**Identifying the cellular pathophysiology of chronic trigeminal neuropathic pain**

**TNP pathophysiology**

**CONFIDENTIAL**

This document is confidential and the property of Professor Luke Henderson

No part of it may be transmitted, reproduced, published, or used without prior written authorisation from the institution.

# STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

**Contents**

[STATEMENT OF COMPLIANCE 1](#_heading=h.gjdgxs)

[GLOSSARY OF ABBREVIATIONS 3](#_heading=h.30j0zll)

[1.](#_heading=h.1fob9te) Study Management 3

[2.](#_heading=h.3znysh7) INTRODUCTION AND BACKGROUND 4

[2.2](#_heading=h.2et92p0) Research Question 5

[2.3](#_heading=h.3dy6vkm) Rationale for Current Study 5

[3](#_heading=h.1t3h5sf) STUDY OBJECTIVES 7

[4.](#_heading=h.4d34og8) STUDY DESIGN 8

[5.](#_heading=h.2s8eyo1) STUDY TREATMENTS 11

[5.1](#_heading=h.17dp8vu) Treatment Arms 11

[5.2](#_heading=h.lnxbz9) Preparation and administration of Study Drug Dispensing and Product Accountability 11

[5.3](#_heading=h.35nkun2) Measurement of participant compliance 11

[5.4](#_heading=h.1ksv4uv) Excluded medications and treatments 11

[6.](#_heading=h.44sinio) PARTICIPANT ENROLMENT AND RANDOMISATION 11

[6.3](#_heading=h.2jxsxqh) Informed Consent Process 12

[7.](#_heading=h.z337ya) STUDY VISITS AND PROCEDURES SCHEDULE 13

[Study Flow Chart 13](#_heading=h.3j2qqm3)

[8.](#_heading=h.1y810tw) CLINICAL AND LABORATORY ASSESSMENTS 13

[9.](#_heading=h.4i7ojhp) ADVERSE EVENT REPORTING 14

[9.1](#_heading=h.2xcytpi) Serious Adverse Event Reporting 14

[9.1.2](#_heading=h.1ci93xb) SUSARs 15

[10.](#_heading=h.3whwml4) STATISTICAL METHODS 15

[10.1](#_heading=h.2bn6wsx) Sample Size Estimation 15

[10.2](#_heading=h.qsh70q) Population to be analysed 15

[10.3](#_heading=h.3as4poj) Statistical Analysis Plan 15

[10.4](#_heading=h.49x2ik5) Interim Analyses 17

[11.](#_heading=h.2p2csry) DATA MANAGEMENT 17

[12.](#_heading=h.3o7alnk) ADMINISTRATIVE ASPECTS 18

[13.](#_heading=h.23ckvvd) USE OF DATA AND PUBLICATIONS POLICY 18

[14.](#_heading=h.ihv636) REFERENCES 19

[15.](#_heading=h.1hmsyys) APPENDICES 21

PROTOCOL SYNOPSIS

| Title | **Identifying the cellular pathophysiology of chronic trigeminal neuropathic pain** |
| --- | --- |
| Objectives | **Primary:** Determine astrocytic and glial binding patterns within the brain of individuals with chronic neuropathic pain.**Secondary:** Determine if these altered binding patterns relate to symptomatology, genetic, endocrine, and/or immune function.  |
| Study Design | Cross-sectional investigation |
| Planned Sample Size | **80 – 40 healthy control, 40 diagnosed with chronic trigeminal neuropathic pain** |
| Selection Criteria | **Inclusion:**1. Chronic trigeminal neuropathic pain as diagnosed according to the International Classification of Orofacial Pain (ICOP) section 4. 2. Over 18 years of age and providing informed consent **Exclusion:**3. Extensive dental prosthesis (>6 teeth) 4. Undergoing dental/orthodontic treatment 5. Psychiatric conditions or traumatic brain injury 6. Does not meet standard MRI/PET inclusion criteria – e.g. metallic implants7. Current pregnancy or thought to be pregnant in the absence of contraception since last normal menstrual period |
| Study Procedures | **The study will take place over two sessions.** **Session 1:**1) Trigeminal neuropathic pain assessment and screening for eligibility2) Psychological assessment (Questionnaires)3) Magnetic Resonance Imaging (MRI)4) Positron Emission Tomography (PET) – [18F]SMBT1 radiotracer**Session 2:**5) Electroencephalography (EEG) recording 6) Positron Emission Tomography (PET) – [18F]PBR06 radiotracer |
| Statistical ProceduresSample Size Calculation:Analysis Plan: | **Statistical procedures:** Behavioural analysis in Graphpad Prism – two sample t-tests and one-way ANOVA. MRI image analysis in MATLAB version 2023a and SPM12. PET image analysis in XXX |
| Duration of the study | **48 months** |

# GLOSSARY OF ABBREVIATIONS

| **ABBREVIATION** | **TERM** |
| --- | --- |
| PET | Positron Emission Tomography |
| MRI | Magnetic Resonance Imaging |
| [18F]SMBT1 | (S)-(2-methylpyrid-5-yl)-6-[(3-[18F]fluoro-2-hydroxy)propoxy]quinoline |
| [18F]PBR06 | [N-(2,5-dimethoxybenzyl)-2-(18)F-fluoro-N-(2-phenoxyphenyl) acetamide] |
| SPM12 | Statistical Parametric Mapping version 12 |
| mSv | Millisieverts  |

# Study Management

* 1. Principal Investigator

Professor Luke Henderson | luke.henderson@sydney.edu.au (email); +612 9351 7063 (Tel) | 94 Mallett Street, Camperdown, 2050, NSW, Australia.

* 1. Associate Investigators

Professor Kevin Keay | kevin.keay@sydney.edu.au (email); +61293514132 (Tel) | 94 Mallett Street, Camperdown, 2050, NSW, Australia.

Professor Christopher Peck | chris.peck@sydney.edu.au (email) | 9 Lower Kent Ridge Road, Level 10, National University Centre for Oral Health, Singapore, S119085

Dr Lewis Crawford | lewis.crawford@sydney.edu.au (email); +61422567299 (Tel) | 94 Mallett Street, Camperdown, 2050, NSW, Australia.

* 1. Internal Trial Committees - NA
	2. Independent Safety and Data Monitoring Committee - NA
	3. Sponsor

The University of Sydney Human Research Ethics Committee 3 | Associate Professor Haryana Dillon

* 1. Funding and resources

This investigation received funding under a National Health and Medical Research Council Grant (Grant ID ID 1130280)

# INTRODUCTION AND BACKGROUND

* 1. Background Information

Chronic trigeminal neuropathic pain is a debilitating neuropathic pain condition defined as pain attributed to a lesion or disease of the trigeminal nerve in the mouth or face region (International Classification of Orofacial Pain).

Trigeminal neuropathic pain is characterised by transient shooting and constant burning pain (Gustin et al. 2011) and often has a severe detrimental effect on an individual's quality of life. Trigeminal neuropathic pain can occur following simple oral procedures such as a tooth extraction or can present with no apparent physical cause. Once established it can be exacerbated by everyday activities such as talking or eating and often responds poorly to current standard treatment regimens. Given its poor response to treatments, individuals with trigeminal neuropathic pain often suffer with the condition for years or even decades. Despite its high prevalence and debilitating consequences, the mechanisms underlying this orofacial pain remain largely unknown.

One potential mechanism which may be underlying the pathophysiology of trigeminal neuropathic pain is the influence of microglia and astrocytes – two non-neuronal (glia) cell types which collectively maintain CNS homeostasis, form myelin, assist synaptogenesis, and facilitate the synthesis, release, and reuptake of neurotransmitters within the brain (Tiwari et al. 2014). Recent evidence suggests that abnormal activation and structural integrity of brain microglia and astrocytes may contribute to the unique symptomatology of chronic pain conditions. In rodent models, microglial stimulation induces allodynia and pain hypersensitivity, which can be attenuated by intrathecal injection of p38 MAPK – a microglial inhibitor (Inoue and Tsuda 2018). Additionally, recent human brain imaging studies have shown elevated TSPO in both Fibromyalgia and chronic low back pain within canonical pain processing and evaluative regions (primary somatosensory, insula, cingulate cortices), suggesting a microgliopathic origin (Loggia et al. 2015; Albrecht et al. 2019).

Further, preclinical and clinical studies have indicated that chronic pain involves long-term changes in brain structure and function. There is substantial evidence that abnormal activity within the central nervous system (CNS) may initiate or maintain trigeminal neuropathic pain, through altered descending modulation (Mills et al. 2018), altered higher cortical processing (Youssef et al. 2014) and altered thalamic anatomy and activity, potentially resulting in disturbed thalamocortical circuits (Henderson et al. 2013). Indeed, we have recently shown altered functional connectivity of brainstem pain-modulation circuits in individuals with trigeminal neuropathic pain, between areas including the rostral ventromedial medulla (RVM), ventrolateral periaqueductal gray (vlPAG), locus coeruleus (LC), and the region that receives orofacial nociceptor afferents, the spinal trigeminal nucleus (SpV) (Mills et al. 2018). We also identified increased functional connectivity between these brainstem regions and higher cortical regions in individuals with trigeminal neuropathic pain, suggesting altered top-down regulation (Mills et al. 2018). Higher brain regions including the thalamus, somatosensory, insular and cingulate cortices have been found to be differentially activated in different chronic pain conditions (Youssef et al. 2014). In a previous study, we showed that neuropathic pain was associated with decreases in regional cerebral blood flow (CBF) in the thalamus and primary somatosensory cortex (S1), whilst chronic non-neuropathic (temporomandibular disorder; TMD) was associated with increases in regions of the anterior cingulate and dorsolateral prefrontal cortices (Youssef et al. 2014). This is not surprising given that trigeminal neuropathic pain is thought to occur as a result of damage to the trigeminal nerve whilst TMD results primarily from peripheral nociceptor activation (Wilcox et al. 2013). Therefore, it is possible that a unique neurobiological signature exists that underlies and perpetuates the trigeminal neuropathic pain experience.

We propose that trigeminal neuropathic pain is associated with altered microglial and astrocyte activation, increased infra-slow neural oscillations, reduced thalamic inhibition, altered thalamocortical rhythms, and constant pain. Using both PET and functional MRI, electro-encephalography (EEG), and blood diagnostics we seek to identify the neuro-anatomical, -functional, and -immune/endocrine biomarkers in humans who have transitioned to chronic pain following nerve injury. Our previous investigations did not determine whether microglial and astrocytic abnormalities are present in trigeminal neuropathic pain, and, if these markers are present, how they interact with changes in brain function or any present genetic, inflammatory, or endocrine abnormalities. This knowledge is essential to better characterize the disorder, by considering the involvement of specialized cell groups known to be capable of influencing pain processing and chronicity.

* 1. Research Question

The overall goal of the proposed study is to determine whether trigeminal neuropathic pain related disability is associated with the extent of brain, genetic, endocrine, inflammatory, and neuroglial function.

* 1. Rationale for Current Study

Using full band electroencephalography (EEG), resting state and pain-evoked functional magnetic resonance imaging (fMRI), and positron emission tomography (PET), it is possible to explore brain activity, anatomy, and neuroglial properties in individuals with trigeminal neuropathic pain. Whether a relationship between these variables and genetic / inflammatory information found in blood samples is unknown. It may be that greater astrocyte or microglial activation may form the basis of a pro-nociceptive brain state which persists the chronic symptoms of trigeminal neuropathic pain.

Indeed, there is a consensus that over-active microglia (as quantified through TSPO) facilitate pain signalling, and that these up-regulations in specific neural sites (such as the thalamus) linearly relate to clinical pain scores (Tiwari et al. 2014; Loggia et al. 2015). However, TSPO is known to be non-specific, binding to both microglia and astrocytes within the brain. By combining our two radiotracers – one non-specific and the other astrocyte-specific, we will be able to discern the relative contributions of astrocytes and microglia in the generation and persistence of trigeminal neuropathic pain. Such an investigation has been performed in Fibromyalgia, where Albrecht et al. (2019) combined TSPO with a MAO-B radiotracer (Deprenyl) to demonstrate that microglia, and not astrocytes where the most pronounced contributor to the pathophysiology. Such a study in orofacial chronic pain disorders combined with an updated and bio-kinetically superior MAO-B radiotracer (SMBT-1) has not been performed.

The TSPO radiotracer [18F]PBR06 is a second generation TSPO radiotracer, and similarly to [11C]PBR28 (Owen et al. 2012) requires genotyping for the Ala147Thr TSPO polymorphism in the TSPO gene (rs6971). Second generation radiotracers have the advantage of binding to the TSPO protein with a higher ratio of specific-to-non-specific binding, but the binding affinity does vary between subjects depending on the rs6971 polymorphism. Since the low-affinity binder phenotype is consistent across all tissues within the same subject, testing for the Ala147Thr polymorphism has been suggested to predict low affinity for second generation TSPO radiotracers in all organs, including the brain. High or Mixed affinity binders (Ala/Ala or Ala/Thr) will be considered eligible, whereas the Low affinity binders (Thr/Thr) will be considered ineligible for this PET scan.

Further, since we have previously characterized changes in coupling between discrete brainstem nuclei that relay ascending and descending pain signals in individuals suffering chronic pain, pain-evoked fMRI during both its perception and modulation (via offset analgesia and conditioned pain modulation) in the present study will enable us to assess whether similar sites which show altered neuroglial activation also display dysfunctions in blood oxygen level dependent (BOLD) signal and coupling in specifically trigeminal neuropathic pain. Previous investigations have shown that chronic pain sufferers display an impaired conditioned pain modulation response (Lewis et al. 2012; Morris et al. 2016), suggesting that the altered coupling we previously observed may also relate to impaired endogenous pain modulation capacity in individuals suffering trigeminal neuropathic pain.

There is also increasing evidence that the immune system/inflammatory mediators as well as endocrine markers are implicated in neuropathic pain. Pro-inflammatory cytokines are known to increase during chronic pain, causing inflammation in the CNS (Staats Pires, Heng, et al. 2020), whilst immune and glial cell inhibitors can reduce facial pain (Wieseler et al. 2017; Su and Yu 2018). An association has been established between neuroinflammation and several different chronic pain conditions including neuropathic pain triggered by diabetes, nerve and spinal cord injury and others (Staats Pires, Heng, et al. 2020). Unfortunately, the mechanisms of how neuroinflammation supports the transition from acute to chronic pain is not well understood. Therefore, we propose a systematic immunophenotyping study of both innate (i.e. monocytes) and adaptive immune cells, using a panel of 40 phenotypic and functional state markers (e.g. NFkB), taking advantage of the latest mass cytometry technology. We have recently used a similar panel of markers to provide evidence of specific immune cell activation signatures in complex regional pain syndrome and diabetic neuropathic pain (Russo et al. 2019; Staats Pires, Tan, et al. 2020). It is hoped that through a thorough evaluation of immune system changes in individuals with trigeminal neuropathic pain, this study can provide deeper insight into the complex pathophysiology of trigeminal neuropathic pain, as well as uncover novel therapeutic targets. Furthermore, correlating these immune changes with the muscle changes, CNV and brain changes, and psychophysical components of the project could demonstrate never before seen associations in trigeminal neuropathic pain.

In addition, analysis and interpretation of the levels of endocrine markers in plasma/serum may provide an excellent set of biomarkers. Ongoing pain and acute painful stimuli each alter the activity of specific hypothalamic nuclei that regulate the activity of pituitary gland, which secretes releasing hormones that control the circulating levels of adrenal (HPA axis), thyroid (HPT axis), and gonadal hormones (HPG axis). For example, chronic pain patients whose pain relief is sub-optimal often show elevated serum levels of adrenocorticotrophic hormone (ACTH) from the pituitary, cortisol and pregnenolone from the adrenal, and testosterone from the gonadal glands. If this state persists, other hormones may become depleted, and their levels drop below resting homeostatic levels. These 2 phases — stimulation and depletion — potentially provide a set of biomarkers to diagnose ongoing pain and its impacts. The re-normalization of serum hormones by an intervention may therefore provide an excellent determinant of pain treatment success, which would provide an independent diagnostic verification tool. Therefore, we will quantify and characterise plasma/serum levels of (i) adrenocorticotrophic hormone (ACTH); cortisol (CORT); pregnenolone (PRG); dehydroepiandrosterone (DHEA) as markers of HPA axis activity, (ii) testosterone (T) Progesterone (P); and Estrogen (E) as markers of HPG axis activity and, (iii) Thyroid stimulating hormone (TSH) Thyroxine (T4); free Thyroxine (fT4) and Triiodothyronine (T3) as markers of HPT axis activity.

In addition to immune cell changes and altered endocrine regulation, a number of genetic polymorphisms have been identified that may explain the variations in pain sensitivity and pain threshold in individuals with chronic pain conditions (Smith and Muralidharan 2012; Young et al. 2012). The most extensively investigated are catechol-O-methyltransferase (COMT); mu-opioid receptor (OPRM1); ATP Binding Cassette Subfamily B Member 1 (ABCB1); melanocortin receptor (MC1R); Cytochrome P450 2D6 (CYP2D6); (Diatchenko et al. 2005; Somogyi et al. 2007; Young et al. 2012; Olesen et al. 2018) in addition to the previously approved Fatty Acid Amide hydrolase (FAAH). COMT is an enzyme with many biological functions including the regulation of catecholamines and enkephalin levels, and COMT polymorphism has long been associated with human pain perception and chronic pain (Diatchenko et al. 2005). COMT activity was found to significantly influence pain sensitivity and the risk of developing temporomandibular joint disorder (Diatchenko et al. 2005). The exact mechanisms by which COMT can identify susceptible individuals for the development of pain conditions is still unknown, however it is well-established that COMT polymorphisms contribute to the variability in pain sensitivity experienced by different people (Belfer and Segall 2011). An important gene of the endogenous opioid system, OPRM1 polymorphisms are also associated with pain sensitivity variability (Walter and Lötsch 2009). OPRM1 has been specifically implicated in the perception of thermal and chemical pain in animal models (Martin et al. 2003). Inversely, polymorphisms in ABCB1, a transporter protein that prevents intracellular accumulation of cytotoxic drugs, have been associated with variability in cold pain thresholds in human males (Zahari et al. 2017).

Also playing a role in pain sensitivity are MC1R polymorphisms. The role of MC1R has been widely investigated for producing the pigment melanin, that regulates the colour of skin, hair and eyes; however, it also plays an important role in pain sensitivity (Andresen et al. 2011). Mutations in MC1R can result in pale skin and red hair, which has been found to modulate pain, for instance, in a study that showed that red haired females were less sensitive to topical capsaicin induced pin-prick hyperalgesia compared with blond/dark haired females (Andresen et al. 2011). In other words, dysfunction to MC1R causes greater analgesia. CYP2D6 is an enzyme involved in the metabolism of active drugs in the central nervous system, and variations in CYP2D6 may significantly affect pain levels and explain for the wide variations in responsivity to opioids seen on an interindividual level (Zahari and Ismail 2014). In fact, ultra-rapid CYPD2D6 metabolisers was shown to have increased endogenous pain modulation and less need for exogenous morphine compared to other CYP2D6 metaboliser groups in the acute post-operative period (Candiotti et al. 2009). A critical role for the endocannabinoid system in pain processing is highlighted by a recent case study in which a genetic polymorphism that reduced the expression of FAAH; the enzyme that breaks down anandamide and related fatty acids; was associated with elevated endocannabinoid concentrations and pain insensitivity (Habib et al. 2019). Lessened FAAH function is also associated with reduced need for postoperative analgesia in women undergoing breast cancer surgery (Cajanus et al. 2016). Each of these polymorphisms are involved in the modulation of analgesic efficacy. Therefore, we would like to identify expression levels of polymorphisms in these and other pain-related genes, and to ascertain whether they can predict pain sensitivity in trigeminal neuropathic pain and healthy subjects. Polymorphisms in these genes do not predict any medical condition; they reflect the natural variability in sensitivity to different analgesic compounds.

# STUDY OBJECTIVES

* 1. Primary Objective

The overall goal of the proposed study is to determine astrocytic and glial binding patterns within the brain of individuals with chronic trigeminal neuropathic pain.

* 1. Secondary Objectives
1. To characterise the relationship between regional glial binding and brain, genetic, endocrine and immune system function in individuals with trigeminal neuropathic pain.

# STUDY DESIGN

* 1. Type of Study

This investigation is a cross-sectional observational study making comparisons in brain structure, function, and cellular pathophysiology between healthy controls and chronic orofacial neuropathic pain sufferers.

* 1. Study Design

The study will be conducted over two independent sessions, each separated by at least a 2-week interval. Component 1 will be conducted prior to study session 1. Study session 1 will comprise components 2-4, and study session 2 comprises components 5-6.

1) Trigeminal neuropathic pain assessment:

Participant documentation will be recorded and assessed by Professor Peck to determine whether they meet the diagnostic criteria for Trigeminal Neuropathic Pain signs or symptoms. Their responses to the web-survey will additionally be assessed to ensure they score ≥ 1.

2) Psychological Assessment (questionnaires)

All participants will rate their current pain, average pain at the muscles of mastication and list any current treatments. They will complete the following psychological questionnaires via REDCap. We will ask them to complete them within the week prior to the study session to save time on the day. However, they will also have the option to complete them during the study session if they prefer. All participants will receive an email with an online link to the following surveys.

A) Graded Chronic Pain Scale (Schiffman et al. 2014)

B) Jaw functional limitation scale 20 (Schiffman *et al.* 2014)

C) Patient health questionnaire 15 (Schiffman *et al.* 2014)

D) General Anxiety disorder 7 (GAD 7) (Schiffman *et al.* 2014)

E) The oral behaviour checklist (Schiffman *et al.* 2014).

F) the State-Trait Anxiety Inventory

G) the Somatosensory Amplification Scale (Barsky et al. 1988)

H) the Beck Depression Inventory (Beck et al. 1961)

I) the Pain Catastrophizing questionnaire (Sullivan et al. 1995)

J) PTN screening questionnaire

K) Study session questions

Control participants will be asked to fill questionnaires: C, D, E, F, G, H, I. Questionnaire K will be administered on the day of each of the two sessions.

3) Magnetic resonance imaging (MRI):

Numerous MRI scans, covering the entire brain will then be collected using a 7 Tesla Siemens MAGNETOM MRI system, with a combined single-channel transmit and 32-channel receive head coil (Nova Medical):

1. T1-anatomical (0.78mm3 voxels)
2. Resting state fMRI (134 volumes, TR2.5s, 1mm3 voxels)
3. Diffusion Weighted Imaging (DWI) set (103 gradient directions)
4. Arterial Spin Labelling (ASL)
5. fMRI: i) during noxious heat stimuli applied to the skin of the right volar forearm, ii) during an offset analgesia paradigm, ii) during a conditioned pain modulation paradigm.

Neural activation during noxious heat stimuli: in all participants we will use a 6.6x4cm Peltier-element thermode to induce thermal pain to the face. During an initial calibration period, in each participant the thermode will be placed onto the left forearm and temperatures will be raised and maintained for 10 seconds through a Thermal Sensory Analyzer (QST Labs). The temperature will be raised and lowered within a 46-50 degree range and the temperature that evokes a moderate pain will be determined in each individual. The participant will use a computerised visual analogue scale to record their pain intensity ratings to each thermal stimulus (from 0-100, where 0 indicates “no pain” and 100 is the “worst pain imaginable”). Moderate pain will be defined as temperature eliciting a pain intensity rating of 50-60/100.

Participants will then be placed into the MRI scanner in the supine position. With participants relaxed and at rest, we will first collect a T1-weighted anatomical image set, resting state fMRI, DWI, and ASL series. No movement or activity is required and there is no injection or thermal pain applied in any of these scans.

Following the collection of these scans, we will collect three additional fMRI scans.

1. During the first of these series, the thermode will be attached to the right volar forearm, and the moderate intensity pain temperature will be applied. This scan will consist of a 90 second baseline, followed by eight individual 20 second periods in which the thermode temperature will be raised from 32 ℃ to the temperature that evokes a moderately painful response over 2.5 seconds. This moderate temperature is maintained for 15 seconds and then returned to 32℃ over 2.5 seconds. Moderate pain will only be maintained for 15 seconds if the temperature is no higher than 49℃. This 15 second “on” period is followed by a 15 second “off” period during which the thermode is maintained at 32℃. This “on-off” pattern is then repeated 7 times for a total of 8 on and 8 off periods. During this scan, the participant will use a visual analogue scale (VAS) to record their pain intensity ratings online to each thermal stimulus.
2. During the sixth fMRI series, participants will undergo an offset analgesia paradigm. Much like in the previous scan, the thermode will be placed on the right volar forearm. An identical “on” “off” paradigm will be followed as the previous series. However, during the 15 seconds of the “on” period where noxious stimuli plateau at the moderate stimulus intensity, the temperature will be raised to a temperature which elicits a 7/10 pain rating response for the middle 5 seconds of the plateau. That is, temperatures will initially be raised from 32℃ and be raised to the moderate pain intensity over 2.5 seconds. This moderate intensity will be held for 5 seconds, and then raise again to a temperature that elicits a 7/10 pain intensity rating for 5 seconds, before once again being lowered to the moderate intensity for 5 seconds. The thermode temperature is then returned to 32℃ over 2.5 seconds. This pattern is then repeated 7 times for a total of 8 on and 8 off periods. During this scan, the participant will use a VAS to record their pain intensity ratings online to each thermal stimulus. A successful offset analgesia response occurs when the mean pain elicited across the 15 second stimulus where temperatures are raised and lowered (fMRI series 2) are lower than the mean pain elicited when temperatures are kept at a constant moderate intensity (fMRI series 1).
3. During the final MRI series, participants will undergo a conditioned pain modulation paradigm. A small gauge butterfly cannula will be inserted into the right tibialis anterior muscle of participants, with extension tubing connected to a 5% hypertonic saline solution. The thermode device will once again be placed on the right volar forearm. At the commencement of the final scan, the hypertonic saline solution will be injected into the muscle belly, which elicits a moderate intensity cramping sensation lasting roughly 8 minutes. An identical noxious heat paradigm as in scan five will then be applied to the right forearm as the MRI scan is being recorded. Participants will be instructed to rate their pain experienced *only* on their forearm. A successful CPM response is defined as one where the mean pain intensity felt on the forearm during scan 7 is lower than that experienced during scan 5 – that is, the pain inhibits pain phenomenon.

Following the collection of these 7 MRI scans, participants will be removed from the MRI machine, and instructed to take a short break while the first radioisotope is prepared. Participants will be able to move freely though the imaging suite, rehydrate, and use the restrooms at their leisure.

4) Positron Emission Tomography (PET) – [18F]SMBT1 radiotracer

Participants will be placed into a high-resolution PET/CT scanner (Siemens Biograph Vision 68 PET/CT scanner, MBCIU) that is located in a room adjacent to the MRI scanner room. A small bolus of [18F]SMBT1 will be injected into the antecubital vein (or other arm or hand vein should the antecubital be inaccessible) at the beginning of the PET scan using an intravenous catheter into the antecubital vein (or other arm or hand vein should the antecubital be inaccessible). PET images will be acquired for approximately 90 minutes. Dynamic and static PET images will be reconstructed, using the attenuation correction map from the CT image. All appropriate corrections will be applied including motion-correction and regional quantitative analysis performed to determine differences in binding between PTN and control participants.

A blood sample (approximately 20 ml) will be collected after the scanning session to perform metabolite correction. It will need to be kept on ice and stabilized with sodium fluoride and cold SMBT1 to prevent further metabolism by blood esterases. Once, the radioactivity is decayed, the plasma will be separated and high-performance liquid chromatography (HPLC) will be used for the analysis, to obtain the percentage of metabolites.

5) Positron Emission Tomography (PET) – [18F]PBR06 radiotracer

Participants will be placed into a high-resolution PET/CT scanner (Siemens Biograph Vision 68 PET/CT scanner, MBCIU) and a bolus of [18F]PBR06 injected into the antecubital vein (or other arm or hand vein should the antecubital be inaccessible) at the beginning of the PET scan. PET images will be acquired for approximately 90 minutes. Dynamic and static PET images will be reconstructed, using the attenuation correction map from the CT image. All appropriate corrections will be applied including motion-correction and regional quantitative analysis performed to determine differences in binding between PTN and control participants.

6) Electroencephalography (EEG) recording

Following this, an EEG cap will be fitted. EEG signals will be recorded continuously for 10 minutes with eyes closed, and 10 minutes eyes open with the participant relaxed.

* 1. Number of Participants

80 participants total will be recruited – 40 individuals with diagnosed chronic orofacial neuropathic pain and 40 age- and gender-matched healthy controls.

* 1. Study sites

All experimental components of the study will be conducted at the Melbourne Brain Centre Imaging Unit – 30 Royal Park Parade, Parkville, VIC, 3052. Brain imaging analysis will be conducted in the laboratory of Professor Luke Henderson – 94 Mallett Street Camperdown, NSW, 2050. Blood sample analysis will be conducted in the laboratory of Professor Kevin Keay - 94 Mallett Street Camperdown, NSW, 2050. Study participants will only ever attend the Melbourne Brain Centre Imaging Unit.

* 1. Expected Duration of Study

Total study duration = 48 months.

Expected Start Date = 6th of May 2024.

Expected Stop Date = 6th of May 2028.

Recruitment will be ongoing throughout the study duration.

There is no follow up component of this study.

* 1. Primary and Secondary Outcome Measures

Primary Outcome = to determine differences in regional PET radiotracer binding to microglia and astrocytes between controls and individual with trigeminal neuropathic pain

Secondary Outcomes = determine the relationship between regional gliosis and brain, genetic, endocrine and immune system function in individuals with chronic trigeminal neuropathic pain.

# STUDY TREATMENTS

* 1. Treatment Arms

 5.1.1 Description – Injection of radiotracers

[18F]-SMBT1 and [18F]-PBR06, in separate sessions at least 2 weeks apart. These are not treatment arms but rather all participants will receive both injections sequentially as long as they qualify as high affinity binders to PBR-06.

 5.1.2 Dosage and Route of Administration

Dosage determined by a Medical Physicist and Radiation Safety Officer – Paul Einsiedel. 3.5mSv per scan for a total of 7mSv.

* 1. Preparation and administration of Study Drug Dispensing and Product Accountability

Both radiotracers will be dispensed by a staff member at the Melbourne Brain Centre Imaging Unit who holds an active Radiation Management Licence.

* 1. Measurement of participant compliance

 NA

* 1. Excluded medications and treatments

NA

# PARTICIPANT ENROLMENT AND RANDOMISATION

* 1. Recruitment

Participants will be recruited via social media where we will share the attached recruitment flyers seeking both healthy controls and those suffering chronic orofacial neuropathic pain. Trigeminal neuropathic pain participants will be recruited via making contact with Professors Henderson or Peck after seeing either recruitment flyers. Recruitment of participants will be conducted using a web-survey (via REDCAP). Participants reporting to have had pain in the jaw or temple area, and with score ≥ 1 in the survey, and that are willing to participate in the study will be contacted via phone / email to verify exclusion criteria.

* 1. Eligibility Criteria
		1. Inclusion Criteria

1. Chronic trigeminal neuropathic pain as diagnosed according to the International Classification of Orofacial Pain (ICOP) section 4.

2. Over 18 years of age and providing informed consent

* + 1. Exclusion Criteria

1. Extensive dental prosthesis (>6 teeth)

2. Undergoing dental/orthodontic treatment

3. Psychiatric conditions or traumatic brain injury

4. Does not meet standard MRI/PET inclusion criteria – e.g. metallic implants

5. Current pregnancy or thought to be pregnant in the absence of contraception since last normal menstrual period

## Informed Consent Process

Verbal consent will be obtained from participants prior to the first experimental session when each procedure is explained to them by Professor Henderson. Verbal and written consent will be re-confirmed and re-attained prior to each of the two sessions of the study commencing. Researchers will make clear to participants that they are under no obligation to complete all study procedures and sessions and can withdraw their consent at any time should they feel uncomfortable by any specific study procedure. This study does not involve children. This study involves obtaining written and verbal informed consent.

* 1. Enrolment and Randomisation Procedures

The participant will be enrolled into the study after the informed consent has been completed and the participant has met all inclusion and none of the exclusion criteria to participate as either a healthy control or chronic orofacial neuropathic pain participant. The participant will receive a code and be immediately de-identified in any electronic or hard copy records. This code will only be re-identifiable by Professor Henderson. This number will be used on all further study documents.

* 1. Blinding Arrangements

NA – none of the procedures proposed require any blinding.

* 1. Breaking of the Study Blind

NA – there is no blinding in this study

* 1. Participant Withdrawal
		1. Reasons for withdrawal

Participants may withdraw from the study due to feeling uncomfortable within the confined space of either the MRI or PET scanners. Within each of these scanners, participants are equipped with emergency buzzers which they can squeeze at any time to conclude scanning and be immediately removed from the scanner. Researchers will advise participants that they can do this at any time without prejudice. Participants may also withdraw from the study between sessions 1 and 2, in which case we will have reduced power in our analysis of glial binding relating to chronic orofacial neuropathic pain and we may seek to recruit additional participants to reach our required sample size of 40 participants in either experimental group.

* + 1. Handling of withdrawals and losses to follow-up

If a participant has ticked “yes” to the box asking if their partial data can be used on the initial consent form, then we will endeavour to use their data collected to the time in which they withdrew for statistical analysis. If this is not the case their data will be securely discarded.

* + 1. Replacements

As we are not conducting a clinical trial and can replace participants who drop out as required.

* 1. Trial Closure

No specific follow-up is required beyond ensuring participants feel comfortable to travel at the conclusion of experimental procedures. The tracers used are in extremely low doses and have a short half-life. That being said, participants are encouraged to contact Professor Luke Henderson should they experience any adverse reaction in the days following the procedures, who will then assist in liaising with their primary care physician for follow-up.

* 1. Continuation of therapy

NA – this study proposes no therapy.

# STUDY VISITS AND PROCEDURES SCHEDULE

## Study Flow Chart



# CLINICAL AND LABORATORY ASSESSMENTS

*Blood collection:*

The blood collection component requires participants to have blood drawn intravenously using the same cannulation site inserted immediately prior to PET scanning commencing. The cannula is inserted by a trained phlebotomist and nuclear medicine specialist, and participants are encouraged to fast for at least four hours prior to blood collection. The participant will feel a minor pinprick, with no additional discomfort after the cannula is attached. Sometimes blood collection can lead to small hematomas at the location of the cannula. No more than 50mL total will be taken, before and after PET imaging. 20mL of this blood sample will be used for analyses relating to inflammatory/endocrine/genetic markers, and the remainder for assessing genetic polymorphisms that influence radiotracer binding to ensure the accuracy of PET imaging analysis. The blood analyses have the potential to reveal important information about currently unknown interactions between inflammatory/endocrine/genetic markers and neural findings recorded in MRI/PET/EEG data. It is also important for correcting quantitative measurements for PET, with post-hoc radiometabolite assessment. Nevertheless, if a participant is not comfortable with either providing a blood draw or fasting, they do not have to consent to this component of the study, or they can choose to withdraw from this component of the study at any time.

*Blood transport and analysis:*

Immediately following blood collection, all tubes will be labelled with a subject ID and will be therefore immediately de-identified. The following blood fractions will be prepared and frozen in a -80℃ freezer located securely at the MBIU. Three different processing procedures will be used:

1. For immune cell analysis, a 5 ml sample will be transferred to 10 x 1.5ml tubes, where 0.5ml of blood will be transferred into each tube containing 0.7ml of proteomic stabliser buffer (Smart Tube Inc, CA, USA), and mixed by inverting 2-3 times. Samples will then be incubated in proteomic stabliser buffer at room temperature for 10 minutes, before being transferred to a -80℃ freezer.
2. For endocrine measures using commercially available ELISA or RIA kits, 5 ml of the blood sample will be left at room temperature for 15 minutes and allowed to clot, before being centrifuged at 2,000 × g for 10 minutes. Serum will then be transferred to 10 x 0.5ml tubes before being transferred to a -80℃ freezer. An additional 5 mls of the blood sample will be collected in anticoagulant, before being centrifuged and plasma collected which will then be transferred to 10 x 0.5ml tubes before being transferred to a -80℃ freezer.
3. For genotyping, a final 5 ml of the blood sample will be collected in anticoagulant, before being centrifuged and the buffy coat and plasma collected which will then be transferred to 10 x 0.5ml tubes before being transferred to a -80℃ freezer. These samples will be used to process COMT, ORPM1, ABCB1, MC1R, CYP2D6 and FAAH and other pain-related genotyping. Also, tested for the second generation TSPO binding affinity, via rs6971 polymorphism. Time of blood collection will be noted.

All blood samples will be transported on dry ice to the laboratory of Professor Keay at the Brain and Mind Centre, University of Sydney. Immune marker measurements will be run at Sydney Cytometry core facility, which is located in CPC (D17) Level 5. Genomic DNA extraction using will be conducted using standard techniques (Psifidi et al. 2015), and genotyping will be performed both at the BMC and at the Ramaciotti Centre for Genomics using the Fluidigm platform. Professor Keay’s laboratory is locked at all times and access to the building and floor restricted to personnel in the laboratory. Any remaining blood un-analysed from the initial sample will be stored in the laboratory of Professor Keay for 20 years, after which it will be safely destroyed in line with the university of sydney’s hazardous waste program.

# ADVERSE EVENT REPORTING

* 1. Serious Adverse Event Reporting
		1. SAEs

Throughout the protocol, a trained and qualified nursing staff member appointed by the scanning facility - Carly Beveridge will be present to oversee and act in the case of any serious adverse event and has up to date first aid training. Technologists who oversee the MRI and PET scanning - Tudor Sava and Rob Williams are advanced life support trained. The scanning facility has a resuscitation bay and crash cart available for use in the case of albeit unlikely serious adverse event.

In rare instances during tracer injection the needle can miss the vessel and cause extravasation at the site. As mentioned above Carly Beveridge is first aid trained to respond to and monitor the alleviation of this SAE should it occur. If mild reactions occur during the injection processs, these will be reported and monitored or treated if required by Dr Crawford and Carly Beveridge until symptoms alleviate.

Should any serious adverse event occur, we will immediately report these to the clinical trials sub committee, our independent physician who will be performing periodic reviews of the study, and the TGA as required

* + 1. SUSARs

Suspected Unexpected Serious Adverse Reaction (SUSAR)

* 1. Specific Safety Considerations (eg Radiation, Toxicity)

All participants enrolled in this study will be exposed to either 3.5 or 7 mSv of radiation in the form of either one or two PET scans (if a participant is a low affinity binder they will only partake in the first experimental session as described in the method section above). Participants are being exposed to ionizing radiation as these tracers are the safest and most effective way to determine the magnitude of neuroglial activity in small brain regions, which may relate to the severity and symptomatology of chronic orofacial neuropathic pain.

To keep radiation exposure to a minimum, the test to assess whether someone is a low, medium, or high affinity binder will be conducted immediately after session 1 concludes to ensure no participant that is a low affinity binder is exposed to the second PET scan unnecessarily. Additionally, in line with the recommendations received within our Diagnostic Medical Physics Risk Assessment performed by doctor Paul Einsiedel (attached to this application) we have included the following statement to the participant information statement: “During your enrolment in this research you will undergo imaging exams of your body involving the use of radiation that you would not normally receive. These procedures involve exposure to a small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisievert (mSv)

each year. The effective dose from these extra examinations is approximately 7 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. If you have been involved in any other research studies that involve radiation, please inform us. Please keep this Patient Information and Consent Form that includes information about your exposure to radiation in this study for at least five years. You will be required to provide this information to researchers of any future research studies involving exposure to radiation.”

This protocol was assessed by a Medical Physicist and Radiation Safety Officer at the Royal Melbourne Hospital (Paul Einsiedel) who deemed the study to fall under low risk – category IIb. His recommendations were further independently assessed and agreed with by physician Josh Varcoe.

# STATISTICAL METHODS

* 1. Sample Size Estimation

In a previous series of investigations in chronic neuropathic pain we assessed resting state connectivity and infra-slow oscillations. On average we found that regions displaying significant differences such as the thalamus, had an effect size of at least 0.83 (e.g., thalamus ISO power Cohen’s D = 0.83). We expect that these thalamic changes may relate to altered activation or density of neuroglia – since altered astrocytic engagement induce changes in infra-slow oscillations (Hughes et al. 2011). A power analysis suggests that we will need approximately 30 subjects in each group to detect similar effect sizes for the brain imaging part of the proposal. Given this, we are confident that 40 trigeminal neuropathic pain and 40 control participants will provide more than enough power to detect significant differences in the MRI/PET measures in this proposal. The additional participants will ensure that we have enough power to detect more subtle changes in indices such as genetic and immune markers, as well as counteract any dropout between sessions 1 and 2.

* 1. Population to be analysed

Our dataset will form two groups which we will draw direct comparisons between – 40 healthy controls age and gender matched with 40 chronic orofacial neuropathic pain sufferers.

* 1. Statistical Analysis Plan

Data from this investigation forms three streams: behavioural, neuroimaging-based, and genetic/endocrine markers derived from blood.

Behavioural:

Psychological questionnaires listed in the above sections will be scored and compared between our two groups for statistical differences (two-sample t-tests, p<0.05 Bonferroni corrected). Additionally, within our TNP sample, characteristics such as symptom severity and length of time since diagnosis will be compared with individual scores on these same questionnaires for linear or non-linear relationships between these variables (linear regression R-squared values / Principal component regression and multiple linear regression rho values).

fMRI Imaging:

The fMRI images will be processed using statistical parametric mapping (SPM) and custom software. Image analysis including brainstem specific analysis procedures, will include removal of cardiac and respiratory frequencies, motion correction, global signal and movement correction, spatial normalization and smoothing procedures. For the resting state fMRI, two analysis procedures will be used to determine significant differences between trigeminal neuropathic pain and control groups: i) resting connectivity within the descending pain modulation system and higher cortical regions, ii) pain- and task-specific connectivity of the descending pain modulatory circuit. For the pain alone, offset and conditioned pain modulation (CPM) scans, significant changes in signal intensity during each pain period will be determined using a repeated box-car model. To assess the brain circuitry underlying offset and CPM analgesia, significant differences between the first pain-only scan and the offset and CPM scans will be determined for each individual. The resultant brain maps will then be placed into a second level, between group analysis to determine differences in offset and CPM analgesia in PTN compared with controls (random effects, p<0.05, corrected).

The ASL images will be used to create cerebral blood flow maps. These maps will spatially normalized to a standard template, smoothed using a Gaussian filter and significant differences between PTN and control groups determined using second level, between group analysis (random effects, p<0.05, corrected). DWI images will be motion corrected and mean diffusivity and fractional anisotropy maps calculated. These diffusion maps will spatially normalized to a standard template, smoothed using a Gaussian filter and significant differences between PTN and control groups determined using second level, between group analysis (random effects, p<0.05, corrected).

PET data analysis:

For each participant, using the PET data analysis software PMOD (Zurich, Switzerland; pmod.com), semi-quantitative analysis will be performed by calculating regional Standard Uptake Values (SUVs) and normalised SUV (SUVratios) and a voxel-by-voxel level. Furthermore, kinetic modelling, using appropriate tissue compartment models will be performed using PMOD to provide more accurate, quantitative voxel values. Using SPM12, these brain maps will spatially normalized to a standard template, smoothed using a Gaussian filter and significant differences between PTN and control groups determined using second level, between group analysis (random effects, p<0.05, corrected).

EEG data analysis:

Using SPM, EEG data will be filtered, artefacts removed and full-band (0.1–25Hz) FFT transformed, power values calculated, logged, plotted and differences between groups over the entire frequency band and within infra-slow, theta, alpha, and beta ranges determined (p<0.05, one-way ANOVA). Differences in power across each EEG channel will be determined (p<0.05, p t-tests). Changes in power during noxious stimuli will also be determined and relationships between pain intensity, EEG power, resting state thalamocortical connectivity, resting infra-slow oscillations as well as markers of astrocyte activation determined.

Blood transport and analysis:

Immediately following blood collection, all tubes will be labelled with a subject ID and will be therefore immediately de-identified. The following blood fractions will be prepared and frozen in a -80℃ freezer located securely at the MBIU. Three different processing procedures will be used:

1. For immune cell analysis, a 5 ml sample will be transferred to 10 x 1.5ml tubes, where 0.5ml of blood will be transferred into each tube containing 0.7ml of proteomic stabliser buffer (Smart Tube Inc, CA, USA), and mixed by inverting 2-3 times. Samples will then be incubated in proteomic stabliser buffer at room temperature for 10 minutes, before being transferred to a -80℃ freezer.
2. For endocrine measures using commercially available ELISA or RIA kits, 5 ml of the blood sample will be left at room temperature for 15 minutes and allowed to clot, before being centrifuged at 2,000 × g for 10 minutes. Serum will then be transferred to 10 x 0.5ml tubes before being transferred to a -80℃ freezer. An additional 5 mls of the blood sample will be collected in anticoagulant, before being centrifuged and plasma collected which will then be transferred to 10 x 0.5ml tubes before being transferred to a -80℃ freezer.
3. For genotyping, a final 5 ml of the blood sample will be collected in anticoagulant, before being centrifuged and the buffy coat and plasma collected which will then be transferred to 10 x 0.5ml tubes before being transferred to a -80℃ freezer. These samples will be used to process COMT, ORPM1, ABCB1, MC1R, CYP2D6 and FAAH and other pain-related genotyping. Also, tested for the second generation TSPO binding affinity, via rs6971 polymorphism. Time of blood collection will be noted.
	1. Interim Analyses

Given this is a cross-sectional cohort proposal we will not be performing interim analyses apart from data quality assessments.

# DATA MANAGEMENT

* 1. Data Collection

Psychological questionnaires will be collected via personal links to a secured REDCAP server being sent to participants elected email address. This REDCAP project is only accessible by investigators listed on this project and participant codes are used for de-identification. Neural imaging data will be collected on site at the Melbourne Brain Centre Imaging Unit (99 Commercial Road Parkville) on their in house 7-Tesla MRI (Siemens MAGNETOM) and their PET/CT scanner (Siemens Biograph Vision 68 PET/CT)

* 1. Data Storage

All MRI and PET images and EEG recordings acquired at the Melbourne Brain Centre Imaging Unit are immediately deidentified and acquired on the database DARIS server. They are then transferred securely to the University of Sydney’s RDS server. The deidentified data is accessed and processed only on computers in Professor Henderson’s Neural Imaging Laboratory at the University of Sydney Brain and Mind Centre. Participants shall be coded by number and each participant’s name and contact details will be kept in a locked filing cabinet in the Neural Imaging Laboratory at the Brain and Mind Centre (BMC), 94 Mallett Street Camperdown, building M02A-04 room A4.20, The University of Sydney and accessed only by the Chief Investigators. Individual participants will not be identified in any publications. The questionnaires will be recorded on REDCAP and electronically stored. All data files and questionnaires will be kept at the University for at least 20 years, after which they will be deleted when the primary data is no longer required. The blood samples will be immediately de-identified and transported securely to the University of Sydney where they will be stored and processed in Professor Keay’s secure laboratory.

* 1. Data Confidentiality

All data will be kept in re-identifiable formatting. Participants will be coded by number on initial recruitment by Professor Henderson who will keep a record of these codes matched to participants in a locked filing cabinet to which he holds the only key.

* 1. Study Record Retention

We are collecting MRI and PET images, EEG recordings, pain rating data and psychological questionnaires and have chosen the 20-year storage period as required. After this period all electronic records will be deleted, and any hard copy records shredded.

# ADMINISTRATIVE ASPECTS

* 1. Independent HREC approval

This study has been approved by the University of Sydney Human Research Ethics Committee 3 (HREC3), reference number 2023/585.

* 1. Amendments to the protocol

Any amendments will be submitted to the HREC for review prior to implementation as per HREC guidelines.

* 1. Protocol deviations

Any protocol deviations will be submitted to the HREC for review.

* 1. Participant reimbursement

Participants will be reimbursed $100 for each experimental session ($200 total) in the form of pre-paid VISA gift cards to cover their parking/transport and incidental expenses related to taking part in the study.

* 1. Financial disclosure and conflicts of interest

No member of the research team possesses any apparent or perceived conflict of interest in conducting or being listed as part of this study.

# USE OF DATA AND PUBLICATIONS POLICY

Results of the study will be published in scientific journals and will be reported at scientific conferences. In addition, the results will form part of Honours and/or PhD theses. In publications arising from this study the Melbourne brain centre imaging unit will receive acknowledgement for their services provided during data collection. A plain language summary of the results of each study will be provided to each participant via either an electronic or a mailed-out newsletter upon request.

# REFERENCES

Albrecht DS, Forsberg A, Sandström A, Bergan C, Kadetoff D, Protsenko E, Lampa J, Lee YC, Höglund CO, Catana C, Cervenka S, Akeju O, Lekander M, Cohen G, Halldin C, Taylor N, Kim M, Hooker JM, Edwards RR, Napadow V, Kosek E, Loggia ML. 2019. Brain glial activation in fibromyalgia - A multi-site positron emission tomography investigation. Brain Behav Immun. 75:72-83.

Andresen T, Lunden D, Drewes AM, Arendt-Nielsen L. 2011. Pain sensitivity and experimentally induced sensitisation in red haired females. Scand J Pain. 2:3-6.

Barsky AJ, Goodson JD, Lane RS, Cleary PD. 1988. The amplification of somatic symptoms. Psychosom Med. 50:510-519.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. 1961. An inventory for measuring depression. Arch Gen Psychiatry. 4:561-571.

Belfer I, Segall S. 2011. COMT genetic variants and pain. Drugs Today (Barc). 47:457-467.

Cajanus K, Holmström EJ, Wessman M, Anttila V, Kaunisto MA, Kalso E. 2016. Effect of endocannabinoid degradation on pain: role of FAAH polymorphisms in experimental and postoperative pain in women treated for breast cancer. Pain. 157:361-369.

Candiotti KA, Yang Z, Rodriguez Y, Crescimone A, Sanchez GC, Takacs P, Medina C, Zhang Y, Liu H, Gitlin MC. 2009. The Impact of CYP2D6 Genetic Polymorphisms on Postoperative Morphine Consumption. Pain Medicine. 10:799-805.

Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W. 2005. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum Mol Genet. 14:135-143.

Garcia-Hernandez R, Carpena A, Drakesmith M, Koller K, Jones D, Canals S, De Santis S. 2020. Imaging Microglia and Astrocytes non-invasively using Diffusion MRI.

Gustin SM, Wilcox SL, Peck CC, Murray GM, Henderson LA. 2011. Similarity of suffering: Equivalence of psychological and psychosocial factors in neuropathic and non-neuropathic orofacial pain patients. PAIN®. 152:825-832.

Habib AM, Okorokov AL, Hill MN, Bras JT, Lee MC, Li S, Gossage SJ, van Drimmelen M, Morena M, Houlden H, Ramirez JD, Bennett DLH, Srivastava D, Cox JJ. 2019. Microdeletion in a FAAH pseudogene identified in a patient with high anandamide concentrations and pain insensitivity. Br J Anaesth. 123:e249-e253.

Henderson LA, Peck CC, Petersen ET, Rae CD, Youssef AM, Reeves JM, Wilcox SL, Akhter R, Murray GM, Gustin SM. 2013. Chronic Pain: Lost Inhibition? The Journal of Neuroscience. 33:7574.

Hughes SW, Lorincz ML, Parri HR, Crunelli V. 2011. Infraslow (<0.1 Hz) oscillations in thalamic relay nuclei basic mechanisms and significance to health and disease states. Prog Brain Res. 193:145-162.

Inoue K, Tsuda M. 2018. Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nature Reviews Neuroscience. 19:138-152.

Lewis GN, Rice DA, McNair PJ. 2012. Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. J Pain. 13:936-944.

Loggia ML, Chonde DB, Akeju O, Arabasz G, Catana C, Edwards RR, Hill E, Hsu S, Izquierdo-Garcia D, Ji R-R, Riley M, Wasan AD, Zürcher NR, Albrecht DS, Vangel MG, Rosen BR, Napadow V, Hooker JM. 2015. Evidence for brain glial activation in chronic pain patients. Brain. 138:604-615.

Martin M, Matifas A, Maldonado R, Kieffer BL. 2003. Acute antinociceptive responses in single and combinatorial opioid receptor knockout mice: distinct mu, delta and kappa tones. Eur J Neurosci. 17:701-708.

Mills EP, Di Pietro F, Alshelh Z, Peck CC, Murray GM, Vickers ER, Henderson LA. 2018. Brainstem Pain-Control Circuitry Connectivity in Chronic Neuropathic Pain. The Journal of Neuroscience. 38:465.

Morris MC, Walker LS, Bruehl S, Stone AL, Mielock AS, Rao U. 2016. Impaired conditioned pain modulation in youth with functional abdominal pain. Pain. 157:2375-2381.

Olesen AE, Nielsen LM, Feddersen S, Erlenwein J, Petzke F, Przemeck M, Christrup LL, Drewes AM. 2018. Association Between Genetic Polymorphisms and Pain Sensitivity in Patients with Hip Osteoarthritis. Pain Pract. 18:587-596.

Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A, Rhodes C, Pulford DJ, Bennacef I, Parker CA, StJean PL, Cardon LR, Mooser VE, Matthews PM, Rabiner EA, Rubio JP. 2012. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. J Cereb Blood Flow Metab. 32:1-5.

Russo MA, Fiore NT, van Vreden C, Bailey D, Santarelli DM, McGuire HM, Fazekas de St Groth B, Austin PJ. 2019. Expansion and activation of distinct central memory T lymphocyte subsets in complex regional pain syndrome. Journal of Neuroinflammation. 16:63.

Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, List T, Svensson P, Gonzalez Y, Lobbezoo F, Michelotti A, Brooks SL, Ceusters W, Drangsholt M, Ettlin D, Gaul C, Goldberg LJ, Haythornthwaite JA, Hollender L, Jensen R, John MT, De Laat A, de Leeuw R, Maixner W, van der Meulen M, Murray GM, Nixdorf DR, Palla S, Petersson A, Pionchon P, Smith B, Visscher CM, Zakrzewska J, Dworkin SF. 2014. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network\* and Orofacial Pain Special Interest Group†. J Oral Facial Pain Headache. 28:6-27.

Smith MT, Muralidharan A. 2012. Pharmacogenetics of pain and analgesia. Clin Genet. 82:321-330.

Somogyi AA, Barratt DT, Coller JK. 2007. Pharmacogenetics of opioids. Clin Pharmacol Ther. 81:429-444.

Staats Pires A, Heng B, Tan VX, Latini A, Russo MA, Santarelli DM, Bailey D, Wynne K, O’Brien JA, Guillemin GJ, Austin PJ. 2020. Kynurenine, Tetrahydrobiopterin, and Cytokine Inflammatory Biomarkers in Individuals Affected by Diabetic Neuropathic Pain. Front Neurosci. 14.

Staats Pires A, Tan VX, Heng B, Guillemin GJ, Latini A. 2020. Kynurenine and Tetrahydrobiopterin Pathways Crosstalk in Pain Hypersensitivity. Front Neurosci. 14:620-620.

Su M, Yu S. 2018. Chronic migraine: A process of dysmodulation and sensitization. Molecular Pain. 14:1744806918767697.

Sullivan MJL, Bishop SR, Pivik J. 1995. The Pain Catastrophizing Scale: Development and validation. Psychological Assessment. 7:524-532.

Tiwari V, Guan Y, Raja SN. 2014. Modulating the delicate glial-neuronal interactions in neuropathic pain: promises and potential caveats. Neurosci Biobehav Rev. 45:19-27.

Villemagne VL, Harada R, Doré V, Furumoto S, Mulligan R, Kudo Y, Burnham S, Krishnadas N, Bourgeat P, Xia Y, Laws S, Bozinovski S, Huang K, Ikonomovic MD, Fripp J, Yanai K, Okamura N, Rowe CC. 2022. Assessing Reactive Astrogliosis with (18)F-SMBT-1 Across the Alzheimer Disease Spectrum. J Nucl Med. 63:1560-1569.

Walter C, Lötsch J. 2009. Meta-analysis of the relevance of the OPRM1 118A>G genetic variant for pain treatment. Pain. 146:270-275.

Wieseler J, Ellis A, McFadden A, Stone K, Brown K, Cady S, Bastos LF, Sprunger D, Rezvani N, Johnson K, Rice KC, Maier SF, Watkins LR. 2017. Supradural inflammatory soup in awake and freely moving rats induces facial allodynia that is blocked by putative immune modulators. Brain Res. 1664:87-94.

Wilcox SL, Gustin SM, Eykman EN, Fowler G, Peck CC, Murray GM, Henderson LA. 2013. Trigeminal Nerve Anatomy in Neuropathic and Non-neuropathic Orofacial Pain Patients. The Journal of Pain. 14:865-872.

Young EE, Lariviere WR, Belfer I. 2012. Genetic basis of pain variability: recent advances. J Med Genet. 49:1-9.

Youssef AM, Gustin SM, Nash PG, Reeves JM, Petersen ET, Peck CC, Murray GM, Henderson LA. 2014. Differential brain activity in subjects with painful trigeminal neuropathy and painful temporomandibular disorder. PAIN®. 155:467-475.

Zahari Z, Ismail R. 2014. Influence of Cytochrome P450, Family 2, Subfamily D, Polypeptide 6 (CYP2D6) polymorphisms on pain sensitivity and clinical response to weak opioid analgesics. Drug Metab Pharmacokinet. 29:29-43.

Zahari Z, Lee CS, Ibrahim MA, Musa N, Mohd Yasin MA, Lee YY, Tan SC, Mohamad N, Ismail R. 2017. Relationship Between ABCB1 Polymorphisms and Cold Pain Sensitivity Among Healthy Opioid-naive Malay Males. Pain Pract. 17:930-940.

# APPENDICES

Attached with this application are the up-to-date Participant information statement for both Control and Orofacial Neuropathic Pain participants, consent form, each questionnaire given to participants as a PDF exported from REDCAP as it will appear to participants, the reimbursement form participants will sign and date at the conclusion of each experimental session, advertisement flyers for controls and Neuropathic Pain participants. In addition, we have attached investigator brochures for both radiotracers proposed for use in this study, as well as our Diagnostic Medical Physics Risk Assessment completed by Dr Paul Einsiedel.