure 7-5A). Two of these clusters were localized to the right IC, and two were in the right external/extreme capsule adjacent to the insula. Other significant clusters were at the junction of the right internal and external/extreme capsules, adjacent to the vIPFC, and in the white matter adjacent to right S1 and M1. The largest cluster included the bilateral anterior body of the corpus callosum, and extended to the left cingulum, the bilateral anterior corona radiata, the bilateral
ICs, the left external/extreme capsules, the fornix, the left and right thalamus and the brainstem. Although the thalamus is mostly a gray matter structure, white matter does course through it and we identified a cluster within the right thalamus with significantly lower FA in patients with TMD, compared to controls. Individual clusters are shown in Figure 7-6.

Because one of our findings encompassed a large, diffuse region, we performed a secondary analysis to test for more focal, highly significant group differences in FA. To do so, we used a cluster-mass correction that requires significant clusters to reach a specified threshold, in this case \( t(32) = 2.30 \). This analysis revealed a single focal cluster in the corpus callosum (peak voxel MNI coordinates: -4, 26, 9, \( t(32) = 2.31 \), cluster size 353 voxels) (see Figure 7-5B).

We also determined whether the white matter tracts with lower FA in patients with TMD also showed changes in \( MD \), \( RD \) and \( \lambda_1 \), indicative changes in axonal diffusion, permeability and myelination. We found that there was a significant between-group difference in these microstructural variable (Pillai’s trace = 0.958; \( F(7,26) = 6.182; p = 0.009 \)). Bonferroni post-hoc tests revealed that all of the clusters with lower FA showed significantly increased \( MD \) and \( RD \) \((p < 0.05)\), but there were no significant differences in \( \lambda_1 \) (see Table 7-3).

### 7.3.4. Probabilistic tractography

Because the corpus callosum is a large structure that contains interhemispheric fibres we used tractography to elucidate the connectivity of our finding of lower FA in the corpus callosum. We evaluated the relationship between FA and connectivity by determining the connectivity of a region with lower FA in patients. To do so, we used multi-fibre probabilistic tractography to determine pathways passing through the corpus callosum finding from the cluster-mass correction analysis (see Figure 7-5B and 7-7A). We first performed a qualitative analysis to determine the connectivity of the corpus callosum finding within each group. As shown in Figure 7-7A, there were denser connections between the corpus callosum and the bilateral dIPFC in controls, compared to patients. Conversely, connections between the corpus callosum and the bilateral frontal pole were quite dense in patients, but sparse in controls. Quantification of these connection probabilities verified that significantly fewer voxels from the corpus callosum connect with the left and right frontal poles in controls, compared to patients with TMD (see Figure 7-7B and C). We also found that patients with TMD have significantly higher number of samples \((i.e.,\) connection probability\) between the corpus callosum and the left frontal pole \((p <
There were no significant group differences in the connection probabilities of the corpus callosum and the right frontal pole. Additionally, we found that patients had significantly lower connection probability between the corpus callosum and the right dlPFC (p < 0.05), but not the left dlPFC (p > 0.05) (see Figure 7-7C).

7.3.5. White matter FA related to TMD pain characteristics

We tested whether each of the clusters with significant group differences in FA was correlated with TMD characteristics (TMD pain intensity, unpleasantness and duration). We found that three of these clusters showed significant FA-TMD correlations. Specifically, the FA within the cluster at the junction of the internal and external/extreme capsules (adjacent to the vlPFC) was significantly negatively correlated with TMD pain intensity (r = -0.49, p = 0.046) (see Figure 7-8A). We also identified a significant negative correlation between FA in the thalamic cluster and TMD pain intensity (r = -0.59, p = 0.013) (see Figure 7-8B). Finally, we identified a significant negative correlation between a cluster in the right IC and both pain intensity (r = -0.72, p = 0.001) and pain unpleasantness (r = -0.63, p = 0.007) (See Figure 7-8C).
**Table 7-1:** Group differences in whole brain skeletonised white matter. Group means ± standard deviations (SD) are presented for each group. Bonferroni post-hoc $p$-values are calculated based on independent-samples $t$-tests.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th></th>
<th>Patients</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (x10^{-4} mm^2/s)</td>
<td>SD (x10^{-4} mm^2/s)</td>
<td>Mean (x10^{-4} mm^2/s)</td>
<td>SD (x10^{-4} mm^2/s)</td>
<td>$p$-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>7.3</td>
<td>0.16</td>
<td>7.6</td>
<td>0.12</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RD</td>
<td>5.4</td>
<td>0.19</td>
<td>5.6</td>
<td>0.10</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>11.2</td>
<td>0.22</td>
<td>11.4</td>
<td>0.23</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MD – mean diffusivity; RD – radial diffusivity; $\lambda_1$ – axial diffusivity
**Table 7-2:** White matter regions in patients with TMD with significantly lower fractional anisotropy compared to controls. Mean and standard deviations of FA values, \( T \)-scores, percent decrease and MNI coordinates are reported for the peak voxel.

<table>
<thead>
<tr>
<th>Regions</th>
<th>FA</th>
<th>TMD</th>
<th># Voxels</th>
<th>% decrease</th>
<th>MNI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td>0.371</td>
<td>0.32</td>
<td>305</td>
<td>13.8</td>
<td>17 -15</td>
</tr>
<tr>
<td>WM adjacent to S1/M1</td>
<td>0.428</td>
<td>0.356</td>
<td>129</td>
<td>16.8</td>
<td>32 -22</td>
</tr>
<tr>
<td>IC(_{AL})</td>
<td>0.564</td>
<td>0.526</td>
<td>3</td>
<td>-11.1</td>
<td>14 12 -2</td>
</tr>
<tr>
<td>EC/ExC</td>
<td>0.373</td>
<td>0.339</td>
<td>4</td>
<td>9.1</td>
<td>33 6 3</td>
</tr>
<tr>
<td>EC/ExC</td>
<td>0.399</td>
<td>0.366</td>
<td>3</td>
<td>8.3</td>
<td>32 9 1</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>0.599</td>
<td>0.558</td>
<td>581</td>
<td>6.8</td>
<td>11 1 3</td>
</tr>
<tr>
<td>IC(_{AL}) and EC/ExC</td>
<td>0.513</td>
<td>0.456</td>
<td>55</td>
<td>6.6</td>
<td>26 24 11</td>
</tr>
<tr>
<td>Diffuse(^1)</td>
<td>0.853</td>
<td>0.786</td>
<td>10283</td>
<td>7.8</td>
<td>-5 26 10</td>
</tr>
</tbody>
</table>

\(^1\)Diffuse cluster includes white matter in the corpus callosum, corticospinal tracts, internal capsule, external/extreme capsules, fornix, cingulum, anterior corona radiata, cerebellar peduncle, and pontine tracts.

All clusters are significant at \( p < 0.05 \), voxel-wise, mask-wide, FWE corrected after threshold-free cluster enhancement. Voxels are 1mm\(^3\).

Abbreviations: IC – internal capsule; WM – white matter; S1 – primary somatosensory cortex; M1 – primary motor cortex; IC\(_{AL}\) – anterior limb of the internal capsule; EC/ExC – external/extreme capsules.
**Table 7-3:** Group differences in mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (λ₁) in clusters with significant group differences in FA. Mean DTI metric values and *p*-values are shown for two-tailed t-tests for MD, RD and λ₁ values in each cluster from Table 7-2 and the cluster-mass corrected cluster. n.s. indicates that the t-test did not reach significance (*p* ≥ 0.05).

<table>
<thead>
<tr>
<th>Cluster</th>
<th>MD (x10^4 mm^2/s)</th>
<th>RD (x10^4 mm^2/s)</th>
<th>λ₁ (x10^3 mm^2/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>TMD</td>
<td><em>p</em></td>
</tr>
<tr>
<td>IC₈</td>
<td>8.0</td>
<td>8.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EC/ExC</td>
<td>7.5</td>
<td>7.9</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>EC/ExC</td>
<td>7.6</td>
<td>8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IC₈/EC/ExC</td>
<td>7.2</td>
<td>7.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>S1/M1</td>
<td>6.7</td>
<td>6.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Thalamus</td>
<td>7.5</td>
<td>7.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IC</td>
<td>7.0</td>
<td>7.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Diffuse¹</td>
<td>7.6</td>
<td>7.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Corpus callosum²</td>
<td>8.2</td>
<td>8.8</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

¹see Table 7-2 for description of the diffuse cluster.

²Corpus callosum cluster is from the cluster-mass correction.

Abbreviations: IC – internal capsule; IC₈ – anterior limb of the internal capsule; EC/ExC – external/extreme capsules; S1 – primary somatosensory cortex; M1 – primary motor cortex
Figure 7-2: Colour orientation maps of the trigeminal nerves. Axial images of three controls and three patients with the primary eigenvector of the tensor model ($\lambda_1$) within each voxel. The eigenvector is colour-coded (green - anterior-posterior, red - left-right, blue - in the inferior-superior plane) and modulated to the FA map. The location of the trigeminal nerves is indicated by the yellow arrowheads.
Figure 7-3: Trigeminal nerve fractional anisotropy abnormalities in TMD. (A) The trigeminal nerve roots (within the blue boxes) at the pontine level are shown on an axial slice from a high-resolution T1-weighted MRI scan. The magnified view of the right nerve is from a diffusion-weighted scan. The direction of the primary vector of the tensor model ($\lambda_1$) within each voxel is color-coded (green - anterior-posterior, red - left-right, blue - in the inferior-superior plane) and
the primary vector shown within each voxel. (B) patients with TMD have lower fractional anisotropy in bilateral trigeminal nerves, compared to controls. (C) Fractional anisotropy is negatively correlated with TMD duration. (D) Group differences in trigeminal nerve mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (λ1). * = p < 0.05; ** = p < 0.01 *** = p < 0.005. CNV = trigeminal nerve.
Figure 7-4: Group differences in mean fractional anisotropy (FA) between patients with TMD and controls. Patients had significantly lower whole brain FA (left panel) and skeleton FA (right panel). Asterisks (*) indicate $p < 0.05$. 
**Figure 7-5:** Group differences in fractional anisotropy between patients with TMD and controls. (A) Blue regions indicate areas showing significant reduction in fractional anisotropy in TMD compared to controls using threshold-free cluster enhancement (TFCE, corrected $p < 0.01$) and overlaid on the white matter skeleton (shown in green) (significant clusters have been thickened for enhanced visualization). Widespread abnormalities are observed in bilateral internal (IC) and external/extreme capsules (EC/ExC), corpus callosum (CC), cingulum (Cing), thalamic and brainstem white matter. (B) 3D rendering of the region of reduced FA in the corpus callosum of patients compared to controls using cluster-mass thresholding ($t > 2.3$, corrected $p < 0.05$), the cluster-mass corrected result in the corpus callosum (CC). A = anterior.
Figure 7-6: Clusters with significant group differences in fractional anisotropy. Blue clusters (filled for visualization purposes) indicate regions of decreased FA in patients compared to controls. Significant clusters (TFCE, $p < 0.01$) are displayed on separate brains in the (A) anterior limb of the internal capsule (IC$_{AL}$), (B and C) external/capsules (EC/ExC), (D) junction of the IC$_{AL}$ and the EC/ExC, (E) white matter adjacent to the primary somatosensory and primary motor cortices (S1/M1), (F) white matter in the thalamus, (G) internal capsule (IC) and (H) in a diffuse cluster that includes brainstem white matter (cerebellar peduncles, pontine tracts, corticospinal tracts), IC, EC/ExC, corpus callosum (CC), cingulum (Cing), the fornix and white matter within the orbitofrontal cortex (OFC).
Figure 7-7: Abnormal white matter connectivity in TMD. Qualitative analysis of probabilistic tractography of the cluster-mass corrected (t > 2.3, p < 0.05) cluster in the left corpus callosum revealed that (A) this abnormal white matter region has different connections (yellow arrowhead).
in TMD and controls. The right panel shows a subtraction map that reveals that patients have sparser connections between the corpus callosum seed and the frontal pole (blue box), and denser connections to the dorsolateral prefrontal cortex (dIPFC; green box). The colour bar indicates the number of subjects (between 5 and 17) contributing to the cluster at each voxel (controls are in blue-light blue, and patients are in red-yellow). Quantitative tractography (B and C) revealed that more voxels from the seed region (in green) in the corpus callosum project to the frontal pole in the patients, compared to controls. The colour bar in (B) represents the proportion of subjects with projections to the frontal pole in each voxel (controls are in blue-light blue, and patients are in red-yellow). (C) Also, controls have a higher connection probability between the corpus callosum and the right dIPFC, whereas patients have a higher probability of connection between the corpus callosum and the left frontal pole. Graphs show mean number of samples (± SE) that reach the target in each group (top panel), and the mean number of voxels (± SE) in the seed mask that have samples that project to the target masks. * = p < 0.05.
Figure 7-8: Regions with group differences in fractional anisotropy are also correlated with TMD characteristics: (A) the junction between the right internal and external/external capsules (IC\textsubscript{AL}/EC/ExC) adjacent to the ventrolateral prefrontal cortex (vPFC), (B) thalamic WM (white matter) and the (C) right internal capsule are negatively correlated with TMD pain intensity. The right internal capsule is also negatively correlated with TMD unpleasantness. Controls’ mean ± SE FA for each region is shown in green. * = p < 0.05; ** = p < 0.01
7.4. Discussion

This study is the first report to show that both peripheral nerve and CNS white matter abnormalities contribute to TMD pain. In support of a peripheral contribution, we found that patients with TMD had lower FA in both CNVs and FA in the right CNV was negatively correlated with TMD duration. In support of a central component to TMD, we found that patients with TMD had (1) widespread abnormalities in the microstructure of white matter tracts related to sensory, motor, cognitive and pain functions, including a focal area of the corpus callosum; (2) stronger connectivity between the corpus callosum and the frontal pole, but sparser connectivity with the dLPFC, compared to controls; (3) FA abnormalities that correlated with TMD characteristics, and (4) reduced brain FA that were associated with greater MD and RD, markers of inflammation and œdema.

7.4.1. Trigeminal nerve abnormalities

This is the first study to assess the CNV white matter integrity in TMD. We found that patients with TMD had significantly lower FA and higher MD and RD in both CNVs. We also found that the FA in the right CNV was negatively correlated with TMD duration. Previously, TMD was considered idiopathic because there were no observable or major peripheral abnormalities in the TM joint or muscles [757]. However, the DTI-based imaging technology used here clearly shows microstructural abnormalities in the CNV. In line with peripheral abnormalities in TMD, one study has demonstrated that the jaw-jerk reflex is abnormal on the painful side in TMD [332], and another study found reduced cortical LEPs and reflexive masticatory muscular activity when the CNV was stimulated in TMD, compared to controls [743]. Therefore, increased nociceptive firing either from the periphery or from aberrant firing patterns (see below) over time could affect the microstructure of the CNV and could also contribute to central abnormalities along the ascending nociceptive system. This concept is supported by findings of increased pain sensitivity within and outside the trigeminal region [313,491,563-565,841]. Alternatively, there is evidence of abnormalities in orofacial motor function in TMD [46]. Recent evidence suggests that nociceptive input can modulate motor output (see Section 2.2.3.2). Therefore, it is possible that the microstructural abnormalities in the trigeminal nerve may be related to altered motor output from the CNS.
7.4.2. Abnormal sensorimotor tracts in TMD

In addition to the peripheral nerve findings, we also found that patients have lower FA in the brainstem, white matter coursing through the thalamus, the IC, and tracts adjacent to S1/M1. Therefore, decreases in FA and increases in MD and RD along the ascending nociceptive pathways could be induced or maintained by aberrant peripheral input from the CNV, or reduced antinociceptive activity in the CNS. In support of this concept, are our previous study showing that in patients with TMD there is a positive correlation between gray matter volume in the thalamus and TMD duration, and thicker cortex in the orofacial region of S1 (See Chapter 5) [617], suggesting that increased activity may lead to structural brain changes over time.

Given that DTI cannot distinguish between ascending and descending tracts [63], it is possible that the abnormal white matter tracts contain descending projection fibres (the corticofugal tracts) and corticothalamic tracts. The corticofugal tracts comprise the CST, corticobulbar, corticoreticular, corticopontine tracts [625]. These tracts largely contain motor efferents and so abnormalities in these tracts may underlie motor abnormalities in TMD, such as the masticatory muscle hyperactivity, and abnormal jaw motor function under cortical control [837,839]. Furthermore, patients with TMD have been shown to have increased cortical activity in motor regions and sluggish reaction times during a cognitive task [928].

7.4.3. Cognitive interference of pain and decreased modulation in TMD

We also identified lower FA in the cingulum bundle (adjacent to the mid-cingulate cortex), white matter tracts coursing to the OFC and sgACC, tracts adjacent to the vIPFC, the anterior corona radiata (in the IC\textsubscript{AL}, which projects to the prefrontal cortex) and the EC/ExC adjacent to the right mid-insula. The so-called medial pain system is comprised of ascending nociceptive fibres that project to the medial thalamus and further to the cingulate cortex, the insula and the PFC [234,505,868]. It is believed that these regions contribute to the cognitive-affective dimension of pain. The insula, mid-cingulate cortex and vIPFC have been implicated in pain perception, negative emotion, and cognitive function [23,94,108,116,183,221,220,222,227,304,447,752,790,898]. The OFC and the sgACC are thought to contribute to the descending pain modulation [95]. Patients with TMD have widespread pain [305,563,565,883], abnormal DNIC [113,491,756], and have been shown to
have sluggish reaction times to a cognitive task [928]. Therefore, the observed microstructural abnormalities in both the ascending medial pain system and descending modulatory system provide a plausible neural substrate for abnormal cognitive and anti-nociceptive function in TMD.

7.4.4. Abnormal connectivity in TMD

Another main finding of this study is that we identified a significant decrease in FA in the corpus callosum (identified using cluster-mass correction). This is a region that connects interhemispheric prefrontal regions [966], and this portion of the corpus callosum is also connected to the cingulate cortex. Similarly, a study examining white matter abnormalities in complex regional pain syndrome reported decreased FA in a similar region of the corpus callosum of patients, compared to controls [356].

In our study, probabilistic tractography revealed that patients had differential connectivity between the corpus callosum and two regions that have been implicated in executive control and pain perception and/or modulation: the frontal polar cortex and the dIPFC [19,47,105,144,173,403,404,457,496,497,503,552,620,834,875,876,908,922]. In line with these findings, we have previously identified structural and functional abnormalities in these regions [617,928]. Further, studies have shown that pain interferes with cognitive processes [529]. Given the possibility that cognitive resources are limited, these competing demands can interfere with one another, and impede cognitive performance. Evidence for the cognitive interference of pain comes from studies that have shown that acute pain can modulate performance in a cognitive task [291,529,687,697]. Further evidence comes from studies that have reported that chronic pain patients have slower reaction times in cognitive tasks [370,379,526,850,928]. Within the context of these findings, our results may represent that FA differences in the corpus callosum are related to differential connectivity in the brain, and potentially related to interaction between executive cognitive function and pain in TMD.

7.4.5. Intense and prolonged TMD may drive plasticity in white matter

We have previously reported that both pre-existing and chronic pain-driven factors contribute to gray and white matter brain abnormalities in patients with IBS (see: [98,156]. In the current study, the intensity and unpleasantness of TMD pain also correlated with regions with lower FA
in patients. There are two possible explanations of the finding of reduced FA correlated with pain intensity. Firstly, it is conceivable that abnormalities in white matter existed prior to the onset of TMD and, therefore, could influence the degree of TMD pain. Secondly, more intense, unpleasant or longer TMD pain may induce white matter plasticity, reflected as changes in FA. Evidence that pain induces or is associated with structural brain plasticity comes from recent studies that have reported that gray matter abnormalities in chronic pain are reversible, once the source of the pain has been resolved [384, 741, 782], which suggests that pain can potentially induce gray matter plasticity. In this study, decreased FA in the right CNV was related to the duration of TMD, which provides support for the latter possibility – that is, pain-induced plasticity. Similarly, the relationship between regions with abnormal FA in the brain (i.e., tracts adjacent to the vIPFC, within the IC and coursing through the thalamus) and TMD pain intensity suggest that TMD pain may be driving the decrease in FA, and the observed group difference. As mentioned above, these findings suggest that increased peripheral firing or abnormal firing in the CNV of patients with TMD or decreased dysfunctional central antinociceptive systems may alter its microstructure over time. This represents a particularly novel finding given the emphasis of a central ætiology for TMD [757].

7.4.6. Cellular basis of changes in FA

The diffuse pattern of white matter abnormality is likely due to the multidimensional nature of chronic pain that impacts several anatomical pathways. However, our diffuse white matter findings need to be interpreted with caution due to the global difference in FA we identified between patients and controls.

The factors that contribute to reduced FA may be macrostructural, such as increased branching, more crossing fibres or larger tracts (more axons) and/or microstructural changes such as cell swelling (œdema), changes to protein filaments (neurofilament phosphorylation), disruptions to the cell membranes, and, to a certain extent, decreased myelin [66, 67]. Recent studies have suggested that chronic pain may induce neuroinflammation in the brain [238, 381, 920], which can lead to changes in both gray matter and white matter that can be detected by MRI. Therefore, the observed changes in white matter may, in fact, be related to neuroinflammation rather than increases or decreases in the number or size of cells and/or axons present in the fields being studied. Our study has examined other measures of white matter microstructure (MD, RD and
The neuroinflammation model is supported by our finding that regions with lower FA in TMD have higher MD and RD, which are markers of inflammation and/or œdema [63,66].

7.4.7. Study limitations

The patients in this study did not self-report any previous diagnosis of depression or anxiety, although these exist in some patients with TMD [283,808,850]. Thus, the contribution of these factors in white matter abnormalities in TMD in general cannot be excluded. Another limitation of the current study is that microstructural abnormalities in white matter tracts cannot definitively be attributed to functional abnormalities, as this study only investigates structural abnormalities. For example, the CNV is a mixed cranial nerve, with motor and sensory fibres. It was not possible to disentangle whether the structural abnormalities are related to sensory abnormalities, motor abnormalities, or both. The resolution of DTI analysis based on voxels that are much larger than the width of a single axon. The current methods allow generalized inferences to be made only about the local microstructure within a voxel, but cannot differentiate which tracts are affected.

7.5. Conclusion

This is the first study to identify abnormalities in the CNVs of patients with TMD, suggesting a peripheral contribution to TMD. We have also demonstrated widespread white matter brain abnormalities in patients with TMD in sensory, motor and cognitive-affective regions, consistent with a central component to TMD. Finally, we have identified that patients with TMD have abnormal connectivity of cognitive-affective regions of the brain. In sum, we provide structural findings consistent with observed behavioural and functional finding in TMD, supporting a model of central abnormalities. While also present novel evidence of a potential peripheral contribution to TMD.
Chapter 8
General Discussion

This is the first comprehensive study of both gray and white matter structural abnormalities in idiopathic TMD, and the first to investigate focal age-related gray matter abnormalities in chronic pain. The main findings of this thesis are that patients with TMD show:

1) Abnormal gray matter in brain areas implicated in somatosensory, motor, cognitive and modulatory functions, including a) increased cortical thickness in the S1 and frontal cortex, b) chronic pain intensity or unpleasantness-dependent cortical thinning in the cingulate, motor, and orbitofrontal cortices, c) pain chronicity-dependent thalamic gray matter increases, d) abnormal impact of neuroticism on orbitofrontal gray matter;

2) Abnormal gray matter aging in focal brain regions of pain and motor systems, some of which is impacted by chronic pain chronicity;

3) Widespread brain white matter abnormalities, including a) in tracts containing connections for sensory, motor, cognitive and pain functions, b) prominently stronger corpus callosum-frontal pole connectivity and weaker dlPFC connectivity, c) chronic pain/unpleasantness-dependent abnormalities, d) abnormally high MD and RD levels, markers of brain neuroinflammation; and

4) Trigeminal nerve abnormalities, exacerbated by TMD chronicity.

Together, these findings suggest that idiopathic TMD are complex, and are accompanied by both central and peripheral abnormalities, some of which may be pre-existing abnormalities that could predispose persons to developing TMD.

Several factors can contribute to the observed structural abnormalities in the brains of patients with TMD. The first possibility is that these changes are “all-or-none”, where having TMD is accompanied with these brain abnormalities. Another possibility is that the observed changes occur over time, the prolonged effect of pain progressively affects brain structure. Another possibility is also related to duration, but different in that the magnitude of pain (intensity or unpleasantness) can affect the magnitude of change in the brain: higher pain intensity drives changes in the structure of the brain. Together, these possibilities may be referred to as pain-
driven abnormalities. Finally, in contrast to pain-driven abnormalities, there could be pre-existing brain abnormalities, possibly related to stable personality traits that can predispose persons to developing TMD. The relative contribution of these factors are discussed below.

8.1. Pain-driven abnormalities along nociceptive pathways

In this thesis, structural abnormalities were identified at several locations along the ascending nociceptive pathway. This pathway begins with the CNV in the periphery. Nociceptive information from the TMJ and the muscles of mastication is carried to the CNS via the CNV. Nociceptive C- and Aδ-fibres project to second-order NS and WDR neurones in the VBSNC [784]. These cells ascend to various brainstem nuclei, and the thalamus via the TTT, where they project to third-order neurones, which form thalamocortical tracts [784]. This thesis has reported decreased FA, and increased \( MD \), and RD along the CNV of patients with idiopathic TMD. These data suggest that there is increased permeability in the membrane or decreased myelin insulation [67] (see below). Further, these abnormalities are negatively correlated to TMD duration in the right CNV—patients with relatively shorter TMD durations have relatively normal FA values, and these values deviate from normal values as TMD duration increases. These data suggest that chronicity of TMD pain contributes to changes in the microstructure of the nerve. Therefore, there may be increased nociceptive input from the periphery, or decreased/dysfunctional central antinociceptive systems, or progressive abnormalities along the nerve root, which may cause aberrant firing patterns. In line with the revised pain adaptation model [431], changes in the recruitment of motor units, over time, can themselves become a source of pain. Therefore, it is possible that increased nociceptive activity over time alters facial motor activity. Prolonged alterations in motor activity from the orofacial region can itself become pathological and a source of nociceptive input. This prolonged input, in turn, may alter CNV microstructure and structures along the ascending nociceptive pathways.

In support of this hypothesis is the finding that gray matter volume in the thalamus positively correlated to TMD duration. Similar to our finding in the CNV, with increased TMD chronicity, patients showed increased gray matter in the thalamus. The age-by-group study confirmed the finding that thalamic gray matter changes are driven by duration. It is therefore likely that ascending nociceptive pathways undergo progressive change with long-term TMD. Similarly, several studies reported pain-driven gray matter structural abnormalities in chronic pain.
[27,98,206,268,303,356,490,502,740,764-766,772,888,889,919,963] (see Table 2-3). Support for the concept of chronic pain-driven gray matter changes comes from a study by Teutsch and colleagues [852], who reported increased S1 and MCC gray matter volume after eight consecutive days of 20-minute paradigm of acute nociceptive stimulation to the volar forearm. Subjects had thicker S1 and MCC. Additionally, this thesis reports abnormalities along white matter tracts connecting the various levels of the ascending trigeminal nociceptive system – the TTT, white matter coursing through the thalamus, TCT coursing through the somatomotor region of the IC and adjacent to the somatosensory and motor cortices. Furthermore, the white matter tracts within the thalamus and the motor region of the IC are related to pain intensity and/or unpleasantness. For a summary schematic of abnormalities along the ascending sensory (and nociceptive) pathway, see Figure 8-1.

In sum, this thesis has demonstrated structural abnormalities at several points along the ascending nociceptive pathway – including key gray matter regions implicated in nociception, and the tracts that connect them.
8.2. Abnormalities in motor regions

The pain and motor systems interact at several levels within the nervous system (see Section 2.1.6.). This thesis reports pain-related structural abnormalities in gray matter and white matter associated to motor function in patients with TMD. Specifically, the thickness of the orofacial region of M1 was negatively correlated to pain intensity in patients with TMD. Similarly, patients with TMD had lower FA in white matter tracts associated to motor regions, and FA in these regions was negatively correlated to pain intensity. Furthermore, our lab has previously reported that the same group of patients with TMD had increased cortical activity in motor regions and sluggish reaction times during a cognitive task [928]. Finally, the age-by-group analysis revealed abnormal aging of the PMC and the dorsal striatum in TMD, compared to healthy controls.
There are three possible mechanisms that may explain the observed abnormalities in motor regions and tracts (see Figure 8-2): (1) nociceptive input dampens motor output to prevent further damage; (2) motor cortex modulates pain intensity; or (3) reduced movement to avoid pain may affect motor cortex thickness. Future work should specifically address the pain-motor interaction in TMD to determine which of these mechanisms is occurring in TMD.

Previous studies have reported that nociceptive input inhibited motor cortex excitability [3,157,301,302,638,856], and that motor cortex stimulation modulated the perceived intensity of pain in chronic pain [103,117,118,301,331,342,344,345,535,548,657,689]. Furthermore, a study by Boudreau and colleagues [109] reported that task training-related motor cortex plasticity was inhibited by noxious stimulation of the intraoral cavity. Additionally, several studies have reported abnormal stimulus-evoked fMRI activity in motor regions of chronic pain patients, although the implication of these abnormalities is not often discussed (see: [23]). For instance, Kirveskari et al. [492] reported that patients with CRPS had weaker M1 reactivity with increased spontaneous pain ratings, and a negative correlation between pain intensity and M1 activation. Therefore, studies in experimental pain paradigms and in chronic pain patients have demonstrated that nociceptive input modulates M1 function. Furthermore, evidence suggests that M1 activity can modulate pain perception.

Alternatively, the observed relationship between pain intensity and M1 thickness may be related to the possibility that patients with TMD make fewer jaw movements to avoid eliciting pain. Based on the concepts of use-dependent plasticity (or in this case disuse-dependent plasticity), this reduction in jaw activity may lead to atrophy of the orofacial region of the motor cortex [576].
Figure 8-2: Proposed model for the neural basis of motor abnormalities in TMD. Altered nociceptive input inhibits motor cortex output in an intensity dependent manner, and reduces FA in corticocortical tracts connecting S1 and M1. Decreased motor output causes disuse-related plasticity along corticofugal tracts (including corticobulbar tracts) and the motor portion of the CNV.

8.3. Abnormalities in cognitive-modulatory brain regions

This thesis has demonstrated structural abnormalities in brain areas that have been implicated in cognitive/modulatory functions, providing a structural framework that could underlie the negative impact of chronic pain on cognitive abilities, including concentration, learning, memory, and attentional focus [26,250,275,365,369,455,489,666,928]. Specifically, we have reported thicker cortex in the vlPFC. Furthermore, patients with TMD had reduced measures of white matter integrity in white matter adjacent to the vlPFC. The vlPFC has been implicated in various aspects of pain, including anticipation [752] and modulation [909], as well as behavioural inhibition [32]. Furthermore, studies have reported hypervigilance in patients with TMD [435,808], which is related to increased pain anticipation. Therefore, the observed structural abnormalities in the vlPFC may be related to patients’ hypervigilance and increased anticipation to pain (see Figure 8-3).
Another key finding of this thesis was that the left frontal polar cortex was thicker in patients with TMD, than in controls. Additionally, probabilistic tractography revealed that the frontal polar cortices were more connected interhemispherically – the right and left frontal poles were more connected in TMD than in pain free controls. The frontal pole can be activated during spontaneous pain in patients with chronic pain [47]. Additionally, the frontal pole has also been implicated in a number of complex executive cognitive functions such as learning behavioural routines [457,496,834], cognitive branching (the ability to put a pending task on hold to execute an ongoing one) [497], behavioural flexibility/adaptability [105] and post-hoc monitoring or evaluating decisions based on feedback [875]. The concept of cognitive branching would suggest that there is a limited resource of working memory, and that tasks that are more behaviourally-relevant (or salient) are prioritized [44,45,155,495,497,498]. Specifically, the frontal polar cortex monitors the relevant salience (or value) of a given stimulus and select behavioural outcomes accordingly. Given that pain is an inherently salient stimulus [265,530,933], it is conceivable that pain engages the frontal pole. Further, a study by Harman and Ruyak [409] reported that people with persistent low-level pain related to their work habits had cognitive deficits, indicative of an interference effect of pain. Therefore, the frontal pole may process the cognitive dimension of pain, which suggests that pain has a cognitive load. In the short term, as in cases of acute pain, the frontal pole may direct behaviour to protect the subject. However, in chronic pain conditions, this region may be continuously engaged. Therefore, when a person with chronic pain is distracted, or performing a demanding cognitive task, the frontal pole may be “putting the pain on hold”, allocating resources to the other, competing cognitive demands (see Figure 8-3).

Accordingly, Eccleston and colleagues [289] showed that chronic pain patients could briefly be disengaged from pain during attentional switches. Further support for this hypothesis comes from studies that demonstrate that distraction can reduce pain perception [123,430,584,588,687,864,888,891,935,955]. These studies have demonstrated that distraction-based modulation of pain requires the recruitment of antinociceptive brain regions. However, these systems may not be effective in patients with TMD in two ways: (1) there is evidence of dysfunctional antinociceptive systems (see Section 2.2.3.1) [305,491], and (2) the cognitive branching model suggests that cognitive resources are limited – there are only so many tasks that can be juggled [497]. Therefore, the cognitive load of chronic pain could impact the a patient’s ability to properly complete tasks [529]. In support of this idea, we have recently shown that patients with TMD have sluggish reaction times to low and high conflict cognitive interference
tasks, but not to a simple sensorimotor task [928]. Therefore, in the context of use-dependent plasticity [576], one possible explanation for the observed thickening of the frontal pole and the increased white matter connectivity between the bilateral frontal poles is that chronic TMD pain bears a cognitive load that the brain needs to ‘put on hold’ in order to address more immediate environmental demands.

Figure 8-3: Proposed model of the neural basis for cognitive abnormalities in TMD. Altered nociceptive activity alters the structure and connectivity of cognitive brain regions, including the vIPFC and the frontal pole.

Additionally, this thesis found that patients with TMD have abnormal gray matter aging in the aMCC/pgACC. Furthermore, these patients have lower FA in the cingulum bundle (adjacent to the MCC). This region receives orofacial nociceptive input from thalamic nuclei that are part of the STT and TTT systems [189,276]. The aMCC/pgACC is a complex, multimodal region that has been implicated in a number of functions [69]. For instance, this region has been identified as
a node in the salience network, and has been implicated in aspects of salience [213,229,262,263,774,849,927], and pain [230,231,259,265,440,508,528,632,933]. Furthermore, the cingulate cortex has also been implicated in the cognitive and affective processing of pain [218,230,529,716,776,777,934,936], and the MCC is involved in action selection and modulation of motor output in response to aversive stimuli [790,898,904]. Therefore, it is possible that these structural abnormalities in the cingulate cortex are related to cognitive and attentional abnormalities in TMD.

Patients with TMD also had widespread white matter abnormalities in white matter tracts related to pain-modulatory brain regions. Specifically, patients had lower FA and increased MD and RD in white matter tracts coursing to the OFC and sgACC, the anterior corona radiata (in the ICAL, which projects to the prefrontal cortex) and the external/extreme capsule adjacent to the right mid-insula. The insula, mid-cingulate cortex have been implicated in pain perception, negative emotion, and cognitive function [23,94,108,116,183,222,227,304,447,752,790,898]. The OFC and sgACC are thought to contribute to descending pain modulation [95]. Furthermore, patients with TMD had abnormal connectivity to the dIPFC, a region implicated in pain modulation [552], selecting information about the sensory environment (attention, self-monitoring, personality, working memory) and decision-making [54,700] (see Figure 8-4). Patients with TMD have widespread acute pain hypersensitivity [305,563,565,883], abnormal DNIC [113,491,756], while having sluggish reaction times to a cognitive task [928].

In sum, this thesis provides a potential structural neural basis for cognitive deficits and dysfunctional pain-modulatory circuits in patients with idiopathic TMD.

8.4. Neuroticism and TMD

It is possible that some factors predispose persons to develop TMD. A recent prospective study by Fillingim and colleagues [315] reported that certain psychological traits could, to some extent, predict TMD onset, including neuroticism. In the current thesis, we report that in patients with TMD there is a positive correlation between neuroticism and cortical thickness in the left vmPFC (part of the OFC); an area that normally shows a negative correlation between gray matter and neuroticism [954]. Neuroticism has been defined as a stable personality trait associated with heightened sensitivity and/or processing of negative affective stimuli [178,179,907]. Therefore, it is not surprising that patients with high neuroticism scores have lower pain threshold and pain
tolerance, and are more distressed by pain in experimental settings and chronic pain [408]. Neuroticism has been associated with pain-related suffering [35,408], pain sensitivity [177,373,907], nerve injury outcomes and the development of neuropathic pain [848], and TMD [315]. However, the mechanism by which inherent personality-related factors reduce the brain’s capacity to effectively modulate nociceptive input and pain perception, remains unclear. Furthermore, not all chronic pain patients have high neuroticism scores, and not all persons with neuroticism have chronic pain [179]. Therefore, neuroticism alone is not sufficient to develop chronic pain, however it can contribute to the severity of pain in chronic pain patients. Therefore, our observation highlights the contribution of neuroticism to abnormal gray matter in the OFC of patients with TMD and, perhaps, to the development of TMD (see Figure 8-4). Similarly, in IBS patients, neuroticism correlated to FA in the left MD thalamus, but not in controls [156]. An alternative interpretation is that chronic pain may have modified the relationship between neuroticism and OFC thickness. However, the lack of correlation of TMD duration and neuroticism scores in our patients with TMD does not support this hypothesis.

We also found that unpleasantness is negatively correlated to gray matter in an adjacent region of the OFC. The OFC has been implicated in cognitive reappraisal and emotional regulation [723] related to interoception and somatovisceral stimuli, for directing our behaviour appropriately [57,935]. The OFC is also thought to play a role in mental flexibility and adaptability (for a critical review, see: [770]. Therefore, our finding suggests that patients with TMD may have abnormal emotional regulation and reappraisal, which may lead to pain behaviours and exaggerated affective responses to pain.
The relationship of neuroticism and cortical thickness in the OFC alters the structure and antinociceptive function in pain modulatory brain regions, including the sgACC. The dlPFC is also has reduced white matter connectivity in patients with idiopathic TMD, which may be another substrate for dysfunctional pain modulation networks in TMD.

8.5. Basis of structural brain abnormalities

The cellular and molecular bases of MRI-detectable gray and white changes in chronic pain are not known but there is now evidence to support several hypotheses. Mechanisms of gray matter change include neuronal and/or glial death [575], but recent evidence suggests that, to some extent, gray matter losses are likely related to density of small dendritic spines [278,608], and the remodeling of neuronal processes [537]. Alternatively, reversible gray matter changes in chronic pain may be caused by neuroinflammation [238,381,920], and induce MRI-detectable increases in gray matter. This mechanism could explain abnormal thickening (or maintenance) of gray matter volume/thickness. For the observed gray matter losses, however, we cannot rule out that cell death is not occurring – healthy populations lose neurones as they age, and persons with neurodegenerative diseases suffer increased rates of atrophy related to cell death.

Figure 8-4: Proposed model for the neural basis of modulatory system abnormalities in TMD.
The factors that contribute to reduced FA may be macrostructural, such as increased branching, more crossing fibres or larger tracts (more axons) and/or microstructural changes such as cell swelling (œdema), changes to protein filaments (neurofilament phosphorylation), disruptions to the cell membranes, and, to a certain extent, decreased myelin [66,67]. Furthermore, the neuroinflammation hypothesis for gray matter changes can also apply to white matter changes. Therefore, the observed changes in white matter may, in fact, be related to neuroinflammation rather than increases or decreases in the number or size of cells and/or axons present in the fields being studied. This thesis has examined other measures of white matter microstructure (MD, RD and $\lambda_1$) to better characterize group differences in FA. The neuroinflammation model is supported by the finding that regions with lower FA in TMD have higher MD and RD, which are markers of inflammation and/or œdema [63,66].

8.6. Study limitations

A few limitations in study design and results should be considered in interpretation of the data reported in this thesis. Some of the limitations specific to each study have been discussed within the relevant chapters.

First, the patients that were recruited for this project were deemed to have TMD based on longstanding criteria used by the TMD clinicians at the Mount Sinai Hospital Pain Clinic and represent a spectrum of idiopathic TMD seen clinically. However, this categorization does not strictly adhere to the standard TMD-RDC, as described by Dworkin and LeResche [285].

Second, it is not known whether there is an impact of long-term use of medications in some of the patients on gray and white matter plasticity. Recent evidence suggests that medications commonly used to manage pain, such as anti-inflammatory medications and opiates, can impact brain structure [916,962]. Only one subject was taking an opiate, hydromorphone, and so this is of less concern to this study, but considering that thirteen of seventeen of the patients included in this study were taking NSAIDs, it is possible that the observed age-related findings – especially those of gray matter maintenance, may be related to the effect of these drugs.

Furthermore, it is not possible to totally rule out a contribution of ventricular volume to the VBM analysis of subcortical structures. Additionally, some of the patients had co-morbid chronic pains (see Table 5-1), and these pains (mostly related to TMD) may have contributed to our findings.
Another limitation is that duration (and intensity) may not fully describe the effect of pain on gray and white matter structure. Due to the nature of TMD pain, other factors that were not collected, such as the number of days of pain in a month may have more explanatory variance. Patients also did not self-report any previous diagnosis of depression or anxiety, although these exist in some patients with TMD [283,808,850]. Thus the contribution of these factors to gray and white matter abnormalities in TMD cannot be ruled out.

Most of the patient sample had bilateral pain, but our findings tended to be unilateral. These inconsistencies can simply be due to statistical thresholds. However, future studies should limit patient recruitment to unilateral pain to determine the laterality of CNS abnormalities.

Another limitation of this thesis is that it is a cross-sectional examination of structural abnormalities in TMD. Therefore, the interpretations of “pain-driven” and “pre-existing” abnormalities are limited, and should be taken with caution. Furthermore, quantitative sensory testing was not performed on the patient group recruited for this study, and so we cannot directly relate our findings of structural abnormalities to these behavioural abnormalities.

Despite these limitations, this thesis does provide considerable evidence in line with previous findings in the literature about gray and white matter abnormalities in chronic pain.

8.7. Future directions

This thesis has clearly demonstrated structural gray and white matter abnormalities in the brain of patients with TMD. Future studies could build on these findings to provide more insight into the potential underlying mechanisms. For example, behavioural measure of pain sensitivity in these patients (obtained through quantitative sensory testing) would be useful to link the structural abnormalities with perceptual abnormalities. Another hypothesis put forward by this thesis is that patients with TMD (and potentially all chronic pain patients) have deficient cognitive abilities due to the cognitive load of pain overloading the cognitive branching system. Therefore, future studies could conduct cognitive tests that have been used to establish the presence of a cognitive branching system in the brain to assess whether patients with TMD do have deficient branching.

It was also demonstrated that pain intensity is related to abnormal structure in the motor cortex. However, whether this abnormality is related to function has not been determined. Therefore, in
the future, motor function could be tested in the patients with TMD in the orofacial region and elsewhere in the body. Future studies could also investigate the functional brain abnormalities related to abnormal motor behaviour. In line with this experiment, we have clearly demonstrated abnormalities along the CNV of patients with TMD. Nerve conduction studies could also be used to test whether the sensory or motor aspect of the nerve is affected, and how these structural abnormalities affect nerve function.

Furthermore, other imaging modalities can be used to assess the molecular basis of structural abnormalities in the brain. For instance, magnetic resonance spectroscopy can be used to assess whether there are differences in markers of specific neural structure and viability, and relate these to structural abnormalities.

Animal models of TMD should be used to investigate the relationship between MRI-detectable structural changes in the brain, and pain-related behaviours. These structural abnormalities could be further characterized with genetic, cellular, electrophysiological and molecular methods.

Functional brain imaging with arterial-spin labeling, an imaging modality that allows baseline imaging, like PET but not invasive, could be used to assess spontaneous pain in patients with TMD. This would provide insight into which brain regions are implicated in pain processing, and abnormally activated in TMD pain.

Prospective studies of TMD could also provide insight about the risk factors and development of TMD. Patients with TMD could be followed over time to assess whether they respond to treatment, or not. The factors that contribute and/or predict whether patients are treatment-responders and non-responders could be assessed. Neuroimaging at several time points could elucidate how the structure and function of the brain are affected by the cumulative effect of pain, and how these abnormalities may vary between the groups.

Fewer patients with idiopathic TMD respond to treatment, compared to patients with post-traumatic TMD. Therefore, another study could compare whether the same functional and structural abnormalities occur in patients with idiopathic and post-traumatic TMD. The study could provide insight about peripheral pain-driven abnormalities (in post-traumatic TMD) and central mechanisms that contribute to TMD.
8.8. Conclusions

In sum, this thesis provides novel evidence for both peripheral nerve and CNS abnormalities that contribute to TMD pain. In support of a peripheral contribution, it was found that patients with TMD had lower FA in both CNVs and FA in the right CNV was negatively correlated with TMD duration. In support of CNS abnormalities, structural abnormalities in gray and white matter were found along the ascending nociceptive, descending pain-modulatory, motor and cognitive pathways. Furthermore, it was demonstrated that some, but not all of the observed structural abnormalities are pain-driven. Other brain abnormalities do not show a relationship to pain characteristics, but rather to factors independent of pain, such as age and neuroticism, a stable personality trait. The latter findings suggest the presence of pre-existing abnormalities that may be a predisposing risk factor to the development of TMD.
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Appendices
Appendix I: Recruitment Letter

To: Mount Sinai Hospital Dental Clinic patients  
From: Drs. Davis, Goldberg and Tenerbaum

My co-workers and I are interested in how chronic pain affects the brain. As part of this research program, we are conducting a research study to examine the relationship between temporomandibular disorder (TMD or TMJ dysfunction) and organization of your brain and its function. We invite you to participate in this important research project.

There are two parts to the project, which may take place on separate days or a single day, and will require approximately 2 hours in total. In the first part, we will ask about your health, your pain and also conduct some very basic personality and cognitive tests to measure your brain’s ability to process information. This will take approximately 30-40 minutes. The second part includes some additional questionnaires and an MRI scan (at the Toronto Western Hospital) to take pictures of your brain while lying still. The MRI will take approximately 1-1.5 hours and does not involve any x-rays or dangerous radiation at all.

There are no major risks to you, and you may withdraw at any time. To compensate for your time you will receive remuneration of $75 for the completed study.

If you require further information, please leave a message for Dr. Karen Davis at 416-603-5502.

Yours sincerely,

Karen D. Davis, PhD.

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Appendix II: Consent forms

Toronto Western Hospital
University Health Network

Pain, Temperature and Tactile Perceptions in Normal Subjects and in Patients With Neurological Disorders Investigators: K.D. Davis, D.J. Mikulis

Consent Form

You are being asked to take part in a research study. Before agreeing to participate in this study, it is important that you read and understand the following study procedures. Please ask the study doctor or study staff to explain any words you don’t understand and make sure all your questions have been answered before signing this document.

Purpose: We are studying the brain mechanisms of touch, temperature, pain sensation and visual and auditory perception. Therefore, we will assess your perception of various stimuli presented and also obtain images of your brain with the magnetic resonance imaging (MRI) scanner during the stimulation.

Procedures: If you agree to participate, you will be trained to rate the intensity of a stimulus and to describe its quality. In some sessions the ratings will consist of a verbal or written indication of the stimulus. In other sessions you may be trained to use a computer to rate the stimulus. You may also be asked to watch visual images or listen to sounds. Prior to applying the stimuli, site(s) on your skin may be marked with water soluble ink. This is to allow us to deliver each stimulus to the same location(s) on your skin. A series of mechanical, thermal and/or electrical stimuli will then be applied to a small area of your skin. Following each stimulus you will be asked for a rating. The mechanical (pressure) stimuli will consist of one or more of several types such as slender nylon filaments, cotton swabs, tuning forks and pins. The thermal stimuli will be delivered with a probe that is precisely controlled and may be warm, cold or painful. You may be asked to place you hand into a cold water bath. The electrical stimuli will be delivered by electrodes placed on your skin. The visual images will be presented on a screen and the auditory stimuli will be heard through headphones. To image your brain, you will be placed into the narrow tube of the MRI machine. The MRI makes loud banging noises when it is taking pictures. At any time, if you are experiencing distress, you can freely communicate with the MR technician through a microphone in the MRI. You are free to terminate the procedure if you feel extreme claustrophobia. We may also ask you to fill out some questionnaires about your feelings towards pain and other life experiences. Each session will take about 1-2 hours. You will receive remuneration for your time at $20/hour. Parking expenses at TWH will be reimbursed if your study participation required parking.

Risks: There are no major risks associated with the experimental protocols other than the discomfort of the painful stimuli. Your participation in this study is voluntary. At any time during these sessions, you are free to withdraw from any stimulus and are free to terminate a session. Your participation or withdrawal from this study in no way affects your current or future medical care. If you have any questions regarding these procedures please contact Dr. Karen D. Davis at 416-603-5662 or Dr. David Mikulis at 416-603-5612. For any questions regarding your rights as a research subject please contact Dr. Heslegrave, (Chair, UHN Research Ethics Board) at 416-340-4557.

All information obtained during the study will be held in strict confidence. No names or identifying information will be used in any publication or presentations.

Authorization: I have read and understand the above description of the study. I understand that there is no direct personal benefit to me. I also understand that I may withdraw from this study at any time, without affecting in any way my medical treatment at this time or in the future. Although the records from this study are kept confidential, I understand that the results may be presented at scientific conferences and published in professional journals. I will receive a copy of the consent form.

subject’s name - please print
subject’s signature
(date)

(investigator obtaining consent - signature ver. 5/2004)
Consent Form

Title: Pain, Temperature and Tactile Perceptions in Normal Subjects and in Patients with Neurological Disorders

Investigators: K.D. Davis, Tel.: 416-603-5662

Co-Investigators: H.C. Tenenbaum
M. Goldberg

Introduction:

You are being asked to take part in a research study. Please read this explanation about the study and its risks and benefits before you decide if you would like to take part. You should take as much time as you need to make your decision. You should ask the study doctor or study staff to explain anything that you do not understand and make sure that all of your questions have been answered before signing this consent form. Before you make your decision, feel free to talk about this study with anyone you wish. Participation in this study is voluntary.

Purpose:

We are studying the brain mechanisms of touch, temperature, pain sensation and visual and auditory perception. Therefore, we will assess your perception of various stimuli presented and also obtain images of your brain with the magnetic resonance imaging (MRI) scanner during the stimulation.

Procedures:

There are two parts to the project, which may take place on separate days or a single day, and will require approximately 2 hours of your time in total. In the first part, we will ask about your health, your pain and also conduct some very basic personality and cognitive tests to measure your brain’s ability to process information. This will take approximately 30-40 minutes. The second part includes some additional
questionnaires and a MRI (magnetic resonance imaging) scan (at the Toronto Western Hospital) to take pictures of your brain while lying still and also while viewing words that may be related to your pain. The MRI will take approximately 1-1.5 hours.

As part of these procedures you will be trained to rate the intensity of a stimulus and to describe its quality. In some sessions the ratings will consist of a verbal or written indication of the stimulus. In other sessions you may be trained to use a computer to rate the stimulus. You may also be asked to watch visual images or listen to sounds. You may have your skin marked with water soluble ink in studies in which we deliver mechanical, thermal and/or electrical stimuli to a small area of your skin. Following each stimulus you will be asked for a rating. For those studies, mechanical (pressure) stimuli will consist of one or more of several types such as slender nylon filaments, cotton swabs, tuning forks and pins. The thermal stimuli will be delivered with a probe that is precisely controlled and may be warm, cold or painful. You may be asked to place you hand into a cold water bath. The electrical stimuli will be delivered by electrodes placed on your skin. The visual images will be presented on a screen and the auditory stimuli will be heard through headphones.

To image your brain, you will be placed into the narrow tube of the MRI machine. The MRI makes loud banging noises when it is taking pictures. At any time, if you are experiencing distress, you can freely communicate with the MR technician through a microphone in the MRI. You are free to terminate the procedure if you feel extreme claustrophobia.

Risks:

There are no major risks associated with the experimental protocols other than the discomfort of the painful stimuli. At any time during these sessions, you are free to withdraw from any stimulus and are free to terminate a session.

Benefits:

You will not receive any direct benefit from being in this study. Information learned from this study may help other people with chronic pain in the future.

Voluntary Participation:

Your participation in this study is voluntary. You may decide not to be in this study, or to be in the study now and then change your mind later. You may leave the study at any time without affecting your current or future medical care.
Confidentiality:

If you agree to join this study, the study doctor and his/her study team will look at your personal health information and collect only the information they need for the study. Personal health information is any information that could be used to identify you and includes your:

- name,
- address,
- date of birth,
- new or existing medical records, that includes types, dates and results of medical tests or procedures.

The information that is collected for the study will be kept in a locked and secure area by the study doctor for 7 years. Only the study team or the people or groups listed below will be allowed to look at your records. Your participation in this study also may be recorded in your medical record at this hospital.

Representatives of the Mount Sinai Hospital Research Ethics Board may look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines.

All information collected during this study, including your personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law. You will not be named in any reports, publications, or presentations that may come from this study.

If you decide to leave the study, the information about you that was collected before you left the study will still be used. No new information will be collected without your permission.

**In Case You Are Harmed in the Study:**

If you become ill, injured or harmed as a result of taking part in this study, you will receive care. The reasonable costs of such care will be covered for any injury, illness or harm that is directly a result of being in this study. In no way does signing this consent form waive your legal rights nor does it relieve the investigators, sponsors or involved institutions from their legal and professional responsibilities. You do not give up any of your legal rights by signing this consent form.

Expenses Associated with Participating in the Study:
You will receive remuneration for your time at approx. $30-40/hour or $75/study.

Questions:

If you have any questions regarding these procedures please contact Dr. Karen D. Davis at 416-603-5662. For any questions regarding your rights as a research subject please contact Dr. Heslegrave, (Chair, UHN and Mount Sinai Hospital Research Ethics Boards) at 416-340-4557 or 416-586-4875. This person is not involved with the research project in any way and contacting him will not affect your participation in the study.

Consent:

This study has been explained to me and any questions I had have been answered.

I know that I may leave the study at any time. I agree to take part in this study.

_________________________  __________________________  __________
Print Study Participant’s Name      Signature          Date

(You will be given a signed copy of this consent form)

My signature means that I have explained the study to the participant named above. I have answered all questions.

_________________________  __________________________  __________
Print Name of Person Obtaining Consent      Signature          Date
Handedness Questionnaire

Instructions

Please indicate your preferences in the use of hands in the following activities. If you are really indifferent, select “Either”. Where the preference is so strong that you would never try to use the other hand select “No”.

<table>
<thead>
<tr>
<th>When:</th>
<th>Which hand do you prefer?</th>
<th>Do you ever use the other hand?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Writing:</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Drawing:</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Throwing:</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using scissors:</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a toothbrush:</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a knife (without fork):</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a spoon:</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a broom (upper hand):</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Striking a match:</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
<tr>
<td>Opening a box (lid):</td>
<td>L R Either</td>
<td>Yes No</td>
</tr>
</tbody>
</table>

Thank you for your responses.

Adapted from: Mark S. Cohen

Based on: Oldfield, R.C. 1971
Appendix IV: Pain Catastrophizing Scale

Client No.: _______  Age: _____  Sex: M(____)  F(____)  Date: ________________

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

0 – not at all  1 – to a slight degree  2 – to a moderate degree  3 – to a great degree  4 – all the time

When I’m in pain …

1. I worry all the time about whether the pain will end.
2. I feel I can’t go on.
3. It’s terrible and I think it’s never going to get any better.
4. It’s awful and I feel that it overwhelms me.
5. I feel I can’t stand it anymore.
6. I become afraid that the pain will get worse.
7. I keep thinking of other painful events.
8. I anxiously want the pain to go away.
9. I can’t seem to keep it out of my mind.
10. I keep thinking about how much it hurts.
11. I keep thinking about how badly I want the pain to stop.
12. There’s nothing I can do to reduce the intensity of the pain.
13. I wonder whether something serious may happen.

…Total
Appendix V: TMD pain assessment

Date: ……/……/……

Patient Name:

Patient No.:

Date of Birth: ……/……/……

What is your highest level of Education: ES HS UNI M

Phase of the menstrual cycle

1 2 3 4

What day did your last period start: ……/……/……

Oral contraceptive Y N

Site and Radiation:

<table>
<thead>
<tr>
<th></th>
<th>Preauricular</th>
<th>Intra-auricular</th>
<th>Temporal</th>
<th>Massetric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. How long have you had TMD? _________________________

2. Describe how your pain feels? _________________________

3. McGill Pain Questionnaire

4. Frequency: Constant-…………

    Occasional-………………… per week

    …………………… per day

Pain History:

Worse in a.m.? Y  N

Worse in p.m.?  Y  N

Responsive to analgesics?  Y  N

Current medication/treatment  Y  N  _________________________________

Other Pain Conditions?  Y  N  arthritis, fibromyalgia, IBS____________________

Aggravating factors?  Y  N  eating, chewing, yawning, talking,_____________________

Relieving factors?  Y  N  rest, warmth, cold, chewing,_______________________
Other symptoms? Y N clicking, locking, limited mouth opening, ___________________

Ear symptoms? Y N pop _________________________

Sleep affected? Y N Prevention, disturbance, wake early a.m. _________________________

Clenching/Bruxism Y N

Do you have pain in regions in other parts of your body? Y N Specify _________________________

Current Pain Rating: NPS 0 (no pain)-10 (most intense pain imaginable)

Intensity- _________

NPS (0 (not unpleasant)-10 (most unpleasant pain imaginable)

Unpleasantness- _________

History of symptoms: Since onset, pain has improved, stayed the same, worsened?

Has the nature of pain changed since onset?
Appendix VI: McGill Pain Questionnaire

<table>
<thead>
<tr>
<th>Patient’s name: ____________________________</th>
<th>ID: __________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test site ________________________________</td>
<td>Date __________</td>
</tr>
<tr>
<td>SP CH MH VH other: _________________________</td>
<td></td>
</tr>
</tbody>
</table>

A) How strong is your pain?
(0) no pain  (1) mild  (2) discomforting  (3) distressing  (4) horrible  (5) excruciating

B) What does your pain feel like?
Please circle the words which describe your pain:

1) flickering  2) jumping  3) pricking  4) sharp
   quivering     flashing     shooting     cutting
   pulsing       gruelling    cruel        blinding
   throbbing     vicious      vicious      wretched
   beating       killing      killing      annoying
   pounding      sickening   sickening   troublesome
                        
5) pinching   6) tugging   7) hot        8) tingling
   pressing     pulling     burning      itchy
   gnawing      wrenching   scalding     smarting
   cramping     squeezing    searing      stinging
                        
9) dull       10) tender    11) tiring    12) sickening
   sore        taut        exhausting   suffocating
   hurting      rasping     splitting
   aching      splitting
   heavy
                        
13) fearful    14) punishing  15) wretched  16) annoying
   frightful    gruelling    blinding    troublesom
   terrifying   cruel        intense
                        
17) spreading  18) tight     19) cool     20) nagging
   radiating    numb        cold        nauseating
   penetrating  drawing     freezing    agonizing
   piercing     squeezing    tearing     dreadful
                        

## Appendix VII: McGill Pain Questionnaire results for Patients with TMD

Table A-1: McGill Pain Questionnaire result for patients with TMD

<table>
<thead>
<tr>
<th>Sub</th>
<th>Sensory (Q.1-10)</th>
<th>Affective (Q.11-15)</th>
<th>Evaluative (Q.16)</th>
<th>Miscellaneous (Q17-20)</th>
<th>Total</th>
<th>Pain Intensity</th>
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<tr>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>I</td>
<td>3</td>
<td>15</td>
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<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>7</td>
<td>2</td>
<td>3</td>
<td>ATM</td>
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<td>13</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>1</td>
<td>0</td>
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<td>2</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>ATI</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
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<td>9</td>
<td>1</td>
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<td>9</td>
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<td>0</td>
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<td>AT</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
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<td>11</td>
<td>0</td>
<td>0</td>
<td>4</td>
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<td>0</td>
<td>3</td>
<td>ATI</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

Abbreviations for “Evaluative” words selected: A – annoying; T – troublesome; M – miserable; I – intense; U - unbearable
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