2022

THETA BURST BRAIN STIMULATION IN PAINFUL DIABETIC NEUROPATHY PATIENTS: INVESTIGATING NEURAL MECHANISMS

BHUSHAN THAKKAR
Virginia Commonwealth University

THETA BURST BRAIN STIMULATION IN PAINFUL DIABETIC NEUROPATHY PATIENTS: INVESTIGATING NEURAL MECHANISMS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

By

Bhushan S. Thakkar
Master of Science, MGH Institute of Health Professions, 2011
Bachelor of Physiotherapy, Manipal University, India, 2009

Director: Edmund O. Acevedo, Ph.D.
Professor, Kinesiology and Health Sciences
College of Humanities and Sciences

Virginia Commonwealth University
Richmond, Virginia
December 2022

Table of Contents

List of Tables…… iv

List of Figures…… v

List of Abbreviations…… vi

Abstract…… vii

Chapter 1: Introduction…… 1

Chapter 2: BDNF as a Biomarker for Neuropathic Pain: Consideration of Mechanisms of Action and Associated Measurement Challenges. *Under Review*…… 13
- Abstract…… 16
- Introduction…… 17
- BDNF related mechanism of action in NP…… 23
  - Spinal dorsal horn, Dorsal root ganglia (DRG) and Microglia mediated action…… 24
  - NMDA- Glutamate- GABA receptor mediated action…… 25
  - Supraspinal Involvement…… 26
- Measurement of BDNF…… 27
  - Factors affecting the measurement of serum and plasma BDNF…… 31
    - Role of Gender, Age and Genetics…… 31
    - Influence of Diurnal Variations and Circadian Rhythms…… 32
    - Psychological/Psychiatric Disorders…… 32
    - Physical Activity and Exercise Training…… 33
    - Storage Conditions…… 34
    - Impact of platelet activation on plasma BDNF levels…… 35
- Conclusion…… 37
- References…… 39

Chapter 3: Short Term Effects of Prolonged Continuous Theta Burst Stimulation Targeting M1 and DLPFC in Patients with Painful Diabetic Neuropathy…… 49
- Abstract…… 52
- Introduction…… 53
- Materials and Methods…… 55
  - Participants…… 55
  - Randomization…… 56
  - Treatment: Prolonged Continuous Theta Burst Stimulation (pcTBS) …… 57
  - Identification of the Cortical Hotspot for DLPFC…… 58
  - Self-report measures of pain perception (SRMP) …… 58
  - Statistical Analysis…… 61
- Results…… 62
  - Demographics…… 62
  - Changes in BEEP scores…… 63
  - Changes in BPI-DN scores…… 64
  - Changes in DASS-21 scores…… 65
  - Changes in Norfolk-QOL-DN scores…… 66
  - Changes in Pain Intensity scores on BPI-DN…… 67
  - Reporting of Adverse Events…… 67
  - Blinding…… 68
- Discussion…… 68
- Conclusion…… 71
- References…… 72

Chapter 4: Changes in Neural Mechanisms following Prolonged Continuous Theta Burst Stimulation Targeting M1 and DLPFC in Patients with Neuropathic pain……87
- Abstract……90
- Introduction……92

Materials and Methods……96
Participants…… 96
Randomization……97
pcTBS Treatment……98
Identification of the Cortical Hotspot for DLPFC……98
Measurement of Intracortical Inhibition (ICI) ……98
Psychophysical pain protocols (PPP) ……99
Analysis of pcTBS data……100
Brief Pain Inventory for patients with diabetic neuropathy……100
Blinding Questionnaire……100
Statistical Analysis……101
Results……102
Demographics……102
Changes in BPI-DN scores……103
Changes in PPP……103
Changes in CE……103
Changes in ICI……104
Relationship between CE and CI on BPI-DN……105
Blinding……105
Discussion……105
Conclusion…… 111
References……113

Chapter 5: Discussion……130

References…… 139
Appendices ……156
Appendix A: IRB Approval Letter and Consent Form…… 157
Appendix B: Screening Forms and Self-report Measures of Pain……168
Appendix C: Curriculum Vita……190

List of Tables

Chapter 2

Table 1a: Study characteristics and findings for studies examining baseline group differences in BDNF in healthy volunteers, patients with chronic pain and patients with neuropsychiatric disorders……..……...20

Table 1b: Study characteristics and findings for studies examining group differences in BDNF across time in healthy volunteers, patients with chronic pain and patients with neuropsychiatric disorders…….…… 21

Table 2: Factors affecting measurement of serum and plasma BDNF…….. 29

Chapter 3

Table 1: Demographic Data for all Participants……..…… 80

Chapter 4

Table 1: Demographic Data for all Participants……..…….122

List of Figures

Chapter 2

Figure 1: List of studies that support the role of BDNF in NP with the associated site for the mechanism of action…… 48

Chapter 3

Figure 1: Study flow chart describing the eligibility and recruitment process …..…… 79

Figure 2: Data collection protocol for the two groups ……..…… 82

Figure 3: Effects of pcTBS at M1 and DLPFC on BEEP scores across the three time points for the M1 and DLPFC group brain regions…….…… 83

Figure 4: Effects of pcTBS at M1 and DLPFC on BPI-DN scores across the three time points for the M1 and DLPFC group brain regions…….…… 84

Figure 5: Effects of pcTBS at M1 and DLPFC on DASS-21 scores across the three time points for the M1 and DLPFC group brain regions……..…… 85

Figure 6: Effects of pcTBS at M1 and DLPFC on QOL-DN across the three time points for the M1 and DLPFC group brain regions…….…… 86

Chapter 4

Figure 1: Neural mechanisms that modulate pain perception in patients with NP……..……123

Figure 2: Data collection protocol for the two groups……..……..……..……..……..……125

Figure 3: Effects of pcTBS at M1 and DLPFC on PPP across the two time points for the M1 and DLPFC group brain regions……..……..……..……..……126

Figure 4: Effects of pcTBS at M1 and DLPFC on MEP across the three time points for the M1 and DLPFC group brain regions……..……..……..……..…127

Figure 5: Effects of pcTBS at M1 and DLPFC on ICI across the two time points for the M1 group and DLPFC group brain regions……..……..…… 128

ABBREVIATIONS

CP: Chronic pain

NP: Neuropathic pain

pDN: Painful diabetic neuropathy

TMS: Transcranial magnetic stimulation

HF-rTMS: High frequency Repetitive TMS

M1: Primary motor cortex

DLPFC: The Dorsolateral Prefrontal Cortex

TBS: Theta burst stimulation

pcTBS: Prolonged continuous theta burst stimulation

CE: Corticospinal excitability

ICI: Intracortical Inhibition

MEP: Motor evoked potential

GABA: Gamma-aminobutyric acid

SRMP: Self-report measures of pain

PPP: Psychophysical pain protocol

CPM: Conditioned pain modulation

TSP: Temporal summation of pain

SICI: Short interval cortical inhibition

LICI: Long interval cortical inhibition

PD-Q: painDETECT questionnaire

BEEP: Bodily and emotional perception of pain

BPI-DN: Brief Pain Inventory for Diabetic Neuropathy

DASS-21: Depression Anxiety Stress Scale

QOL-DN: Norfolk Quality of Life-Diabetic Neuropathy

ADL: Activities of daily living

GHS: Generic health status

RMANOVA: two-way repeated measures mixed model ANOVA

ABSTRACT

THETA BURST BRAIN STIMULATION IN PAINFUL DIABETIC NEUROPATHY PATIENTS: INVESTIGATING NEURAL MECHANISMS

By Bhushan S. Thakkar, Ph.D.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, at Virginia Commonwealth University.

Virginia Commonwealth University, 2022.

Director: Edmund O. Acevedo, Ph.D.

Professor, Kinesiology and Health Sciences

College of Humanities and Sciences

Virginia Commonwealth University

Richmond, VA 23284-3021

Chronic pain (CP) is a significant contributor to disability and disease burden globally. In 2019, approximately 50.2 million adults (20.4% of the US population) experienced chronic pain, contributing to $560-635 billion in direct medical costs. In addition, the worldwide prevalence of diabetes mellitus has reached epidemic proportions and is set to increase to 629 million by 2045. Almost 50% of patients with diabetes present with diabetic neuropathy (DN), and one in five patients with diabetes presents with painful DN (pDN) which is the most common cause of neuropathic pain (NP) in the US. Symptomatic treatment is the mainstay of management for pDN due to the paucity of disease-modifying therapies targeting the irreversible nerve damage from DN. Noninvasive brain stimulation using transcranial magnetic stimulation (TMS) has been utilized as a therapeutic tool in patients with neuropsychiatric disorders, and has only been used in CP patients for research purposes. Previous studies have consistently reported the analgesic

effects of high frequency repetitive TMS (HF-rTMS) via stimulation of the primary motor cortex (M1) in patients with NP. Another cortical target that has been studied using rTMS is the Dorsolateral Prefrontal Cortex (DLPFC). More recently, rTMS paradigms such as theta burst stimulation (TBS) have been developed that require less stimulation time (1-4 minutes) and lower stimulation intensities than conventional HF-rTMS protocols. TBS can be provided using either the intermittent or continuous paradigms. A prolonged form of continuous TBS (pcTBS) produces facilitatory and analgesic effects similar to HF-rTMS. No study has examined the analgesic effects of pcTBS targeted at the M1 and DLPFC brain regions in pDN patients, and concomitantly evaluated neural mechanisms of pain perception. Therefore, the central aim of this dissertation is to examine the effectiveness of pcTBS as an intervention in pDN patients by targeting the M1 and DLPFC regions of the brain, and to investigate the neural mechanisms that may explain the changes in pain perception. Therefore, Study 1 (Chapter 3) examined the efficacy of pcTBS targeted at the M1 and DLPFC brain regions as an intervention in pDN patients with a single session, prospective, single-blind, sham-controlled, randomized clinical trial. Study 2 (Chapter 4) investigated the neural mechanisms that could potentially explain the effects of pcTBS targeted at the M1 and DLPFC brain regions on pain perception in patients with pDN; (a) psychophysical mechanisms that comprise of the descending and ascending endogenous pain modulatory systems (b) neurophysiological mechanisms of corticospinal excitability, and (c) intracortical inhibition measures linked to GABA activity. The main findings from this dissertation are that pcTBS targeted at M1 or DLPFC may constitute an effective analgesic treatment for pDN and neurophysiological mechanisms related to corticospinal excitability and neurochemical mechanisms linked to intracortical inhibition may explain the analgesic response to pcTBS stimulation at the M1 and DLPFC brain regions in patients with pDN. Chapter 2 presents a review of the literature on brain derived neurotrophic factor (BDNF), focusing on its role as a biomarker, its mechanism of action in NP, and a critical analysis of the quantification of BDNF in serum and plasma.

CHAPTER 1

INTRODUCTION

Neuropathic Pain in Patients with Painful Diabetic Neuropathy

Approximately 50.2 million adults in the United States annually experience chronic pain (CP) accounting for $560 to 635 billion in direct medical costs. Diabetic neuropathy (DN), a type of nerve damage that can occur with diabetes, can lead to CP and approximately half of the estimated 425 million patients with diabetes worldwide are affected by this chronic complication of diabetes mellitus. [19,54,66]. One fifth of these patients develop chronic painful DN (pDN). [25,54]. pDN has debilitating consequences, with a major impact on morbidity and quality of life [1,62]. It is also the most common cause of neuropathic pain (NP), defined as “pain caused by a lesion or disease of the somatosensory nervous system,”[31]. NP can arise from a variety of causes including stroke, spinal cord injury, radiculopathy, phantom limb, diabetic neuropathy [17]. NP symptoms include spontaneous continuous pain, shooting pain, allodynia, and hyperalgesia with sensory deficits [8,42]. Current NP treatment options include pharmacological and non-pharmacological approaches that predominantly target clinical symptoms instead of causative factors [8,47]. Primary treatment with analgesic medication leaves 30–40% of patients without clinical improvement [3,9]. Furthermore, current interventions designed to alleviate chronic NP have demonstrated limited success due in part to the lack of understanding of the neurophysiological, psychophysical and neurochemical mechanisms that regulate this complex human condition [17,47,55].

Transcranial Magnetic Stimulation

Over the last two decades, the most established non-invasive technique to stimulate the brain is transcranial magnetic stimulation (TMS). TMS is used to (1) study central nervous system physiology and (2) as a therapeutic tool to treat neuropsychiatric disorders and CP [10,33]. High frequency repetitive TMS (HF-rTMS) to the primary motor cortex (M1) has been utilized to modulate corticospinal excitability (CE) [24,63] and to induce analgesia in patients with experimentally induced pain [38,67], NP [28,34,39,51] and other CP [67] conditions such as

fibromyalgia. Using HF-rTMS at the dorsolateral prefrontal cortex (DLPFC) has also exhibited similar reductions in experimentally induced pain and various CP conditions including NP [2,34,61]. Differing from the M1, the DLPFC is linked to the pain experience and plays an important role in the modulation of its cognitive and emotional aspects. Interestingly, at least 50% of patients with CP have co-morbid depression [22,58], and previous research has explored the bidirectional nature of CP and depression, especially in the elderly [22,45,58]. Although the mechanisms explaining the HF-rTMS analgesic effects are unclear, previous studies have demonstrated that M1 and DLPFC stimulation separately modulate nociceptive pain processing via activation of descending pain modulation systems [4,50,61,67] and alterations in intracortical excitability [18,24]. The potential of DLPFC region activation for the alleviation of pain and improvement in quality of life (via its emotional and cognitive effects) highlight the potential value of the DLPFC region as a more advantageous target for noninvasive brain stimulation compared to M1.

Theta Burst Stimulation

Conventional HF-rTMS requires 20 to 30 min of stimulation time, making some experimental and clinical applications logistically challenging. More recently developed rTMS paradigms, such as theta burst stimulation (TBS), require less stimulation time (1-4 minutes) and lower stimulation intensities (bursts of three pulses at 30 Hz or 50 Hz, repeated five times per second with 600 pulses in total) than conventional rTMS protocols [30,60], thus investigators have initiated investigations into the effectiveness of rTMS. TBS can either depress (when applied as continuous TBS; cTBS) or increase (when applied as intermittent TBS) cortical excitability [15,30,60]. However, a prolonged form of cTBS (pcTBS) with twice the number of stimuli (1,200 pulses) produces a facilitatory effect similar to that of intermittent TBS [15,20,60]. The effects of TBS, and especially pcTBS, have been investigated in healthy subjects [32,41,44,48] only, and these studies have demonstrated similar and greater increases in pain thresholds for pcTBS compared to rTMS lasting up to 24 hours post stimulation. No study has examined the analgesic

effects of pcTBS targeted at the M1 and DLPFC brain regions in patients with CP, in particular NP patients, and concomitantly evaluated the changes in emotional and cognitive measures of pain perception.

Self-Report Measures of Pain Perception (SRMP)

CP is regarded as a multidimensional pain experience with sensory-discriminative (location, quality and intensity), affective-motivational (unpleasantness) and cognitive-evaluative components (beliefs, attitudes, intention). These components can be measured using self-report measures of pain (SRMP). SRMP provide a quantitative measure of pain perception and overall health to evaluate the impact of pDN on daily life.

Therefore, Study 1 (Chapter 3) examined the efficacy of pcTBS targeted at the M1 and DLPFC brain regions as an intervention in pDN patients with a single session, prospective, single-blind, sham-controlled, randomized clinical trial. Forty-two subjects with pDN were randomized to receive either pcTBS targeted at M1 or DLPFC and completed SRMP on an Ipad at three time points (baseline, post-pcTBS and 24 h post-pcTBS) using REDCap. Statistically significant improvements over the three time points in all the SRMPs revealed a response indicative of an alleviation of pain. Thus, pcTBS targeted at M1 or DLPFC may constitute an effective analgesic treatment for pDN. Headache (n=8), 24hrspost-pcTBS, was the most common side effect in 18% of the study participants followed by neck pain (n=6) in 11% of the study participants.

Mechanisms of Pain Perception

Role of descending and ascending pain modulatory systems

To understand the varying benefits of pcTBS for patients with pDN, it is important to examine the psychophysical, neurophysiological, and neurochemical mechanisms that regulate pain perception. Psychophysical pain mechanisms are characterized by the interactions between the descending and ascending endogenous pain modulatory systems. The descending endogenous pain system is inhibitory and composed of communications between the cortico-limbic structures and brain stem nuclei [21,52]. The conditioned pain modulation (CPM) paradigm can

be used as a psychophysical pain protocol (PPP) to assess the activity of the descending pain systems [37,65]. An impaired CPM characterizes inefficient functioning of these descending inhibitory pathways [23,40,69]. Typically, a CPM protocol consists of two remote noxious stimuli with one, the 'conditioning stimulus', inhibiting the other, the 'test stimulus' [68]. A thermal contact stimulation, mechanical pressure, or electrical stimuli are used for the test stimulus and cold or hot water immersion is most commonly used for the conditioning stimuli [37,40]. Temporal summation of pain (TSP) is used to quantify the activity of the ascending endogenous pain system. It is the increase in pain rating after application of a repeated brief noxious stimuli (e.g., electrical, thermal, mechanical), and is correlated with the 'wind-up' phenomenon [13,26,27]. In patients with chronic pain, several studies have demonstrated an enhanced TSP response compared with asymptomatic controls [14,70]. Furthermore, TSP has been found to be facilitated in patients with NP, and in patients with pDN CPM has been shown to be impaired. However, no study has evaluated these PPPs (CPM and TSP) and in patients with NP.

Role of Corticospinal Excitability (CE) and Intracortical Inhibition (ICI)

In order to evaluate the neurophysiological effects of rTMS targeted at M1, quantification of different CE measures have been performed. TMS administered over the cortical representation of a specific muscle at M1 generates an action potential which induces descending volleys in the pyramidal tract projecting on the spinal motoneurons [10,33,56] that evokes a biphasic response termed a motor evoked potential (MEP). This results in a twitch in a contralateral muscle that can be measured using electromyography [33,56]. Previous studies, utilizing HF-rTMS in healthy subjects and patients with chronic pain, have demonstrated reductions in CE (decrease in MEP amplitude). An increase in MEP amplitude indicating an increase in CE has been observed post HF-rTMS (in healthy subjects and patients with chronic pain) and pcTBS (in healthy subjects only). Thus, pain exerts an inhibitory modulation on CE, reducing MEP amplitude that can be reversed using HF-rTMS and pcTBS.

Another neurochemical mechanism that plays an important role in pain perception, is gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system involved in pain transmission and perception [6,43]. GABA is a significant mediator of intracortical inhibition (ICI) [5,29,43]. GABA modulates neural excitability at the level of the dorsal horn in the spinal cord, via the activity of the GABA- A receptors and GABA-B receptors Thus, in patients with chronic pain, inhibition of GABA-A and GABA-B activity promotes pain transmission at the level of the dorsal horn in the spinal cord [6,43]. Using paired pulse TMS, previous studies have examined neurophysiological correlates linked to GABA-A activity, in particular, short intracortical inhibition (SICI) [5,11,12,44]. In addition, long-interval intracortical inhibition (LICI) has been used as a marker of GABA-B activity [46,71]. Significant reductions in SICI and LICI have been observed in patients with CP [5,12] especially NP [35,57,64]. Thus, changes in SICI and LICI as markers linked to GABAergic activity could delineate mechanisms of TMS induced analgesia.

Therefore, *Study 2* (Chapter 4) investigated the neural mechanisms that could potentially explain the effects of pcTBS targeted at the M1 and DLPFC brain regions on pain perception in patients with pDN. Forty-two subjects with pDN were randomized to receive either pcTBS targeted at M1 or targeted at DLPFC. PPP, CE and CI were examined at baseline and post-pcTBS. Statistically significant increases in CE and CI post-active pcTBS targeted at the M1 and DLPFC brain regions were observed. Despite these mechanistic changes, neither CE nor ICI predicted responses to BPI-DN at baseline.

Role of BDNF as a biomarker

In the two studies above, SRMP were utilized as a measure of pain perception and previous studies have highlighted how subjective self-reporting of pain has played a key role in the diagnosis and treatment of NP [8,49]. However, this assessment is complicated by individual differences in sensitivity [16] and the lack of reliability in these measures that often include the

evaluation of the impact of NP on activities of daily living and quality of life [59]. This highlights the critical need for objective data to assess pain and support the management of pain perception. The identification of a biomarker(s) that could complement patient reporting and serve as a correlate to the neurobiological processes underlying pDN and NP could be an important tool in identifying effective treatments.

For approximately two decades, brain derived neurotrophic factor (BDNF) has attracted attention as a potential biomarker for NP because of its role in promoting neuronal growth, maintenance, survival and neurogenesis [7,36,53]. Although BDNF has been proposed as a candidate biomarker of chronic pain, especially NP, there remains a significant gap in the understanding of the physiological mechanisms that lead to changes in BDNF levels measured peripherally. This is partially due to the difficulty in assessing the influence of central nervous system BDNF levels on BDNF levels assessed from the periphery. At present, more than 95% of the studies in the literature that have evaluated factors involved in the measurement of BDNF, analyze either serum BDNF and/or plasma BDNF. In addition, previous studies have documented inconsistent results across studies between plasma BDNF and serum BDNF

Chapter 2 presents a review of the literature on BDNF, focusing on its role as a biomarker, its mechanism of action in NP, and a critical analysis of the quantification of BDNF in serum and plasma. The section regarding quantification of BDNF highlights factors that may contribute to the discrepancy in results between plasma and serum BDNF values and presents a case for the most reliable and valid techniques. Although evidence from studies utilizing animal models provide a clear rationale for utilizing BDNF as a biomarker for CNS activity, the studies that have used BDNF as a potential biomarker in healthy volunteers, patients with chronic pain and in patients with neuropsychiatric disorders have presented inconsistent findings. To elucidate the role of BDNF in the periphery, it is suggested that serum BDNF levels versus plasma BDNF levels be utilized because of their stability and sensitive to changes.

References

[1] Alleman CJM, Westerhout KY, Hensen M, Chambers C, Stoker M, Long S, van Nooten FE. Humanistic and economic burden of painful diabetic peripheral neuropathy in Europe: A review of the literature. Diabetes Res Clin Pract 2015;109:215–225.

[2] de Andrade DC, Mhalla A, Adam F, Texeira MJ, Bouhassira D. Neuropharmacological basis of rTMS-induced analgesia: The role of endogenous opioids. Pain 2011;152:320–326. doi:10.1016/j.pain.2010.10.032.

[3] Attal N, Cruccu G, Haanpää M, Hansson P, Jensen TS, Nurmikko T, Sampaio C, Sindrup S, Wiffen P. EFNS guidelines on pharmacological treatment of neuropathic pain. Eur J Neurol 2006;13:1153–1169.

[4] Ayache SS, Ahdab R, Chalah MA, Farhat WH, Mylius V, Goujon C, Sorel M, Lefaucheur JP. Analgesic effects of navigated motor cortex rTMS in patients with chronic neuropathic pain. European Journal of Pain (United Kingdom) 2016;20:1413–1422.

[5] Barr MS, Farzan F, Davis KD, Fitzgerald PB, Daskalakis ZJ. Measuring GAB aergic inhibitory activity with TMS-EEG and its potential clinical application for chronic pain. Journal of Neuroimmune Pharmacology 2013;8:535–546.

[6] Benson C, Mifflin K, Kerr B, Jesudasan SJB, Dursun S, Baker G. Biogenic amines and the amino acids GABA and glutamate: Relationships with pain and depression. Mod Trends Pharmacopsychiatry 2015;30:67–79.

[7] Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors 2004;22:123–131.

[8] Bouhassira D. Neuropathic pain: Definition, assessment and epidemiology. Rev Neurol (Paris) 2019;175:16–25.

[9] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain 2008;136:380–387.

[10] Burke MJ, Fried PJ, Pascual-Leone A. Transcranial magnetic stimulation: Neurophysiological and clinical applications. Handb Clin Neurol 2019;163:73–92.

[11] Cardinal TM, Antunes LC, Brietzke AP, Parizotti CS, Carvalho F, de Souza A, da Silva Torres IL, Fregni F, Caumo W. Differential neuroplastic changes in fibromyalgia and depression indexed by up-regulation of motor cortex inhibition and disinhibition of the descending pain system: An exploratory study. Front Hum Neurosci 2019;13:1–13.

[12] Caumo W, Deitos A, Carvalho S, Leite J, Carvalho F, Dussán-Sarria JA, Tarragó M da GL, Souza A, Torres IL da S, Fregni F. Motor cortex excitability and BDNF levels in chronic musculoskeletal pain according to structural pathology. Front Hum Neurosci 2016;10:1–15.

[13] Cheng JC, Erpelding N, Kucyi A, Desouza DD, Davis KD. Individual differences in temporal summation of pain reflect pronociceptive and antinociceptive brain structure and function. Journal of Neuroscience 2015;35:9689–9700.

[14] Cheng JC, Erpelding N, Kucyi A, Desouza DD, Davis KD. Individual differences in temporal summation of pain reflect pronociceptive and antinociceptive brain structure and function. Journal of Neuroscience 2015;35:9689–9700.

[15] Chung SW, Hill AT, Rogasch NC, Hoy KE, Fitzgerald PB. Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis. Neurosci Biobehav Rev 2016;63:43–64. doi:10.1016/j.neubiorev.2016.01.008.

[16] Coghill RC, Eisenach J. Individual differences in pain sensitivity: implications for treatment decisions. Anesthesiology 2003;98:1312–1314.

[17] Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB, Eccleston C, Kalso E, Bennett DL, Dworkin RH, Raja SN. Neuropathic pain. Nat Rev Dis Primers 2017;3:1–19.

[18] Dall'Agnol L, Medeiros LF, Torres ILS, Deitos A, Brietzke A, Laste G, de Souza A, Vieira JL, Fregni F, Caumo W. Repetitive transcranial magnetic stimulation increases the corticospinal inhibition and the brain-derived neurotrophic factor in chronic myofascial pain syndrome: An explanatory double-blinded, randomized, sham-controlled trial. Journal of Pain 2014;15:845–855.

[19] Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, Bril V, Russell JW, Viswanathan V. Diabetic neuropathy. Nat Rev Dis Primers 2019;5. doi:10.1038/s41572-019-0092-1.

[20] Gamboa OL, Antal A, Moliadze V, Paulus W. Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. Exp Brain Res 2010;204:181–187.

[21] Gebhart GF. Descending modulation of pain. Neurosci Biobehav Rev 2004;27:729–737.

[22] Grady K, Severn A, Eldridge P. Depression and Pain. Key Topics in Pain Management, Third Edition 2006;163:69–72.

[23] Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, Yarnitsky D. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? Pain 2008;136:142–149.

[24] Granovsky Y, Sprecher E, Sinai A. Motor corticospinal excitability: A novel facet of pain modulation? Pain Rep 2019;4:1–7.

[25] Hicks CW, Selvin E. Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. Current Diabetes Reports 2019 19:10 2019;19:1–8. doi:10.1007/S11892-019-1212-8.

[26] Hoeger Bement M, Petersen KK, Sørensen LB, Andersen HH, Graven-Nielsen T. Temporal aspects of endogenous pain modulation during a noxious stimulus prolonged for 1 day. European Journal of Pain (United Kingdom) 2020;24:752–760.

[27] Horn-Hofmann C, Kunz M, Madden M, Schnabel EL, Lautenbacher S. Interactive effects of conditioned pain modulation and temporal summation of pain-the role of stimulus modality. Pain 2018;159:2641–2648.

[28] Hosomi K, Sugiyama K, Nakamura Y, Shimokawa T, Oshino S, Goto Y, Mano T, Shimizu T, Yanagisawa T, Saitoh Y. A randomized controlled trial of 5 daily sessions and continuous trial of 4 weekly sessions of repetitive transcranial magnetic stimulation for neuropathic pain. Pain 2020;161:351–360.

[29] Huang YJ, Grau JW. Ionic plasticity and pain: The loss of descending serotonergic fibers after spinal cord injury transforms how GABA affects pain. Exp Neurol 2018;306:105–116.

[30] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–206.

[31] Jensen TS, Baron R, Haanpää M, Kalso E, Loeser JD, Rice ASC, Treede RD. A new definition of neuropathic pain. Pain 2011;152:2204–2205.

[32] Klírová M, Hejzlar M, Kostýlková L, Mohr P, Rokyta R, Novák T. Prolonged Continuous Theta Burst Stimulation of the Motor Cortex Modulates Cortical Excitability But not Pain Perception. Front Syst Neurosci 2020;14:1–9.

[33] Klomjai W, Katz R, Lackmy-Vallée A. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). Ann Phys Rehabil Med 2015;58:208–213.

[34] Lefaucheur J-P, Aleman A, Baeken C, Benninger DH, Brunelin J, di Lazzaro V, Filipović SR, Grefkes C, Hasan A, Hummel FC, Jääskeläinen SK, Langguth B, Leocani L, Londero A, Nardone R, Nguyen J-P, Nyffeler T, Oliveira-Maia AJ, Oliviero A, Padberg F, Palm U, Paulus W, Poulet E, Quartarone A, Rachid F, Rektorová I, Rossi S, Sahlsten H, Schecklmann M, Szekely D, Ziemann U. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clinical Neurophysiology 2020;131:474–528. doi:https://doi.org/10.1016/j.clinph.2019.11.002.

[35] Lefaucheur JP, Drouot X, Ménard-Lefaucheur I, Keravel Y, Nguyen JP. Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. Neurology 2006;67:1568–1574.

[36] Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde Y-A. Molecular cloning and expression of brain-derived neurotrophic factor. Nature 1989 341:6238 1989;341:149–152.

[37] Leone C, Truini A. The CPM Effect: Functional Assessment of the Diffuse Noxious Inhibitory Control in Humans. Journal of Clinical Neurophysiology 2019;36:430–436.

[38] Leo RJ, Latif T. Repetitive Transcranial Magnetic Stimulation (rTMS) in Experimentally Induced and Chronic Neuropathic Pain: A Review. Journal of Pain 2007;8:453–459.

[39] Leung A, Shirvalkar P, Chen R, Kuluva J, Vaninetti M, Bermudes R, Poree L, Wassermann E, Kopell B, Levy R. Transcranial Magnetic Stimulation for Pain, Headache, and Comorbid Depression: INS-NANS Expert Consensus Panel Review and Recommendation. Neuromodulation 2020;2019:267–290.

[40] Lewis GN, Rice DA, McNair PJ. Conditioned pain modulation in populations with chronic pain: A systematic review and meta-analysis. Journal of Pain 2012;13:936–944. doi:10.1016/j.jpain.2012.07.005.

[41] Li C, Zhang N, Han Q, Zhang L, Xu S, Tu S, Xie Y, Wang Z. Prolonged Continuous Theta Burst Stimulation Can Regulate Sensitivity on Aβ Fibers: An Functional Near-Infrared Spectroscopy Study. Front Mol Neurosci 2022;15.

[42] Luana Colloca1, Taylor Ludman1, Didier Bouhassira2, Ralf Baron3, Anthony H. Dickenson4, David Yarnitsky5, Roy Freeman6, Andrea Truini7, Nadine Attal8, Nanna B. Finnerup9, Christopher Eccleston10, 11, Eija Kalso12, David L. Bennett13, Robert H. Dworkin14 and SNR. Neuropathic Pain. Nat Rev Dis Primers 2017:749–757.

[43] Malcangio M. GABAB receptors and pain. Neuropharmacology 2018;136:102–105.

[44] de Martino E, Fernandes AM, Galhardoni R, de Oliveira Souza C, Ciampi De Andrade D, Graven-Nielsen T. Sessions of Prolonged Continuous Theta Burst Stimulation or High-frequency 10 Hz Stimulation to Left Dorsolateral Prefrontal Cortex for 3 Days Decreased Pain Sensitivity by Modulation of the Efficacy of Conditioned Pain Modulation. Journal of Pain 2019;20:1459–1469.

[45] Meyer T, Cooper J, Raspe H. Disabling Low Back Pain and Depressive Symptoms in the Community-Dwelling Elderly. Spine (Phila Pa 1976) 2007;32:2380–2386.

[46] Michelle N Mcdonnell, Orekhov Y, Ziemann U. The role of GABA B receptors in intracortical inhibition in the human motor cortex. Exp Brain Res 2006;173:86–93.

[47] Moisset X, Bouhassira D, Couturier JA, Alchaar H, Conradi S, Delmotte MH, Lanteri-Minet M, Lefaucheur JP, Mick G, Piano V, Pickering G, Piquet E, Regis C, Salvat E, Attal N. Practice guidelines Pharmacological and non-pharmacological treatments for neuropathic pain: Systematic review and French recommendations. Rev Neurol (Paris) 2020;176:325–352.

[48] Moisset X, Goudeau S, Poindessous-Jazat F, Baudic S, Clavelou P, Bouhassira D. Prolonged continuous theta-burst stimulation is more analgesic than “classical” high frequency repetitive transcranial magnetic stimulation. Brain Stimul 2015;8:135–141. doi:10.1016/j.brs.2014.10.006.

[49] Mulvey MR, Bennett MI, Liwowsky I, Freynhagen R. The role of screening tools in diagnosing neuropathic pain. Pain Manag 2014;4:233–243.

[50] Mylius V, Borckardt JJ, Lefaucheur JP. Noninvasive cortical modulation of experimental pain. Pain 2012;153:1350–1363.

[51] Nardone R, Höller Y, Langthaler PB, Lochner P, Golaszewski S, Schwenker K, Brigo F, Trinka E. RTMS of the prefrontal cortex has analgesic effects on neuropathic pain in subjects with spinal cord injury. Spinal Cord 2017;55:20–25.

[52] Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronification of pain. Curr Opin Support Palliat Care 2014;8:143–151.

[53] Park H, Poo M. Neurotrophin regulation of neural circuit development and function. Nature Reviews Neuroscience 2013 14:1 2012;14:7–23. doi:10.1038/nrn3379.

[54] Pop-Busui R, Boulton AJM, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D. Diabetic neuropathy: A position statement by the American diabetes association. Diabetes Care 2017;40:136–154.

[55] Price TJ, Gold MS. From mechanism to cure: Renewing the goal to eliminate the disease of pain. Pain Medicine (United States) 2018;19:1525–1549.

[56] Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, di Iorio R, di Lazzaro V, Ferreri F, Fitzgerald PB, George MS, Hallett M, Lefaucheur JP, Langguth B, Matsumoto H, Miniussi C, Nitsche MA, Pascual-Leone A, Paulus W, Rossi S, Rothwell JC, Siebner HR, Ugawa Y, Walsh V, Ziemann U. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee. Clinical Neurophysiology 2015;126:1071–1107. doi:10.1016/j.clinph.2015.02.001.

[57] Schwenkreis P, Scherens A, Rönnau AK, Höffken O, Tegenthoff M, Maier C. Cortical disinhibition occurs in chronic neuropathic, but not in chronic nociceptive pain. BMC Neurosci 2010;11.

[58] Shpaner M, Tulipani LJ, Bishop JH, Naylor MR. The Vicious Cycle of Chronic Pain in Aging Requires Multidisciplinary Non-pharmacological Approach to Treatment. Curr Behav Neurosci Rep 2017;4:176–187.

[59] Smith SM, Amtmann D, Askew RL, Gewandter JS, Hunsinger M, Jensen MP, Mcdermott MP, Patel K V., Williams M, Bacci ED, Burke LB, Chambers CT, Cooper SA, Cowan P, Desjardins P, Etropolski M, Farrar JT, Gilron I, Huang IZ, Katz M, Kerns RD, Kopecky EA, Rappaport BA, Resnick M, Strand V, Vanhove GF, Veasley C, Versavel M, Wasan AD, Turk DC, Dworkin RH.

Pain intensity rating training: Results from an exploratory study of the ACTTION PROTECCT system. Pain 2016;157:1056–1064.

[60] Suppa A, Huang YZ, Funke K, Ridding MC, Cheeran B, di Lazzaro V, Ziemann U, Rothwell JC. Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. Brain Stimul 2016;9:323–335.

[61] Taylor JJ, Borckardt JJ, Canterberry M, Li X, Hanlon CA, Brown TR, George MS. Naloxone-Reversible Modulation of Pain Circuitry by Left Prefrontal rTMS. Neuropsychopharmacology 2013;38:1189–1197. doi:10.1038/npp.2013.13.

[62] Tesfaye S, Vileikyte L, Rayman G, Sindrup SH, Perkins BA, Baconja M, Vinik AI, Boulton AJM, on behalf of The Toronto Expert Panel on Diabetic Neuropathy. Painful diabetic peripheral neuropathy: consensus recommendations on diagnosis, assessment and management. Diabetes Metab Res Rev 2011;27:629–638. doi:https://doi.org/10.1002/dmrr.1225.

[63] Thibaut A, Zeng D, Caumo W, Liu J, Fregni F. Corticospinal excitability as a biomarker of myofascial pain syndrome. Pain Rep 2017;2:1–8.

[64] Turgut N, Altun BU. Cortical disinhibition in diabetic patients with neuropathic pain. Acta Neurol Scand 2009;120:383–388.

[65] van Wijk G, Veldhuijzen DS. Perspective on Diffuse Noxious Inhibitory Controls as a Model of Endogenous Pain Modulation in Clinical Pain Syndromes. Journal of Pain 2010;11:408–419.

[66] Williams R, Karuranga S, Malanda B, Saeedi P, Basit A, Besançon S, Bommer C, Esteghamati A, Ogurtsova K, Zhang P, Colagiuri S. Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract 2020;162:108072.

[67] Yang S, Chang MC. Effect of Repetitive Transcranial Magnetic Stimulation on Pain Management: A Systematic Narrative Review. Front Neurol 2020;11.

[68] Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): Its relevance for acute and chronic pain states. Curr Opin Anaesthesiol 2010;23:611–615.

[69] Yarnitsky D. Role of endogenous pain modulation in chronic pain mechanisms and treatment. Pain 2015;156:S24–S31.

[70] Yarnitsky D, Granot M, Granovsky Y. Pain modulation profile and pain therapy: between pro- and antinociception. Pain 2014;155:663–665.

[71] Ziemann U. Pharmaco-transcranial magnetic stimulation studies of motor excitability. 1st ed. Elsevier B.V., 2013 doi:10.1016/B978-0-444-53497-2.00032-2.

CHAPTER 2

BDNF as a Biomarker for Neuropathic Pain: Consideration of Mechanisms of Action and Associated Measurement Challenges.

In review with Brain and Behavior.

Bhushan Thakkar, Edmund Acevedo.

BDNF as a Biomarker for Neuropathic Pain: Consideration of Mechanisms of Action and Associated Measurement Challenges

Bhushan Thakkar [1] and Edmund O. Acevedo [2]

1 Department of Physical Therapy, Virginia Commonwealth University, Richmond, VA

2 Department of Kinesiology and Health Sciences, Virginia Commonwealth University, Richmond, VA

Corresponding Author:

Bhushan Thakkar

1101 East Marshall Street, 11th Floor, Room 11-027

Box #980034,

Richmond, VA 23298

Email: thakkarbs2@vcu.edu

Phone: 804-300-5578

Keywords: BDNF, Neuropathic Pain, Biomarker, Mechanisms, Measurement

Running Title: BDNF role in NP: Mechanisms and Measurement

Acknowledgment: None.

Conflict of Interest: The authors declare no conflict of interest.

Brain and Behavior
Open Access

BDNF as a Biomarker for Neuropathic Pain: Consideration of Mechanisms of Action and Associated Measurement Challenges

Journal:	*Brain and Behavior*
Manuscript ID	BRB3-2022-07-0657
Wiley - Manuscript type:	Review
Date Submitted by the Author:	16-Jul-2022
Complete List of Authors:	THAKKAR, BHUSHAN; Virginia Commonwealth University College of Health Professions, Acevedo, Edmund; Virginia Commonwealth University
Search Terms:	neuroprotection, Neuroscience, neuropsychopharmacology, pain
Abstract:	Introduction: The primary objective of this paper is to (1) provide a summary of human studies that have used brain derived neurotrophic factor (BDNF) as a biomarker, (2) review animal studies that help to elucidate the mechanistic involvement of BDNF in the development and maintenance of Neuropathic Pain (NP), and (3) provide a critique of the existing measurement techniques to highlight the limitations of the methods utilized to quantify BDNF in different biofluids in the blood (i.e., serum and plasma) with the intention of presenting a case for the most reliable and valid technique. Lastly, this review also explores potential moderators that can influence the measurement of BDNF and provides recommendations to standardize its quantification to reduce the inconsistencies across studies. Methods: In this manuscript we examined the literature on BDNF, focusing on its role as a biomarker, its mechanism of action in NP, and critically analyzed its measurement in serum and plasma to identify factors that contribute to the discrepancy in results between plasma and serum BDNF values. Results: A large heterogenous literature was reviewed that detailed BDNF's utility as a potential biomarker in healthy volunteers, patients with chronic pain and patients with neuropsychiatric disorders but demonstrated inconsistent findings. The literature provides insight in to the mechanism of action of BDNF at different levels of the central nervous system using animal studies. We identified multiple factors that influence the measurement of BDNF in serum and plasma and based on current evidence, we recommend assessing serum BDNF levels to quantify peripheral BDNF as they are more stable and sensitive to changes than plasma BDNF. Conclusion: Although mechanistic studies clearly explain the role of BDNF, results from human studies are inconsistent. More studies are needed to evaluate the methodological challenges in using serum BDNF as a biomarker in NP.

Abstract

Introduction: The primary objective of this paper is to (1) provide a summary of human studies that have used brain derived neurotrophic factor (BDNF) as a biomarker, (2) review animal studies that help to elucidate the mechanistic involvement of BDNF in the development and maintenance of Neuropathic Pain (NP), and (3) provide a critique of the existing measurement techniques to highlight the limitations of the methods utilized to quantify BDNF in different biofluids in the blood (i.e., serum and plasma) with the intention of presenting a case for the most reliable and valid technique. Lastly, this review also explores potential moderators that can influence the measurement of BDNF and provides recommendations to standardize its quantification to reduce the inconsistencies across studies.

Methods: In this manuscript we examined the literature on BDNF, focusing on its role as a biomarker, its mechanism of action in NP, and critically analyzed its measurement in serum and plasma to identify factors that contribute to the discrepancy in results between plasma and serum BDNF values.

Results: A large heterogenous literature was reviewed that detailed BDNF's utility as a potential biomarker in healthy volunteers, patients with chronic pain and patients with neuropsychiatric disorders but demonstrated inconsistent findings. The literature provides insight in to the mechanism of action of BDNF at different levels of the central nervous system using animal studies. We identified multiple factors that influence the measurement of BDNF in serum and plasma and based on current evidence, we recommend assessing serum BDNF levels to quantify peripheral BDNF as they are more stable and sensitive to changes than plasma BDNF.

Conclusion: Although mechanistic studies clearly explain the role of BDNF, results from human studies are inconsistent. More studies are needed to evaluate the methodological challenges in using serum BDNF as a biomarker in NP.

1 Introduction

The International Association for the Study of Pain defines neuropathic pain (NP) as "pain caused by a lesion or disease of the somatosensory nervous system" [68]. It can be initiated by nerve, brain or spinal cord injury and represents a broad category of pain syndromes encompassing a wide variety of peripheral of central disorders. Previous epidemiological studies have revealed that NP affects 7%–10% of the general population [18,30,52,113], accounting for almost 20-25% of patients with chronic pain [18,33]. It is more frequent in older individuals (>60 years old), more common in women than in men and characterized by unpleasant symptoms, such as shooting or burning pain, numbness, and allodynia [17,18,113]. It is also associated with a high level of disability [4,38,44] and has a high socio-economic cost [4,52,56,101]. Most importantly, current drug treatment is inadequate due to both poor efficacy and tolerability [6,25,108]. A recent report by Maher and colleagues using clinical trial data from the last 20 years reported that the probability of successful drug treatment for NP was only 7.1% [72]. Identifying effective treatments to address the associated severe pain and disability is limited by the lack of understanding of the underlying pathophysiological mechanisms [25,42,92,107]. The identification of a biomarker that links the signs and symptoms of NP to pathophysiological mechanism, would provide information relevant for drug-discovery and development. This review examines the literature on brain derived neurotrophic factor (BDNF) to determine the physiological validity for utilizing BDNF as a biomarker, and the possibility of a pragmatic approach to measuring BDNF peripherally in the blood.

The subjective self-reporting of pain has played a key role in the diagnosis and treatment of NP [17,78]. However, this assessment is complicated by individual differences in sensitivity [29] and the lack of reliability in these measures that often include the evaluation of the impact of NP on activities of daily living and quality of life [103]. This highlights the critical need for objective data to assess pain and support the management of pain perception. The identification

of a biomarker(s) that could complement patient reporting and serve as a correlate to the neurobiological processes underlying painful conditions would be an important tool in identifying effective treatments. This could also support the aim of reliably diagnosing NP. Furthermore, biomarkers that are directly related to the presence and severity of NP could lead to (a) successful mechanism-based treatment approaches to alleviate the need for long-term use of opioids, (b) significant reduction in the healthcare costs worldwide, and (c) improvements in the quality of life of NP patients.

The FDA [41] describes a biomarker as a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions". Types of biomarkers that have been studied in NP include plasma and cerebrospinal fluid biomarkers (lipid mediators, nerve growth factor, BDNF, tumor necrosis factor alpha, interleukins and neurotransmitters like gamma-aminobutyric acid and glutamate) [16,49], skin biopsy [16,99,104], genetic biomarkers (point mutations in the gene encoding of TRPV1 and TRPA1, SCN10A and SCN11A), [16,99], sensory biomarkers (quantitative sensory testing) [99,104], and imaging biomarkers (resting-state brain activity, evoked activity with ongoing clinical pain) [76,99,104].

For approximately two decades, BDNF has attracted attention as a potential biomarker for NP because it promotes neuronal growth, maintenance, survival and neurogenesis [7,13,64,84,131]. BDNF is a member of the neurotrophic factor family [131], has been identified as an important pain modulator [75,85,120] regulates central and peripheral synaptic plasticity [13,82,84,131]. BDNF synthesis is initiated from pre-pro-BDNF, which is cleaved to mature BDNF, and is secreted both by presynaptic and postsynaptic terminals with its secretion dependent on neuronal activity [5,13,84]. It has also been implicated in neuropathic [36,85,86,109,126,128,129] and inflammatory pain mechanisms [53,98,132] because of its

important role in sensory neurotransmission in spinal and supraspinal level nociceptive pathways.

It is plausible that BDNF initiates compensatory processes that facilitate recovery or alleviate the adverse chronic effects of injury or disease to the central and peripheral nervous system. Furthermore, BDNF can act as a pain mediator and modulator at different sites in the central nervous system including dorsal root ganglion, spinal cord, and supra-spinal sites. Lastly, because of its involvement at the dorsal horn level, previous studies have also implicated its role in central sensitization [1,12,98,120]. Furthermore, long-term BDNF exposure increases the excitability of the dorsal horn and mediates central sensitization of the dorsal horn, which initiates changes in synaptic functioning that may be responsible for the generation of NP [34,58,102].

Although BDNF has been proposed as a candidate biomarker of chronic pain, especially NP, there remains a significant gap in our understanding of the physiological mechanisms that lead to changes in BDNF levels measured peripherally. This is partially due to the difficulty in assessing central nervous system BDNF level. In addition, an understanding of this multi-factorial experience could lead to the more effective use of personalized medicine approaches to pain management. This purpose of this review is to (a) summarize current findings from studies that have utilized BDNF as a potential biomarker, (b) briefly outline the role of BDNF in NP by summarizing results from animal studies, and c) provide a critique of the existing measurement techniques used to assess BDNF with the intention of presenting a case for the most reliable and valid techniques.

As an initial step, Table 1 provides the study characteristics and findings for articles that have investigated group differences at baseline (Table 1a) and group differences across time following an intervention (Table 1b) utilizing BDNF as a biomarker in healthy volunteers, patients with chronic pain and patients with neuropsychiatric disorders.

Table 1a: Study characteristics and findings for studies examining baseline group differences in BDNF in healthy volunteers, patients with chronic pain and patients with neuropsychiatric disorders.

Author(s)	**Study population**	**Source of BDNF measurement**	**Mean ± SD BDNF values in pg/mL or ng/ml (as noted)**	**p-values Sig or Not Sig**
Stefani et al., 2019	Fibromyalgia (n=117), Osteoarthritis (n=88), Endometriosis (n=36), Chronic tensional type headache (n=33) and Healthy controls (n=41)	Serum	*Osteoarthritis: 24.85, Endometriosis: 23.71 Fibromyalgia: 38.60, Chronic tensional type headache: 37.22 and Healthy controls: 22.85	Sig
Jasim et al., 2020	Chronic temporomandibular disorders myalgia (TMD) (n=39) and Healthy controls (n=39)	Salivary Plasma	TMD group: 3.57 ± 1.47 and Healthy controls 4.62 ± 2.51 TMD group: 263.33 ± 245.13 and Healthy controls 151.81	Sig Sig
Rocha et al., 2017	Ovarian endometrioma (n=11), other benign ovarian tumors (n=11), deep endometriosis (n=9) and uterine fibroids (n=4)	Plasma	Ovarian endometrioma:1063 ± 157, other benign ovarian tumors: 537 ± 131, deep endometriosis: 584 ± 138, and uterine fibroids: 216 ± 129	Sig
Pillai et al., 2010	Male patients with Schizophrenia (n=15), Female patients with Schizophrenia (n=19), Male Healthy controls (n=13) and Female Healthy controls (n=23)	Plasma CSF	**Lower in patients with Schizophrenia than controls **Lower in patients with Schizophrenia than controls	Sig Sig
Baumeister et al., 2019	Fibromyalgia (n=89), Healthy controls (n=36)	Plasma	**No differences between the two groups	Not Sig
Lang et al.2004	Male (n=64) and female (n=54) healthy volunteers	Serum	***Males: 16.1±7.2 and Females: 16.5±7.4	Not Sig
Haas et al., 2010	Fibromyalgia (n=30), Healthy controls (n=30)	Plasma	Fibromyalgia: 167.1 ± 171.2 Healthy controls: 113.8 ± 149.6	Sig
Caumo et al., 2016	Fibromyalgia (n=19), osteoarthritis (n=27), myofascial pain syndrome (n=54) and healthy controls (n=14)		***Fibromyalgia: 50.78±16.06, osteoarthritis: 17.91±7.27, myofascial pain syndrome: 29.28±20.01 and healthy controls: 19.00±8.79	Sig

Deitos et al., 2015	Central sensitivity syndrome absent of structural pathology (n=81), Central sensitivity syndrome with persistent nociception (n=59) and healthy controls (n=37)	Serum	***Central sensitivity syndrome absent of structural pathology: 49.87±31.86, Central sensitivity syndrome with persistent nociception 20.44±8.30 and healthy controls:14.09±11.80	Not reported
Gasparin et al., 2020	Male (n=32) and female (n=24) healthy volunteers	Serum	***Males 33.06 ± 11.87 and females 23.71 ± 13.71	Sig

SD - Standard deviation; Sig - Significant difference between the two groups with $p<0.05$; Not Sig - No significant difference between the two groups; * - No SD values reported; ** - BDNF values not reported; *** - BDNF measured in ng/ml

Table 1b: Study characteristics and findings for studies examining group differences in BDNF across time in healthy volunteers, patients with chronic pain and patients with neuropsychiatric disorders.

Author	**Study population**	**Source of BDNF measurement**	**Mean ± SD BDNF values in pg/mL or ng/ml (as noted)**	**p-values Sig or Not Sig**
Gomes et al., 2014	Knee osteoarthritis	Plasma	Before exercise: 7.69 ± 4.45 Immediately following exercise: 12.24 ± 3.80	Sig
Hanoglu et al.,2021	Alzheimer's disease (N=15)	Serum	Pre rTMS: 372.01 ± 42.41 Post rTMS: 508.61 ± 47.55	Sig
Cho et al., 2012	Male Healthy volunteers (N=18)	Plasma Serum Platelet	At rest: 3376.87 ± 319.45, **Increase post exercise At rest: 22944.54 ± 9116.57, **Increase post exercise At rest: 77.32 ± 33.89, **Increase post exercise	Sig Sig Sig
Naegelin et al., 2018	Male (n=81) and Female (n=178) Healthy volunteers	Serum	***At baseline, Males: 32.34 ± 7.82, Females: 32.85 ± 8.57. ***At 12 months, Males: 32.95 ± 8.19, Females: 32.98 ± 8.47	Not Sig Not Sig
Gaede et al.2014	Healthy volunteers (n=39)	Serum	***Pre: 8.2 ± 2.7 ***Post: 5.6 ± 3.2	Sig
Zhao et al.	Patients with refractory depression receiving rTMS (n=29), Patients acting as controls not	Serum	Patients receiving rTMS: 4.24±1.12, **Increase post rTMS	Sig

	receiving rTMS (n=29), Healthy volunteers (n=30)		Patients acting as controls: 4.31±1.14, **Increase post rTMS Healthy volunteers: 16.77±1.07	Sig
Lang et al.2008	Male (n=19) and female (n=23) healthy volunteers	Serum	***Males: Pre rTMS: 10.05±2.6 vs Post rTMS 10.01± 3.68 ***Females: Pre rTMS: 11.25±4.27 vs Post rTMS 11.38±4.16	Not Sig
[100]	Male Healthy volunteers (N=13)	Serum Plasma	**Increase post exercise **Increase post exercise	Sig Not Sig

SD - Standard deviation; rTMS - Repetitive Transcranial Magnetic Stimulation; Sig -Significant difference between the two groups with $p<0.05$; Not Sig- No significant difference between the two groups; ** - BDNF values not reported; *** - BDNF measured in ng/ml.

It is evident from the information in Table 1a and 1b that BDNF is typically measured either in serum or in plasma. In addition, studies quantify concentrations of BDNF in pg/ml or ng/ml, and these studies present inconsistent findings with a number of studies demonstrating differences at rest between various groups and across time in healthy volunteers, gender, patients with chronic pain, and patients with neuropsychiatric disorders, whereas other studies have not demonstrated these differences. The perplexing nature of these data demand a more intricate examination beginning with the evidence supporting BDNF as a possible mechanistic biomarker for pain perception and then a more sophisticated review of the measurement techniques utilized.

2 BDNF related mechanism of action in NP

BDNF acts as a pain mediator (factor that contributes to the initiation and development of pain) and modulator (factor that regulates pain) and performs its biological functions through two receptors: p75 neurotrophin (pan-selective p75 neurotrophin receptor) and the TrkB receptor (tropomyosin receptor kinase B or tyrosine receptor kinase B) [3,13,26]. BDNF is released in response to peripheral inflammation and is known as a nociceptive modulator for both pain perception and sensitization at both spinal and supraspinal levels[75,86]. p75 is a low affinity receptor while the tropomyosin receptor kinase B (TrkB) receptor is a high affinity receptor [13], and is upregulated in chronic pain states [102,112,122]. Spinal BDNF-TrkB signaling has been implicated in studies that have investigated pathological mechanisms for NP [22,32,81,105,112,122]. This BDNF-TrkB signaling can modulate neurotransmission and enhance synaptic efficacy both via presynaptic and postsynaptic mechanisms [13,86]. Furthermore, the pronociceptive role of BDNF–TrkB is responsible for the persistent increase in excitability of second order neurons in the spinal dorsal horn contributing to allodynia, hyperalgesia, spontaneous pain and causalgia that characterize NP and central sensitization [12,96,124,131]. Because the focus of this article is on NP (pain induced by injury to the nervous system) and the

associated role of BDNF in promoting neuronal growth, survival and neurogenesis in the nervous system, animal studies that describe the prevalent role of BDNF in the initiation and maintenance of NP at the spinal, peripheral and central levels will be discussed. Figure 1 provides a depiction of the role of BDNF in NP and the site of involvement for its mechanism of action with citations of the supporting literature.

2.1 Spinal dorsal horn, Dorsal root ganglia (DRG) and Microglia mediated action

Previous studies utilizing NP models have demonstrated that elevated BDNF levels in the spinal dorsal horn contributes to hyperalgesia and central sensitization [1,34,130]. Evidence from preclinical studies that utilize peripheral nerve injury models have also revealed that BDNF is synthesized by dorsal horn neurons and causes hyperexcitation of dorsal horn neurons, which results in pain hypersensitivity [32,36,98,133,134], an important contributor to NP. Lu et al. 2007 and 2009 describe the role of BDNF in NP using chronic constriction injury models in the rat dorsal horn to illustrate the increased excitability in the dorsal horn. These investigators demonstrated that the excitatory and inhibitory neurons in the substantia gelatinosa of the dorsal horn exhibited altered behavior due to changes in synaptic drive mediated by the release of BDNF with increased excitatory synaptic drive to excitatory neurons and a decrease in the synaptic drive to the inhibitory interneurons. It is critical to consider that central sensitization is an activity-dependent increase in excitability of dorsal horn neurons [62,125], and BDNF expression facilitates this process by promoting a slowly developing increase in excitability and synaptic activity in the dorsal horn. From here, TrkB receptors are activated on second order neurons or primary afferent endings which in turn activate spinal reflexes and primary afferents [34,58,70,122] causing allodynia, hyperalgesia and spontaneous pain, defining characteristics of NP.

Microglia are the resident immune cells in the central nervous system, and their activation following peripheral nerve injury leads to the release of BDNF via the purinergic receptors which facilitate the excitability of dorsal horn neurons contributing to NP [12,32,60,116]. In the microglia, BDNF can also activate PI3K and ERK kinase pathways that are fundamental for the development of neuropathic pain [116].

Elevated levels of BDNF at the level of the dorsal root ganglion (DRG) facilitates pain transmission and contributes to pain hypersensitivity and central sensitization [126]. Both in NP and inflammatory pain models, increased levels of BDNF in the DRG neurons are correlated with increase in the BDNF levels in the spinal dorsal horn [86,126]. Therefore, at the spinal level, BDNF is expressed in microglia, in the neurons and in nociceptors of the DRG and in the dorsal horn neurons. This BDNF release is maladaptive in that it contributes to central sensitization and NP.

2.2 NMDA- Glutamate- GABA receptor mediated action

BDNF also exerts its effects via interactions with other receptors and ion channels. Previous studies have described the interaction of BDNF-TrkB signaling with N-methyl-d-aspartate (NMDA) receptors as an underlying mechanism that contributes to central sensitization of spinal neurons [58,62,117]. Animal models of NP have revealed that BDNF-TrkB signaling promotes the upregulation of NR2B, a subunit of NMDA receptors via activation of the mTOR pathway (Zhang et al., 2018). In addition, BDNF plays a major role in modulating the contributions of the glutamatergic and GABAergic mechanisms responsible for long-term potentiation of the glutamatergic transmission both presynaptically and postsynaptically [14,75,119]. BDNF facilitates excitatory transmission at the dorsal horn by attenuating GABAergic inhibitory neurotransmission that causes a disequilibrium in GABA (y-aminobutyric

acid) levels [45,70,71,127]. This disinhibition is an important contributor to central sensitization and NP [73]

In addition, elevated BDNF contributes to decreased expression of KCC2. KCC2 is a potassium/chloride cotransporter that controls intracellular chloride concentrations in these neurons causing disruption of neuronal chloride homeostasis. This contributes to spinal disinhibition and promotes the development of pain hypersensitivity and mechanical allodynia, which is commonly observed in inflammatory pain and NP models [22,116,129,132]. The increase in chloride concentrations shifts the chloride equilibrium potential to a less negative value, and this also contributes to GABA disinhibition [130]. This BDNF–KCC2–GABA attenuation leads to NP and central sensitization [27,34,102]. Thus, altered BDNF levels in NP, perturb the balance in potentiation between glutamatergic and GABAergic synapses in the CNS that contributes to an imbalance in excitatory/inhibitory neurotransmission.

2.3 Supraspinal Involvement

Due to its role in brain signaling and synaptic plasticity, coupled with its involvement in emotional comorbidities like memory, decision making and depression, cerebral BDNF in brain areas including the hippocampus, prefrontal cortex and reward centers including the mesocorticolimbic system [77,80,128], has been proposed as a marker of nociception in chronic pain. Brainstem areas like the rostroventral medulla and the nucleus raphe magnus involved in descending pain modulation also contribute to the BDNF-KCC2-GABA impairment in the development of chronic NP [31,34]. The nucleus raphe magnus activates the descending pain pathways due to BDNF mediated KCC2 downregulation causing GABAergic disinhibition which plays an important role in the process of central sensitization during the development of chronic pain [34,130].

[Insert Figure 1 here]

Figure 1 includes a list of the studies that address the corresponding site of activity for BDNF at the supraspinal, spinal and receptor level. Therefore, increased levels of BDNF at different locations in the central nervous system including the spinal dorsal horn, the microglia, and the brain, coupled with its involvement at the receptor level and its connections to neurotransmitters like Glutamate and GABA, suggests that enhanced BDNF signaling mediates the pathophysiology of chronic NP. Therefore, these BDNF contributions to the processing of pain offer clues to the mechanisms of central sensitization, hyperalgesia and mechanical allodynia, and support the proposition that BDNF levels may serve as a biomarker for chronic pain.

3 Measurement of BDNF

BDNF can be quantified in peripheral whole blood, serum, or plasma, and is stored in the platelets. In addition, the brain is potentially a major contributor to circulating blood levels [93] since BDNF freely crosses the blood–brain barrier [83]. Thus, serum and plasma BDNF are highly correlated with central nervous system BDNF [59,61,83,88]. For example, in a study on rats, Karege et al. found a positive correlation ($r=0.81$, $P<0.01$) between serum and cortical BDNF concentrations [66]. Therefore, peripheral blood BDNF levels (serum or plasma) have been used as a proxy for central (brain) BDNF levels. However, several studies have demonstrated discrepant results between plasma and serum BDNF values within the same subjects (see Table 1a and Table 1b), while other studies have presented relatively high correlations between serum and plasma BDNF levels [40,59,90,114]. This highlights the challenge of assessing reliable BDNF concentrations in the periphery. Furthermore, more than 90% of blood BDNF is stored in the platelets [43] and released from the platelets to serum during the clotting process, explaining in part, the differences in serum and plasma BDNF levels (serum BDNF level is about 100-200 fold higher than that of plasma) [19,43]. Radka et al. also showed that there is a strong correlation between serum serotonin, a marker for platelet

activation, and serum concentration of BDNF, thus highlighting the release of BDNF during the clotting process described above. Previous studies have presented a broad range of correlations between plasma and serum BDNF concentrations ranging from r = 0.2 to r = 0.70 [15,63,110].

Moreover, circulating BDNF levels measured using conventional enzyme-linked immunosorbent assay (ELISA) kits, lack of standardization has likely contributed to the poor reproducibility of results. Polacchini et al., (2015) analyzed five different assays in healthy adults and found interassay variations of 5% to 20%. In addition, there were differences in the form of BDNF that the kits were measuring with some kits selectively recognizing mature BDNF, while the others reacted with both pro-BDNF and mature BDNF. Lastly, Bus et al. (2011) has demonstrated the ability of platelets to release BDNF and sequester BDNF from blood. This activity may result in differences between serum and plasma BDNF levels. Other considerations that can affect the measurement of BDNF in the plasma and serum include (1) gender [9,69], genetics [23,39,111] and age (2) the timing of measurement (accounting for diurnal variations) [10,54,89]; (3) psychological/psychiatric disorders [15,65,91,121]; (4) physical activity [28,48,100]; (5) duration of the sample storage period [20,79,115,118] and (6) role of platelet activation [11,57]. Each of these factors can negatively influence the consistency of results. Table 2 provides a summary of the studies that have examined factors that influence the measurement of serum and plasma BDNF.

Table 2: Factors affecting measurement of serum and plasma BDNF

Factor	Author	Study population	Serum BDNF Measurement	Plasma BDNF Measurement
Gender differences	Begliuomini et al., 2007	Fertile ovulatory women (n= 20), amenorrhoeic women (n= 15) and postmenopausal women (n= 25)	Not measured	Lower levels in amenorrhoeic and postmenopausal women compared to fertile ovulatory women
	Lommatzsch et al., 2005	140 healthy adults (72 men (n= 72), women (n= 68)	No gender differences when matched by weight	No gender differences when matched by weight
Age	Begliuomini et al., 2007	Fertile ovulatory women (n= 20), amenorrhoeic women (n= 15) and postmenopausal women (n= 25)	Not measured	Decrease with age
	Lommatzsch et al., 2005	140 healthy adults (72 men (n= 72), women (n= 68)	No difference in the two groups	Decrease with age
Diurnal Variations	Begliuomini et al., 2008	Healthy Males (n=34)	Not measured	Elevated BDNF levels in males in the morning with lowest levels at midnight.
	Piccinni et al., 2008	Healthy volunteers, men (n=14) and women (n=14)	No impact of diurnal variation in serum BDNF level in both men and women	Elevated BDNF levels in males at 8am with lowest levels at 10pm.
	Cain et al., 2017	Healthy volunteers, men (n=23) and women (n=16)	Not measured	Significant circadian rhythms in 12/16 women and 12/23 men
	Pluchino et al., 2009	fertile ovulatory women (n=10), women undergoing oral contraceptive therapy	Not measured	Diurnal variation in BDNF levels and changes with hormonal status.

		(n=10) and post-menopausal women(n=10)		
Medications	Polyakova et al., 2015	Systematic review and meta-analysis	Increase in BDNF levels post treatment with antidepressants	No difference
	Ventriglia et al., 2013	624 subjects (266 Patients with Alzheimer's Disease (n=266), Patients with frontotemporal dementia (n=28), Patients with Lewy body dementia(n=40),Patients with vascular dementia(n=91), Patients with Parkinsons disease (n=30), and controls(n=169)	Increased BDNF levels post treatment with mood stabilizers/antiepileptics and L-DOPA and decrease in levels post benzodiazepines	Not measured
	Leyhe et al., 2008	Patients with Alzheimer's disease (n=19) and age-matched healthy controls (n=20)	Post treatment with AChE-inhibitors there was increase in BDNF levels	Not measured
Storage conditions	Amadio et al., 2017	Healthy subjects including males (n=3) and females (n=3)	No plateau in levels after 60 min of clotting at room temperature, but a constant increase for 120 min.	Not measured
	Trajkovska et al., 2007b	206 healthy subjects (122 women, 84 men)	Storage at −20 ◦C led to decrease in BDNF levels up to 5 years	Not measured
	Tsuchimine et al., 2014	10 healthy volunteers	No change in BDNF levels over time	Increase in BDNF levels over time and changes with storage temperature

3.1 Factors affecting the measurement of serum and plasma BDNF

At present, more than 95% of the studies in the literature that have evaluated factors involved in the measurement of BDNF, analyze either serum BDNF and/or plasma BDNF. Below is a summary of the studies that have examined factors that influence the measurement of serum and plasma BDNF.

3.1.1 Role of Gender, Age and Genetics

Begliuomini et al. (2007) examined changes in plasma BDNF circulating levels in 60 women (20 fertile ovulatory women, 15 amenorrhoeic women and 25 postmenopausal women) and discovered that women with regular ovulatory cycles present with higher BDNF levels than amenorrhoeic or postmenopausal women ($p < 0.001$) [9]. Lommatzsch et al. (2005) also observed in their sample of 68 women, that platelet levels of BDNF were found to be higher in the second half of the menstrual cycle and in the postmenopausal period [69]. In the same study, an analysis of weight-matched groups found that women had significantly lower BDNF levels in platelets than men, but no difference was observed for plasma levels. Both studies also noted that plasma BDNF levels for postmenopausal women decreased significantly with increasing age (number of years following menopause). Similar results were observed for serum BDNF by Bus et al. (2011) who found an age-related elevation of serum BDNF in premenopausal women and an age-related decrease in postmenopausal women.

Fillingim et al. (2008) and Hempstead et al. (2015) have previously described the negative influence of BDNF polymorphisms especially Val66Met polymorphism on the BDNF/TrkB signaling pathways, with reduced TrkB activation causing impaired secretion of BDNF in patients with neuropsychiatric disorders. In a meta-analysis of 11 studies on healthy individuals that evaluated the relationship between the BDNF Val66Met variant and BDNF levels, Terracciano et al. (2013) concluded that there was no correlation between the BDNF Val66Met variant and serum, plasma and whole blood BDNF levels.

3.1.2. Influence of Diurnal Variations and Circadian Rhythms

In another study by the Begliuomini group (2008), males demonstrated elevated plasma BDNF concentrations in the morning, followed by a substantial decrease throughout the day with lowest values observed at midnight [10]. Piccini et al. (2008) examined plasma and serum BDNF levels at three different times during the day (08:00 h, 14:00 h, and 22:00 h), and similar to Begliuomini and colleagues, noted significant diurnal variation in plasma BDNF levels in men, with peak values in the morning for men and decreasing levels throughout the day with lowest values at 22:00 h. For women, no significant diurnal variations were observed in plasma BDNF levels. In addition, for serum BDNF, Piccinni and colleagues (2008) observed no changes across the three time points and there were no sex differences [87]. Pluchino et al. (2009) investigated the influence of circadian rhythm and hormonal status on plasma BDNF levels in fertile ovulatory women, women on oral contraceptive therapy, and post-menopausal women. He and colleagues detected significant differences in BDNF levels among the three groups. In fertile women, plasma BDNF levels were significantly higher during the luteal phase compared to the follicular phase, whereas for post-menopausal women, BDNF was significantly lower in the follicular phase [89]. Concerning circadian variations, in all the three groups, plasma BDNF levels decreased during the day. To summarize, in both men and women, plasma BDNF can vary greatly across the day. Thus, when assessing plasma BDNF, it may be beneficial to take multiple samples over a 24-hour period in consideration of diurnal variations in both men and women, and control for hormonal changes in women. Serum BDNF levels are likely resistant to the impact of diurnal, however, no study has evaluated the influence of circadian rhythms on serum BDNF levels.

3.1.3 Psychological/Psychiatric Disorders

Studies have demonstrated stress-induced alterations in BDNF levels with acute stress causing an increase in serum BDNF levels and chronic stress being associated with reduced

serum BDNF levels. [67,74] In two separate studies in healthy participants, examined serum BDNF levels utilizing an acute psychosocial stress paradigm, the Trier Social Stress Test and the results demonstrated an elevated serum BDNF response compared to baseline and a control group. No studies have examined changes in plasma BDNF.

In a meta-analysis of 57 studies in human subjects comparing serum and plasma BDNF levels in patients with major depressive disorder, bipolar disorder and healthy control subjects, Polyakova et al. (2015) reported that at baseline, serum and plasma BDNF levels were reduced in these patients with major depressive disorder and bipolar disorder compared to healthy controls. In the same article, Polyakova and colleague performed a second meta-analysis that included 553 patients with major depressive disorder who received treatment for 2-8 weeks. They concluded that serum BDNF levels were significantly higher in treatment responders and remitters compared to non-responders. Only seven studies reported plasma BDNF levels and no differences were observed in the treatment responders and non-responders [91].

In a study looking at changes in serum BDNF levels in patients with Alzheimer's disease before and after 15 months of treatment with acetylcholinesterase inhibitors, Leyhe et al. (2007), reported that serum BDNF levels were higher post treatment. In a subsequent study by Ventriglia et al. (2013), treatment with mood stabilizers/antiepileptics and L-DOPA, increased serum BDNF levels, whereas patients administered benzodiazepine demonstrated a decrease in serum BDNF. These results highlight the importance of controlling for the use of medications.

3.1.4 Physical Activity and Exercise Training

A majority of the studies evaluating the changes in BDNF levels post exercise have measured serum BDNF. These studies consistently demonstrate an increase in serum BDNF following an acute bout of exercise in healthy individuals. The fact that circulating BDNF is a good surrogate for changes in CNS plasticity and cognition has led some investigators to

propose this as a mechanism for explaining the relationship of physical activity and cognitive function. Several studies have demonstrated increases in both serum and plasma BDNF post exercise [37,93,100]. In an interesting study, Slusher et al. (2018) investigated the role of plasma and serum BDNF following high intensity interval training on executive function in healthy college aged males, and revealed a significant increase in serum BDNF concentrations post exercise but no difference in plasma BDNF [100]. Reycraft et al. (2020) only measured plasma BDNF levels after exercise at different intensities (including moderate-intensity continuous training at 65%VO_{2max}, vigorous-intensity continuous training at 85%VO_{2max}, and sprint interval training) and observed that plasma BDNF levels increased immediately after exercise for all the groups with the greatest increase seen in the sprint interval training group [94]. These increases in plasma BDNF levels were short-lived with plasma concentrations recovering 30 to 90 min post-exercise for all the groups. In a previous study, Gilder et al. (2014) demonstrated that serum BDNF levels recover more quickly than plasma BDNF levels (30 minutes vs 90 minutes) in individuals with high compared with low-fat free mass post completion of an incremental graded exercise test. This study suggests that the time required for BDNF recovery post exercise is dependent on the biofluid from which the BDNF was quantified, namely serum and plasma and body composition.

3.1.5 Storage Conditions

The time from blood sample collection to processing and the temperature at which the sample is stored can influence BDNF levels. During the coagulation process, activation of platelets causes a rapid release of BDNF from platelets into serum within the first hour at room temperature. This suggests that the length of clotting time constitutes a critical methodological issue when measuring the concentration of BDNF, in particular in serum. Therefore, it is important to evaluate pre-analysis conditions (e.g., preparation time and temperature) to ensure that BDNF analyses across studies assess similar physiological events. Gejl et al. (2019) noted

that BDNF levels measured in serum samples increased significantly with time during the first hour between collection and centrifugation, and subsequently became relatively stable [47]. Similar results were seen in a study by Tsuchimine et al. (2014), where BDNF measured in serum increased during the first hour of coagulation at 25 °C and were relatively stable with a clotting time between one and 48 hours. In contrast, a study by Amadio and colleagues (2017), demonstrated that serum BDNF samples incubated at 37 degrees °C, reached a plateau after 30 min, whereas 120 min were necessary to obtain similar BDNF levels at room temperature. Furthermore, Wessels and colleagues (2020) noted that the type of plasma separator tube, storage duration, and number of freeze–thaw cycles can impact the quantification of plasma BDNF concentration. In addition, plasma stored at − 80 °C compared to - 20 °C tends to have less variability in mean BDNF concentrations. More specifically, storing plasma BDNF for up to 6 months at either − 20 or − 80 °C was shown to have reproducible results [123]. In addition, Trajovska et al. (2007) demonstrated that serum BDNF levels were stable up to one year after being stored at -20 °C, but the levels significantly decreased after 5 years of storage and Bus et al. (2011) observed similar decreases after 3.5 years when it was stored at – 85 °C. Thus, a possible disadvantage of measuring BDNF in serum may be a decline in BDNF levels after long-term storage of serum, which may not occur for BDNF stored in plasma.

3.1.6 Impact of platelet activation on plasma BDNF levels

A possible confounder in the blood that can impact plasma levels of BDNF is clotting and platelet activation due to the storage of BDNF in platelets. Platelet activation or clotting can release large quantities of BDNF into the bloodstream, which causes the release of platelet factor 4 and the surface expression of P-selectin. Belanger et al. (2021) demonstrated that higher platelet activity measured using soluble P-selectin in plasma was associated with significantly higher plasma BDNF concentrations in individuals with and without coronary artery disease. In a study in patients with depression, Karege et al. 2005 investigated whether serum

BDNF levels are dependent on platelet reactivity and determined that serum BDNF is independent of platelet reactivity but plasma BDNF levels were accompanied by increase in platelet factor 4, a marker of platelet reactivity. Schneider et al. 1997 examined the role of coagulation factors on platelet activation by evaluating the binding of coagulation factors to the platelet surface and observed that, anticoagulants such as heparin, sodium citrate, and oxalate can influence platelet activation which can influence BDNF release. Another factor that can cause increase BDNF release from platelets is presence of agonists like thrombin, collagen, Ca^{2+} and shear stress [97]. It is critical to keep in mind that even with agonist stimulation, only 30-40% of BDNF in platelets is secreted and the other 70% that is present in cytoplasm is never released (Fujimura et al.2002). Lastly, comorbidities like depression and cardiac abnormalities can influence platelet activation, thus platelet reactivity, assessed by examining platelet factor 4 and or P-selectin, should be examined in these patients when quantifying BDNF. Galeano et al. (2015) demonstrated that when corrected for hemoconcentration, BDNF levels increased in the whole blood and in the serum 24 hours after exercise compared to baseline, but plasma levels did not significantly change at baseline and at 24 hours post exercise. Furthermore, correlation analyses revealed that serum BDNF levels were highly correlated to whole blood levels whereas plasma levels were not.

To summarize, a majority of studies have utilized measurement of serum BDNF as a marker of BDNF levels in healthy controls and in patients with neuropsychiatric conditions, in response to stress, following exercise, and the following the administration of medication. In addition, serum BDNF is more stable and reproducible than plasma BDNF, in particular when considering the impact of diurnal and circadian variations, psychotropic medications, and blood volume changes in response to exercise on plasma BDNF levels. Both serum and plasma levels are sensitive to changes with age, bodyweight, and menstrual cycle phase (hormonal influences in women). It is also important to consider that alterations in serum BDNF have been observed

with various clotting times and storage temperatures. Genetic associations linked to the Val66Met polymorphism have not been found to influence the measurement of serum and plasma BDNF.

Below is a list of factors to consider when deciding upon a valid and reliable research protocol.

1. Sex and gender differences (hormonal status for women)
2. Age (older individuals, especially women present with lower BDNF concentrations at baseline)
3. Diurnal variations and Circadian rhythms (report time of the day and consider collecting samples at different time points during the day)
4. Assess the use of medication(s)
5. The use of 1 hour for clotting time and store samples at -20 to -80 °C
6. If measuring in a clinical population with platelet impairment, measure platelet factor 4 and or P-selectin to evaluate platelet reactivity.

4 Conclusion

Concerning chronic pain, there is no conclusive evidence supporting the notion that changes in BDNF levels are causative or a consequence of chronic pain conditions, including NP and musculoskeletal pain in humans. In addition, the documented inconsistent results across studies between plasma BDNF and serum BDNF may be attributable to differences in the constitution of plasma and serum. More specifically, BDNF is largely stored in platelets and is released from activated platelets to the serum during the clotting process. This explains the lower concentration of BDNF in plasma compared to serum, and the timing of the changes in serum and plasma BDNF following activities that activate platelets. Based on current evidence, we would recommend assessing serum BDNF levels to quantify peripheral BDNF as they are more stable and sensitive to changes than plasma BDNF. Future studies should clarify serum and plasma responses to various stimuli, and define a standard protocol for the measurement of peripheral BDNF. In addition, large prospective studies are needed to address the

methodological confounds and generalizability for utilizing serum BDNF as a biomarker for chronic pain, and specifically for NP diagnosis and response to treatment.

References

[1] Alles SRA, Odem MA, Lu VB, Cassidy RM, Smith PA. Chronic BDNF simultaneously inhibits and unmasks superficial dorsal horn neuronal activity. Sci Rep 2021;11:1–14. doi:10.1038/s41598-021-81269-6.

[2] Amadio P, Sandrini L, Ieraci A, Tremoli E, Barbieri SS. Effect of clotting duration and temperature on BDNF measurement in human serum. Int J Mol Sci 2017;18.

[3] Andero R, Choi DC, Ressler KJ. BDNF–TrkB Receptor Regulation of Distributed Adult Neural Plasticity, Memory Formation, and Psychiatric Disorders. Prog Mol Biol Transl Sci 2014;122:169–192.

[4] Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. The specific disease burden of neuropathic pain: Results of a French nationwide survey. Pain 2011;152:2836–2843.

[5] Autry AE, Monteggia LM. Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 2012;64:238–258.

[6] Bannister K, Sachau J, Baron R, Dickenson AH. Neuropathic Pain: Mechanism-Based Therapeutics. https://doi.org/101146/annurev-pharmtox-010818-021524 2020;60:257–274.

[7] Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J 1982;1:549–553.

[8] Baumeister D, Eich W, Saft S, Geisel O, Hellweg R, Finn A, Svensson CI, Tesarz J. no evidence for altered plasma nGf and BDNF levels in fibromyalgia patients Methods. n.d. doi:10.1038/s41598-019-49403-7.

[9] Begliuomini S, Casarosa E, Pluchino N, Lenzi E, Centofanti M, Freschi L, Pieri M, Genazzani AD, Luisi S, Genazzani AR. Influence of endogenous and exogenous sex hormones on plasma brain-derived neurotrophic factor. Human Reproduction 2007;22:995–1002.

[10] Begliuomini S, Lenzi E, Ninni F, Casarosa E, Merlini S, Pluchino N, Valentino V, Luisi S, Luisi M, Genazzani AR. Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. Journal of Endocrinology 2008;197:429–435. doi:10.1677/JOE-07-0376.

[11] Bélanger J-C, Bouchard V, le Blanc J, Starnino L, Welman M, Chabot-Blanchet M, Busseuil D, Chertkow H, D'Antono B, Lordkipanidzé M. Brain-Derived Neurotrophic Factor Mitigates the Association Between Platelet Dysfunction and Cognitive Impairment. Front Cardiovasc Med 2021;0:1060.

[12] Biggs JE, Lu VB, Stebbing MJ, Balasubramanyan S, Smith PA. Is BDNF sufficient for information transfer between microglia and dorsal horn neurons during the onset of central sensitization? Mol Pain 2010;6:1–14.

[13] Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors 2004;22:123–131.

[14] Bliss TVP, Cooke SF. Long-term potentiation and long-term depression: A clinical perspective. Clinics 2011;66:3–17.

[15] Bocchio-Chiavetto L, Bagnardi V, Zanardini R, Molteni R, Gabriela Nielsen M, Placentino A, Giovannini C, Rillosi L, Ventriglia M, Riva MA, Gennarelli M. Serum and plasma BDNF levels in major depression: a replication study and meta-analyses. World J Biol Psychiatry 2010;11:763–773. doi:10.3109/15622971003611319.

[16] Bönhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D. Emerging biomarkers, tools, and treatments for diabetic polyneuropathy. 2018.

[17] Bouhassira D. Neuropathic pain: Definition, assessment and epidemiology. Rev Neurol (Paris) 2019;175:16–25.

[18] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain 2008;136:380–387.

[19] Brigadski T, Leßmann V. The physiology of regulated BDNF release. n.d. doi:10.1007/s00441-020-03253-2/Published.

[20] Bus BAA, Molendijk ML, Penninx BJWH, Buitelaar JK, Kenis G, Prickaerts J, Elzinga BM, Voshaar RCO. Determinants of serum brain-derived neurotrophic factor. Psychoneuroendocrinology 2011;36:228–239.

[21] Cain SW, Chang AM, Vlasac I, Tare A, Anderson C, Czeisler CA, Saxena R. Circadian rhythms in plasma brain-derived neurotrophic factor differ in men and women. J Biol Rhythms 2017;32:75–82.

[22] Cao T, Matyas JJ, Renn CL, Faden AI, Dorsey SG, Wu J. Function and Mechanisms of Truncated BDNF Receptor TrkB.T1 in Neuropathic Pain. Cells 2020;9.

[23] Cash RFH, Udupa K, Gunraj CA, Mazzella F, Daskalakis ZJ, Wong AHC, Kennedy JL, Chen R. Influence of BDNF Val66Met polymorphism on excitatory-inhibitory balance and plasticity in human motor cortex. Clinical Neurophysiology 2021;132:2827–2839.

[24] Caumo W, Deitos A, Carvalho S, Leite J, Carvalho F, Dussán-Sarria JA, Tarragó M da GL, Souza A, Torres IL da S, Fregni F. Motor cortex excitability and BDNF levels in chronic musculoskeletal pain according to structural pathology. Front Hum Neurosci 2016;10:1–15.

[25] Cavalli E, Mammana S, Nicoletti F, Bramanti P, Mazzon E. The neuropathic pain: An overview of the current treatment and future therapeutic approaches: https://doi.org/101177/2058738419838383 2019;33.

[26] Chao M v., Hempstead BL. p75 and Trk: A two-receptor system. Trends Neurosci 1995;18:321–326.

[27] Chen W, Walwyn W, Ennes HS, Kim H, McRoberts JA, Marvizón JCG. BDNF released during neuropathic pain potentiates NMDA receptors in primary afferent terminals. European Journal of Neuroscience 2014;39:1439–1454. doi:https://doi.org/10.1111/ejn.12516.

[28] Cho HC, Kim J, Kim S, Son YH, Lee N, Jung SH. The concentrations of serum, plasma and platelet BDNF are all increased by treadmill VO 2max performance in healthy college men. Neurosci Lett 2012;519:78–83. doi:10.1016/j.neulet.2012.05.025.

[29] Coghill RC, Eisenach J. Individual differences in pain sensitivity: implications for treatment decisions. Anesthesiology 2003;98:1312–1314.

[30] Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB, Eccleston C, Kalso E, Bennett DL, Dworkin RH, Raja SN. Neuropathic pain. Nat Rev Dis Primers 2017;3:1–19.

[31] Costigan M, Scholz J, Woolf CJ. Neuropathic pain: A maladaptive response of the nervous system to damage. Annu Rev Neurosci 2009;32:1–32.

[32] Coull JAM, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, de Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 2005;438:1017–1021.

[33] Dahlhamer JM, Lucas J, Zelaya C, Nahin R, Mackey S, Debar L, Kerns R, von Korff M, Porter L, Helmick C. Prevalence of chronic pain and high-impact chronic pain among adults — United States, 2016. Morbidity and Mortality Weekly Report 2018;67:1001–1006.

[34] Dai S, Ma Z. BDNF-trkB-KCC2-GABA pathway may be related to chronic stress-induced hyperalgesia at both the spinal and supraspinal level. Med Hypotheses 2014;83:772–774.

[35] Deitos A, Dussán-Sarria JA, de Souza A, Medeiros L, da Graça Tarragô M, Sehn F, Chassot M, Zanette S, Schwertner A, Fregni F, Torres ILS, Caumo W. Clinical value of serum neuroplasticity mediators in identifying the central sensitivity syndrome in patients with chronic pain with and without structural pathology. Clinical Journal of Pain 2015;31:959–967.

[36] Ding X, Cai J, Li S, Liu XD, Wan Y, Xing GG. BDNF contributes to the development of neuropathic pain by induction of spinal long-term potentiation via SHP2 associated GluN2B-containing NMDA receptors activation in rats with spinal nerve ligation. Neurobiol Dis 2015;73:428–451. doi:10.1016/j.nbd.2014.10.025.

[37] Dinoff A, Herrmann N, Swardfager W, Lanctôt KL. The effect of acute exercise on blood concentrations of brain-derived neurotrophic factor in healthy adults: a meta-analysis. European Journal of Neuroscience 2017;46:1635–1646. doi:10.1111/EJN.13603.

[38] Doth AH, Hansson PT, Jensen MP, Taylor RS. The burden of neuropathic pain: A systematic review and meta-analysis of health utilities. Pain 2010;149:338–344.

[39] Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met Polymorphism Affects Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function and its val/met polymorphism in human memory and hippocampal function and suggest val/met exerts these effects by impacting intracellular trafficking and activity-dependent secretion of BDNF. 2003.

[40] Elfving B, Plougmann PH, Wegener G. Detection of brain-derived neurotrophic factor (BDNF) in rat blood and brain preparations using ELISA: Pitfalls and solutions. J Neurosci Methods 2010;187:73–77. doi:10.1016/j.jneumeth.2009.12.017.

[41] FDA-NIH Biomarker Working Group. BEST (Biomarkers , EndpointS , and other Tools) Resource. Silver Spring: Food and Drug Administration (US)/ Co-published by National Institutes of Health (US), Bethesda (MD)., 2016 Available: https://www.ncbi.nlm.nih.gov/books/NBK326791/.

[42] Finnerup NB, Kuner R, Jensen TS. Neuropathic Pain: From Mechanisms to Treatment. https://doi.org/101152/physrev000452019 2020;101:259–301.

[43] Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi JI, Sun B, Tandon NN. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb Haemost 2002;87:728–734.

[44] Gálvez R, Marsal C, Vidal J, Ruiz M, Rejas J. Cross-sectional evaluation of patient functioning and health-related quality of life in patients with neuropathic pain under standard care conditions. European Journal of Pain 2007;11:244–255.

[45] Gao J, Wang H, Liu Y, Li Y, Chen C, Liu L, Wu Y, Li S, Yang C. Glutamate and GABA imbalance promotes neuronal apoptosis in hippocampus after stress. Medical Science Monitor 2014;20:499–512.

[46] Gasparin A, Zortea M, dos Santos VS, Carvalho F, Torres ILS, de Souza A, Fregni F, Caumo W. Brain-Derived Neurotrophic Factor Modulates the Effect of Sex on the Descending Pain Modulatory System in Healthy Volunteers. Pain Medicine 2020;0:1–9.

[47] Gejl AK, Enevold C, Bugge A, Andersen MS, Nielsen CH, Andersen LB. Associations between serum and plasma brain-derived neurotrophic factor and influence of storage time and centrifugation strategy. Scientific Reports 2019 9:1 2019;9:1–9. doi:10.1038/s41598-019-45976-5.

[48] Gomes WF, Lacerda ACR, Mendonça VA, Arrieiro AN, Fonseca SF, Amorim MR, Teixeira AL, Teixeira MM, Miranda AS, Coimbra CC, Brito-Melo GEA. Effect of exercise on the plasma BDNF levels in elderly women with knee osteoarthritis. Rheumatol Int 2014;34:841–846.

[49] Gunn J, Hill MM, Cotten BM, Deer TR. Observational Study An Analysis of Biomarkers in Patients with Chronic Pain. Pain Physician 2020;23:E41–E49. Available: www.painphysicianjournal.com.

[50] Haas L, Portela LVC, Böhmer AE, Oses JP, Lara DR. Increased plasma levels of brain derived neurotrophic factor (BDNF) in patients with fibromyalgia. Neurochem Res 2010;35:830–834.

[51] Hanoglu L, Velioglu HA, Hanoglu T, Yulug B. Neuroimaging-Guided Transcranial Magnetic and Direct Current Stimulation in MCI: Toward an Individual, Effective and Disease-Modifying Treatment. Clin EEG Neurosci n.d.;0:15500594211052816. doi:10.1177/15500594211052815.

[52] van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: A systematic review of epidemiological studies. Pain 2014;155:654–662. doi:10.1016/J.PAIN.2013.11.013.

[53] Ishikawa T, Yasuda S, Minoda S, Ibuki T, Fukuhara K, Iwanaga Y, Ariyoshi T, Sasaki H. Neurotropin® Ameliorates Chronic Pain via Induction of Brain-Derived Neurotrophic Factor. Cell Mol Neurobiol 2015;35:231–241.

[54] Jasim H, Ghafouri B, Carlsson A, Hedenberg-Magnusson B, Ernberg M. Daytime changes of salivary biomarkers involved in pain. J Oral Rehabil 2020;47:843–850.

[55] Jasim H, Ghafouri B, Gerdle B, Hedenberg-Magnusson B, Ernberg M. Altered levels of salivary and plasma pain related markers in temporomandibular disorders. n.d. doi:10.1186/s10194-020-01160-z.

[56] Jensen MP, Chodroff MJ, Dworkin RH. The impact of neuropathic pain on health-related quality of life: Review and implications. Neurology 2007;68:1178–1182.

[57] Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G. Low Brain-Derived Neurotrophic Factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol Psychiatry 2005;57:1068–1072.

[58] Kerr BJ, Bradbury EJ, Bennett DLH, Trivedi PM, Dassan P, French J, Shelton DB, McMahon SB, Thompson SWN. Brain-Derived Neurotrophic Factor Modulates Nociceptive Sensory Inputs and NMDA-Evoked Responses in the Rat Spinal Cord. The Journal of Neuroscience 1999;19:5138. doi:10.1523/JNEUROSCI.19-12-05138.1999.

[59] Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, Knudsen GM, Aznar S. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. International Journal of Neuropsychopharmacology 2011;14:347–353.

[60] Kohno K, Tsuda M. Role of microglia and P2X4 receptors in chronic pain. Pain Rep 2021;6:e864. doi:10.1097/PR9.0000000000000864.

[61] Lang UE, Hellweg R, Seifert F, Schubert F, Gallinat J. Correlation Between Serum Brain-Derived Neurotrophic Factor Level and An In Vivo Marker of Cortical Integrity. Biol Psychiatry 2007;62:530–535.

[62] Latremoliere A, Woolf CJ. Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. J Pain 2009;10:895–926.

[63] Lee BH, Kim YK. Reduced platelet BDNF level in patients with major depression. Prog Neuropsychopharmacol Biol Psychiatry 2009;33:849–853.

[64] Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde Y-A. Molecular cloning and expression of brain-derived neurotrophic factor. Nature 1989 341:6238 1989;341:149–152.

[65] Leyhe T, Stransky E, Eschweiler GW, Buchkremer G, Laske C. Increase of BDNF serum concentration during donepezil treatment of patients with early Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci 2008;258:124–128. doi:10.1007/s00406-007-0764-9.

[66] Licien Karege F, le Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. n.d. Available: www.elsevier.com/locate/neulet.

[67] Linz R, Puhlmann LMC, Apostolakou F, Mantzou E, Papassotiriou I, Chrousos GP, Engert V, Singer T. Acute psychosocial stress increases serum BDNF levels: an antagonistic relation to cortisol but no group differences after mental training. Neuropsychopharmacology 2019;44:1797–1804.

[68] Loeser JD, Treede R-D. The Kyoto protocol of IASP Basic Pain Terminology. Pain 2008;137:473–477.

[69] Lommatzsch M, Zingler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-Werner P, Virchow JC. The impact of age, weight and gender on BDNF levels in human platelets and plasma. Neurobiol Aging 2005;26:115–123.

[70] Lu VB, Ballanyi K, Colmers WF, Smith PA. Neuron type-specific effects of brain-derived neurotrophic factor in rat superficial dorsal horn and their relevance to "central sensitization." Journal of Physiology 2007;584:543–563.

[71] Lu VB, Colmers WF, Smith PA. Long-term effects of brain-derived neurotrophic factor on the frequency of inhibitory synaptic events in the rat superficial dorsal horn. Neuroscience 2009;161:1135–1143.

[72] Maher DP, Wong CH, Siah KW, Lo AW. Estimates of Probabilities of Successful Development of Pain Medications: An Analysis of Pharmaceutical Clinical Development Programs 2000-2020. Anesthesiology 2022. doi:10.1097/ALN.0000000000004265.

[73] Malcangio M. GABAB receptors and pain. Neuropharmacology 2018;136:102–105.

[74] Meng D, Wu TC, Rao U, North CS, Xiao H, Javors MA, Adinoff B. Serum NPY and BNDF response to a behavioral stressor in alcohol-dependent and healthy control participants. Psychopharmacology (Berl) 2011;218:59–67.

[75] Merighi A, Salio C, Ghirri A, Lossi L, Ferrini F, Betelli C, Bardoni R. BDNF as a pain modulator. Prog Neurobiol 2008;85:297–317.

[76] Miesen MM van der, Lindquist MA, Wager TD. Neuroimaging-based biomarkers for pain: state of the field and current directions. Pain Rep 2019;4. doi:10.1097/PR9.0000000000000751.

[77] Mitsi V, Zachariou V. Modulation of pain, nociception, and analgesia by the brain reward center. Neuroscience 2016;338:81–92.

[78] Mulvey MR, Bennett MI, Liwowsky I, Freynhagen R. The role of screening tools in diagnosing neuropathic pain. Pain Manag 2014;4:233–243.

[79] Naegelin Y, Dingsdale H, Säuberli K, Schädelin S, Kappos L, Barde Y-A. Measuring and Validating the Levels of Brain-Derived Neurotrophic Factor in Human Serum. eNeuro 2018;5.

[80] Navratilova E, Xie JY, Okun A, Qu C, Eyde N, Ci S, Ossipov MH, King T, Fields HL, Porreca F. Pain relief produces negative reinforcement through activation of mesolimbic reward–valuation circuitry. Proceedings of the National Academy of Sciences 2012;109:20709–20713.

[81] Ohira K, Hayashi M. A New Aspect of the TrkB Signaling Pathway in Neural Plasticity. Curr Neuropharmacol 2009;7:276–285.

[82] Panja D, Bramham CR. BDNF mechanisms in late LTP formation: A synthesis and breakdown. Neuropharmacology 2014;76:664–676.

[83] Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. Transport of brain-derived neurotrophic factor across the blood–brain barrier. Neuropharmacology 1998;37:1553–1561.

[84] Park H, Poo M. Neurotrophin regulation of neural circuit development and function. Nature Reviews Neuroscience 2013 14:1 2012;14:7–23. doi:10.1038/nrn3379.

[85] Pezet S, Malcangio M, Mcmahon SB. BDNF: a neuromodulator in nociceptive pathways? 2002 Available: www.elsevier.com/locate/brainresrev.

[86] Pezet S, McMahon SB. Neurotrophins: Mediators and modulators of pain. Annu Rev Neurosci 2006;29:507–538.

[87] Piccinni A, Marazziti D, del Debbio A, Bianchi C, Roncaglia I, Mannari C, Origlia N, Catena Dell'Osso M, Massimetti G, Domenici L, Dell'Osso L. Diurnal variation of plasma brain-derived neurotrophic factor (BDNF) in humans: An analysis of sex differences. Chronobiol Int 2008;25:819–826.

[88] Pillai A, Kale A, Joshi S, Naphade N, Raju MSVK, Nasrallah H, Mahadik SP. Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology. International Journal of Neuropsychopharmacology 2010;13:535–539.

[89] Pluchino N, Cubeddu A, Begliuomini S, Merlini S, Giannini A, Bucci F, Casarosa E, Luisi M, Cela V, Genazzani AR. Daily variation of brain-derived neurotrophic factor and cortisol in women with normal menstrual cycles, undergoing oral contraception and in postmenopause. Human Reproduction 2009;24:2303–2309. doi:10.1093/HUMREP/DEP119.

[90] Polacchini A, Metelli G, Francavilla R, Baj G, Florean M, Mascaretti LG, Tongiorgi E. A method for reproducible measurements of serum BDNF: Comparison of the performance of six commercial assays. Sci Rep 2015;5:1–10.

[91] Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML. BDNF as a biomarker for successful treatment of mood disorders: A systematic & quantitative meta-analysis. J Affect Disord 2015;174:432–440.

[92] Price TJ, Gold MS. From mechanism to cure: Renewing the goal to eliminate the disease of pain. Pain Medicine (United States) 2018;19:1525–1549.

[93] Rasmussen P, Brassard P, Adser H, Pedersen M v., Leick L, Hart E, Secher NH, Pedersen BK, Pilegaard H. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. Exp Physiol 2009;94:1062–1069.

[94] Reycraft JT, Islam H, Townsend LK, Hayward GC, Hazell TOMJ, MacPherson REK. Exercise Intensity and Recovery on Circulating Brain-derived Neurotrophic Factor. Med Sci Sports Exerc 2020;52:1210–1217. doi:10.1249/MSS.0000000000002242.

[95] Rocha AL, Vieira EL, Ferreira MC, Maia LM, Teixeira AL, Reis FM. Plasma brain-derived neurotrophic factor in women with pelvic pain: a potential biomarker for endometriosis? Biomark Med 2017;11:313–317. doi:10.2217/bmm-2016-0327.

[96] Sandkühler J. Models and Mechanisms of Hyperalgesia and Allodynia. https://doi.org/101152/physrev000252008 2009;89:707–758. doi:10.1152/PHYSREV.00025.2008.

[97] Serra-Millàs M. Are the changes in the peripheral brain-derived neurotrophic factor levels due to platelet activation? World J Psychiatry 2016;6:84.

[98] Sikandar S, Minett MS, Millet Q, Santana-Varela S, Lau J, Wood JN, Zhao J. Brain-derived neurotrophic factor derived from sensory neurons plays a critical role in chronic pain. Brain 2018;141:1028–1039.

[99] Sisignano M, Lötsch J, Parnham MJ, Geisslinger G. Potential biomarkers for persistent and neuropathic pain therapy. Pharmacol Ther 2019;199:16–29.

[100] Slusher AL, Patterson VT, Schwartz CS, Acevedo EO. Impact of high intensity interval exercise on executive function and brain derived neurotrophic factor in healthy college aged males. Physiol Behav 2018;191:116–122.

[101] Smith BH, Torrance N, Bennett MI, Lee AJ. Health and quality of life associated with chronic pain of predominantly neuropathic origin in the community. Clinical Journal of Pain 2007;23:143–149.

[102] Smith PA. BDNF: No gain without pain? Neuroscience 2014;283:107–123. doi:10.1016/j.neuroscience.2014.05.044.

[103] Smith SM, Amtmann D, Askew RL, Gewandter JS, Hunsinger M, Jensen MP, Mcdermott MP, Patel K V., Williams M, Bacci ED, Burke LB, Chambers CT, Cooper SA, Cowan P, Desjardins P, Etropolski M, Farrar JT, Gilron I, Huang IZ, Katz M, Kerns RD, Kopecky EA, Rappaport BA, Resnick M, Strand V, Vanhove GF, Veasley C, Versavel M, Wasan AD, Turk DC, Dworkin RH. Pain intensity rating training: Results from an exploratory study of the ACTTION PROTECCT system. Pain 2016;157:1056–1064.

[104] Smith SM, Dworkin RH, Turk DC, Baron R, Polydefkis M, Tracey I, Borsook D, Edwards RR, Harris RE, Wager TD, Arendt-Nielsen L, Burke LB, Carr DB, Chappell A, Farrar JT, Freeman R, Gilron I, Goli V, Haeussler J, Jensen T, Katz NP, Kent J, Kopecky EA, Lee DA, Maixner W, Markman JD, McArthur JC, McDermott MP, Parvathenani L, Raja SN, Rappaport BA, Rice ASC, Rowbotham MC, Tobias JK, Wasan AD, Witter J. The Potential Role of Sensory Testing, Skin Biopsy, and Functional Brain Imaging as Biomarkers in Chronic Pain Clinical Trials: IMMPACT Considerations. Journal of Pain 2017;18:757–777. doi:10.1016/j.jpain.2017.02.429.

[105] Soril LJJ, Ramer LM, McPhail LT, Kaan TKY, Ramer MS. Spinal brain-derived neurotrophic factor governs neuroplasticity and recovery from cold-hypersensitivity following dorsal rhizotomy. Pain 2008;138:98–110.

[106] Stefani LC, Leite FM, da Graça L. Tarragó M, Zanette SA, de Souza A, Castro SM, Caumo W. BDNF and serum S100B levels according the spectrum of structural pathology in chronic pain patients. Neurosci Lett 2019;706:105–109. doi:https://doi.org/10.1016/j.neulet.2019.05.021.

[107] Szok D, Tajti J, Nyári A, Vécsei L. Therapeutic Approaches for Peripheral and Central Neuropathic Pain. Behavioural Neurology 2019;2019.

[108] Taneja A, Della Pasqua O, Danhof M. Challenges in translational drug research in neuropathic and inflammatory pain: the prerequisites for a new paradigm. Eur J Clin Pharmacol 2017;73:1219–1236.

[109] Tateiwa H, Kawano T, Nishigaki A, Yamanaka D, Aoyama B, Shigematsu-Locatelli M, Eguchi S, Locatelli FM, Yokoyama M. The role of hippocampal brain-derived neurotrophic factor in age-related differences in neuropathic pain behavior in rats. Life Sci 2018;197:56–66.

[110] Terracciano A, Lobina M, Piras MG, Mulas A, Cannas A, Meirelles O, Sutin AR, Zonderman AB, Uda M, Crisponi L, Schlessinger D. Neuroticism, depressive symptoms, and serum BDNF. Psychosom Med 2011;73:638–642. doi:10.1097/PSY.0B013E3182306A4F.

[111] Terracciano A, Piras MG, Lobina M, Mulas A, Meirelles O, Sutin AR, Chan W, Sanna S, Uda M, Crisponi L, Schlessinger D. Genetics of serum BDNF: Meta-analysis of the Val66Met and genome-wide association study. World Journal of Biological Psychiatry 2013;14:583–589.

[112] Thibault K, Lin WK, Rancillac A, Fan M, Snollaerts T, Sordoillet V, Hamon M, Smith GM, Lenkei Z, Pezet S. Development/Plasticity/Repair BDNF-Dependent Plasticity Induced by Peripheral Inflammation in the Primary Sensory and the Cingulate Cortex Triggers Cold Allodynia and Reveals a Major Role for Endogenous BDNF As a Tuner of the Affective Aspect of Pain. 2014.

[113] Torrance N, Smith BH, Bennett MI, Lee AJ. The Epidemiology of Chronic Pain of Predominantly Neuropathic Origin. Results From a General Population Survey. Journal of Pain 2006;7:281–289.

[114] Trajkovska V, Marcussen AB, Vinberg M, Hartvig P, Aznar S, Knudsen GM. Measurements of brain-derived neurotrophic factor: Methodological aspects and demographical data. Brain Res Bull 2007;73:143–149.

[115] Trajkovska V, Marcussen AB, Vinberg M, Hartvig P, Aznar S, Knudsen GM. Measurements of brain-derived neurotrophic factor: Methodological aspects and demographical data. Brain Res Bull 2007;73:143–149.

[116] Trang T, Beggs S, Salter MW. Brain-derived neurotrophic factor from microglia: A molecular substrate for neuropathic pain. Neuron Glia Biol 2012;7:99–108.

[117] Trang T, Beggs S, Salter MW. Brain-derived neurotrophic factor from microglia: a molecular substrate for neuropathic pain. Neuron Glia Biol 2011;7:99–108.

[118] Tsuchimine S, Sugawara N, Ishioka M, Yasui-Furukori N. Preanalysis storage conditions influence the measurement of brain-derived neurotrophic factor levels in peripheral blood. Neuropsychobiology 2014;69:83–88.

[119] Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. From Acquisition to Consolidation: On the Role of Brain-Derived Neurotrophic Factor Signaling in Hippocampal-Dependent Learning. Learning & Memory 2002;9:224–237.

[120] Vanelderen P, Rouwette T, Kozicz T, Roubos E, Zundert J Van, Heylen R, Vissers K. The role of brain-derived neurotrophic factor in different animal models of neuropathic pain. European Journal of Pain 2010;14:473.e1-473.e9. doi:10.1016/j.ejpain.2009.09.006.

[121] Ventriglia M, Zanardini R, Bonomini C, Zanetti O, Volpe D, Pasqualetti P, Gennarelli M, Bocchio-Chiavetto L. Serum Brain-Derived Neurotrophic Factor Levels in Different Neurological Diseases. Biomed Res Int 2013;2013. doi:10.1155/2013/901082.

[122] Wang X, Ratnam J, Zou B, England PM, Basbaum AI. TrkB signaling is required for both the induction and maintenance of tissue and nerve injury-induced persistent pain. Journal of Neuroscience 2009;29:5508–5515.

[123] Wessels JM, Agarwal RK, Somani A, Verschoor CP, Agarwal SK, Foster WG. Factors affecting stability of plasma brain-derived neurotrophic factor. Scientific Reports 2020 10:1 2020;10:1–13. doi:10.1038/s41598-020-77046-6.

[124] Woolf CJ. Central sensitization: uncovering the relation between pain and plasticity. Anesthesiology 2007;106:864.

[125] Woolf CJ, Thompson SWN. The induction and maintenance of central sensitization is dependent on N-methyl-d-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. Pain 1991;44:293–299.

[126] Wu Y, Shen Z, Xu H, Zhang K, Guo M, Wang F, Li J. BDNF Participates in Chronic Constriction Injury-Induced Neuropathic Pain via Transcriptionally Activating P2X7 in Primary Sensory Neurons. Mol Neurobiol 2021;58:4226–4236.

[127] Zafra F, Castrén E, Thoenen H, Lindholm D. Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. Proceedings of the National Academy of Sciences 1991;88:10037–10041. doi:10.1073/PNAS.88.22.10037.

[128] Zhang H, Qian YL, Li C, Liu D, Wang L, Wang XY, Liu MJ, Liu H, Zhang S, Guo XY, Yang JX, Ding HL, Koo JW, Mouzon E, Deisseroth K, Nestler EJ, Zachariou V, Han MH, Cao JL. Brain-Derived Neurotrophic Factor in the Mesolimbic Reward Circuitry Mediates Nociception in Chronic Neuropathic Pain. Biol Psychiatry 2017;82:608–618. doi:10.1016/j.biopsych.2017.02.1180.

[129] Zhang X, Wang J, Zhou Q, Xu Y, Pu S, Wu J, Xue Y, Tian Y, Lu J, Jiang W, Du D. Brain-derived neurotrophic factor-activated astrocytes produce mechanical allodynia in neuropathic pain. Neuroscience 2011;199:452–460.

[130] Zhang Z, Wang X, Wang W, Lu YG, Pan ZZ. Brain-derived neurotrophic factor-mediated downregulation of brainstem K+-Cl- cotransporter and cell-type-specific GABA impairment for activation of descending pain facilitation. Mol Pharmacol 2013;84:511–520.

[131] Zhao H, Alam A, San C, Eguchi S, Chen Q, Lian Q, Ma D. Molecular mechanisms of brain-derived neurotrophic factor in neuro-protection: Recent developments. Brain Res 2017;1665:1–21.

[132] Zhao J, Seereeram A, Nassar MA, Levato A, Pezet S, Hathaway G, Morenilla-Palao C, Stirling C, Fitzgerald M, McMahon SB, Rios M, Wood JN. Nociceptor-derived brain-derived neurotrophic factor regulates acute and inflammatory but not neuropathic pain. Molecular and Cellular Neuroscience 2006;31:539–548.

[133] Zhou LJ, Yang T, Wei X, Liu Y, Xin WJ, Chen Y, Pang RP, Zang Y, Li YY, Liu XG. Brain-derived neurotrophic factor contributes to spinal long-term potentiation and mechanical hypersensitivity by activation of spinal microglia in rat. Brain Behav Immun 2011;25:322–334.

[134] Zhou W, Xie Z, Li C, Xing Z, Xie S, Li M, Yao J. Driving effect of BDNF in the spinal dorsal horn on neuropathic pain. Neurosci Lett 2021;756.

Figure 1: List of studies that support the role of BDNF in NP with the associated site for the mechanism of action. Created with BioRender.com

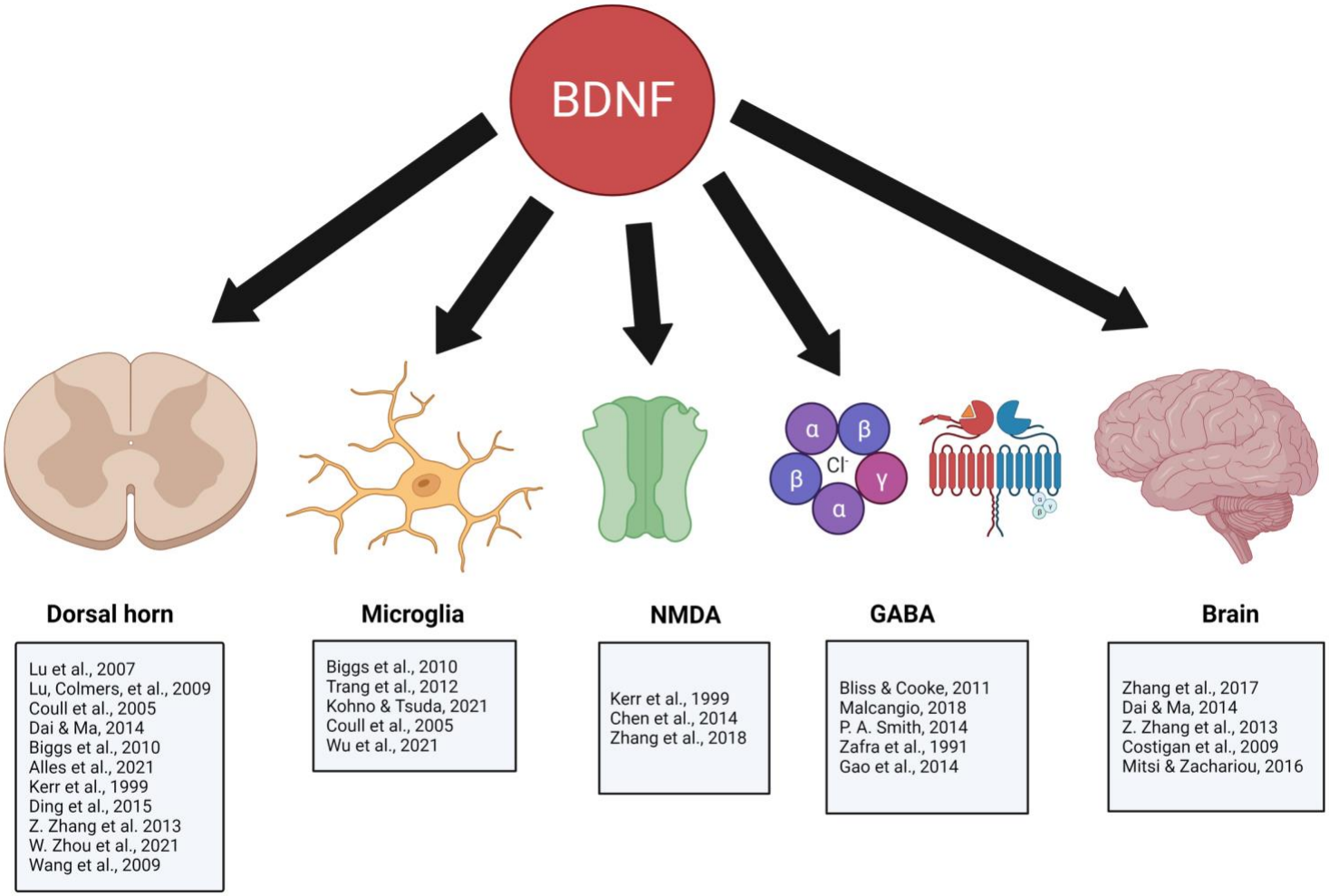


Figure 1: List of studies that support the role of BDNF in NP with the associated site for the mechanism of action. Created with BioRender.com

CHAPTER 3

Short Term Effects of Prolonged Continuous Theta Burst Stimulation Targeting M1 and DLPFC in Patients with Painful Diabetic Neuropathy.

To be submitted to the PAIN Journal

Bhushan Thakkar, Carrie L. Peterson and Edmund Acevedo

Short Term Effects of Prolonged Continuous Theta Burst Stimulation Targeting M1 and DLPFC in Patients with Painful Diabetic Neuropathy

Bhushan Thakkar [1] Carrie L. Peterson [2] and Edmund O. Acevedo [3]

1 Department of Physical Therapy, Virginia Commonwealth University, Richmond, VA

2 Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA

3 Department of Kinesiology and Health Sciences, Virginia Commonwealth University,

Richmond, VA

Pages: 38

Tables: 1

Figures: 6

Corresponding Author:

Bhushan Thakkar

1101 East Marshall Street, 11th Floor, Room 11-027

Box #980034,

Richmond, VA 23298

Email: thakkarbs2@vcu.edu

Phone: 804-300-5578

Funding: This study was partially supported by a Grant in Aid of Research from Sigma Xi, The Scientific Research Honor Society. The project [REDCap and Research Datasets] was supported by CTSA award No. UL1TR002649 from the National Center for Advancing Translational Sciences. Its contents are solely the responsibility of the authors and do not necessarily represent official views of the National Center for Advancing Translational Sciences or the National Institutes of Health.

Acknowledgements: All the authors declare no conflicts of interest.

To be submitted to the PAIN Journal as a Research Report

The manuscript must contain an Abstract (unstructured, 250 words), Introduction (500 words), Methods (no word limit), Results (no word limit), Discussion (1,500 words), Acknowledgments, and References.

Abstract

Painful Diabetic Neuropathy (pDN) is the most common cause of neuropathic pain (NP) in the United States. Prolonged continuous theta burst stimulation (pcTBS), a newer form of repetitive transcranial magnetic stimulation (rTMS), takes less time (1-4 minutes) and is more tolerable for most individuals than rTMS, but similar to rTMS can modulate pain thresholds in healthy participants. However, its effects on patients with chronic pain are still unclear. The primary purpose of this paper is to investigate the effects of pcTBS targeted at the M1 and DLPFC brain regions on a set of self-report measures of pain perception (SRMP) that assess the (a) sensory-discriminative, (b) affective-motivational and (c) cognitive-evaluative components of the pain experience. For this prospective, single-blind, sham-controlled study, forty-two participants with pDN were randomized to receive either pcTBS targeting the M1 or the DLPFC brain regions. SRMP were completed at baseline, post pcTBS and 24h-post pcTBS. Statistically significant improvements from baseline to post pcTBS and baseline to 24h-post pcTBS for different subscales within each SRMP demonstrate the feasibility of pcTBS targeted at M1 or DLPFC to be utilized as a clinical tool to alleviate pDN. Headache at 24h-post pcTBS, was the most common side effect in 18% (n=8) of patients followed by neck pain in 11% (n=5) of patients in this study. Future studies should consider utilizing multiple sessions of pcTBS to evaluate its long-term effects on pain perception, safety and tolerability in patients with chronic pain. (ClinicalTrials.gov identifier: NCT04988321)

1.0 Introduction

In 2019, approximately 50.2 million adults (20.4% of the US population) experienced chronic pain (CP), contributing to $560 to $635 billion in direct medical costs [94,95], lost productivity [47,48,68], and disability [39,47,68]. For those reporting CP, approximately 20% are thought to have neuropathic pain (NP) [11,12]. Diabetic neuropathy, a type of nerve damage that can occur with diabetes, can lead to CP [26,75,87]. One in five patients with diabetic neuropathy develop painful diabetic neuropathy (pDN), the most common cause of NP in the United States [41,75]. pDN manifests with a 'stocking and glove' distribution, whereby the hands and lower limbs are commonly affected. In addition, it has debilitating consequences with a major impact on morbidity and quality of life [32,75,87]. There are no medications that target the pathophysiology of pDN in an attempt to reverse the course of this neuropathy. Therefore, symptomatic treatment is the mainstay of management for pDN with only three US Food and Drug Administration (FDA) approved therapies; pregabalin, duloxetine, and tapentadol [2,75]. Furthermore, the estimated increase in the prevalence of diabetes mellitus to 629 million cases by 2045 [91] and the associated increase of pDN in the US and across the globe, highlight the urgent need to develop new therapeutic approaches.

Repetitive transcranial magnetic stimulation (rTMS), a noninvasive form of brain stimulation, has been utilized as a therapeutic tool in patients with depression, migraine, and neuropsychiatric disorders such as schizophrenia and obsessive-compulsive disorder [22,52,54,58]. rTMS has also been investigated as a form of treatment for NP [51,52,65]. rTMS involves the application of TMS pulses using an electromagnetic coil applied to the scalp. A magnetic field is directed to a specific region(s) of the brain with different patterns and frequencies that modulate brain activity to produce immediate and long-term effects through changes in neuroplasticity [5,35,50]. Previous studies have consistently reported its feasibility and safety in the treatment of neuropsychiatric disorders with only a few contraindications

[71,78]. With regards to CP and specifically NP, high frequency repetitive TMS (HF-rTMS) via stimulation of the primary motor cortex (M1) has consistently demonstrated analgesic effects [31,52,72]. Another cortical target for NP that has been studied using rTMS is the Dorsolateral Prefrontal Cortex (DLPFC) [18,85,86]. This is also the primary target site for alleviating depression [9,28,70]. In patients with NP, activation of DLPFC has been linked to pain perception, in particular through the modulation of the cognitive and emotional aspects of pain processing [18,74,80].

The analgesic, cognitive and emotional aspects of pain perception can be characterized using the biopsychosocial model of pain [27,30,89]. This model regards chronic pain as a multidimensional experience with sensory-discriminative (location, quality and intensity), affective-motivational (unpleasantness) and cognitive-evaluative components (beliefs, attitudes, intention) [4,27,30]. These components can be measured using self-report measures of pain (SRMP) that are valid and reliable tools, albeit subjective, to evaluate the pain experience and are considered the gold standard to assess chronic pain outcomes in clinical trials [25,27,82].

Conventional HF-rTMS requires 20 to 30 min of stimulation time to achieve its full effect, which can make experimental and clinical applications logistically challenging. A recently developed rTMS paradigm, theta burst stimulation (TBS) [43], is more time efficient and can reduce patient discomfort. It requires significantly less stimulation time (1-4 minutes) and lower stimulation intensities (bursts of three pulses at 30 Hz or 50 Hz, repeated five times per second with 600 pulses in total) [19,43,83]. TBS can either depress (when applied as continuous TBS; cTBS) or increase (when applied as intermittent TBS) cortical excitability; the strength of the response of cortical neurons to a given stimulation [43]. In addition, a prolonged form of cTBS (pcTBS) with twice the number of stimuli (1,200 pulses) produces a facilitatory increase similar to that of intermittent TBS and HF-rTMS [19,43,83].

The effects of TBS, in particular pcTBS on pain modulation, have been investigated only in healthy subjects. Moisset et al. [66], Li et al. [56] and Klirova et al. [49] evaluated the analgesic effects of pcTBS targeted at M1, and De Martino et al. [61] examined the effects of pcTBS targeted at the DLPFC region. Two of these studies [56,61] found a similar increase in pain threshold for pcTBS compared to HF-rTMS after one session of pcTBS. De Martino et al. observed a greater increase in pain thresholds after three sessions of pcTBS [66] compared to rTMS, and demonstrated that this greater increase continued up to 24 hours post stimulation.

There are no studies that have examined the analgesic effects of pcTBS targeted at the M1 and DLPFC brain regions in patients with NP, in particular pDN patients. Therefore, the purpose of this study is to examine the effectiveness of pcTBS at the M1 and DLPFC regions of the brain to alleviate pain in patients with pDN. The present study hypothesized that, compared to baseline, a single session of pcTBS targeted at the M1 brain region would lead to improvement in scores on SRMP that evaluate the sensory-discriminative and affective-motivational components of pain. In addition, pcTBS targeted at the DLPFC brain region would lead to similar improvements on SRMP, although the scores on the SRMP that measure the cognitive-emotional aspects of pain and quality of life would be elevated beyond the improvements demonstrated with stimulation at M1.

2.0 Materials and Methods

2.1. Participants

This study was approved by the Virginia Commonwealth University (VCU) Institutional Review Board (HM20021531) and was also registered on the ClinicalTrials.gov website (NCT04988321). All participants provided written informed consent before study participation. Eligible patients were recruited from the VCU Health Hospital Systems in the Richmond area. A dataset of patients who were treated for pDN at VCU Health Hospital Systems in the last two

years (January 1, 2020 to December 5, 2021), was created by the VCU bioinformatics team using the study inclusion criteria provided by the study team. Patients identified in the dataset were either sent postcards or emails with study information. If they did not respond to the initial invitation to participate after 5 days of receipt of the postcard or emails, the study team contacted them via phone to provide them with study information. Figure 1 describes the recruitment for the patients included in the study. Inclusion criteria were: female or males aged over 18 and under 75 years; Type 2 diabetes; pain for at least 6 months; pain of at least 4/10 on the visual analog scale; a score of >19 on the painDETECT Questionnaire (PD-Q) (>19 represents a >90% likelihood of NP); and stable pharmacological treatment for pain at least 1 month before inclusion. Patients were excluded if they had any active contraindications to rTMS (previous severe head trauma, head surgery or concussion, past or current epilepsy, active brain tumor, intracranial hypertension, implanted ferromagnetic devices (e.g. cardiac pacemaker, neurostimulator or cochlear implants, surgical clips or medical pumps); any other form of NP. Participants were also excluded if they were unable to read or interpret instructions due to any language barriers and all females of childbearing age if they were pregnant or looking to be pregnant.

(Insert Figure 1 here)

2.2 Randomization

Upon enrollment, participants were randomized into two groups (M1 or DLPFC) in a 1:1 ratio. Participants in the M1 group received sham pcTBS at M1 followed by active pcTBS at MI and Participants in the DLPFC group received sham pcTBS at DLPFC followed by active pcTBS at DLPFC. Sham stimulation was always presented first with a 35-45 minute gap between sham and active pcTBS stimulation to avoid any carry over effects that could occur if active pcTBS stimulation was provided first. The study participants were blinded to the treatment sequence. Figure 2 describes the data collection protocol for the two groups.

(Insert Figure 2 here)

Each session began with completion of SRMP and identification of cortical hotspots for M1 or DLPFC. Then baseline measures of corticospinal excitability were assessed followed by sham pcTBS stimulation at M1 or DLPFC. Next, active pcTBS (treatment) at M1 or DLPFC was performed. Lastly, SRMP were collected again. Each individual study session took 120-150 minutes to be completed. SRMP were measured twice for every session and collected electronically 24 hours after the study session was completed. In addition, a pcTBS safety questionnaire to evaluate any potential side effects was completed. All participants received a check of $50 as compensation for their time taken to participate in the study.

2.3 Treatment: Prolonged Continuous Theta Burst Stimulation (pcTBS)

Participants were seated in a comfortable reclining chair with a tightly fitted hair dye cap placed over the head. They were instructed to keep their hands as relaxed as possible. pcTBS was performed using a Magstim Super Rapid2 Plus1 stimulator and a 70 mm double air film coil (P/N: 3950-00, Magstim, Whitland, UK). The pcTBS protocol consisted of three pulses at 50 Hz (i.e. 60 ms) repeated 400 times at intervals of 200 ms (total of 1200 pulses in 1 min and 44 s) [43,61,66]. For the sham condition, a sham coil looking identical to the active coil and making a similar noise but without delivering any active stimulation, was placed above the hotspot. Throughout each session, participants' attention was directed to a monitor presenting nature videos to limit focus on the stimulator and treatment. In addition, participants were offered the use of earplugs to limit the noise from the pcTBS treatment. The optimal site for evoking motor responses for the cortical hotspot for M1 was the abductor pollicis brevis (APB). A surface electromyography (EMG) electrode was placed on the right APB muscle. A grounding electrode was placed on the ulnar styloid. EMG signals were amplified (x1000) and bandpass-filtered using an AMT-8 amplifier (Bortec Biomedical) prior to A/D conversion sampled at 2 kHz. Single-pulse TMS was delivered to the contralateral M1 brain region (Left M1) using a Magstim 200

stimulator and a 90 mm figure-of-eight coil. The coil was held tangentially on the scalp with the coil center rotated to induce a posterior-to-anterior current across the central sulcus. Identification of the cortical “hotspot” of the APB and site of subsequent stimulation was the location evoking the largest peak-to-peak motor evoked potential amplitude in the APB at the lowest stimulation intensity. pcTBS stimulation intensity was set to 80% of the resting motor threshold which was determined as the lowest stimulus intensity that induces motor evoked potential with an amplitude of ≥ 50 μV in at least 5 of 10 consecutive stimuli with the APB fully relaxed [50,77]. Stimulus intensity was determined using an adaptive parameter estimation by sequential testing (PEST) software [10]. PEST triangulates to a threshold with fewer stimulations to prevent overstimulation before pcTBS.

2.3.4 Identification of the Cortical Hotspot for DLPFC

The cortical hotspot for Left DLFPC was measured using the Beam F3 location system where F3 stands for hotspot location for DLPFC [6,63]. It utilizes three measurements: head circumference, nasion-inion distance, and left tragus-right tragus distance. An online calculator then provides a polar-coordinate approximation of the F3 site with respect to the scalp vertex [6]. This method accounts for head size and shape and has a higher level of precision and reproducibility compared to other methods [63,88].

2.3.5 Self-report measures of pain perception (SRMP)

All questionnaires were completed by the participants on an iPad using REDCap electronic data capture tools hosted at Virginia Commonwealth University [36,37]. Participants completed four SRMP at baseline (pre pcTBS), post pcTBS (after active pcTBS) and 24h-post pcTBS (24 hours after the treatment session was completed).

1) The **Bodily and Emotional Perception of Pain (BEEP)** questionnaire is a self-report questionnaire measuring the impact of chronic pain on daily life [76]. It has 23 items on a 0-5 Likert scale that assess three pain subscales, namely the emotional reaction to pain, the

limitations to daily life caused by pain and the interference caused by pain in personal and social functioning [76]. BEEP has demonstrated adequate internal consistency reliability (>0.70) both as a global scale and for its three dimensions [63] . The subscales of BEEP evaluate the sensory-discriminative, affective-motivational, cognitive-evaluative constructs of chronic pain

that align with the function of both the M1 and the DLPFC brain regions.

2) The **Brief Pain Inventory (BPI)** for patients with diabetic neuropathy (BPI-DN) is a widely used and validated numeric rating scale that measures severity of pain (4 items), its interference (7 items) with daily function and other aspects of pain (e.g., location of pain, relief from medications) [21,84,96]. Each item uses a 0-10 numeric rating scale anchored at zero for “no pain” and 10 for “pain as bad as you can imagine” for Severity (4 items); and “does not interfere” to “completely interferes” for Interference (7 items) [21]. Zelman et al. 2005 demonstrated that both the severity and interference subscales were distinct scales with sufficient construct validity and criterion validity when compared to other scales including the Medical Outcomes Survey Short Form-12 Version 2; the Hospital Anxiety and Depression Scale; and the Medical Outcomes Survey-Sleep Scale. In addition, both the subscales demonstrated high internal consistency reliability determined by a Cronbach’s alpha of 0.94 [96].

The Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) group in 2008 made recommendations for improving the design, execution, and interpretation of clinical trials of treatments for pain. The recommendations included utilizing the BPI Interference scale to assess the physical functioning domain in patients with chronic pain [24]. It was proposed that a change of 1 point on the BPI Interference Scale indicates a minimally clinically important change [24]. Other recommendations included a decrease in pain intensity on a 0-10 scale (0 being ‘no pain’ and ‘10’ being ‘pain as bad as you can imagine’) of, with a decrease of 10% to 20% (1 or 2 points on the same 10 point scale) which appears to

reflect minimally important changes [24]. Furthermore, a decrease of ≥ 30% appears to reflect at least moderate clinically important differences, and lastly a decrease ≥ 50% appears to reflect substantial improvements in pain intensity [24]. BPI-DN primarily targets the M1 region and assesses the sensory-discriminative constructs.

3) The **Depression, Anxiety and Stress Scale (DASS-21)** is a 21-item scale comprised of three 7-item subscales that measure depression, anxiety and stress. Psychometric analyses conducted primarily with nonclinical samples, has revealed strong support for the internal consistency and convergent and discriminant validity of the three scales [15,59]. Subjects are asked to use 4-point severity/frequency scales (0- did not apply to me at all, 1- applied to me to some degree, or some of the time, 2 -applied to me to a considerable degree, or a good part of time, and 3 -applied to me very much, or most of the time) to rate the extent to which they have experienced each state over the past week. Scores for Depression, Anxiety and Stress are calculated by summing the scores for the relevant items. DASS-21 has demonstrated strong internal consistency (0.96, 0.89 and 0.93 for Depression, Anxiety, and Stress, respectively) and convergent and discriminant validity for the three scales in clinical and nonclinical samples [15,59]. Cronbach's alpha was 0.94 for Depression, 0.87 for Anxiety, and 0.91 for Stress [3]. The DASS-21 also strongly correlates with the Beck Depression Inventory-II and Beck Anxiety Inventory scales [3,15,38]. DASS-21 mainly evaluates the role of the DLPFC brain region by measuring the cognitive-evaluative constructs of chronic pain.

4) The **Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN)** questionnaire is an instrument used to assess QOL in diabetic polyneuropathy [87,90]. It is comprised of 35 items with three subscales (symptoms, activities of daily living (ADL) and generic health status (GHS) [90]. QOL-DN has also demonstrated high internal consistency reliability measured by Cronbach’s alpha (0.77-0.84) [13,90]. QOL-DN provides a quantitative evaluation of the impact of pDN on quality

of life in these patients and measures the cognitive-evaluative constructs of chronic pain associated with the DLPFC brain region.

BEEP, BPI-DN, DASS-21 and QOL-DN provide quantitative evaluation of pain perception and its impact on quality of life in pDN patients. Participants also completed a demographic questionnaire at baseline and a brief assessment of the blinding effectiveness at the end of the treatment session. There is no consensus in the rTMS literature (randomized control trials with active and sham rTMS treatment) on a standardized question or a questionnaire that should be used to assess patient blinding. Incorporating the recommendations from two systematic reviews that evaluated blinding success [7,14], the participants were asked the following questions as parts of the blinding questionnaire:

> "You received two forms of pcTBS treatment during this study, active and inactive, which one do you think you received first?
>
> a. Active pcTBS
>
> b. Inactive pcTBS

There was also a question in the survey about how certain they were with their ability to correctly guess the treatment using a visual analog scale based on the guidelines of Broadbent et al. [14] with 0 being active pcTBS and 100 corresponding to inactive pcTBS. Participants also completed a safety questionnaire 24 hours after the treatment session was completed to record any adverse events to the treatment. The safety questionnaire has been included in the Appendix.

2.3.12 Statistical Analysis

The study sample size was calculated based on data from previous studies. Studies have reported standardized effect sizes of 0.55 for BEEP [76], and 0.44-0.73 for BPI-DN [84,96].

Using an effect size of 0.50 with power of 0.80 at the 0.05 significance level, the appropriate sample size was calculated to be 42. To account for attrition, 90 patients were recruited and 47 participants were enrolled. Twenty-one participants in each group completed the study. Statistical Package for Social Science (SPSS®software, v. 28.1, IBM Corporation) was used for all statistical analysis with significance set at $p < 0.05$. All data from all assessments were normality tested using visual inspection and the Shapiro–Wilk test. Independent sample t-tests were conducted to look for differences in demographic characteristics for the two groups and results were interpreted accounting for Levene's Test for Equality of Variances.

For variables that had a normal distribution, the middle 95% of the data was identified and outliers were eliminated if the data was outside the plus or minus 2 standard deviations of the mean. If the data was not normal, quartiles (Q1, lowest 25% of the data; Median, lowest 50% of data; Q3, lowest 75% of the data) were computed, and if any observation was less than Q1-1.5 X Interquartile range (equal to Q3 – Q1) or if it was greater than Q3+1.5 X Interquartile range, they were eliminated.

The dependent variables for the statistical analysis were the BEEP, BPI-DN, DASS-21, and QOL-DN, and the two independent variables were the two brain regions; M1 and DLPFC. A two-way mixed model repeated measures analysis of variance (RMANOVA; 2 brain regions by 3 time points) was conducted to evaluate the effects of pcTBS stimulation at M1 and DLPFC for each SRMP. The Greenhouse–Geisser approach was used to correct for violations of sphericity if the estimated epsilon (ε) was less than 0.75. Huynh-Feldt correction was used if ε was greater than 0.75. Effect sizes (partial eta-squared [η^2]) are reported for significant effects. Where appropriate, post hoc analyses were performed using a Bonferroni multiple comparison correction.

3. Results

3.1. Demographics

Participant demographics for the two groups are presented in Table 1. With 47 participants enrolled in the study, two participants reported 0 for all the subscales on the DASS-21 at all three time-points and were removed from that that analysis.

(Insert Table 1 here)

3.2 Changes in SRMP

3.2.1 BEEP scores

Figure 3 depicts the changes in the BEEP subscale scores for the two brain regions from baseline to 24h-post pcTBS. A two-way mixed model RMANOVA (2 brain regions by 3 time points) examining the effects of pcTBS stimulation at M1 and DLPFC on the BEEP Emotional Reaction to Pain subscale revealed no significant interaction effects for brain region activation across time, $F(1.79, 68.11) = 0.158$, $p = .831$. However, there was a statistically significant main effect for time for this subscale ($F[1.79, 68.11] = 15.66$, $p = < .001$, partial $\eta 2 = .292$). Bonferroni post hoc analyses revealed that for the M1 group, the BEEP Emotional Reaction to Pain subscale had a significant decrease from baseline to post pcTBS and from baseline to 24h-post pcTBS. For the DLPFC group, the BEEP Emotional Reaction to Pain subscale demonstrated a significant decrease from baseline to post pcTBS. With regards to the effects of pcTBS stimulation at M1 and DLPFC on the BEEP Pain Interference subscale, there was no significant interaction effect for brain region activation across time, $F(2,80) = 0.808$, $p = .449$. There was a statistically significant main effect for time on this subscale ($F[2,80] = 5.876$, $p = .004$, partial $\eta 2 = .128$). Bonferroni post hoc analyses revealed that for the DLPFC group, the BEEP Pain Interference scores had a significant decrease from baseline to 24h-post pcTBS. Lastly, with regards to the effects of pcTBS stimulation at M1 and DLPFC on the BEEP Pain Limitations subscale, there was no significant interaction effect for brain region and time, $F(1.65, 66.09) = 0.476$, $p = .587$. However, there was a statistically significant main effect for

time on this subscale (F [1.65, 66.09] = 4.702, p = .017, partial η2= .105). Bonferroni post hoc analyses revealed that for the DLPFC group, the BEEP Pain Limitations subscale approached significance from baseline to 24h-post pcTBS; mean difference= 0.655, p = .057 and standard error = 0.268.

(Insert Figure 3 here)

3.2.2 BPI-DN scores

Figure 4 depicts the changes in BPI-DN subscale scores for the two brain regions from baseline to 24h-post pcTBS. A two-way RMANOVA analysis revealed no significant interaction effects for brain region activation across time, F (2,80) = 0.186, p = .831, for the BPI-DN Pain Severity subscale. There was a statistically significant main effect for time on this subscale, (F [2,80] = 5.839, p = .004, partial η2= .127). Bonferroni post hoc analyses revealed that for the M1 group this subscale demonstrated a significant decrease from baseline to 24h-post pcTBS. With regard to the effects of pcTBS stimulation at M1 and DLPFC on the BPI-DN Pain Interference subscale, there was no significant interaction effect for brain region activation and time, F (2,80) = 1.762, p = .178. There was a statistically significant main effect for time on this subscale (F [2,80] = 5.457, p = .006, partial η2= 0.120). Bonferroni post hoc analyses were conducted and for the DLPFC group brain region, the BPI-DN Pain Interference subscale demonstrated a significant decrease from baseline to 24h-post pcTBS. In addition, the BPI-DN Pain Interference subscale approached a significant drop from baseline to post pcTBS for the DLPFC group; mean difference= 1.087, p = .073 and standard error = 0.465.

(Insert Figure 4 here)

3.2.3 DASS-21 scores

Figure 5 depicts the changes in DASS-21 subscale scores for the two brain regions from baseline to 24h-post pcTBS. A two-way RMANOVA analysis revealed no significant interaction

effects for brain region activation across time, F (1.70, 62.77) = 0.286, p = .718, for the DASS-21 Depression subscale. There was a statistically significant main effect for time on the DASS-21 Depression subscale (F [1.70, 62.77] = 18.518, p = < .001, partial η2= .334). Bonferroni post hoc analyses revealed that for the M1 group, this subscale demonstrated a significant decrease from baseline to post pcTBS and a significant decrease from baseline to 24h-post pcTBS. For the DLPFC group, the DASS-21 Depression subscale demonstrated a significant decrease from baseline to post pcTBS and from baseline to 24h-post pcTBS. With regard to the effects of pcTBS stimulation at M1 and DLPFC on the DASS-21 Anxiety subscale, there was no significant interaction effect for brain region activation across time, F (1.23, 42.89) = 0.487, p = .535. There was a statistically significant main effect for time on the subscale (F [1.23, 42.89] = 11.752, p = < .001, partial η2= .251). Bonferroni post hoc analyses revealed that for the M1 group, the DASS-21 Anxiety subscale demonstrated a significant decrease from baseline to post pcTBS and from baseline to 24h-post pcTBS. For the DLPFC group, this subscale approached a significant decrease from baseline to 24h-post pcTBS; mean difference= 1.900, p = .069 and standard error = .798 and from post pcTBS to 24h-post pcTBS; mean difference= .750, p = .059 and standard error = .307. Lastly, for the DASS-21 stress subscale, the two-way RMANOVA analysis revealed no significant interaction effect for brain region and time, F (1.52, 58.07) = 1.786, p = .184. There was a statistically significant main effect for time on this subscale (F [1.52, 58.07] = 12.972, p = < .001, partial η2= .254). Bonferroni post hoc analyses revealed that for the M1 group, the DASS-21 stress subscale demonstrated a significant decrease from baseline to post pcTBS and from baseline to 24h-post pcTBS.

(Insert Figure 5 here)

3.2.4 Norfolk-QOL-DN scores

Figure 6 depicts the changes in Norfolk-QOL-DN subscale scores for the two brain regions from baseline to 24h-post pcTBS. A two-way RMANOVA analyses revealed a significant interaction

effect for brain region and time, F (2,76) = 1.819, p = .045, for the Norfolk-QOL-DN Symptoms subscale. Subsequent simple effect analysis revealed no significant differences between groups for the Norfolk-QOL-DN Symptoms subscale. With regards to the effects of pcTBS stimulation at M1 and DLPFC on the Norfolk-QOL-DN Activities of daily living subscale, there was no significant interaction effect for brain region and time, F (2,76) = 0.611, p = .545. However, there was a statistically significant main effect for time on this subscale (F [2.00, 76.00] = 8.212, p = < .001, partial ɳ2= .178). Bonferroni post hoc analyses revealed that for the M1 group, the Norfolk-QOL-DN Activities of daily living subscale demonstrated a significant decrease from baseline to post pcTBS and from baseline to 24h-post pcTBS. With regards to the effects of pcTBS stimulation at M1 and DLPFC on the Norfolk-QOL-DN Generic health status scores, the two-way RMANOVA did not demonstrate an interaction effect for brain region and time, F (1.72, 65.38) = 0.211, p = .778. The main effect for time on this subscale approached significance, F (1.72, 65.38) = 2.737, p = 0.080.

(Insert Figure 6 here)

3.3 Changes in Pain Intensity scores on BPI-DN

Pain intensity was assessed on a 0-10 scale response to the following question on BPI-DN; “Please rate your pain due to your diabetes by sliding to the one number that tells how much pain you have right now”. Results revealed a reduction in pain intensity of 13.53±0.41% om baseline to post pcTBS and a reduction of 15.11±0.41% from baseline to 24h-post pcTBS. Similarly, responses to the following item; "Please rate your pain due to your diabetes by sliding to the one number that best describes your pain at its worst in the last 24 hours", revealed a reduction in pain intensity scores of14.52±0.61% from baseline to post pcTBS and 15.60±0.61% from baseline to 24h-post pcTBS. Of note, the one-point decrease in the BPI-DN Pain interference subscale score is considered a minimally important improvement [23,24]. Likewise, the 0.54 point decrease from baseline to post pcTBS and the 1.22 point decrease from baseline

to 24h-post pcTBS also represents minimally important improvement in patients with chronic pain [23,24].

3.4 Reporting of Adverse Events

At 24h-post pcTBS, 11 (25%) participants reported an adverse event (44 participants completed the safety questionnaire). Headache was reported by eight participants, neck pain was reported by six participants, and discomfort was reported by five participants. In addition, one participant reported a toothache, two participants reported an increase in pain, one participant mentioned that they had shoulder pain, and one participant reported nausea. None of these events required medical care and recovery was spontaneous. There were no reports of seizures or hearing impairments by the participants.

3.5 Blinding

The blinding questionnaire was completed by 40 participants. Fifteen participants (37.5%) reported that they received inactive pcTBS (sham pcTBS) first with 55% certainty, whereas 25 participants (63.5%) reported that they received active pcTBS first with 44.44% certainty.

4. Discussion

This is the first study to examine the efficacy of pcTBS targeted at the M1 brain region and the DLPFC brain region to alleviate pain perception in patients with pDN. Both pcTBS targeted at M1 and DLPFC demonstrated significant improvement in SRMP over time that evaluated the sensory-discriminative, affective-motivational, and cognitive-evaluative constructs of the pain experience. Furthermore, the magnitude of reduction in pain intensity (13-16%) on a 0-10 scale across the three time points and the one-point reduction from baseline to 24h-post pcTBS on the BPI-DN pain interference scale revealed “minimally important improvement” [23,24]. These results highlight the possible clinical benefit of pcTBS targeted at the M1 and the DLPFC brain regions as an intervention to alleviate pain for patients with pDN.

As described earlier, the effects of pcTBS on the M1 and DLPFC brain regions have only been examined in healthy subjects to examine pain threshold and cortical excitability [49,56,66].

In addition, previous studies that have utilized HF-rTMS as an intervention in patients with pDN have only targeted the M1 brain region and revealed short-term pain relief and improvement in quality of life [1,17,73]. For example, Yang et al. (2022) in patients with pDN who were randomized either to an HF-rTMS group or a sham stimulation group completed five sessions targeting the M1 brain region [17]. Participants in the HF-rTMS group reported improvement in scores both at one day and one-week post treatment on the Short Form 36 scale, a measure of quality of life, and on a 0-10 numeric rating scale that measured pain intensity [17]. Utilizing a protocol similar to Yang and colleagues, Onesti et al. [73] and Abdelkader et al [1] targeted the M1 brain region for five sessions and observed a reduction in pain scores immediately post treatment [1,73] and for up to 3 weeks post treatment in pDN patients [73]. Results from the present study utilizing pcTBS demonstrated a reduction in pain scores across time on subscales of the BEEP and BPI-DN inventories, and are consistent with previous studies utilizing HF-rTMS. In addition, this study provides unique information regarding the use of pcTBS at DLPFC as a possible intervention for pDN.

Changes in the SRMP revealed that for the subscales measuring the sensory-discriminative and affective-motivational aspects of the pain experience in patients with pDN, pcTBS targeting the DLPFC brain region resulted in a significant decrease in the BEEP subscales of pain interference and pain limitations from baseline to 24h-post pcTBS. A decrease was also observed for the BPI-DN score of pain interference from baseline to post pcTBS. pcTBS targeting the M1 resulted in a significant decrease only for the pain severity subscale of the BPI-DN. Neuroimaging studies have revealed that the cortical areas associated with the sensory-discriminative and affective-motivational dimensions of pain include the somatosensory cortex and the anterior cingulate cortex [4,29,57,60]. Thus, pcTBS targeted at the DLPFC brain region modulates the activation of these brain regions to decrease the sensory and affective dimensions of the pain experience. Similarly, the cortical areas associated with the cognitive-evaluative dimensions of pain experience include the DLPFC, hippocampus, limbic

system and the insula [57,69,80,86]. As a result of the changes observed in SRMP subscales linked to the cognitive-evaluative dimensions of pain (emotional reaction to pain scores on BEEP, DASS-21 subscales of anxiety, depression and stress), it can be postulated that pcTBS targeted at the M1 and DLPFC brain regions decreases activation of these brain regions in these patients resulting in improvement of symptoms linked to emotional and cognitive aspects of the pain experience. Future studies combining structural MRI assessment before and after pcTBS to identify and examine functional connectivity approaches that delineate the specific changes in the activation of these brain regions involved in modulation of pain perception will provide the mechanistic link to pcTBS stimulation and cortical activation patterns in different brain regions related to the sensory-discriminative, affective-motivational and cognitive-evaluative constructs of the pain experience.

In addition, the symptom subscale of Norfolk-QOL-DN, which measures quality of life in patients with pDN and provides a subjective perception of symptoms associated with nerve damage revealed a significant interaction, although there were no group differences, with the DLPFC group reporting a decrease in scores across the three time points. This subscale is composed of 7 items and participants rate the presence of nerve fiber-related symptoms (numbness, tingling/pins and needles, electric shocks, superficial peripheral pain, deep pain, weakness, and other symptoms) in their feet, legs, hands, and/or arms during the past 4 weeks. Future studies should incorporate clinical screening tools that assess sensory function and examine its correlation with these subjective patient perceptions of their neuropathy symptoms before and after pcTBS.

The mechanism of action for pcTBS targeted at the M1 and DLPFC brain regions are unclear, although previous studies have highlighted synaptic plasticity [16,43,44,92]; distinct neurophysiological [33,34], neurochemical [44,83,92]; and endogenous mechanisms acting cortically, supraspinally and spinally [20,64,85,86,93] as possible explanations. The effects of

pcTBS and HF-rTMS are dependent on the frequency of stimulation used to induce synaptic plasticity [40,42,79]. In addition, the neuroplasticity induced changes in the cortical circuits outlast the period of stimulation, a characteristic of long-term potentiation (LTP) and long-term depression (LTD) [8,43,45]. TBS consists of pulses applied in bursts of three at 50 Hz with an inter-burst interval at 5 Hz and with pcTBS these pulses are repeated 400 times at intervals of 200 ms resulting in 1,200 pulses. With pcTBS induced synaptic plasticity, there are specific alterations in neuronal calcium concentrations that dictate its after effects [42,45,55]. Greater calcium influx leads to LTP and a decrease in calcium influx contributes to LTD [16,45,92]. The changes in calcium concentrations are dependent on the action of the N-Methyl-D-aspartate receptors, γ- aminobutyric acid receptor activity, glutamate receptors and the and the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor activity [16,43,83]. To analyze the changes in the glutamate and γ- aminobutyric acid receptor activity, future studies should utilize neuroimaging using MRI spectroscopy and or indirectly measure their activity using paired pulse TMS to assess intracortical inhibition.

None of the participants in this study reported any adverse events during the study intervention or any treatment related adverse events 24 hours after the session was completed. Thus, confirming the safety and tolerability of pcTBS. In addition, the side effects that study participants reported (headache for 8 participants and neck pain for 6 participants) were similar in type and frequency to what has been reported in previous studies [46,71,78] that utilized HF-rTMS targeted at the M1 and DLPFC brain regions.

rTMS and pcTBS are noninvasive, painless and safe techniques to activate different brain regions and cause changes that can last from a few days to weeks depending on the stimulus frequency and number of sessions. For example, the effects of single session of HF-rTMS targeted at the M1 region lasts up to 5-8 days [53,67] and the analgesic effect of five to ten sessions can last up to 2-4 weeks following the last session [62,69,81]. Future studies are needed to determine the long-term efficacy of pcTBS targeted at M1 and DLPFC by including a

greater number of sessions and longer follow-up time. Future investigations should also consider a double-blind protocol and the use of a neuronavigational system to optimize the identification of cortical hotspots; In the protocol for this study, the sham stimulation was delivered prior to active pcTBS stimulation for both the brain regions to prevent any potential response to active pcTBS stimulation from influencing the sham response, it is possible this sequence resulted in an order effect.

A particular strength of this study is the homogeneity of its participants. These patients with pDN likely share a similar physiological mechanism responsible for their NP, whereas previous studies that have evaluated the efficacy of HF-rTMS on NP have included patients with multiple etiologies and symptoms. It is quite possible that the mechanisms for different types of NP and chronic pain may respond differently to the pcTBS intervention, and thus lead to differences in the analgesic and emotional aspects of the pain experience.

5.0 Conclusion

DN is the most common complication of diabetes mellitus and pDN is the most common cause of NP. pDN is largely irreversible, and management is mainly supportive with the goal of limiting progression of symptoms when medications no longer provide sufficient analgesia. pcTBS is a safe, non-invasive brain stimulation technique that can stimulate different brain regions by producing brief magnetic pulses to induce changes in brain networks and areas that modulate the sensory, affective and emotional aspects of pain processing. The present study results found that a single session of pcTBS targeted at either the M1 or the DLPFC brain region in patients with pDN resulted in improvement on the affective, sensory, quality of life, emotional, and cognitive aspects of the pain experience. In addition, pcTBS demonstrated excellent tolerability and feasibility and future studies should consider utilizing multiple sessions of pcTBS to evaluate its long-term effects on pain perception.

References

[1] Abdelkader AA, Gohary AME, Mourad HS, Salmawy DAE. Repetitive tms in treatment of resistant diabetic neuropathic pain. Egyptian Journal of Neurology, Psychiatry and Neurosurgery 2019;55:1–9.

[2] Alam U, Sloan G, Tesfaye S. Treating Pain in Diabetic Neuropathy: Current and Developmental Drugs. Drugs 2020;80:363–384. doi:10.1007/s40265-020-01259-2.

[3] Antony MM, Cox BJ, Enns MW, Bieling PJ, Swinson RP. Psychometric properties of the 42-item and 21-item versions of the Depression Anxiety Stress Scales in clinical groups and a community sample. Psychol Assess 1998;10:176–181.

[4] Auvray M, Myin E, Spence C. The sensory-discriminative and affective-motivational aspects of pain. Neurosci Biobehav Rev 2010;34:214–223.

[5] Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet 1985;1:1106–1107. doi:10.1016/S0140-6736(85)92413-4.

[6] Beam W, Borckardt JJ, Reeves ST, George MS. An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. Brain Stimul 2009;2:50–54. doi:10.1016/j.brs.2008.09.006.

[7] Berlim MT, Broadbent HJ, van den Eynde F. Blinding integrity in randomized sham-controlled trials of repetitive transcranial magnetic stimulation for major depression: A systematic review and meta-analysis. International Journal of Neuropsychopharmacology 2013;16:1173–1181.

[8] Bliss TVP, Cooke SF. Long-term potentiation and long-term depression: A clinical perspective. Clinics 2011;66:3–17.

[9] Blumberger DM, Vila-Rodriguez F, Thorpe KE, Feffer K, Noda Y, Giacobbe P, Knyahnytska Y, Kennedy SH, Lam RW, Daskalakis ZJ, Downar J. Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial. The Lancet 2018;391:1683–1692. doi:https://doi.org/10.1016/S0140-6736(18)30295-2.

[10] Borckardt JJ, Nahas Z, Koola J, George MS. Estimating resting motor thresholds in transcranial magnetic stimulation research and practice: A computer simulation evaluation of best methods. Journal of ECT 2006;22:169–175. doi:10.1097/01.yct.0000235923.52741.72.

[11] Bouhassira D. Neuropathic pain: Definition, assessment and epidemiology. Rev Neurol (Paris) 2019;175:16–25.

[12] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain 2008;136:380–387.

[13] Bredfeldt C, Altschuler A, Adams AS, Portz JD, Bayliss EA. Patient reported outcomes for diabetic peripheral neuropathy. J Diabetes Complications 2015;29:1112–1118.

[14] Broadbent HJ, van den Eynde F, Guillaume S, Hanif EL, Stahl D, David AS, Campbell IC, Schmidt U. Blinding success of rTMS applied to the dorsolateral prefrontal cortex in randomised sham-controlled trials: A systematic review. World Journal of Biological Psychiatry 2011;12:240–248.

[15] Brown TA, Chorpita BF, Korotitsch W, Barlow DH. Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples. Behaviour Research and Therapy 1997;35:79–89.

[16] Cárdenas-Morales L, Nowak DA, Kammer T, Wolf RC, Schönfeldt-Lecuona C. Mechanisms and applications of theta-burst rTMS on the human motor cortex. Brain Topogr 2010;22:294–306.

[17] Chang MC, Yang S, Kwak SG, Choi G-S. Short-term Effect of Repetitive Transcranial Magnetic Stimulation on Diabetic Peripheral Neuropathic Pain. n.d. Available: www.painphysicianjournal.comwww.painphysicianjournal.com.

[18] Che X, Cash RFH, Luo X, Luo H, Lu X, Xu F, Zang YF, Fitzgerald PB, Fitzgibbon BM. High-frequency rTMS over the dorsolateral prefrontal cortex on chronic and provoked pain: A systematic review and meta-analysis. Brain Stimul 2021;14:1135–1146.

[19] Chung SW, Hill AT, Rogasch NC, Hoy KE, Fitzgerald PB. Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis. Neurosci Biobehav Rev 2016;63:43–64. doi:10.1016/j.neubiorev.2016.01.008.

[20] Ciampi De Andrade D, Mhalla A, Adam F, Texeira MJ, Bouhassira D. Repetitive transcranial magnetic stimulation induced analgesia depends on N-methyl-d-aspartate glutamate receptors. Pain 2014;155:598–605. doi:10.1016/j.pain.2013.12.022.

[21] Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. Ann Acad Med Singap 1994;23:129–138.

[22] Cohen SL, Bikson M, Badran BW, George MS. A visual and narrative timeline of US FDA milestones for Transcranial Magnetic Stimulation (TMS) devices. Brain Stimul 2022;15:73–75.

[23] Dworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP, Kerns RD, Stucki G, Allen RR, Bellamy N, Carr DB, Chandler J, Cowan P, Dionne R, Galer BS, Hertz S, Jadad AR, Kramer LD, Manning DC, Martin S, McCormick CG, McDermott MP, McGrath P, Quessy S, Rappaport BA, Robbins W, Robinson JP, Rothman M, Royal MA, Simon L, Stauffer JW, Stein W, Tollett J, Wernicke J, Witter J. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain 2005;113:9–19.

[24] Dworkin RH, Turk DC, Wyrwich KW, Beaton D, Cleeland CS, Farrar JT, Haythornthwaite JA, Jensen MP, Kerns RD, Ader DN, Brandenburg N, Burke LB, Cella D, Chandler J, Cowan P, Dimitrova R, Dionne R, Hertz S, Jadad AR, Katz NP, Kehlet H, Kramer LD, Manning DC, McCormick C, McDermott MP, McQuay HJ, Patel S, Porter L, Quessy S, Rappaport BA, Rauschkolb C, Revicki DA, Rothman M, Schmader KE, Stacey BR, Stauffer JW, von Stein T, White RE, Witter J, Zavisic S. Interpreting the Clinical Importance of Treatment Outcomes in Chronic Pain Clinical Trials: IMMPACT Recommendations. J Pain 2008;9:105–121.

[25] Edwards RR, Dworkin RH, Sullivan MD, Turk DC, Wasan AD. The Role of Psychosocial Processes in the Development and Maintenance of Chronic Pain. Journal of Pain 2016;17:T70–T92.

[26] Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, Bril V, Russell JW, Viswanathan V. Diabetic neuropathy. Nat Rev Dis Primers 2019;5. doi:10.1038/s41572-019-0092-1.

[27] Fillingim RB, Loeser JD, Baron R, Edwards RR. Assessment of Chronic Pain: Domains, Methods, and Mechanisms. Journal of Pain 2016;17:T10–T20.

[28] Fitzgerald PB, Brown TL, Marston NAU, Daskalakis ZJ, de Castella A, Kulkarni J. Transcranial Magnetic Stimulation in the Treatment of Depression: A Double-blind, Placebo-Controlled Trial. Arch Gen Psychiatry 2003;60:1002–1008. doi:10.1001/archpsyc.60.9.1002.

[29] Garcia-Larrea L, Peyron R. Pain matrices and neuropathic pain matrices: A review. Pain. Elsevier B.V., 2013, Vol. 154.

[30] Gatchel RJ, Peng YB, Peters ML, Fuchs PN, Turk DC. The Biopsychosocial Approach to Chronic Pain: Scientific Advances and Future Directions. Psychol Bull 2007;133:581–624.

[31] Gatzinsky K, Bergh C, Liljegren A, Silander H, Samuelsson J, Svanberg T, Samuelsson O. Repetitive transcranial magnetic stimulation of the primary motor cortex in management of chronic neuropathic pain: A systematic review. Scand J Pain 2021;21:8–21.

[32] Girach A, Julian TH, Varrassi G, Paladini A, Vadalouka A, Zis P. Quality of life in painful peripheral neuropathies: A systematic review. Pain Res Manag 2019;2019.

[33] Goldsworthy MR, Vallence AM, Hodyl NA, Semmler JG, Pitcher JB, Ridding MC. Probing changes in corticospinal excitability following theta burst stimulation of the human primary motor cortex. Clinical Neurophysiology 2016;127:740–747.

[34] Granovsky Y, Sprecher E, Sinai A. Motor corticospinal excitability: A novel facet of pain modulation? Pain Rep 2019;4:1–7.

[35] Hallett M. Transcranial Magnetic Stimulation: A Primer. Neuron 2007;55:187–199.

[36] Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform 2019;95.

[37] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377–381.

[38] Henry JD, Crawford JR. The short-form version of the Depression anxiety stress scales (DASS-21): Construct validity and normative data in a large non-clinical sample. British Journal of Clinical Psychology 2005;44:227–239.

[39] Henschke N, Kamper SJ, Maher CG. The epidemiology and economic consequences of pain. Mayo Clin Proc 2015;90:139–147.

[40] He W, Fong PY, Leung TWH, Huang YZ. Protocols of non-invasive brain stimulation for neuroplasticity induction. Neurosci Lett 2020;719.

[41] Hicks CW, Selvin E. Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. Current Diabetes Reports 2019 19:10 2019;19:1–8. doi:10.1007/S11892-019-1212-8.

[42] Huang YZ, Chen RS, Rothwell JC, Wen HY. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clinical Neurophysiology 2007;118:1028–1032.

[43] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–206.

[44] Huang YZ, Lu MK, Antal A, Classen J, Nitsche M, Ziemann U, Ridding M, Hamada M, Ugawa Y, Jaberzadeh S, Suppa A, Paulus W, Rothwell J. Plasticity induced by non-invasive transcranial brain stimulation: A position paper. Clinical Neurophysiology 2017;128:2318–2329.

[45] Huang YZ, Rothwell JC, Chen RS, Lu CS, Chuang WL. The theoretical model of theta burst form of repetitive transcranial magnetic stimulation. Clinical Neurophysiology 2011;122:1011–1018.

[46] Jiang X, Yan W, Wan R, Lin Y, Zhu X, Song G, Zheng K, Wang Y, Wang X. Effects of repetitive transcranial magnetic stimulation on neuropathic pain: A systematic review and meta-analysis. Neurosci Biobehav Rev 2022;132:130–141.

[47] Kassebaum NJ. Global, regional, and national burden of diseases and injuries for adults 70 years and older: Systematic analysis for the Global Burden of Disease 2019 Study. The BMJ 2022;376.

[48] Kawai K, Kawai AT, Wollan P, Yawn BP. Adverse impacts of chronic pain on health-related quality of life, work productivity, depression and anxiety in a community-based study. Fam Pract 2017;34:656–661.

[49] Klírová M, Hejzlar M, Kostýlková L, Mohr P, Rokyta R, Novák T. Prolonged Continuous Theta Burst Stimulation of the Motor Cortex Modulates Cortical Excitability But not Pain Perception. Front Syst Neurosci 2020;14:1–9.

[50] Klomjai W, Katz R, Lackmy-Vallée A. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). Ann Phys Rehabil Med 2015;58:208–213.

[51] Lefaucheur JP. Cortical neurostimulation for neuropathic pain: State of the art and perspectives. Pain 2016;157:S81–S89.

[52] Lefaucheur J-P, Aleman A, Baeken C, Benninger DH, Brunelin J, di Lazzaro V, Filipović SR, Grefkes C, Hasan A, Hummel FC, Jääskeläinen SK, Langguth B, Leocani L, Londero A, Nardone R, Nguyen J-P, Nyffeler T, Oliveira-Maia AJ, Oliviero A, Padberg F, Palm U, Paulus W, Poulet E, Quartarone A, Rachid F, Rektorová I, Rossi S, Sahlsten H, Schecklmann M, Szekely D, Ziemann U. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clinical Neurophysiology 2020;131:474–528. doi:https://doi.org/10.1016/j.clinph.2019.11.002.

[53] Lefaucheur JP, Drouot X, Nguyen JP. Interventional neurophysiology for pain control: duration of pain relief following repetitive transcranial magnetic stimulation of the motor cortex. 2001 Available: www.elsevier.fr/direct/nc-cn.

[54] Leung A, Shirvalkar P, Chen R, Kuluva J, Vaninetti M, Bermudes R, Poree L, Wassermann E, Kopell B, Levy R. Transcranial Magnetic Stimulation for Pain, Headache, and Comorbid Depression: INS-NANS Expert Consensus Panel Review and Recommendation. Neuromodulation 2020;2019:267–290.

[55] Li CT, Huang YZ, Bai YM, Tsai SJ, Su TP, Cheng CM. Critical role of glutamatergic and GABAergic neurotransmission in the central mechanisms of theta-burst stimulation. Hum Brain Mapp 2019;40:2001–2009.

[56] Li C, Zhang N, Han Q, Zhang L, Xu S, Tu S, Xie Y, Wang Z. Prolonged Continuous Theta Burst Stimulation Can Regulate Sensitivity on Aβ Fibers: An Functional Near-Infrared Spectroscopy Study. Front Mol Neurosci 2022;15.

[57] Lindsay NM, Chen C, Gilam G, Mackey S, Scherrer G. Brain circuits for pain and its treatment. Sci Transl Med 2021;13.

[58] Lipton RB, Dodick DW, Silberstein SD, Saper JR, Aurora SK, Pearlman SH, Fischell RE, Ruppel PL, Goadsby PJ. Single-pulse transcranial magnetic stimulation for acute treatment of migraine with aura: a randomised, double-blind, parallel-group, sham-controlled trial. Lancet Neurol 2010;9:373–380.

[59] Lovibond, S.H. & Lovibond PF. Manual for the Depression Anxiety Stress Scales. 2nd ed. Sydney: Psychology Foundation, 1995.

[60] Malfliet A, Coppieters I, van Wilgen P, Kregel J, de Pauw R, Dolphens M, Ickmans K. Brain changes associated with cognitive and emotional factors in chronic pain: A systematic review. European Journal of Pain (United Kingdom) 2017;21:769–786.

[61] de Martino E, Fernandes AM, Galhardoni R, de Oliveira Souza C, Ciampi De Andrade D, Graven-Nielsen T. Sessions of Prolonged Continuous Theta Burst Stimulation or High-frequency 10 Hz Stimulation to Left Dorsolateral Prefrontal Cortex for 3 Days Decreased Pain Sensitivity by Modulation of the Efficacy of Conditioned Pain Modulation. Journal of Pain 2019;20:1459–1469.

[62] Mhalla A, Baudic S, de Andrade DC, Gautron M, Perrot S, Teixeira MJ, Attal N, Bouhassira D. Long-term maintenance of the analgesic effects of transcranial magnetic stimulation in fibromyalgia. Pain 2011;152:1478–1485.

[63] Mir-Moghtadaei A, Caballero R, Fried P, Fox MD, Lee K, Giacobbe P, Daskalakis ZJ, Blumberger DM, Downar J. Concordance between BeamF3 and MRI-neuronavigated target sites for repetitive transcranial magnetic stimulation of the left dorsolateral prefrontal cortex. Brain Stimul 2015;8:965–973.

[64] Moisset X, de Andrade DC, Bouhassira D. From pulses to pain relief: An update on the mechanisms of rTMS-induced analgesic effects. European Journal of Pain (United Kingdom) 2016;20:689–700.

[65] Moisset X, Bouhassira D, Avez Couturier J, Alchaar H, Conradi S, Delmotte MH, Lanteri-Minet M, Lefaucheur JP, Mick G, Piano V, Pickering G, Piquet E, Regis C, Salvat E, Attal N. Pharmacological and non-pharmacological treatments for neuropathic pain: Systematic review and French recommendations. Rev Neurol (Paris) 2020;176:325–352.

[66] Moisset X, Goudeau S, Poindessous-Jazat F, Baudic S, Clavelou P, Bouhassira D. Prolonged continuous theta-burst stimulation is more analgesic than “classical” high frequency repetitive transcranial magnetic stimulation. Brain Stimul 2015;8:135–141. doi:10.1016/j.brs.2014.10.006.

[67] Moisset X, Lefaucheur JP. Non pharmacological treatment for neuropathic pain: Invasive and non-invasive cortical stimulation. Rev Neurol (Paris) 2019;175:51–58.

[68] Murray CJL, Mokdad AH, Ballestros K, Echko M, Glenn S, Olsen HE, Mullany E, Lee A, Khan AR, Ahmadi A, Ferrari AJ, Kasaeian A, Werdecker A, Carter A, Zipkin B, Sartorius B, Serdar B, Sykes BL, Troeger C, Fitzmaurice C, Rehm CD, Santomauro D, Kim D, Colombara D, Schwebel DC, Tsoi D, Kolte D, Nsoesie E, Nichols E, Oren E, Charlson FJ, Patton GC, Roth GA, Dean Hosgood H, Whiteford HA, Kyu H, Erskine HE, Huang H, Martopullo I, Singh JA, Nachega JB, Sanabria JR, Abbas K, Ong K, Tabb K, Krohn KJ, Cornaby L, Degenhardt L, Moses M, Farvid M, Griswold M, Criqui M, Bell M, Nguyen M, Wallin M, Mirarefin M, Qorbani M, Younis M, Fullman N, Liu P, Briant P, Gona P, Havmoller R, Leung R, Kimokoti R, Bazargan-Hejazi S, Hay SI, Yadgir S, Biryukov S, Vollset SE, Alam T, Frank T, Farid T, Miller T, Vos T, Bärnighausen T, Gebrehiwot TT, Yano Y, Al-Aly Z, Mehari A, Handal A, Kandel A, Anderson B, Biroscak B, Mozaffarian D, Ray Dorsey E, Ding EL, Park EK, Wagner G, Hu G, Chen H, Sunshine JE, Khubchandani J, Leasher J, Leung J, Salomon J, Unutzer J, Cahill L, Cooper L, Horino M, Brauer M, Breitborde N, Hotez P, Topor-Madry R, Soneji S, Stranges S, James S, Amrock S, Jayaraman S, Patel T, Akinyemiju T, Skirbekk V, Kinfu Y, Bhutta Z, Jonas JB, Murray CJL. The state of US health, 1990-2016: Burden of diseases, injuries, and risk factors among US states. JAMA - Journal of the American Medical Association 2018;319:1444–1472.

[69] Nardone R, Höller Y, Langthaler PB, Lochner P, Golaszewski S, Schwenker K, Brigo F, Trinka E. rTMS of the prefrontal cortex has analgesic effects on neuropathic pain in subjects with spinal cord injury. 2016. doi:10.1038/sc.2016.87.

[70] Noda Y, Silverstein WK, Barr MS, Vila-Rodriguez F, Downar J, Rajji TK, Fitzgerald PB, Mulsant BH, Vigod SN, Daskalakis ZJ, Blumberger DM. Neurobiological mechanisms of repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex in depression: A systematic review. Psychol Med 2015;45:3411–3432.

[71] Oberman L, Edwards D, Eldaief M, Pascual-Leone A. Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. J Clin Neurophysiol 2011;28:67–74.

[72] O'Connell NE, Marston L, Spencer S, DeSouza LH, Wand BM. Non-invasive brain stimulation techniques for chronic pain. Cochrane Database Syst Rev 2018;4:CD008208.

[73] Onesti E, Gabriele M, Cambieri C, Ceccanti M, Raccah R, di Stefano G, Biasiotta A, Truini A, Zangen A, Inghilleri M. H-coil repetitive transcranial magnetic stimulation for pain relief in patients with diabetic neuropathy. European Journal of Pain (United Kingdom) 2013;17:1347–1356.

[74] Ong W-Y, Stohler CS, Herr DR. Role of the Prefrontal Cortex in Pain Processing. Mol Neurobiol 2019;56:1137–1166.

[75] Pop-Busui R, Boulton AJM, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D. Diabetic neuropathy: A position statement by the American diabetes association. Diabetes Care 2017;40:136–154.

[76] Preti A, Stocchino S, Pinna F, Deidda MC, Musu M, Sancassiani F, Romano F, Machado S, Finco G, Carta MG. BEEP—Bodily and Emotional Perception of Pain. A Questionnaire to Measure Reaction to Pain in Chronic Pain Disorders . Frontiers in Psychology 2019;10:480. Available: https://www.frontiersin.org/articles/10.3389/fpsyg.2019.00480.

[77] Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, di Iorio R, di Lazzaro V, Ferreri F, Fitzgerald PB, George MS, Hallett M, Lefaucheur JP, Langguth B, Matsumoto H, Miniussi C, Nitsche MA, Pascual-Leone A, Paulus W, Rossi S, Rothwell JC, Siebner HR, Ugawa Y, Walsh V, Ziemann U. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee. Clinical Neurophysiology 2015;126:1071–1107. doi:10.1016/j.clinph.2015.02.001.

[78] Rossi S, Antal A, Bestmann S, Bikson M, Brewer C, Brockmöller J, Carpenter LL, Cincotta M, Chen R, Daskalakis JD, di Lazzaro V, Fox MD, George MS, Gilbert D, Kimiskidis VK, Koch G, Ilmoniemi RJ, Pascal Lefaucheur J, Leocani L, Lisanby SH, Miniussi C, Padberg F, Pascual-Leone A, Paulus W, Peterchev A v., Quartarone A, Rotenberg A, Rothwell J, Rossini PM, Santarnecchi E, Shafi MM, Siebner HR, Ugawa Y, Wassermann EM, Zangen A, Ziemann U, Hallett M. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. Clinical Neurophysiology 2021;132:269–306.

[79] Rounis E, Huang YZ. Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease. Exp Brain Res 2020;238:1707–1714.

[80] Seminowicz DA, Moayedi M. The Dorsolateral Prefrontal Cortex in Acute and Chronic Pain. Journal of Pain 2017;18:1027–1035. doi:10.1016/j.jpain.2017.03.008.

[81] Short EB, Borckardt JJ, Anderson BS, Frohman H, Beam W, Reeves ST, George MS. Ten sessions of adjunctive left prefrontal rTMS significantly reduces fibromyalgia pain: A randomized, controlled pilot study. Pain 2011;152:2477–2484. doi:10.1016/j.pain.2011.05.033.

[82] Smith SM, Dworkin RH, Turk DC, McDermott MP, Eccleston C, Farrar JT, Rowbotham MC, Bhagwagar Z, Burke LB, Cowan P, Ellenberg SS, Evans SR, Freeman RL, Garrison LP, Iyengar

S, Jadad A, Jensen MP, Junor R, Kamp C, Katz NP, Kesslak JP, Kopecky EA, Lissin D, Markman JD, Mease PJ, O'Connor AB, Patel K v., Raja SN, Sampaio C, Schoenfeld D, Singh J, Steigerwald I, Strand V, Tive LA, Tobias J, Wasan AD, Wilson HD. Interpretation of chronic pain clinical trial outcomes: IMMPACT recommended considerations. Pain 2020;161:2446–2461. doi:10.1097/j.pain.0000000000001952.

[83] Suppa A, Huang YZ, Funke K, Ridding MC, Cheeran B, di Lazzaro V, Ziemann U, Rothwell JC. Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. Brain Stimul 2016;9:323–335.

[84] Tan G, Jensen MP, Thornby JI, Shanti BF. Validation of the brief pain inventory for chronic nonmalignant pain. Journal of Pain 2004;5:133–137. doi:10.1016/j.jpain.2003.12.005.

[85] Taylor JJ, Borckardt JJ, Canterberry M, Li X, Hanlon CA, Brown TR, George MS. Naloxone-Reversible Modulation of Pain Circuitry by Left Prefrontal rTMS. Neuropsychopharmacology 2013;38:1189–1197. doi:10.1038/npp.2013.13.

[86] Taylor JJ, Borckardt JJ, George MS. Endogenous opioids mediate left dorsolateral prefrontal cortex rTMS-induced analgesia. Pain 2012;153:1219–1225. doi:https://doi.org/10.1016/j.pain.2012.02.030.

[87] Tesfaye S, Vileikyte L, Rayman G, Sindrup SH, Perkins BA, Baconja M, Vinik AI, Boulton AJM, on behalf of The Toronto Expert Panel on Diabetic Neuropathy. Painful diabetic peripheral neuropathy: consensus recommendations on diagnosis, assessment and management. Diabetes Metab Res Rev 2011;27:629–638. doi:https://doi.org/10.1002/dmrr.1225.

[88] Trapp NT, Bruss J, King Johnson M, Uitermarkt BD, Garrett L, Heinzerling A, Wu C, Koscik TR, ten Eyck P, Boes AD. Reliability of targeting methods in TMS for depression: Beam F3 vs. 5.5 cm. Brain Stimul 2020;13:578–581.

[89] Turk DC, Fillingim RB, Ohrbach R, Patel K v. Assessment of Psychosocial and Functional Impact of Chronic Pain. Journal of Pain 2016;17:T21–T49. doi:10.1016/j.jpain.2016.02.006.

[90] Vinik EJ, Hayes RP, Oglesby A, Bastyr E, Barlow P, Ford-Molvik SL, Vinik AI. The development and validation of the Norfolk QOL-DN, a new measure of patients' perception of the effects of diabetes and diabetic neuropathy. Diabetes Technol Ther 2005;7:497–508.

[91] Williams R, Karuranga S, Malanda B, Saeedi P, Basit A, Besançon S, Bommer C, Esteghamati A, Ogurtsova K, Zhang P, Colagiuri S. Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract 2020;162:108072.

[92] Wischnewski M, Schutter DJLG. Efficacy and time course of theta burst stimulation in healthy humans. Brain Stimul 2015;8:685–692.

[93] Yang S, Chang MC. Effect of Repetitive Transcranial Magnetic Stimulation on Pain Management: A Systematic Narrative Review. Front Neurol 2020;11.

[94] Yong RJ, Mullins PM, Bhattacharyya N. Prevalence of chronic pain among adults in the United States. 2021. doi:10.1097/j.pain.0000000000002291.

[95] Zelaya CE, Ph D, Dahlhamer JM, Ph D, Lucas JW, Connor EM. Chronic pain and high-impact chronic pain among U.S. adults, 2019. Hyattsville, MD, 2020 Available: https://www.cdc.gov/nchs/products/index.htm.

[96] Zelman DC, Gore M, Dukes E, Tai KS, Brandenburg N. Validation of a Modified Version of the Brief Pain Inventory for Painful Diabetic Peripheral Neuropathy. J Pain Symptom Manage 2005;29:401–410.

Table 1: Demographic Data for all Participants

	Total (N=47)	pcTBS at M1 (n=23)	pcTBS at DLPFC (n=24)	p-value
Sex, n (%) Male Female	 19 (40.42) 28 (59.38)	 11 (47.7) 12 (52.2)	 8 (33.33) 16 (66.67)	0.16
Race, n (%) • Non-Hispanic Black • Non-Hispanic White • Asian • Hispanic/Latino/Spanish • Mixed • Prefer not to say	 24 (51.10) 18 (38.30) 1 (2.10) 1 (2.10) 2 (4.30) 1 (2.10)	 12 (52.17) 8 (34.78) 0 (0.00) 1 (4.30) 1 (4.30) 1 (4.30)	 12 (50.00) 10 (41.66) 1 (4.23) 0 0 1 (4.23)	0.13
Age (years)	58.65 ± 8.82	59.65 ± 10.23	57.71 ± 7.33	0.46
Duration of pain (months)	67.07 ± 6.51	67.65 ± 58.47	66.50 ± 72.97	0.48
PD-Q score (-1 and 38 range)	22.15 ± 65.55	21.78 ± 2.58	22.50 ± 3.36	0.21
Current pain on VAS (0-10 range)	5.87 ± 1.88	5.91 ± 1.90	5.83 ± 1.90	0.44
BMI, kg/m^2	31.87 ± 6.51	33.26 ± 6.57	30.54 ± 6.30	0.08
Pre_RMT	55.74 ± 8.87	56.17 ± 9.49	55.33 ± 8.44	0.37

BMI: body mass index, VAS: visual analog scale, PD-Q: painDETECT score, RMT: Resting motor threshold

Figure 1

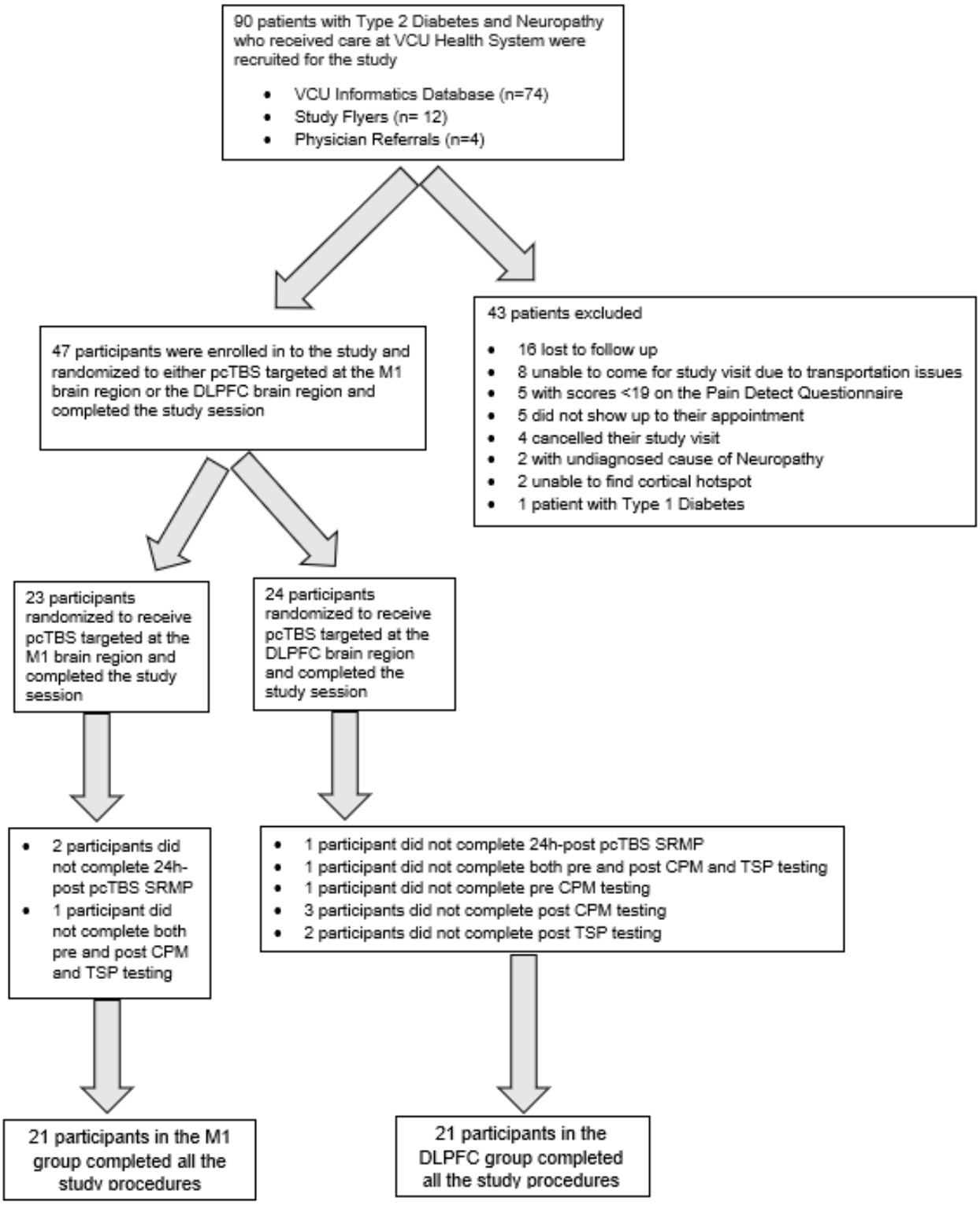


Figure 1: Study flow chart describing the eligibility and recruitment process.

Figure 2

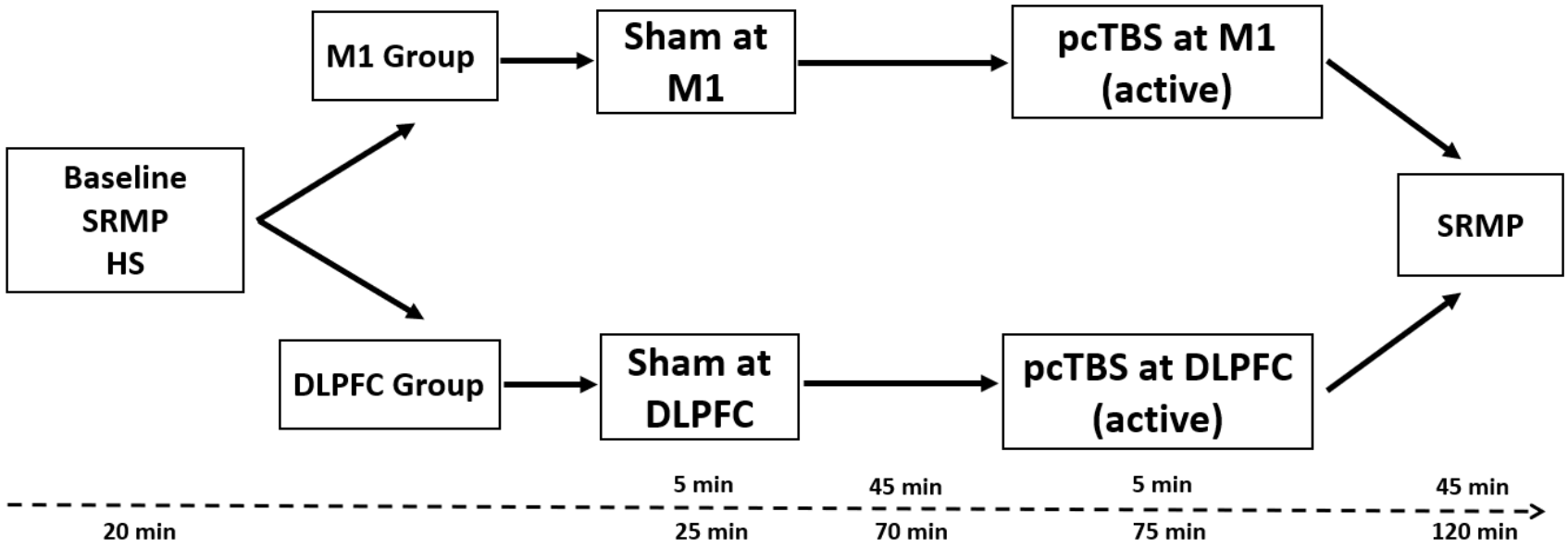
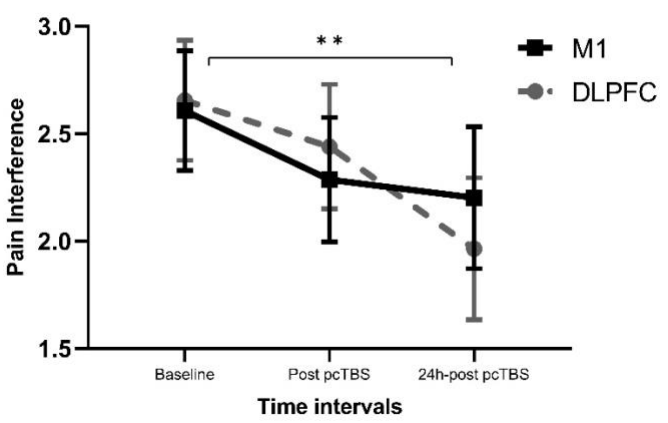
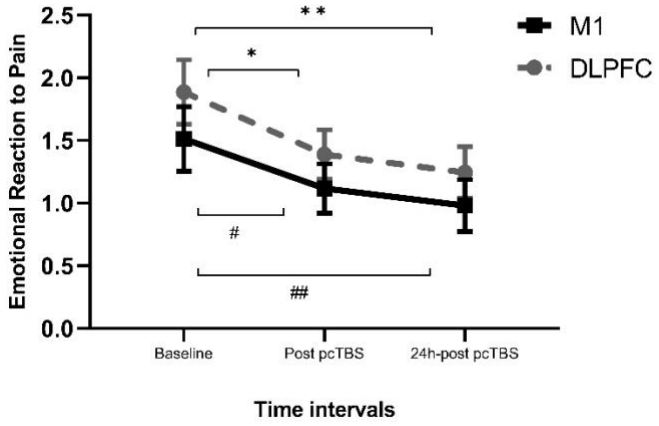


Figure 2: Data collection protocol for the two groups. Prior to collecting data, the cortical hotspots for M1 and DLPFC were identified. Self-report measures of pain perception (SRMP), Identification of Cortical Hotspots (HS).

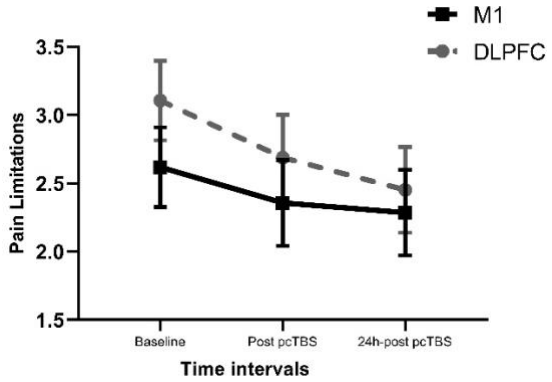
Figure 3



a



b



c

Figure 3: Effects of pcTBS at M1 and DLPFC on BEEP scores across the three time points for the M1 group ($^{\#}$ indicates a significant decrease from baseline to post pcTBS; $^{\#\#}$ indicates significant decrease from baseline to 24h-post pcTBS) and for the DLPFC group (* indicates a significant decrease from baseline to post pcTBS; ** indicates a significant decrease from baseline to 24h-post pcTBS). Panel A: BEEP Emotional Reaction to Pain. Panel B: BEEP Pain Interference. Panel C: BEEP Pain Limitations. There was no interaction effect for all the three BEEP subscales but there was a statistically significant effect of time for all the three subscales. Post hoc Bonferroni analyses revealed a significant decrease for both M1 and DLPFC for the BEEP Emotional Reaction to Pain scores from baseline to post pcTBS and baseline to 24h-post pcTBS. For the BEEP Pain Interference scores, only the DLPFC region demonstrated a significant decrease from baseline to 24h-post pcTBS.

Figure 4

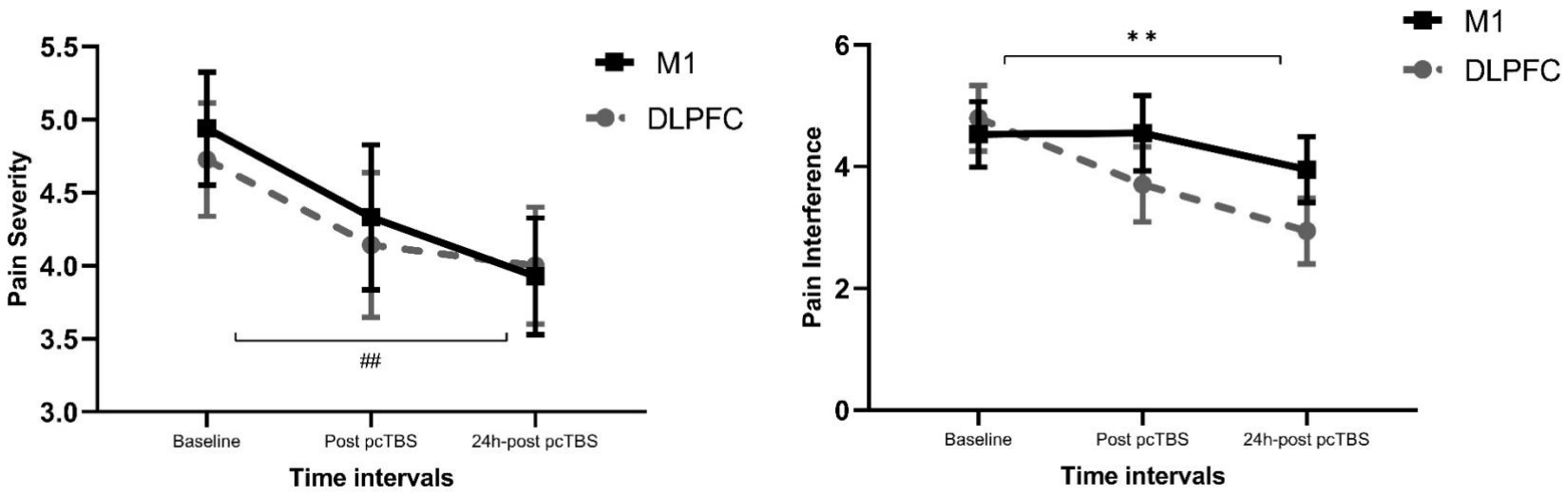
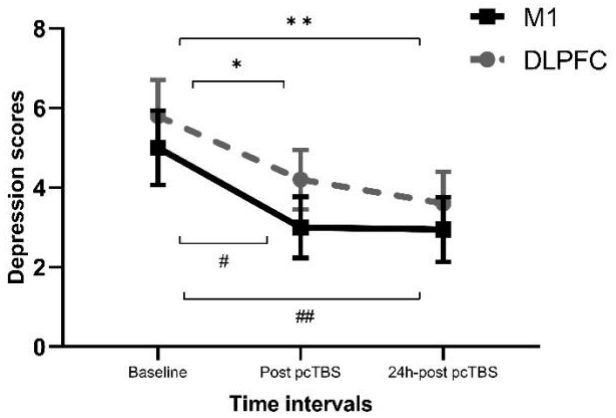
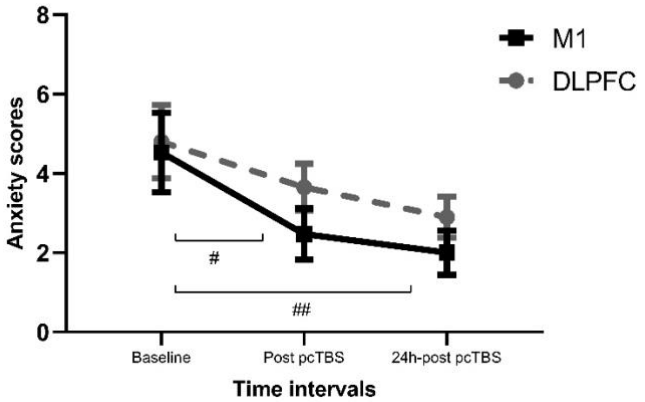


Figure 4: Effects of pcTBS at M1 and DLPFC on BPI-DN scores across the three time points. Panel A: BPI-DN Pain Severity. Panel B: BPI-DN Pain Interference. There was no interaction effect for both the BPI-DN subscales but there was a statistically significant effect of time. Post hoc Bonferroni analyses revealed a significant decrease for the M1 group brain region for Pain Severity scores from baseline to 24h-post pcTBS and for the Pain Interference scores for the DLPFC group brain region from baseline to 24h-post pcTBS.

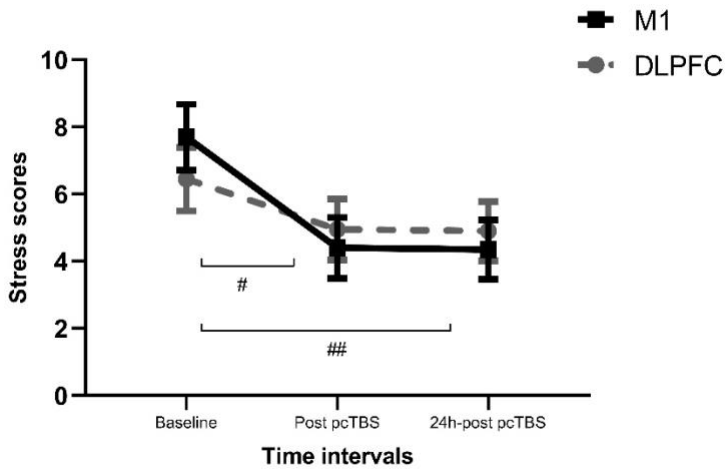
Figure 5



a



b



c

Figure 5: Effects of pcTBS at M1 and DLPFC on DASS-21 scores across the three time points. Panel A: BPI-DN Pain Severity. Panel A: DASS-21 Depression scores. Panel B: DASS-21 Anxiety scores. Panel C: DASS-21 Stress scores. There was no interaction effect for all the three DASS-21 subscales but there was a statistically significant effect of time for all the three subscales. Post hoc Bonferroni analyses revealed a significant decrease for both M1 and DLPFC for the DASS-21 Depression scores from baseline to post pcTBS and baseline to 24h-post pcTBS. For the DASS-21 Anxiety scores, only the DLPFC region demonstrated a significant decrease from baseline to 24h-post pcTBS.

Figure 6

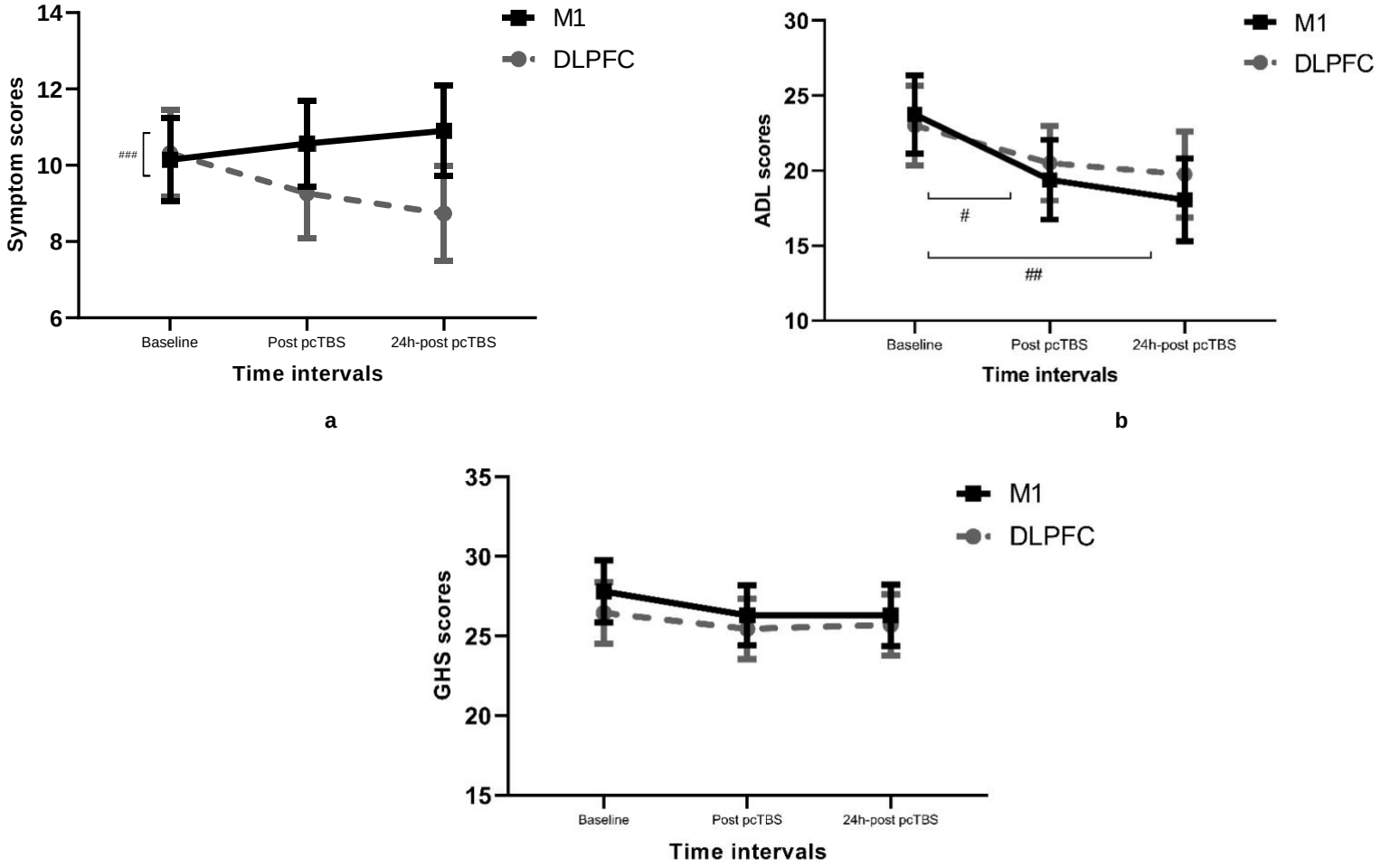


Figure 6: Effects of pcTBS at M1 and DLPFC on QOL-DN (### indicates a significant interaction effect for brain region and time). Panel A: QOL-DN Symptoms. Panel B: QOL-DN Activities of daily living (ADL). Panel C: QOL-DN Generic health status (GHS). There was a significant interaction effect for brain region and time for the QOL-DN-symptom subscale but there were no differences between the two groups. There was no interaction effect for the ADL and GHS subscales but there was a statistically significant effect of time on the ADL subscale. Post hoc Bonferroni analyses revealed a significant decrease for the M1 brain region group from baseline to post pcTBS and baseline to 24h-post pcTBS.

CHAPTER 4

Changes in Neural Mechanisms following Prolonged Continuous Theta Burst Stimulation Targeting M1 and DLPFC in Patients with Neuropathic pain.

To be submitted to The Journal of Pain as an Original Research Report.

Bhushan Thakkar, Carrie L. Peterson and Edmund Acevedo.

Changes in Neural Mechanisms following Prolonged Continuous Theta Burst Stimulation Targeting M1 and DLPFC in Patients with Neuropathic pain

Bhushan Thakkar [1] Carrie L. Peterson [2] and Edmund O. Acevedo [3]

1 Department of Physical Therapy, Virginia Commonwealth University, Richmond, VA

2 Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA

3 Department of Kinesiology and Health Sciences, Virginia Commonwealth University,

Richmond, VA

Pages: 43 with references

Tables: 1

Figures: 5

Corresponding Author:

Bhushan Thakkar

1101 East Marshall Street, 11th Floor, Room 11-027

Box #980034,

Richmond, VA 23298

Email: thakkarbs2@vcu.edu

Phone: 804-300-5578

Funding: This study was partially supported by a Grant in Aid of Research from Sigma Xi, The Scientific Research Honor Society. The project [REDCap and Research Datasets] was also supported by CTSA award No. UL1TR002649 from the National Center for Advancing Translational Sciences. Its contents are solely the responsibility of the authors and do not necessarily represent official views of the National Center for Advancing Translational Sciences or the National Institutes of Health.

'Declarations of interest: none'

To be submitted to The Journal of Pain as an Original Research Report.

An abstract of 200 words or less should describe concisely the purpose of the study, the main findings, and conclusions, all in one paragraph without subheadings. References may not be included in the abstract.

Perspective, this item, limited to 50 words, should appear at the end of the abstract. The perspective presents a synopsis of the work to facilitate understanding of its significance.

Limit the Introduction to 600 words and the Discussion to 1500 words.

Structured abstract

Background

A new paradigm for Transcranial Magnetic Stimulation (TMS), referred to as prolonged continuous theta burst stimulation (pcTBS), has recently received attention in the literature as a possible alternative to high frequency repetitive TMS (HF-rTMS). The clinical advantages of pcTBS include less time per intervention session and the effects appear to be more robust and reproducible than HF-rTMS. Both pcTBS and HF-rTMS have demonstrated analgesic effects, although the mechanisms of action are unclear and pcTBS has been studied primarily in healthy subjects. This study examined three neural mechanisms (the descending and ascending endogenous pain modulatory systems; corticospinal excitability; and intracortical inhibition) that have been proposed to play a role in explaining the effects of TMS by utilizing pcTBS targeted at the M1 and DLPFC brain regions in patients with neuropathic pain (NP).

Objective/Hypothesis

The present study hypothesized that following pcTBS at M1 and DLPFC, diabetic neuropathy patients with NP would report a decrease in pain perception and demonstrate an increase in the efficiency of the descending pain systems, a decrease in the activity of the ascending pain systems, an increase in corticospinal excitability (CE), and an increase in intracortical inhibition (ICI) measures linked to GABA activity.

Methods

Forty-two patients with painful diabetic neuropathy (pDN) were randomized to receive either pcTBS targeted at the M1 or the DLPFC brain region. Assessment of the descending pain modulatory system (conditioned pain modulation; CPM), the ascending pain modulatory system (temporal summation of pain; TSP), CE (motor evoked potential amplitude; MEP) and ICI (short and long intracortical inhibition) were examined at baseline and post-pcTBS stimulation for both

groups (M1 and DLPFC) using a two-way repeated measures mixed model ANOVA. This study was registered on the ClinicalTrials.gov website (ClinicalTrials.gov identifier: NCT04988321).

Results

The Brief Pain Inventory for patients with pDN revealed statistically significant improvements in pain perception following pcTBS at M1 and at DLPFC. In addition, pcTBS targeted at M1 and DLPFC had no effect on the descending and ascending pain modulatory systems (no change in CPM or TSP). CE, measured using MEP amplitude, and ICI measured using SICI and LICI, increased significantly from baseline to post-pcTBS for both the M1 and DLPFC groups.

Conclusion(s)

Results from this study suggested that neurophysiological mechanisms related to CE and neurochemical mechanisms linked to ICI (GABA activity) may explain the analgesic response to pcTBS stimulation at the M1 and DLPFC brain regions in patients with pDN.

Keywords

Chronic pain, neuromodulation, neuropathic pain, Intracortical inhibition, prolonged continuous theta burst stimulation, primary motor cortex, dorsolateral prefrontal cortex, painful diabetic neuropathy

1.0 Introduction

Epidemiological studies have reported that Neuropathic Pain (NP) affects 7%–10% of the general population, and accounts for almost 20-25% of patients with chronic pain [9,109]. NP is caused by a lesion or disease affecting the somatosensory nervous system; has a considerable impact on quality of life; and is associated with substantial morbidity and high economic burden on the individual and society [2,66,102]. Many studies have described how current treatments for NP are ineffective, including pharmacological treatments that are inadequate due to both poor efficacy and tolerability [2,10,109]. Despite the increasing focus on mechanism-based classification approaches, and the identification of disease-based phenotypes [1,43,70,107], the lack of an understanding of the pathophysiological mechanisms has undermined efforts to effectively develop treatment strategies that target the underlying mechanism or cause(s) of NP. To date, pharmacotherapy and non-pharmacological treatments have addressed psychophysical, neurophysiological, and neurochemical mechanisms. This study examines the effectiveness of a non-pharmacological treatment, transcranial magnetic stimulation (TMS), on NP, and variables linked to the aforementioned mechanisms.

TMS is a form of non-pharmacological treatment that has received much attention over the last two decades as a potential treatment for NP partially due to its non-invasive nature, excellent safety profile, and tolerability [71,81,87]. TMS is a form of brain stimulation that uses electromagnetic induction to excite or inhibit activity in a small area of the brain [12,93]. High frequency repetitive TMS (HF-rTMS) to the primary motor cortex (M1) has been utilized to modulate corticospinal excitability (CE), enhance intracortical inhibition (ICI) [37,108] and induce analgesic effects in patients with NP, experimental pain, and chronic pain [29,58,95]. In addition, HF-rTMS at the dorsolateral prefrontal cortex (DLPFC) has been approved by the FDA for the treatment of neurological conditions and neuropsychiatric disorders including depression [27,58,62], schizophrenia, and obsessive-compulsive disorder [20]. DLPFC stimulation has also

exhibited efficacy in reducing experimentally induced pain [100] and in various chronic pain conditions including fibromyalgia [19,51,83,106] and spinal cord injury related pain [84].

Theta burst stimulation (TBS), a newer rTMS paradigm, provides repetitive bursts of magnetic stimuli at a higher frequency, uses relatively lower intensity and the treatment protocol is much shorter (within 1-3 minutes) than HF-rTMS [46,104]. Furthermore, the effects of TBS seem to persist longer and the experience is less averse, suggesting that TBS may be a more effective, efficient, tolerable therapeutic intervention compared to HF-rTMS [18,104].

Although the mechanisms that explain the analgesic effects of HF-rTMS and pcTBS are unclear, previous studies have demonstrated that stimulating M1 and DLPFC separately modulates nociceptive pain processing via activation of descending pain modulation systems [23,56,79,106] and alterations in intracortical excitability [21,37,73]. The descending systems are inhibitory and composed of communications between the cortico-limbic structures and brain stem nuclei [30,88]. Lack of efficiency in these systems has been shown to contribute to chronic pain and NP [90,112–114]. The conditioned pain modulation (CPM) paradigm is a psychophysical pain protocol (PPP) used to assess the activity of the descending systems [64,90,112]. The ascending pain systems are also critical in processing pain stimuli [103]. Temporal summation of pain (TSP) is used to examine the activity of the ascending pain systems and is quantified with an increase in pain ratings after application of a repeated brief noxious stimuli (e.g., electrical, thermal, mechanical) [17]. In chronic pain patients with NP, separate studies have demonstrated an impaired CPM response (lack of efficiency in the descending pain systems), and an enhanced TSP response (diminished efficiency in the ascending pain systems) compared to asymptomatic controls [7,17,31,64,90]. Furthermore, in patients with chronic pain, two studies have examined these mechanisms at the same time and demonstrated impaired CPM and facilitated TSP in patients with chronic pain [39,86] .

Studies that have examined the neurophysiological mechanisms have utilized motor evoked potential (MEP) amplitude as a marker of CE [33,37,108]. MEP is the biphasic response that occurs when TMS is administered at M1 over the cortical representation of a specific muscle [50]. Stimulation generates an action potential which induces descending volleys in the pyramidal tract projecting on the spinal motoneurons [12,50,93]. Patients with chronic pain have demonstrated reductions in MEP amplitude at baseline followed by an increase in MEP amplitude following HF-rTMS [21,89]. Furthermore, in healthy participants following pcTBS, an increase in MEP amplitude following pcTBS has also been observed [8,15,21,25,60]. Thus, chronic pain exerts an inhibitory modulation on CE, reducing MEP amplitude that can be increased using HF-rTMS, and potentially pcTBS.

Lastly, ICI is an indirect marker for Gamma-aminobutyric acid (GABA), an important neurotransmitter in the central nervous system involved in pain transmission and perception [3,67]. GABA is a modulator of neural excitability mainly at the level of the dorsal horn in the spinal cord, via the activity of the GABA-A receptors and GABA-B receptors [54,67,74]. Their inhibitory activity modulates chloride influx and high voltage-gated Ca 2+ channels influx which plays an important role in spinal excitation, pain transmission and central sensitization [53,67]. A decrease in ICI depicts a decrease in the inhibitory neural activity resulting in an increase in pain transmission and pain perception. Figure 1 summarizes the neural mechanisms discussed above that modulate pain perception in patients with NP.

No study has examined pcTBS mechanisms in chronic pain patients and only a few studies have investigated these mechanisms utilizing HF-rTMS. For example, Agnol et al. [21], in patients with chronic myofascial pain syndrome, demonstrated that 10 sessions of HF-rTMS targeted at M1 was associated with a significant reduction in daily pain scores and use of analgesic medication. This effect was mediated by an increase in CE (increase in MEP amplitude). Furthermore, this improvement in pain scores was also mediated by the activity of

the descending pain modulatory systems measured using changes in quantitative sensory testing and CPM [21]. pcTBS was not utilized in this study. In another study in patients with myofascial pain syndrome that did not incorporate HF-rTMS or pcTBS, Botelho et al. [8] demonstrated at baseline that impaired CPM scores were correlated with greater MEP amplitude, decreased short intracortical inhibition (SICI) and lower heat pain thresholds. In a study that did compare the analgesic effects of pcTBS and HF-rTMS (10hz rTMS) targeted at DLPFC, De Martino et al. [69], demonstrated that 24 hours post stimulation both pcTBS and HF-rTMS revealed a similar increase in pain threshold, a decrease in pain sensitivity, and both were correlated with changes in CPM. Furthermore, this study conducted with healthy adults, revealed an increase in MEP and no change in SICI for both treatments [69]. Contrary to those results, Moisset et al [80] and Klirova et al [49] examining pcTBS at M1 in healthy subjects observed an increase in CE, but there were no changes in heat thresholds or CPM levels. These studies highlight the inconsistency in the type of TMS, participant characteristics, assessment techniques, and mechanisms of interest across studies.

A study that addresses these inconsistencies in protocol and includes an analysis of neural mechanisms will help to explain the analgesic effects and clinical benefits of pcTBS in NP. Therefore, the purpose of this study was to examine psychophysical mechanisms of pain modulation, neurophysiological measures of CE, and GABA activity mediated ICI in patients with NP, in response to pcTBS stimulation on the M1 and DLPFC regions of the brain. The present study hypothesized that following pcTBS at M1 and DLPFC, patients with NP would demonstrate an increase in the efficiency of the descending pain systems (measured using CPM), a decrease in the activity of the ascending pain systems (measured using TSP), increases in CE (quantified with MEP), and increases in ICI measures linked to GABA activity (quantified using SICI and LICI). To provide a more homogeneous population, participants diagnosed with type 2 diabetes and painful neuropathy were selected to participate in the study.

2.0 Materials and Methods

2.1. Participants

All participants provided written informed consent prior to participating in this study. This study was approved by the Virginia Commonwealth University (VCU) Institutional Review Board (HM20021531) and was also registered on the ClinicalTrials.gov website (NCT04988321). The inclusion criteria were (a) female or males aged over 18 and under 75 years, (b)Type 2 diabetes diagnosis, (c) pain for at least 6 months, (d) current pain of at least 4/10 on the visual analog scale, (d) fulfilling the criteria for >90% likelihood of NP with a score of >19 on the painDETECT Questionnaire (PD-Q) [28,96,111], and (e) if being treated pharmacologically, then stable pharmacological treatment for pain at least 1 month before inclusion. Patients were excluded if they had any active contraindications to rTMS (previous severe head trauma, head surgery or concussion, past or current epilepsy, active brain tumor, intracranial hypertension, implanted ferromagnetic devices; e.g. cardiac pacemaker, neurostimulator or cochlear implants, surgical clips or medical pumps) or any other form of NP. Participants were also excluded if they were unable to read or interpret instructions due to any language barriers and all females of childbearing age if they were pregnant or looking to be pregnant. Eligible patients were recruited from the VCU Health Hospital System in the Richmond, Virginia area.

2.2 Randomization

Upon enrollment, participants were randomized into two groups (M1 or DLPFC) in a 1:1 ratio. Participants in the M1 group received sham pcTBS at M1 followed by active pcTBS at MI and Participants in the DLPFC group received sham pcTBS at DLPFC followed by active pcTBS at DLPFC. Sham stimulation was always presented first with a 35-45 minute gap between sham and active pcTBS stimulation to avoid any carry over effects that could occur if active pcTBS stimulation was provided first. The study participants were blinded to the treatment sequence.

Figure 2 describes the data collection protocol for the two groups. Each session began with the participant completing of the Brief Pain Inventory for patients with diabetic neuropathy (BPI-DN) and identification of cortical hotspots for M1 or DLPFC. Then baseline measures of CE and ICI were collected, followed by sham pcTBS stimulation at M1 or DLPFC. The CE measures were recorded again and following this, active pcTBS (treatment) at M1 or DLPFC was performed. Lastly, CE and ICI were collected and responses to the BPI-DN were gathered. Each individual study session took 120-150 minutes to be completed. CE measures were recorded three times for every session whereas BPI-DN and ICI were measured twice for every session. All participants received a check of $50 as compensation for their time for participating in the study.

2.3 pcTBS Treatment

Participants were seated in a comfortable reclining chair with a tightly fitted hair dye cap placed over the head. They were instructed to keep their hands as relaxed as possible. pcTBS was performed using a Magstim Super Rapid2 Plus1 stimulator and a 70 mm double air film coil and the pcTBS protocol consisted of three pulses at 50 Hz (i.e. 60 ms) repeated 400 times at intervals of 200 ms (total of 1200 pulses in 1 min and 44 s) [46,69,80]. For the sham condition, a sham coil (Magstim 70mm double air film sham coil) (P/N: 3950-00, Magstim, Whitland, UK), looking identical to the active coil and making a similar noise but without delivering any active stimulation, was applied to the hotspot. Throughout each session participants' attention was redirected to a monitor presenting nature videos to limit focus on the stimulator and treatment. In addition, participants were offered the use of earplugs to limit the noise from the pcTBS treatment. The optimal site for evoking motor responses for the cortical hotspot for M1 was the abductor pollicis brevis (APB). A surface electromyography (EMG) electrode was placed on the right APB muscle. A grounding electrode was placed on the ulnar styloid. EMG signals were amplified (x1000) and bandpass-filtered using an AMT-8 amplifier (Bortec Biomedical) prior to A/D conversion which was sampled at 2 kHz. Single-pulse TMS was delivered to the

contralateral M1 brain region (Left M1) using a Magstim 200 stimulator and a 90 mm figure-of-eight coil. The coil was held tangentially on the scalp with the coil center rotated to induce a posterior-to-anterior current across the central sulcus. Identification of the cortical “hotspot” of the APB and site of subsequent stimulation was the location evoking the largest peak-to-peak motor evoked potential amplitude in the APB at the lowest stimulation intensity. pcTBS stimulation intensity was set to 80% of the resting motor threshold which was determined as the lowest stimulus intensity that induces motor evoked potential with an amplitude of ≥ 50 μV in at least 5 of 10 consecutive stimuli with the APB fully relaxed [50,93]. Stimulus intensity was determined using an adaptive parameter estimation by sequential testing (PEST) software [6]. PEST triangulates to a threshold with fewer stimulations to prevent overstimulation before pcTBS.

2.4 Identification of the Cortical Hotspot for DLPFC

The cortical hotspot for Left DLFPC was measured using the Beam F3 location system where F3 stands for hotspot location for DLPFC [4,75]. It utilizes three measurements: head circumference, nasion-inion distance, and left tragus-right tragus distance and uses an online calculator which then provides a polar-coordinate approximation of the F3 site with respect to the scalp vertex [4]. This method accounts for head size and shape and has a higher level of precision and reproducibility compared to other methods [75,110].

2.5 *Measurement of Intracortical Inhibition (ICI)*

Using paired pulse TMS, paired pulses were delivered randomly at interstimulus intervals of 2 ms and 4 ms for measuring SICI [21,60,80] and at 100 ms and155 ms for LICI [97,101], with the intensity of the first stimulus set at 80% of the RMT and the intensity of the second stimulus at 120% of the RMT [59,69,80]. For each measurement, the results of at least 4 trials were averaged, and the changes in test MEP induced by conditioned stimuli (paired pulses) were

expressed as a percentage of the control MEP (cMEP) amplitude at 120% [59]. SICI and LICI were expressed as the amount of inhibition (ICI=100%-pp(paired-pulses)/ cMEP%).

2.6 *Psychophysical pain protocols (PPP)*

Testing for CPM and TSP was performed on body locations that were without painful sensations: the forearm, trapezius, palm of the hand/wrist or shin area. Baseline pressure pain thresholds (PPT) were assessed using a handheld digital pressure algometer. Mechanical force was applied using a .5-cm^2 probe covered with polypropylene pressure-transducing material. Pressure was increased at a steady rate until the subject indicated that the pressure was "first perceived as painful." For CPM, the cold pressor test (CPT) was used as the conditional stimuli and participants immersed their contralateral (left) hand up to the wrist in a cold-water bath maintained at 4°C; a water temperature used as conditioning stimulus in previous CPM studies [61,64,68]. Twenty to 30 seconds after hand immersion, pressure equivalent to the PPT using the pressure algometer was applied on the contralateral hand (right hand, not in water) and participants again indicated when the increasing pressure stimulation first became painful. The pressure at this point represented their conditioned PPT. For each of the CPM trials, a CPM index was derived by calculating the percent ratio of PPT during CPT to PPT before CPT. Scores from the 2 CPM trials were averaged, and higher CPM scores (an increase in threshold) represents greater pain-inhibitory capacity [112,115].

To assess TSP, using the same pressure algometer device, ten identical pressure stimuli equivalent to a pressure at the individual's PPT level, with 1 s duration and 1 s inter-stimulus interval, participants were asked to rate their pain intensity for each of the 10 pressure stimuli using a VAS. For analysis of TSP, the mean VAS score was calculated in the interval from the first to the end of the fourth stimulus (VAS-I) and in the interval from the eighth to the end of the 10th stimulus (VAS-II). Temporal summation of pain was defined as the difference between

VAS-I and VAS-II (i.e. VAS-II minus VAS-I). This protocol has been used in previous studies that have assessed TSP in healthy subjects and in patients with chronic pain [7,44,82].

2.7 *Analysis of pcTBS data*

Quantification and analysis of CE and CI has been previously described [76–78]. Purpose-written code in MATLAB (MATLAB v 9.7.0.1190202) was used to calculate peak-to-peak MEP amplitudes from the motor target EMG data of each session. The root mean square (RMS) amplitude was then calculated for the evoked response over a 50 ms window (12-62 ms post TMS pulse), and for a 50 ms window prior to the TMS pulse (pre-stimulus). For instances where the pre-stimulus RMS exceeds the evoked response, RMS was excluded [22]. During each time interval in which MEPs was recorded, no more than 15 stimulations were delivered. MEPs were normalized and presented as a percentage of the recorded MVC value. Normalized MEPs (nMEPs) will serve as the measure of CE.

2.8 *Brief Pain Inventory for patients with diabetic neuropathy*

All study participants completed the Brief Pain Inventory (BPI) for patients with diabetic neuropathy (BPI-DN) questionnaire electronically on an iPad using REDCap electronic data capture tools [41,42] hosted at Virginia Commonwealth University. Data was collected at baseline and post-active pcTBS

2.9 *Blinding Questionnaire*

All the participants also completed a demographic questionnaire at baseline and blinding questionnaire at the end of the study session. More information about the questionnaire has been included in the Appendix. Briefly, the participants were asked the following questions as part of the blinding questionnaire:

“You received two forms of pcTBS treatment during this study, active and inactive, which one do you think you received first?

a. Active pcTBS

b. Inactive pcTBS

The participants were also asked a question about how certain they were with their ability to correctly guess the treatment using a visual analog scale based on the guidelines of Broadbent et al. [11] with 0 being active pcTBS and 100 corresponding to inactive pcTBS.

2.10 *Statistical Analysis*

Previous studies have reported standardized effect sizes of 0.45-0.47 for changes in MEP [8,15]. Using an effect size of 0.50 with power of 0.80 at the 0.05 significance level, the appropriate sample size was calculated to be 42. To account for attrition, 90 patients were recruited and 47 participants were enrolled. Twenty-one participants in each group completed the study. Demographic characteristics are presented and normality tests were performed using visual inspection and the Shapiro–Wilk test. Statistical Package for Social Science (SPSS®software, v. 28.1, IBM Corporation) was used for all statistical analysis with significance set at $p < 0.05$. Independent sample t-tests were conducted to look for differences in demographic characteristics for the two groups and results were interpreted using Levene's Test for Equality of Variances.

For variables that had a normal distribution, the middle 95% of the data was identified and outliers were eliminated if the data was outside the plus or minus 2 standard deviations of the mean. If the data was not normal, quartiles (Q1, lowest 25% of the data; Median, lowest 50% of data; Q3, lowest 75% of the data) were computed, and if any observation was less than Q1-1.5 X Interquartile range (equal to Q3 – Q1) or if it was greater than Q3+1.5 X Interquartile range, they were eliminated.

The dependent variables for the statistical analysis were BPI-DN, CPM, TSP, MEP, SICI at 2ms, SICI at 4ms, LICI at 100ms, and LICI at 155ms. The two independent variables were the

two brain regions, M1 and DLPFC, and the three time points of measurement; baseline, post-sham pcTBS and post-active pcTBS. A two-way mixed model repeated measures analysis of variance (RMANOVA; 2 brain regions by 2 time points) was conducted to evaluate the effects of pcTBS stimulation at M1 and DLPFC on BPI-DN. Likewise, a two-way mixed model RMANOVA (2 brain regions by 3 time points) was performed to evaluate the effects of pcTBS stimulation at M1 and DLPFC for MEP. Furthermore, a two-way mixed model RMANOVA (2 brain regions by 2 time points) was also conducted to evaluate the effects of pcTBS stimulation at M1 and DLPFC for each of the following variables: CPM, TSP, SICI at 2ms, SICI at 4ms, LICI at 100ms, and LICI at 155ms. The Greenhouse–Geisser approach was used to correct for violations of sphericity if the estimated epsilon (ε) was less than 0.75. Huynh-Feldt correction was used if ε was greater than 0.75. Effect sizes (partial eta-squared [η^2]) are reported for significant effects. Where appropriate, post hoc analyses were performed using a Bonferroni multiple comparison correction.

3. Results

3.1. Demographics

Participant demographics for the two groups are reported in Table 1. With 47 participants enrolled in the study and randomized to receive either pcTBS at M1 or pcTBS at DLPFC (see Figure 2), one participant was excluded from testing because they could not perceive any pressure anywhere in the hand/forearm/ thighs/shin with the pressure algometer. Another participant did not complete the CPM procedure because they had Raynaud's phenomenon and their symptoms could be exacerbated with exposure to cold. Two participants refused to participate in the post-pcTBS testing of CPM and TSP because of pain. In addition, two participants did not complete the study session due to an inability to locate the cortical hotspot for the M1 brain region.

(Insert Table 1 here)

3.2 Changes in BPI-DN scores

Two-way mixed model RMANOVA analyses examining the effects of pcTBS stimulation at M1 and DLPFC on the BPI-DN pain severity subscale and on the BPI-DN pain interference subscale revealed no significant interaction effects, $F(1,40) = 0.002$, $p = .963$ and $F(1,40) = 2.843$, $p = .100$, respectively. In addition, there was no main effect for time on the BPI-DN pain severity subscale and the BPI-DN pain interference subscale.

3.3 Changes in PPP

Figure 3 depicts the changes in PPP for the two brain regions post-pcTBS. A two-way mixed model RMANOVA examining the effects of pcTBS stimulation at M1 and DLPFC on the CPM scores, revealed no significant interaction effect for brain region and time, $F(1,36) = 0.044$, $p = .834$. Similarly, no effect was observed for pcTBS at M1 and DLPFC on the TSP scores, $F(1,36) = 2.060$, $p = .160$. Furthermore, there was no main effect of time or brain region on the CPM and TSP scores.

(Insert Figure 3 here)

3.4 Changes in CE

Figure 4 depicts the changes in MEP amplitude for the two brain regions from baseline to post-sham pcTBS, post-sham pcTBS to post-active pcTBS and baseline to post-active pcTBS. In contrast to CPM and TSP, the RMANOVA revealed that although there was no significant interaction effect for brain region and time, $F(2,76) = 2.198$, $p = .118$, there was a statistically significant main effect for time ($F[2,76] = 16.144$, $p = <.001$, partial $\eta 2 = .298$). Post hoc Bonferroni analyses revealed that for the M1 group, there was a significant decrease in MEP from baseline to post-sham pcTBS. There was a significant increase in MEP from post-sham pcTBS to post-active pcTBS. With regards to the DLPFC brain region, post hoc Bonferroni

analyses revealed that there was a significant increase in MEP from baseline to post-active pcTBS and from post-sham pcTBS to post-active pcTBS.

(Insert Figure 4 here)

3.5 Changes in ICI

Figure 5 depicts the changes in ICI (SICI at 2ms, SICI at 4ms, LICI at 100ms) for the two brain regions post-pcTBS. A two-way mixed model RMANOVA examining the effects of pcTBS stimulation at M1 and DLPFC on SICI at 2ms revealed no significant interaction effect, $F(1,31) = 2.594$, $p = .117$. There was no main effect for time or brain region on pcTBS stimulation at M1 and DLPFC on SICI at 2ms. In addition, pcTBS stimulation at M1 and DLPFC on SICI at 4ms revealed no significant interaction effect for brain region and time, $F(1,38) = 1.472$, $p = .233$. However, SICI at 4ms did result in a statistically significant effect for time ($F[1,38] = 17.713$, $p = <.001$, partial $\eta 2 = .318$) and for brain region ($F[1,38] = 5.564$, $p = 0.024$, partial $\eta 2 = .128$). Post hoc Bonferroni analyses revealed that for the M1 group there was a significant increase in SICI at 4ms from baseline to post-active pcTBS. Similarly, for the DLPFC group, there was a significant increase in SICI at 4ms from baseline to post-active pcTBS.

The effects of pcTBS stimulation at M1 and DLPFC on LICI at 100ms, revealed no significant interaction effect for brain region and time, $F(1,32) = 1.595$, $p = .216$. Although, there was a statistically significant effect for time ($F[1,32] = 13.586$, $p = <.001$, partial $\eta 2 = .298$) and brain region ($F[1,32] = 6.485$, $p = 0.024$, partial $\eta 2 = .169$) on LICI at 100ms. Post hoc Bonferroni analyses conducted and at both time points (baseline and post active pcTBS) revealed significant differences for LICI at 100ms for the M1 and DLPFC groups. Post hoc Bonferroni analyses for the M1 group also demonstrated a significant increase in LICI at 100ms from baseline to post-active pcTBS. There were no significant differences across time for the DLPFC group. With regards to the effects of pcTBS stimulation at M1 and DLPFC on LICI at 155ms, the RMANOVA revealed no significant interaction effect for brain region and time, $F(1,31) = 1.013$,

p = .322. There was a statistically significant effect for time (F [1,31] =12.48, p = .001, partial ηˆ2= .287) on LICI at 155ms. Post hoc Bonferroni analyses revealed that for the DLPFC group, there was a significant increase in LICI at 155ms from baseline to post-active pcTBS.

3.6 Relationship between CE and CI on BPI-DN

To further examine the statistically significant increases in CE (MEP) and ICI (SICI and LICI) for the two brain regions post pcTBS, a stepwise regression was utilized to investigate if these neural mechanisms, would predict scores on the BPI-DN at baseline for the entire study sample. The dependent variables were the BPI-DN pain severity and BPI-DN pain interference subscale scores and the independent variables were MEP, SICI at 2ms, SICI at 4ms, LICI at 100ms, and LICI at 155ms. The regression analysis revealed that, without including any demographic variables from Table 1, MEP, SICI and LICI did not predict any of the subscales at baseline. Similarly, when including the demographic of age, gender, BMI, race, PDQ score, duration of symptoms and current pain, as covariates, MEP, SICI, and LICI did not predict the BPI-DN subscales of pain severity and pain interference. At baseline, only current pain significantly predicted scores on the BPI-DN pain severity subscale with B= 0.406, standard error= 0.162, t = 2.502 and p= 0.017.

3.5 Blinding

Forty participants completed the blinding questionnaire. Fifteen participants (37.5%) reported that they received inactive pcTBS (sham pcTBS) first with 55% certainty whereas 25 participants (63.5%) reported receiving active pcTBS first with 44.44% certainty.

4.0 Discussion

This is the first study to examine neural mechanisms associated with pcTBS stimulation at the M1 and DLPFC brain regions in patients with NP. Results demonstrated that a single session of pcTBS at M1 for one group and at DLPFC for a second group alleviated pain perception, but did not influence the activity of the ascending and descending pain modulatory systems, measured

by CPM and TSP using a PPP, respectively. In addition, for both groups, stimulation elicited an increase in MEP amplitude, depicting an increase in CE. In addition, enhancement of GABA-A receptor activity measured using SICI at 4ms was observed following pcTBS at M1 and DLPFC, whereas GABA-B receptor activity, measured using LICI, increased for only the M1 brain region at 100ms and for the DLPFC brain region at 155ms.

In a systematic review that examined the effects of non-invasive brain stimulation on CPM and TSP, Giannoni-Luza et al summarized from seven studies that non-invasive brain stimulation techniques had a significant effect on CPM compared to sham, both in healthy subjects and patient populations [32]. Although, only three studies utilized rTMS targeted at the M1 brain region only two studies assessed TSP [32]. To some degree, contrary to that summary, the present study results demonstrated no differences in the activity of the descending pain systems and the ascending pain systems, measured using CPM and TSP, respectively, following a single session of pcTBS targeted at either M1 or DLPFC in patients with pDN.

There are at least two possible explanations for the lack of changes in the endogenous pain systems demonstrated in this study; pcTBS targeted at M1 and DLPFC does not influence the endogenous pain systems and/or patients with pDN have an efficient endogenous pain system at baseline. In regard to the first explanation, De Martino et al [69], in a study in healthy participants utilizing pcTBS targeted at DLPFC, after three sessions did not find any increase in the efficiency of the CPM. Similarly, Moisset et al. [80] targeted the M1 brain region and after one session of pcTBS in healthy participants, there was no change in CPM efficiency. The present study is unique in that CPM was assessed in a clinical population of NP patients and TSP was used to examine the ascending pain systems, whereas past studies have only assessed CPM in healthy subjects, and no study has evaluated changes in TSP at baseline and following pcTBS targeted at M1 and DLPFC.

Across the CPM literature, heterogeneity in the methodology of evoking CPM has also contributed to the discrepancies in results [26,32,34,91]. Pud et al. [91] summarized the methodological differences and proposed that discrepancies were linked to the type of test stimulus (utilizing a tonic, suprathreshold, pain threshold or pain tolerance approach), modality of conditioning and test stimuli (thermal, cold, pressure, ischemic), and the site of testing the conditional and test stimuli (affected vs unaffected body areas) [91]. In a review by Fernandes and colleagues to examine the concurrent validity of CPM with chronic pain, they reported that stimulation site was a critical factor influencing CPM results [26]. More specifically, testing on painful areas seems to alter results due to the level of sensitivity. In the present study, prior to CPM testing, participants were asked to identify non-painful areas. However, the influence of chronic pain, along with the presence of sensory loss, tingling, and numbness cannot be ruled out as possibly altering the measurement of the pressure pain threshold. This raises an important methodological issue to consider when assessing CPM in patients with peripheral neuropathic sensory changes.

Granovsky et al., in two separate studies [35,36], examined endogenous pain modulation using CPM in pDN patients and concluded that patients with pDN have a more efficient CPM compared to patients with nonpainful diabetic neuropathy. They also observed that longer pain duration (more than 2 years) in patients with pDN, was related to a more efficient CPM response and enhanced TSP response [35]. In the present study, the average pain duration was 5.5 years. Thus, it is possible that the duration and chronicity of NP could bring about alterations in the pain modulation systems, such that CPM and TSP no longer indicate changes in the descending and ascending pain system patients with pDN[35,36]. A correlation analysis of the present data for duration of pain and CPM scores for the entire study sample (N=44) was significant at $r = .373$ ($p= 0.013$). There was no relationship between TSP and duration of pain. Lastly, previous studies have also suggested that CPM is more efficient in

younger populations [40,92]. There were no significant correlations between Age and CPM and Age and TSP in the present study, and participants were middle age and older. Future studies should evaluate CPM and TSP in body areas without any alterations in sensory function and account for the chronic pain duration in pDN patients with NP.

Another psychophysical paradigm utilized to assess the descending pain systems is offset analgesia, defined as the disproportionate decrease in pain perception followed by a slight decrease in noxious stimulation [38,116]. This assessment can be complementary to CPM and is associated with a temporal filtering mechanism, while CPM utilizes a spatial filtering mechanism. Another possible advantage of using offset analgesia is that it can only be assessed using thermal stimuli, thus providing a more standardized protocol compared to CPM. Additional studies are needed to evaluate the utility of offset analgesia as a marker of the endogenous descending pain systems. Future studies should also evaluate the effectiveness of TSP and CPM following single and multiple sessions of pcTBS targeted at M1 or DLPFC in NP patients and other chronic pain populations.

Previous studies using pcTBS in healthy subjects have described similar increase in CE after a single session of pcTBS targeted at M1 [49,80] and after three sessions of pcTBS targeted at DLPFC [69]. The present study results highlight the role of facilitation of CE (increase in MEP) as a neurophysiological mechanism to help explain the effects (alleviation of pain) of pcTBS targeted at M1 and DLPFC in patients with NP [37,55]. In chronic pain patients, motor cortex reorganization contributes to the intensity of chronic pain; and pain relief has been correlated with reversal of the cortical reorganization [89]. The increase in CE causes increased excitability of the motor cortex and the corticospinal tract due to changes that potentially contribute to the reversal of cortical reorganization [57,105,106]. Furthermore, this increased excitability exerts inhibitory effects on the top-down endogenous system via activation of cortical and subcortical structures including the limbic region, anterior cingulate cortex, orbitofrontal

cortex, periaqueductal gray, thalamus, subcortical brain regions and the opioidergic systems involved in pain processing and perception [[5,23,55,72].

In previous studies that have demonstrated changes in CE following pcTBS at M1 [80] and DLPFC [69], no changes in in SICI at interstimulus intervals (2ms, 3ms and 4ms) were revealed. The present study results concur with no changes in SICI at 2ms, but with regard to SICI at 4ms, there was a significant increase, suggesting GABAergic inhibition following pcTBS at DLPFC. A decrease in SICI [13] has been observed at baseline in cross-sectional studies in patients with chronic NP [73,99], musculoskeletal pain [13] and experimental pain [98]. Similarly, following a single session of HF-rTMS targeted at the M1 brain region [60] an increase in SICI at different interstimulus intervals (2ms, 3ms and 4ms) from baseline has been demonstrated [59,60]. Only two studies [97,101] have assessed LICI in chronic pain conditions. With a cross-sectional analysis, Salerno (2000) examined LICI and found a significant reduction in LICI at 155ms when comparing patients with fibromyalgia and rheumatoid arthritis to healthy controls [97]. In another study, Siniatchkin et al. evaluated LICI at baseline using different interstimulus intervals (20, 60, 120 ms) in patients with migraine compared to healthy controls and found no differences [101]. The present study is the first study to investigated LICI at 100ms and 155ms at baseline and following a single session of pcTBS targeted at the M1 and DLPFC brain regions. Results suggest an increase in GABA-A receptor activity measured using SICI at 4ms following pcTBS at DLPFC and an increase in GABA-B activity following pcTBS at M1 and DLPFC measured using LICI at 100ms and 155ms. This demonstrates a reversal of the dysfunction in GABAergic inhibition at baseline seen with chronic pDN, following a single session of pcTBS at M1 and DLPFC.

Neuroimaging studies utilizing TMS and rTMS targeted at M1 and DLPFC have observed a correlation between presynaptic GABA levels in different brain regions and ICI markers (SICI and LICI) in healthy subjects [16] and in patients with neuropsychiatric disorders

such as schizophrenia [85], depression [24,63]. These studies also found altered presynaptic levels of GABA in the DLPFC brain region [16,24,63,85]. The results in the present study provide inconsistent results with regards to SICI measured at 2ms and 4ms and LICI at 100ms and 155ms. Future studies measuring SICI and LICI in chronic pain and in healthy subjects should standardize the assessment of the interstimulus intervals and stimulus intensities used to assess ICI.

Stagg et al. (2009) in a study using magnetic resonance spectroscopy revealed that continuous TBS over the primary motor cortex increased GABA levels without altering the levels of glutamate, suggesting that the increase in these presynaptic levels of GABA are driving the changes seen with continuous TBS. Furthermore, Huang et al [45,47,65,94] and Morales et al. [14] have highlighted the role of N-methyl-D-aspartate (NMDA) receptors [45], glutaminergic receptors and GABA receptors [47,65] in intracellular calcium dynamics that induce synaptic plasticity related changes in the mediation of long-term potentiation (quick and rapid influx) and long-term depression (slow and moderate) at the level of the post-synaptic terminal neuron [46,48]. More recently, Larson and Munkacsy (2015) elucidated the role of GABAergic circuits using modeling studies and demonstrated that bursts repeated at theta rhythm induce maximum long-term potentiation by disabling feedforward inhibition that involves presynaptic GABA receptors leadings to GABAergic inhibition [52]. To summarize, CE and ICI are critical in maintaining a balance between the cortical excitability and inhibitory networks in the brain involved in the modulation of pain perception and pcTBS targeted at M1 and DLPFC seems to restore this balance by inducing synaptic neuroplasticity, increasing neurotransmitter levels, and activating other cortical structures and circuits involved in pain perception. Future studies in patients with chronic pain should consider utilizing neuroimaging techniques to examine different brain regions and incorporate multiple sessions of pcTBS to clarify the role of GABA as a mediator of synaptic plasticity.

Although the participants in this study were homogeneous in regard to type of chronic pain, (a methodological advantage), external validity may have been compromised. Thus, additional studies with a larger number of patients who are experiencing various types of chronic pain are needed to assess the potential benefits of pcTBS in various clinical settings with various clinical populations. In addition, this was a single blind study and double-blind studies are necessary. Future studies should also consider utilizing a neuronavigational system to optimize the identification of cortical hotspots for the M1 brain region and the DLPFC brain region. As previously mentioned, future studies should incorporate multiple session to evaluate possible changes in the three mechanisms over time that may explain the efficacy of pcTBS targeted at M1 and DLPFC. Finally, during the experimental session, the sham stimulation was provided first to all the participants prior to active pcTBS for both the brain regions. This was done because a reversal of this order would have made it impractical to assess the effects of active pcTBS, however, an order effect cannot be ruled out and future studies could incorporate a sham session on a different day with greater time between the sham and treatment sessions. Finally, the results from the blinding questionnaire revealed that more than 60% (n=25/40, 63.5%) of the participants guessed the order of stimulation incorrectly, suggesting that patients weren't aware of the study protocol and that sham stimulation was effective.

5.0 Conclusion

Noninvasive brain stimulation using HF-rTMS targeted at the M1 and DLPFC brain regions has demonstrated positive therapeutic outcomes in patients with NP. Recently, a newer form of rTMS has been developed, pcTBS, which is much shorter, taking 1–3 min to apply, and uses lower intensities of stimuli. Thus, this protocol is generally considered more amenable to patients and clinicians. The mechanisms of action for pcTBS are unclear and it has only been utilized in healthy participants to examine its analgesic effects and thus it is critical to gain a better understanding of the cortical and spinal mechanisms underlying the efficacy of pcTBS in

patients with NP. This is of considerable interest to determine why the intervention is effective and identifying the most effective pain management strategies. The present study highlights a link between the neurophysiological mechanisms of CE and GABA activity (utilizing ICI measures) after one session of pcTBS targeted at M1 and DLPFC. Future studies should incorporate multiple treatment sessions, different subject populations and neuroimaging methods to further elucidate the mechanisms that govern the efficacy of pcTBS targeted at M1 and DLPFC.

References

[1] Attal N, Fermanian C, Fermanian J, Lanteri-Minet M, Alchaar H, Bouhassira D. Neuropathic pain: Are there distinct subtypes depending on the aetiology or anatomical lesion? Pain 2008;138:343–353.

[2] Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. The specific disease burden of neuropathic pain: Results of a French nationwide survey. Pain 2011;152:2836–2843.

[3] Barr MS, Farzan F, Davis KD, Fitzgerald PB, Daskalakis ZJ. Measuring GAB aergic inhibitory activity with TMS-EEG and its potential clinical application for chronic pain. Journal of Neuroimmune Pharmacology 2013;8:535–546.

[4] Beam W, Borckardt JJ, Reeves ST, George MS. An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. Brain Stimul 2009;2:50–54. doi:10.1016/j.brs.2008.09.006.

[5] Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J. Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. European Journal of Neuroscience 2004;19:1950–1962.

[6] Borckardt JJ, Nahas Z, Koola J, George MS. Estimating resting motor thresholds in transcranial magnetic stimulation research and practice: A computer simulation evaluation of best methods. Journal of ECT 2006;22:169–175. doi:10.1097/01.yct.0000235923.52741.72.

[7] Bosma RL, Cheng JC, Rogachov A, Kim JA, Hemington KS, Osborne NR, Venkat Raghavan L, Bhatia A, Davis KD. Brain Dynamics and Temporal Summation of Pain Predicts Neuropathic Pain Relief from Ketamine Infusion. Anesthesiology 2018;129:1015–1024.

[8] Botelho LM, Morales-Quezada L, Rozisky JR, Brietzke AP, Torres ILS, Deitos A, Fregni F, Caumo W. A framework for understanding the relationship between descending pain modulation, motor corticospinal, and neuroplasticity regulation systems in chronic myofascial pain. Front Hum Neurosci 2016;10:1–12.

[9] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain 2008;136:380–387.

[10] Bouhassira D, Letanoux M, Hartemann A. Chronic Pain with Neuropathic Characteristics in Diabetic Patients: A French Cross-Sectional Study. PLoS One 2013;8:1–9.

[11] Broadbent HJ, van den Eynde F, Guillaume S, Hanif EL, Stahl D, David AS, Campbell IC, Schmidt U. Blinding success of rTMS applied to the dorsolateral prefrontal cortex in randomised sham-controlled trials: A systematic review. World Journal of Biological Psychiatry 2011;12:240–248.

[12] Burke MJ, Fried PJ, Pascual-Leone A. Transcranial magnetic stimulation: Neurophysiological and clinical applications. Handb Clin Neurol 2019;163:73–92.

[13] Burns E, Chipchase LS, Schabrun SM. Altered function of intracortical networks in chronic lateral epicondylalgia. European Journal of Pain (United Kingdom) 2016;20:1166–1175.

[14] Cárdenas-Morales L, Nowak DA, Kammer T, Wolf RC, Schönfeldt-Lecuona C. Mechanisms and applications of theta-burst rTMS on the human motor cortex. Brain Topogr 2010;22:294–306.

[15] Cardinal TM, Antunes LC, Brietzke AP, Parizotti CS, Carvalho F, de Souza A, da Silva Torres IL, Fregni F, Caumo W. Differential neuroplastic changes in fibromyalgia and depression indexed by up-regulation of motor cortex inhibition and disinhibition of the descending pain system: An exploratory study. Front Hum Neurosci 2019;13:1–13.

[16] Cash RFH, Noda Y, Zomorrodi R, Radhu N, Farzan F, Rajji TK, Fitzgerald PB, Chen R, Daskalakis ZJ, Blumberger DM. Characterization of Glutamatergic and GABA A-Mediated Neurotransmission in Motor and Dorsolateral Prefrontal Cortex Using Paired-Pulse TMS-EEG. Neuropsychopharmacology 2017;42:502–511.

[17] Cheng JC, Erpelding N, Kucyi A, Desouza DD, Davis KD. Individual differences in temporal summation of pain reflect pronociceptive and antinociceptive brain structure and function. Journal of Neuroscience 2015;35:9689–9700.

[18] Chung SW, Hill AT, Rogasch NC, Hoy KE, Fitzgerald PB. Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis. Neurosci Biobehav Rev 2016;63:43–64. doi:10.1016/j.neubiorev.2016.01.008.

[19] Ciampi De Andrade D, Mhalla A, Adam F, Texeira MJ, Bouhassira D. Repetitive transcranial magnetic stimulation induced analgesia depends on N-methyl-d-aspartate glutamate receptors. Pain 2014;155:598–605. doi:10.1016/j.pain.2013.12.022.

[20] Cohen SL, Bikson M, Badran BW, George MS. A visual and narrative timeline of US FDA milestones for Transcranial Magnetic Stimulation (TMS) devices. Brain Stimul 2022;15:73–75.

[21] Dall'Agnol L, Medeiros LF, Torres ILS, Deitos A, Brietzke A, Laste G, de Souza A, Vieira JL, Fregni F, Caumo W. Repetitive transcranial magnetic stimulation increases the corticospinal inhibition and the brain-derived neurotrophic factor in chronic myofascial pain syndrome: An explanatory double-blinded, randomized, sham-controlled trial. Journal of Pain 2014;15:845–855.

[22] Darling WG, Wolf SL, Butler AJ. Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. Exp Brain Res 2006;174:376–385. doi:10.1007/s00221-006-0468-9.

[23] DosSantos MF, Ferreira N, Toback RL, Carvalho AC, DaSilva AF. Potential Mechanisms Supporting the Value of Motor Cortex Stimulation to Treat Chronic Pain Syndromes. Front Neurosci 2016;10:18.

[24] Dubin MJ, Mao X, Banerjee S, Goodman Z, Lapidus KAB, Kang G, Liston C, Shungu DC. Elevated prefrontal cortex GABA in patients with major depressive disorder after TMS treatment measured with proton magnetic resonance spectroscopy. Journal of Psychiatry and Neuroscience 2016;41:E37–E45.

[25] Dussán-Sarria JA, da Silva NRJ, Deitos A, Stefani LC, Laste G, de Souza A, Torres ILS, Fregni F, Caumo W. Higher cortical facilitation and serum BDNF are associated with increased sensitivity to heat pain and reduced endogenous pain inhibition in healthy males. Pain Medicine (United States) 2018;19:1578–1586.

[26] Fernandes C, Pidal-Miranda M, Samartin-Veiga N, Carrillo-De-La-Peña MT. Conditioned pain modulation as a biomarker of chronic pain: A systematic review of its concurrent validity. Pain 2019;160:2679–2690.

[27] Fitzgerald PB, Brown TL, Marston NAU, Daskalakis ZJ, de Castella A, Kulkarni J. Transcranial Magnetic Stimulation in the Treatment of Depression: A Double-blind,

Placebo-Controlled Trial. Arch Gen Psychiatry 2003;60:1002–1008. doi:10.1001/archpsyc.60.9.1002.

[28] Freynhagen R, Baron R, Gockel U, Tölle TR. painDETECT: A new screening questionnaire to identify neuropathic components in patients with back pain. Curr Med Res Opin 2006;22:1911–1920. doi:10.1185/030079906X132488.

[29] Gatzinsky K, Bergh C, Liljegren A, Silander H, Samuelsson J, Svanberg T, Samuelsson O. Repetitive transcranial magnetic stimulation of the primary motor cortex in management of chronic neuropathic pain: A systematic review. Scand J Pain 2021;21:8–21.

[30] Gebhart GF. Descending modulation of pain. Neurosci Biobehav Rev 2004;27:729–737.

[31] Gerhardt A, Eich W, Treede R-D, Tesarz J. Conditioned pain modulation in patients with nonspecific chronic back pain with chronic local pain, chronic widespread pain, and fibromyalgia. Pain 2017;158:430–439.

[32] Giannoni-Luza S, Pacheco-Barrios K, Cardenas-Rojas A, Mejia-Pando PF, Luna-Cuadros MA, Barouh JL, Gnoatto-Medeiros M, Candido-Santos L, Barra A, Caumo W, Fregni F. Non-invasive motor cortex stimulation effects on quantitative sensory testing (QST) in healthy and chronic pain subjects: a systematic review and meta-analysis HHS Public Access. Pain 2020;161:1955–1975.

[33] Goldsworthy MR, Vallence AM, Hodyl NA, Semmler JG, Pitcher JB, Ridding MC. Probing changes in corticospinal excitability following theta burst stimulation of the human primary motor cortex. Clinical Neurophysiology 2016;127:740–747. doi:10.1016/j.clinph.2015.06.014.

[34] Granovsky Y. Conditioned pain modulation: A predictor for development and treatment of neuropathic pain. Curr Pain Headache Rep 2013;17.

[35] Granovsky Y, Nahman-Averbuch H, Khamaisi M, Granot M. Efficient conditioned pain modulation despite pain persistence in painful diabetic neuropathy. Pain Rep 2017;2:1–7.

[36] Granovsky Y, Shafran Topaz L, Laycock H, Zubiedat R, Crystal S, Buxbaum C, Bosak N, Hadad R, Domany E, Khamaisi M, Sprecher E, Bennett DL, Rice A, Yarnitsky D. Conditioned pain modulation is more efficient in patients with painful diabetic polyneuropathy than those with nonpainful diabetic polyneuropathy. Pain 2022;163:827–833. doi:10.1097/J.PAIN.0000000000002434.

[37] Granovsky Y, Sprecher E, Sinai A. Motor corticospinal excitability: A novel facet of pain modulation? Pain Rep 2019;4:1–7.

[38] Grill JD, Coghill RC. Transient analgesia evoked by noxious stimulus offset. J Neurophysiol 2002;87:2205–2208.

[39] Hackett J, Naugle KE, Naugle KM. The Decline of Endogenous Pain Modulation With Aging: A Meta-Analysis of Temporal Summation and Conditioned Pain Modulation. The journal of pain : official journal of the American Pain Society 2019.

[40] Hackett J, Naugle KE, Naugle KM. The Decline of Endogenous Pain Modulation With Aging: A Meta-Analysis of Temporal Summation and Conditioned Pain Modulation. 2020. doi:10.1016/j.jpain.2019.09.005.

[41] Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform 2019;95.

[42] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377–381.

[43] von Hehn CA, Baron R, Woolf CJ. Deconstructing the Neuropathic Pain Phenotype to Reveal Neural Mechanisms. Neuron 2012;73:638–652.

[44] Horn-Hofmann C, Kunz M, Madden M, Schnabel EL, Lautenbacher S. Interactive effects of conditioned pain modulation and temporal summation of pain-the role of stimulus modality. Pain 2018;159:2641–2648.

[45] Huang YZ, Chen RS, Rothwell JC, Wen HY. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clinical Neurophysiology 2007;118:1028–1032.

[46] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–206.

[47] Huang YZ, Rothwell JC, Chen RS, Lu CS, Chuang WL. The theoretical model of theta burst form of repetitive transcranial magnetic stimulation. Clinical Neurophysiology 2011;122:1011–1018.

[48] Huang YZ, Rothwell JC, Lu CS, Wang JJ, Weng YH, Lai SC, Chuang WL, Hung J, Chen RS. The effect of continuous theta burst stimulation over premotor cortex on circuits in primary motor cortex and spinal cord. Clinical Neurophysiology 2009;120:796–801.

[49] Klírová M, Hejzlar M, Kostýlková L, Mohr P, Rokyta R, Novák T. Prolonged Continuous Theta Burst Stimulation of the Motor Cortex Modulates Cortical Excitability But not Pain Perception. Front Syst Neurosci 2020;14:1–9.

[50] Klomjai W, Katz R, Lackmy-Vallée A. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). Ann Phys Rehabil Med 2015;58:208–213.

[51] Larsen DB, Graven-Nielsen T, Hirata RP, Seminowicz D, Schabrun S, Boudreau SA. Corticomotor excitability reduction induced by experimental pain remains unaffected by performing a working memory task as compared to staying at rest. Exp Brain Res 2019;237:2205–2215.

[52] Larson J, Munkácsy E. Theta-burst LTP. Brain Res 2015;1621:38–50.

[53] Latremoliere A, Woolf CJ. Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. J Pain 2009;10:895–926.

[54] di Lazzaro V, Pilato F, Dileone M, Ranieri F, Ricci V, Profice P, Bria P, Tonali PA, Ziemann U. GABAA receptor subtype specific enhancement of inhibition in human motor cortex. Journal of Physiology 2006;575:721–726.

[55] Lefaucheur JP. Cortical neurostimulation for neuropathic pain: State of the art and perspectives. Pain 2016;157:S81–S89.

[56] Lefaucheur JP. Pain. Handb Clin Neurol 2013;116:423–440.

[57] Lefaucheur JP. The use of repetitive transcranial magnetic stimulation (rTMS) in chronic neuropathic pain. Neurophysiologie Clinique 2006;36:117–124.

[58] Lefaucheur J-P, Aleman A, Baeken C, Benninger DH, Brunelin J, di Lazzaro V, Filipović SR, Grefkes C, Hasan A, Hummel FC, Jääskeläinen SK, Langguth B, Leocani L, Londero A, Nardone R, Nguyen J-P, Nyffeler T, Oliveira-Maia AJ, Oliviero A, Padberg F, Palm U,

Paulus W, Poulet E, Quartarone A, Rachid F, Rektorová I, Rossi S, Sahlsten H, Schecklmann M, Szekely D, Ziemann U. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clinical Neurophysiology 2020;131:474–528. doi:https://doi.org/10.1016/j.clinph.2019.11.002.

[59] Lefaucheur JP, Ayache SS, Sorel M, Farhat WH, Zouari HG, Ciampi De Andrade D, Ahdab R, Ménard-Lefaucheur I, Brugières P, Goujon C. Analgesic effects of repetitive transcranial magnetic stimulation of the motor cortex in neuropathic pain: Influence of theta burst stimulation priming. European Journal of Pain (United Kingdom) 2012;16:1403–1413.

[60] Lefaucheur JP, Drouot X, Ménard-Lefaucheur I, Keravel Y, Nguyen JP. Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. Neurology 2006;67:1568–1574.

[61] Leone C, Truini A. The CPM Effect: Functional Assessment of the Diffuse Noxious Inhibitory Control in Humans. Journal of Clinical Neurophysiology 2019;36:430–436.

[62] Leung A, Shirvalkar P, Chen R, Kuluva J, Vaninetti M, Bermudes R, Poree L, Wassermann E, Kopell B, Levy R. Transcranial Magnetic Stimulation for Pain, Headache, and Comorbid Depression: INS-NANS Expert Consensus Panel Review and Recommendation. Neuromodulation 2020;2019:267–290.

[63] Levitt JG, Kalender G, O'Neill J, Diaz JP, Cook IA, Ginder N, Krantz D, Minzenberg MJ, Vince-Cruz N, Nguyen LD, Alger JR, Leuchter AF. Dorsolateral prefrontal γ-aminobutyric acid in patients with treatment-resistant depression after transcranial magnetic stimulation measured with magnetic resonance spectroscopy. Journal of Psychiatry and Neuroscience 2019;44:386–394.

[64] Lewis GN, Rice DA, McNair PJ. Conditioned pain modulation in populations with chronic pain: A systematic review and meta-analysis. Journal of Pain 2012;13:936–944. doi:10.1016/j.jpain.2012.07.005.

[65] Li CT, Huang YZ, Bai YM, Tsai SJ, Su TP, Cheng CM. Critical role of glutamatergic and GABAergic neurotransmission in the central mechanisms of theta-burst stimulation. Hum Brain Mapp 2019;40:2001–2009.

[66] Luana Colloca1, Taylor Ludman1, Didier Bouhassira2, Ralf Baron3, Anthony H. Dickenson4, David Yarnitsky5, Roy Freeman6, Andrea Truini7, Nadine Attal8, Nanna B. Finnerup9, Christopher Eccleston10, 11, Eija Kalso12, David L. Bennett13, Robert H. Dworkin14 and SNR. Neuropathic Pain. Nat Rev Dis Primers 2017:749–757.

[67] Malcangio M. GABAB receptors and pain. Neuropharmacology 2018;136:102–105.

[68] Martel MO, Petersen K, Cornelius M, Arendt-Nielsen L, Edwards R. Endogenous Pain Modulation Profiles Among Individuals With Chronic Pain: Relation to Opioid Use. Journal of Pain 2019;20:462–471. doi:10.1016/j.jpain.2018.10.004.

[69] de Martino E, Fernandes AM, Galhardoni R, de Oliveira Souza C, Ciampi De Andrade D, Graven-Nielsen T. Sessions of Prolonged Continuous Theta Burst Stimulation or High-frequency 10 Hz Stimulation to Left Dorsolateral Prefrontal Cortex for 3 Days Decreased Pain Sensitivity by Modulation of the Efficacy of Conditioned Pain Modulation. Journal of Pain 2019;20:1459–1469.

[70] Max MB. Towards physiologically based treatment of patients with neuropathic pain. Pain 1990;42:131–133.

[71] Meeker TJ, Jupudi R, Lenz FA, Greenspan JD. New Developments in Non-invasive Brain Stimulation in Chronic Pain. Curr Phys Med Rehabil Rep 2020;8:280–292.

[72] Meeker TJ, Keaser ML, Khan SA, Gullapalli RP, Seminowicz DA, Greenspan JD. Non-invasive Motor Cortex Neuromodulation Reduces Secondary Hyperalgesia and Enhances Activation of the Descending Pain Modulatory Network. Front Neurosci 2019;13:467.

[73] Mhalla A, de Andrade DC, Baudic S, Perrot S, Bouhassira D. Alteration of cortical excitability in patients with fibromyalgia. Pain 2010;149:495–500. doi:10.1016/j.pain.2010.03.009.

[74] Michelle N Mcdonnell, Orekhov Y, Ziemann U. The role of GABA B receptors in intracortical inhibition in the human motor cortex. Exp Brain Res 2006;173:86–93.

[75] Mir-Moghtadaei A, Caballero R, Fried P, Fox MD, Lee K, Giacobbe P, Daskalakis ZJ, Blumberger DM, Downar J. Concordance between BeamF3 and MRI-neuronavigated target sites for repetitive transcranial magnetic stimulation of the left dorsolateral prefrontal cortex. Brain Stimul 2015;8:965–973.

[76] Mittal N, Majdic BC, Peterson CL. Intermittent theta burst stimulation modulates biceps brachii corticomotor excitability in individuals with tetraplegia. J Neuroeng Rehabil 2022;19:73. doi:10.1186/s12984-022-01049-9.

[77] Mittal N, Majdic BC, Sima AP, Peterson CL. The effect of intermittent theta burst stimulation on corticomotor excitability of the biceps brachii in nonimpaired individuals. Neurosci Lett 2021;764.

[78] Mittal N, Thakkar B, Hodges CB, Lewis C, Cho Y, Hadimani RL, Peterson CL. Effect of neuroanatomy on corticomotor excitability during and after transcranial magnetic stimulation and intermittent theta burst stimulation. Hum Brain Mapp 2022. doi:10.1002/hbm.25968.

[79] Moisset X, de Andrade DC, Bouhassira D. From pulses to pain relief: An update on the mechanisms of rTMS-induced analgesic effects. European Journal of Pain (United Kingdom) 2016;20:689–700.

[80] Moisset X, Goudeau S, Poindessous-Jazat F, Baudic S, Clavelou P, Bouhassira D. Prolonged continuous theta-burst stimulation is more analgesic than “classical” high frequency repetitive transcranial magnetic stimulation. Brain Stimul 2015;8:135–141. doi:10.1016/j.brs.2014.10.006.

[81] Moisset X, Lefaucheur JP. Non pharmacological treatment for neuropathic pain: Invasive and non-invasive cortical stimulation. Rev Neurol (Paris) 2019;175:51–58.

[82] Nahman-Averbuch H, Martucci KT, Granovsky Y, Weissman-Fogel I, Yarnitsky D, Coghill RC. Distinct brain mechanisms support spatial vs temporal filtering of nociceptive information. Pain 2014;155:2491–2501.

[83] Nahmias F, Debes C, de Andrade DC, Mhalla A, Bouhassira D. Diffuse analgesic effects of unilateral repetitive transcranial magnetic stimulation (rTMS) in healthy volunteers. Pain 2009;147:224–232.

[84] Nardone R, Höller Y, Langthaler PB, Lochner P, Golaszewski S, Schwenker K, Brigo F, Trinka E. rTMS of the prefrontal cortex has analgesic effects on neuropathic pain in subjects with spinal cord injury. 2016. doi:10.1038/sc.2016.87.

[85] Noda Y, Barr MS, Zomorrodi R, Cash RFH, Farzan F, Rajji TK, Chen R, Daskalakis ZJ, Blumberger DM. Evaluation of short interval cortical inhibition and intracortical facilitation from the dorsolateral prefrontal cortex in patients with schizophrenia. Sci Rep 2017;7.

[86] O'Brien AT, Deitos A, Triñanes Pego Y, Fregni F, Carrillo-de-la-Peña MT. Defective Endogenous Pain Modulation in Fibromyalgia: A Meta-Analysis of Temporal Summation and Conditioned Pain Modulation Paradigms. Journal of Pain 2018;19:819–836. doi:10.1016/j.jpain.2018.01.010.

[87] O'Connell NE, Marston L, Spencer S, DeSouza LH, Wand BM. Non-invasive brain stimulation techniques for chronic pain. Cochrane Database Syst Rev 2018;4:CD008208.

[88] Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronification of pain. Curr Opin Support Palliat Care 2014;8:143–151.

[89] Parker RS, Lewis GN, Rice DA, Mcnair PJ. Is Motor Cortical Excitability Altered in People with Chronic Pain? A Systematic Review and Meta-Analysis. Brain Stimul 2016;9:488–500.

[90] Petersen KK, McPhee ME, Hoegh MS, Graven-Nielsen T. Assessment of conditioned pain modulation in healthy participants and patients with chronic pain: manifestations and implications for pain progression. Curr Opin Support Palliat Care 2019;13:99–106.

[91] Pud D, Granovsky Y, Yarnitsky D. The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. Pain 2009;144:16–19.

[92] Riley JL, Cruz-Almeida Y, Staud R, Fillingim RB. Age does not affect sex effect of conditioned pain modulation of pressure and thermal pain across 2 conditioning stimuli. Pain Rep 2019;5:E796. doi:10.1097/PR9.0000000000000796.

[93] Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, di Iorio R, di Lazzaro V, Ferreri F, Fitzgerald PB, George MS, Hallett M, Lefaucheur JP, Langguth B, Matsumoto H, Miniussi C, Nitsche MA, Pascual-Leone A, Paulus W, Rossi S, Rothwell JC, Siebner HR, Ugawa Y, Walsh V, Ziemann U. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee. Clinical Neurophysiology 2015;126:1071–1107. doi:10.1016/j.clinph.2015.02.001.

[94] Rounis E, Huang YZ. Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease. Exp Brain Res 2020;238:1707–1714.

[95] Sacco P, Prior M, Poole H, Nurmikko T. Repetitive transcranial magnetic stimulation over primary motor vs non-motor cortical targets; effects on experimental hyperalgesia in healthy subjects. BMC Neurol 2014;14:1–8.

[96] Sadosky A, Cappelleri J, Bienen EJ, Koduru V. Measurement properties of painDETECT by average pain severity. ClinicoEconomics and Outcomes Research 2014:497.

[97] Salerno A, Thomas E, Olive P, Blotman F, Picot MC, Georgesco M. Motor cortical dysfunction disclosed by single and double magnetic stimulation in patients with fibromyalgia. Clinical Neurophysiology 2000;111:994–1001.

[98] Schabrun SM, Christensen SW, Mrachacz-Kersting N, Graven-Nielsen T. Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain. Cereb Cortex 2016;26:1878–1890. doi:10.1093/CERCOR/BHU319.

[99] Schwenkreis P, Scherens A, Rönnau AK, Höffken O, Tegenthoff M, Maier C. Cortical disinhibition occurs in chronic neuropathic, but not in chronic nociceptive pain. BMC Neurosci 2010;11.

[100] Seminowicz DA, de Martino E, Schabrun SM, Graven-Nielsen T. Left dorsolateral prefrontal cortex repetitive transcranial magnetic stimulation reduces the development of long-term muscle pain. Pain 2018;159:2486–2492. doi:10.1097/J.PAIN.0000000000001350.

[101] Siniatchkin M, Kröner-Herwig B, Kocabiyik E, Rothenberger A. Intracortical inhibition and facilitation in migraine - A transcranial magnetic stimulation study. Headache 2007;47:364–370.

[102] Smith BH, Torrance N, Bennett MI, Lee AJ. Health and quality of life associated with chronic pain of predominantly neuropathic origin in the community. Clinical Journal of Pain 2007;23:143–149.

[103] Staud R. Abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions. Expert Rev Neurother 2012;12:577–585.

[104] Suppa A, Huang YZ, Funke K, Ridding MC, Cheeran B, di Lazzaro V, Ziemann U, Rothwell JC. Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. Brain Stimul 2016;9:323–335.

[105] Taylor JJ, Borckardt JJ, Canterberry M, Li X, Hanlon CA, Brown TR, George MS. Naloxone-Reversible Modulation of Pain Circuitry by Left Prefrontal rTMS. Neuropsychopharmacology 2013;38:1189–1197. doi:10.1038/npp.2013.13.

[106] Taylor JJ, Borckardt JJ, George MS. Endogenous opioids mediate left dorsolateral prefrontal cortex rTMS-induced analgesia. Pain 2012;153:1219–1225. doi:https://doi.org/10.1016/j.pain.2012.02.030.

[107] Themistocleous AC, Crombez G, Baskozos G, Bennett DL. Using stratified medicine to understand, diagnose, and treat neuropathic pain. Pain 2018;159:S31–S42.

[108] Thibaut A, Zeng D, Caumo W, Liu J, Fregni F. Corticospinal excitability as a biomarker of myofascial pain syndrome. Pain Rep 2017;2:1–8.

[109] Torrance N, Smith BH, Bennett MI, Lee AJ. The Epidemiology of Chronic Pain of Predominantly Neuropathic Origin. Results From a General Population Survey. Journal of Pain 2006;7:281–289.

[110] Trapp NT, Bruss J, King Johnson M, Uitermarkt BD, Garrett L, Heinzerling A, Wu C, Koscik TR, ten Eyck P, Boes AD. Reliability of targeting methods in TMS for depression: Beam F3 vs. 5.5 cm. Brain Stimul 2020;13:578–581.

[111] Vollert J, Kramer M, Barroso A, Freynhagen R, Haanpää M, Hansson P, Jensen TS, Kuehler BM, Maier C, Mainka T, Reimer M, Segerdahl M, Serra J, Solà R, Tölle TR, Treede RD, Baron R. Symptom profiles in the painDETECT Questionnaire in patients with peripheral neuropathic pain stratified according to sensory loss in quantitative sensory testing. Pain 2016;157:1810–1818.

[112] Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): Its relevance for acute and chronic pain states. Curr Opin Anaesthesiol 2010;23:611–615.

[113] Yarnitsky D. Role of endogenous pain modulation in chronic pain mechanisms and treatment. Pain 2015;156:S24–S31.

[114] Yarnitsky D, Granot M, Granovsky Y. Pain modulation profile and pain therapy: between pro- and antinociception. Pain 2014;155:663–665.

[115] Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y. Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 2012;153:1193–1198.

[116] Yelle MD, Rogers JM, Coghill RC. Offset analgesia: A temporal contrast mechanism for nociceptive information. Pain 2008;134:174–186.

Table 1: Demographic Data for all Participants

	Total (N=47)	**pcTBS at M1 (n=23)**	**pcTBS at DLPFC (n=24)**	p-value
Sex, n (%) Male Female	 19 (40.42) 28 (59.38)	 11 (47.7) 12 (52.2)	 8 (33.33) 16 (66.67)	0.16
Race, n (%) • Non-Hispanic Black • Non-Hispanic White • Asian • Hispanic/Latino/Spanish • Mixed • Prefer not to say	 24 (51.10) 18 (38.30) 1 (2.10) 1 (2.10) 2 (4.30) 1 (2.10)	 12 (52.17) 8 (34.78) 0 (0.00) 1 (4.30) 1 (4.30) 1 (4.30)	 12 (50.00) 10 (41.66) 1 (4.23) 0 0 1 (4.23)	0.13
Age (years)	58.65 ± 8.82	59.65 ± 10.23	57.71 ± 7.33	0.46
Duration of pain (months)	67.07 ± 6.51	67.65 ± 58.47	66.50 ± 72.97	0.48
PD-Q score (-1 and 38 range)	22.15 ± 65.55	21.78 ± 2.58	22.50 ± 3.36	0.21
Current pain on VAS (0-10 range)	5.87 ± 1.88	5.91 ± 1.90	5.83 ± 1.90	0.44
BMI, kg/m^2	31.87 ± 6.51	33.26 ± 6.57	30.54 ± 6.30	0.08
Pre_RMT (%MSO)	55.74 ± 8.87	56.17 ± 9.49	55.33 ± 8.44	0.37
Post_activepcTBS_RMT (%MSO)	54.39 ± 9.68	53.13 ± 11.02	55.60 ± 8.27	0.38
MVC, mv	37.51 ± 13.09	40.00 ± 14.28	35.03 ± 11.56	0.20

BMI: body mass index, VAS: visual analog scale, PD-Q: painDETECT score, RMT: Resting motor threshold, MVC: Maximum voluntary contraction

Figure 1 Neural mechanisms that modulate pain perception in patients with NP

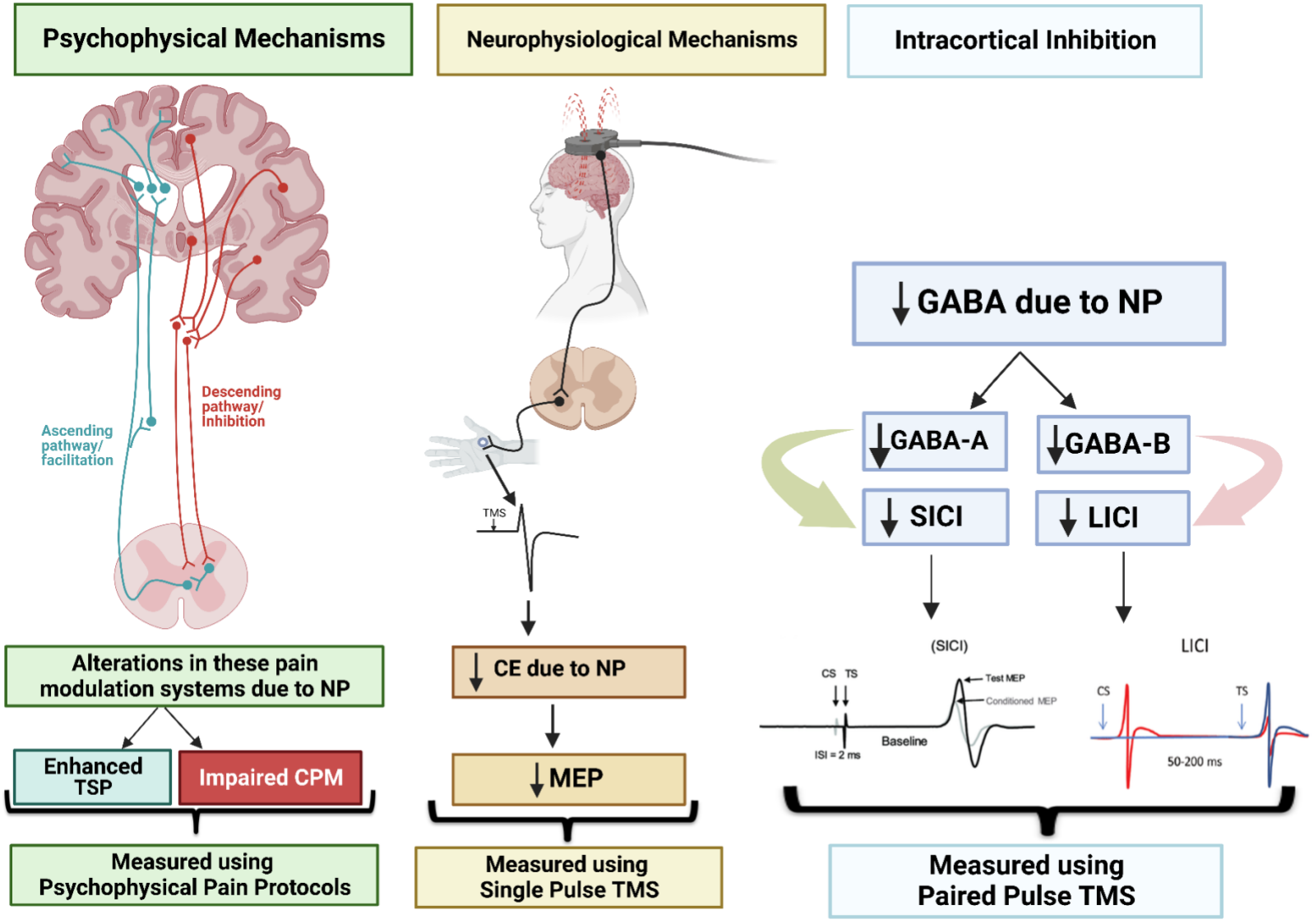


Figure 1 presents (a) the descending pain systems, the ascending pain systems, (b) pathway of CE, and (c) ICI linked to GABAergic activity (SICI= Short Intracortical Inhibition, LICI= Short Intracortical Inhibition, CS= Conditioning Stimulus, TS: Test stimulus, ISI: Interstimulus Intervals). The psychophysical mechanisms are composed of the descending (inhibitory) and the ascending (faciliatory) pain mechanisms. In patients with chronic pain, impaired pain perception is caused by lack of efficiency in the descending pain modulatory systems coupled with facilitation of the ascending pain modulatory systems. The neurophysiological mechanisms evaluated in this study include measurement of CE, which is quantified using the motor evoked potential. This is generated when TMS administered over the cortical representation of a specific muscle at M1 generates an action potential which evokes a biphasic response and results in a twitch in a contralateral muscle measured using electromyography. Lastly, Intracortical inhibition, measured using paired pulse TMS is a marker of GABA mediated activity and in patients with NP there is a decrease in GABA-A and GABA-B receptor activity, which results decrease in SICI and LICI. Interval and NP= Neuropathic pain. Figure created with Biorender.com and adapted from Rogasch et al.2013, Christiansen et al. 2018

Figure 2

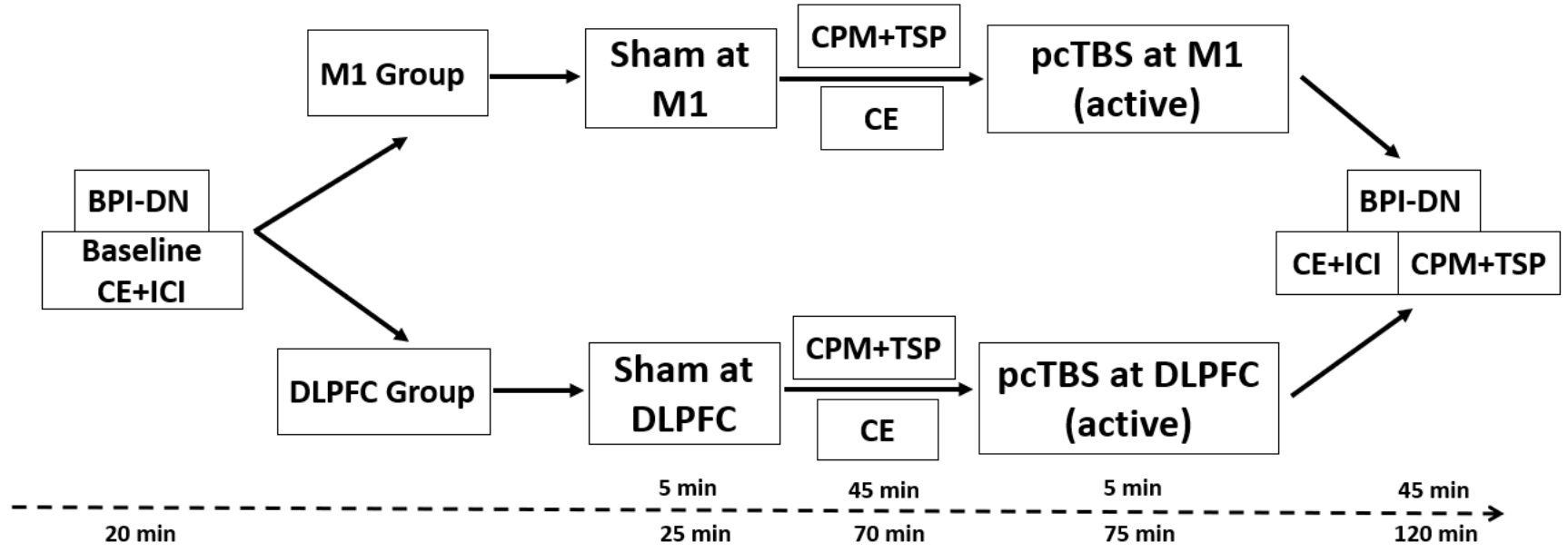


Figure 2: Data collection protocol for the two groups. Prior to collecting data, the cortical hotspots for M1 and DLPFC were identified. Brief Pain Inventory for patients with diabetic neuropathy (BPI-DN), Corticospinal excitability (CE), Intracortical Inhibition (ICI), Conditioned Pain Modulation (CPM), Temporal Summation of Pain (TSP), Primary motor cortex (M1), Dorsolateral prefrontal cortex (DLPFC).

Figure 3

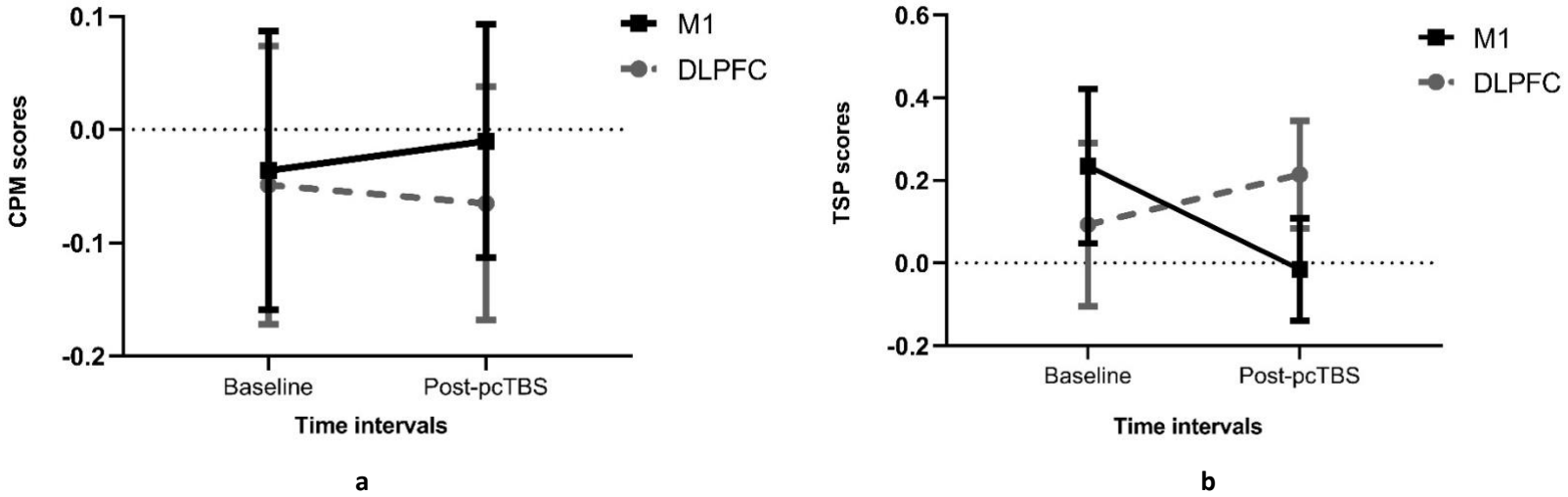


Figure 3: Effects of pcTBS at M1 and DLPFC on PPP across the two time points for the M1 and DLPFC group brain regions. There was no interaction effect for brain region and time and no simple main effect for brain region or time.

Figure 4

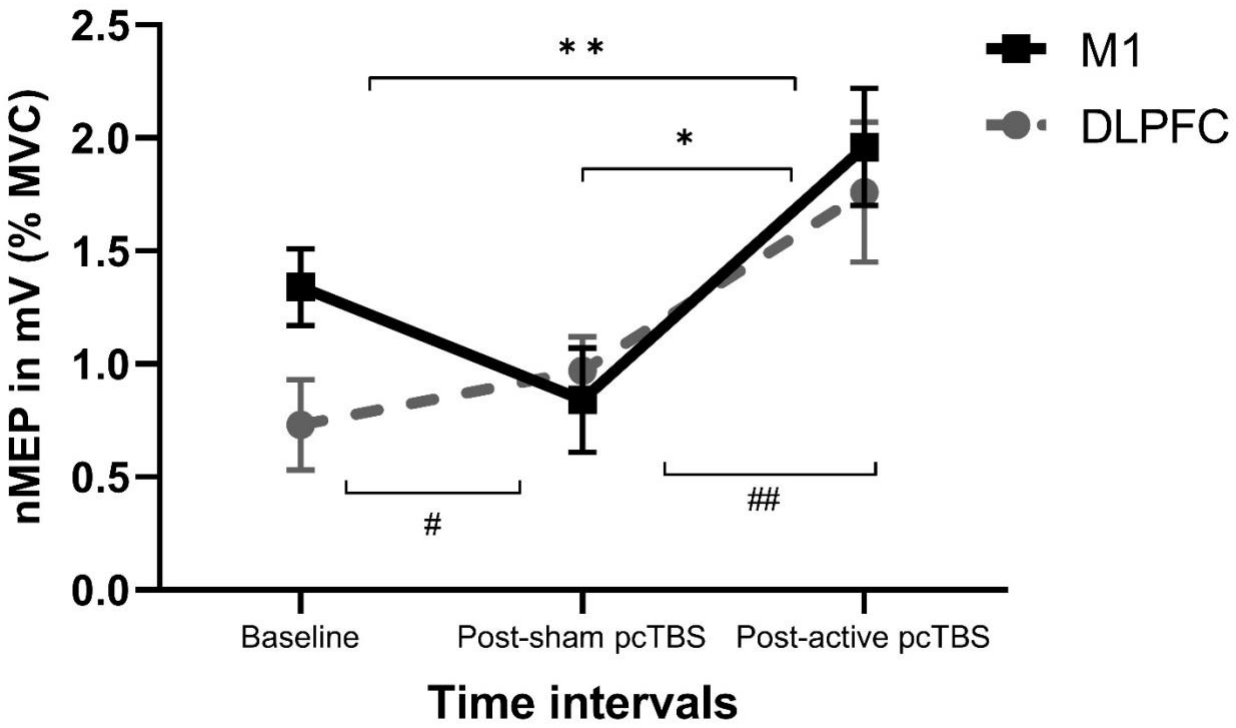


Figure 4: Effects of pcTBS at M1 and DLPFC on MEP across the three time points for the M1 group (# indicates a significant decrease from baseline to post-sham pcTBS; ### indicates significant increase from post-sham pcTBS to post-active pcTBS) and for the DLPFC group (* indicates a significant decrease from post-sham pcTBS to post-active pcTBS; ** indicates a significant increase from baseline to post-active pcTBS). There was no interaction effect for brain region and time but there was a statistically significant effect of time. Post hoc Bonferroni analyses revealed a significant increase for both M1 and DLPFC for MEP from increase in MEP from post-sham pcTBS to post-active pcTBS. For the M1 brain region, there was a significant decrease in MEP from baseline to post-sham pcTBS. For the DLPFC brain region, there was a significant increase in MEP from baseline to post-active pcTBS.

Figure 5

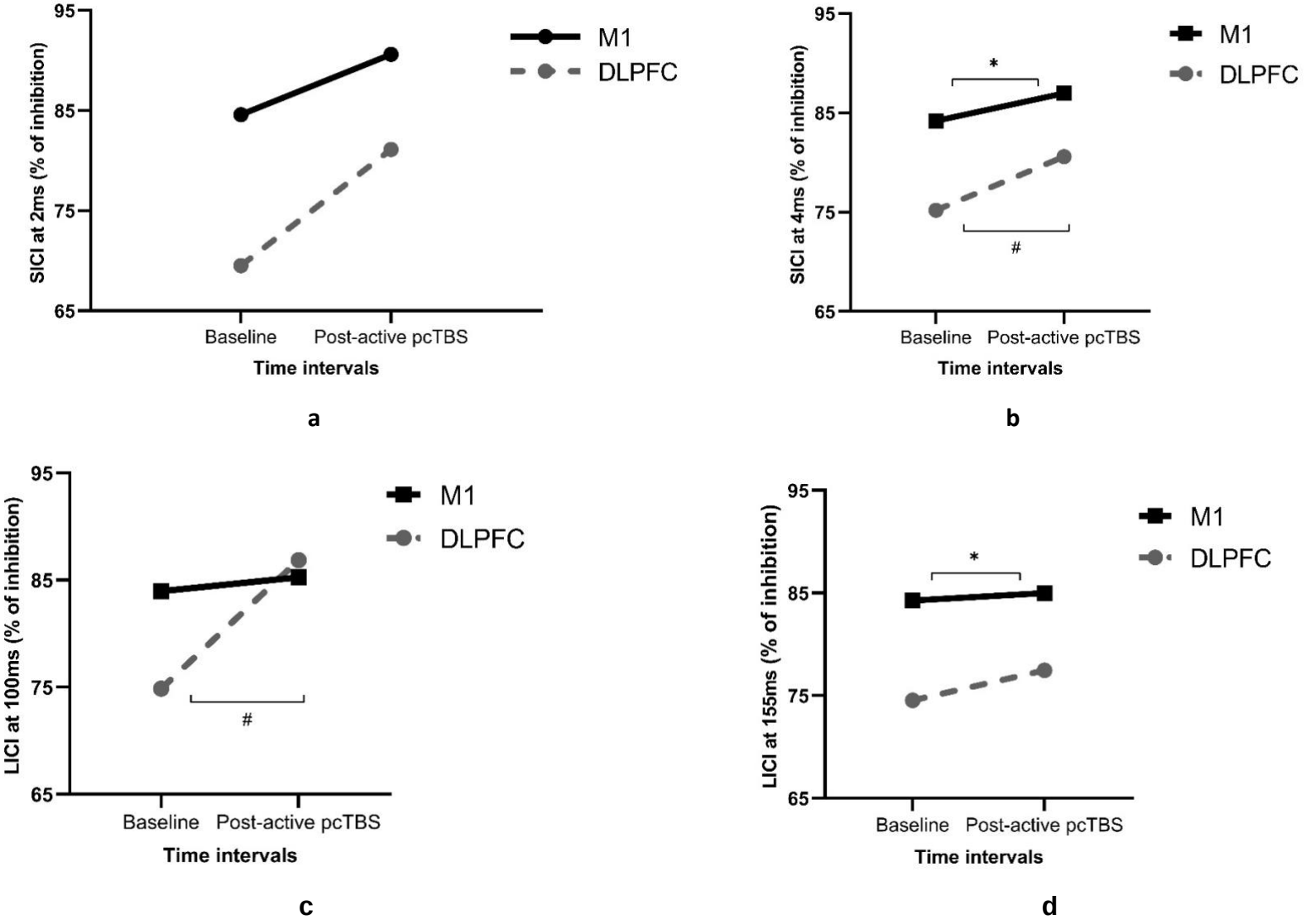

95
85
75
65
SICI at 2ms (% of inhibition)
Baseline
Post-active pcTBS
Time intervals
M1
DLPFC
a
SICI at 4ms (% of inhibition)
*
#
b
LICI at 100ms (% of inhibition)
c
LICI at 155ms (% of inhibition)
d

Figure 5: Effects of pcTBS at M1 and DLPFC on ICI across the two time points for the M1 group (# indicates a significant increase from baseline to post-active pcTBS) and for the DLPFC group (* indicates a significant increase from baseline to post-active pcTBS). Panel A: SICI at 2ms. Panel B: SICI at 4ms. Panel C: LICI at 100ms. Panel D: LICI at 155ms. There was no interaction effect for all the four ICI measures but there was a statistically significant simple main effect of brain region and time for SICI at 4ms and LICI at 100ms. There was a statistically significant simple main effect of time for LICI at 155ms. Post hoc Bonferroni analyses revealed a significant increase for both M1 group brain region and DLPFC group brain region for SICI at 4ms from baseline to post-active pcTBS. There was also a significant increase in ICI measured using LICI at 100 ms for the M1 group brain region and LICI at 155ms for the DLPFC group brain region.

CHAPTER 5

DISCUSSION

The central aim of this dissertation was to examine the effectiveness of pcTBS as an intervention in pDN patients by targeting the M1 and DLPFC regions of the brain, and to investigate the neural mechanisms that may explain the changes in pain perception. Study 1 in Chapter 3 examined the changes in SRMP post pcTBS. To understand the complex and multidimensional pain experience in patients with pDN, SRMP were selected that captured the sensory-discriminative (location, quality and intensity), affective-motivational (unpleasantness) and cognitive-evaluative components (beliefs, attitudes, intention) and aligned with activation of the two brain regions (M1 and DLPFC). The BEEP subscales evaluate the sensory-discriminative, affective-motivational, cognitive-evaluative constructs of CP that align with activation of both the M1 and the DLPFC brain regions while BPI-DN assesses the sensory-discriminative constructs of CP that align with the M1 region. The DASS-21 captures the cognitive-evaluative constructs of CP that align with activation of the DLPFC brain region. Lastly, QOL-DN provides a quantitative evaluation of the impact of pDN on quality of life in these patients.

The results from Study 1 in Chapter 3 revealed that both M1 and DLPFC brain regions exhibited significant improvement in scores across the three time points (baseline, post-pcTBS and 24 h post-pcTBS) for the BEEP emotional reaction to pain subscale and the DASS-21 depression subscale. This suggests that pcTBS targeted at the M1 and DLPFC brain regions modulates the emotional and cognitive aspects constructs of the pain experience measured using SRMP. The present study results also revealed that pcTBS targeted at DLPFC led to improvement in scores on the BEEP pain interference and pain limitations subscales and BPI-DN pain interference subscale. These results provide new information regarding the analgesic effects of HF-rTMS specifically pcTBS targeted at the DLPFC brain region contradicting the results from a recent study [1] that have demonstrated positive analgesic effects of HF-rTMS when targeting the M1 brain region only utilizing SRMP that measure pain intensity, activity

interference, and affective interference. Furthermore, these results provide evidence supporting the utilization of DLPFC as a cortical target to decrease pain in patients with pDN primarily because of its effect on different components of pain perception including pain intensity, limitations and interference and emotional and cognitive measures of pain perception. In addition, for the Norfolk-QOL-DN Symptoms subscale, a significant interaction effect for brain region and time was observed but no differences between the two groups were observed. This subscale captures the subjective nerve related symptoms in the upper and lower extremities and future studies should incorporate clinical screening tools that assess sensory function to correlate these findings before and after pcTBS.

Additionally, the complete study sample (n=42) demonstrated a 13-16% reduction in pain intensity on a 0-10 scale across the three time points and a one-point reduction in scores from baseline to 24 h post-pcTBS on the BPI-DN pain interference subscale revealing a “minimally important improvement” in scores compared to baseline [5,36]. This provides some evidence for the clinical benefit of pcTBS as an intervention targeted at the M1 and the DLPFC brain region. No prior study has investigated the effects of pcTBS on the M1 and DLPFC brain regions in patients with CP and utilized SRMP to evaluate the change in pain perception before and after the treatment. As described earlier, the effects of pcTBS on the M1 and DLPFC brain regions has only been examined in healthy subjects to examine its effect on pain thresholds and cortical excitability [13,21,23,25]. The effects of pcTBS can last from a few days to weeks depending on the frequency of stimulation and number of sessions [15,18,23]. The effects of single session of HF-rTMS targeted at the M1 and DLPFC region lasts up to 5-8 days while the analgesic effect of five to ten sessions could last up to 2-4 weeks after the last session. Future studies are needed to determine the long-term efficacy of pcTBS targeted at the M1 brain region and DLPFC brain region by adding more sessions and a longer follow-up time. It is widely

recognized that repeated sessions are necessary for HF-rTMS and pcTBS to generate an accumulated treatment response and carryover of its analgesic effects in clinical settings.

The mechanisms of action for pcTBS targeted at the M1 and DLPFC brain regions are unclear, although, prior studies have highlighted the role of synaptic plasticity and distinct neurophysiological, neurochemical and endogenous pain modulatory mechanisms acting cortically, supraspinally and at the level of the spinal cord to explain its effects [2,4,11,20,24,32]. The mechanisms of action for pcTBS targeted at DLPFC are focused on the top-down activation of the endogenous system with its effects on the midbrain-thalamus-anterior cingulate cortex pathway and the diffused activation of surrounding brain regions including the limbic system, hippocampus, insula combined with enhanced neurotransmitter activity of dopamine and serotonin involved in pain modulation [3,7,33,34]. This is in contrast to M1, where the mechanism of action is only focused on the top-down effect via the midbrain-thalamus-anterior cingulate cortex pathway [2,4,26,35]. Future studies examining the mechanisms that explain the effects of pcTBS in patients with CP, and specifically NP, at different brain regions should consider the use of neuroimaging methods, human psychophysics testing, and further analysis of corticospinal excitability and intracortical inhibition.

To address the need for a greater understanding of the mechanisms of action, Study 2 in Chapter 4 of this dissertation, examined the three neural mechanisms (the balance between descending and ascending endogenous pain modulatory systems; corticospinal excitability; and intracortical inhibition) that likely play a role in explaining the effects of pcTBS targeted at the M1 and DLPFC brain regions on pain perception in pDN patients. This study exhibited significant increase in CE measured using MEP amplitude and the ICI markers of SICI and LICI, for pcTBS targeted at M1 and DLPFC brain regions, from baseline to post-active pcTBS. No effect of pcTBS targeted at M1 and DLPFC was observed on the descending and ascending endogenous pain modulatory systems, measured using PPP of CPM and TSP. SICI at 2ms and

4ms are markers of GABA-A activity and LICI at 100ms and 155ms serve as markers of GABA-B receptor activity. The present study results concur with changes in MEP amplitude and SICI observed in healthy subjects post pcTBS at M1 and DLPFC [23,25], although differences in SICI were observed only for interstimulus intervals of 4ms and not at 2ms. Furthermore, changes in LICI at 100ms were observed post pcTBS at M1 and for 155ms post pcTBS at DLPFC.

GABAergic inhibition, critical in pain modulation [14,22] decreases in patients with CP represented by decrease in SICI and LICI at different interstimulus intervals [16,17,29]. The increase in SICI and LICI observed in the present study demonstrates enhancement of ICI, which modulates synaptic plasticity cortically and at the level of the spinal cord. Similarly, the increase in CE post pcTBS at M1 and DLPFC activates the top-down endogenous analgesic system and potentially reverses the cortical reorganization due to CP.

Previous studies in healthy subjects have also demonstrated no effect of pcTBS targeted at M1 and DLPFC regions on the descending endogenous pain modulatory systems measured using CPM [23,25]. The present study results concur with these studies. Heterogeneity in the methodology for evoking CPM across different studies, specifically methodological discrepancies linked to the type of test stimulus (utilizing a tonic, suprathreshold, pain threshold or pain tolerance approach), modality of conditioning and test stimuli (thermal, cold, pressure, ischemic) and the site of testing the conditional and test stimuli (affected vs unaffected body areas) may contribute to the lack of differences observed in the present study [6,8,19,27,30]. Another factor contributing to the results of this study may be alterations in sensory function due to neuropathy in pDN patients [9,10,37]. In addition, longer CP duration (>2 years) may potentially cause pDN patients to have more efficient CPM when compared to patients with nonpainful diabetic neuropathy [9]. Future studies should incorporate multiple treatment sessions, different subject populations and neuroimaging-based methods to further elucidate the mechanisms that govern the efficacy of pcTBS targeted at M1 and DLPFC.

Finally, it is important to note that, none of the participants reported any adverse events during the study session or any treatment related adverse events 24 hours after the study session was completed, confirming the excellent safety and tolerability for pcTBS. Headache (18%, 8/44 participants) and neck pain (14%, 6/44 participants) were the common side effects that were reported by the study participants. Previous studies utilizing HF-rTMS targeted at the M1 and DLPFC brain regions have also reported similar side effects [12,28,31].

References

[1] Attal N, Poindessous-Jazat F, de Chauvigny E, Quesada C, Mhalla A, Ayache SS, Fermanian C, Nizard J, Peyron R, Lefaucheur JP, Bouhassira D. Repetitive transcranial magnetic stimulation for neuropathic pain: a randomized multicentre sham-controlled trial. Brain 2021;144:3328–3339. doi:10.1093/BRAIN/AWAB208.

[2] Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J. Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. European Journal of Neuroscience 2004;19:1950–1962.

[3] Brosnan MB, Wiegand I. The Dorsolateral Prefrontal Cortex, a Dynamic Cortical Area to Enhance Top-Down Attentional Control. J Neurosci 2017;37:3445–3446. doi:10.1523/JNEUROSCI.0136-17.2017.

[4] DosSantos MF, Ferreira N, Toback RL, Carvalho AC, DaSilva AF. Potential Mechanisms Supporting the Value of Motor Cortex Stimulation to Treat Chronic Pain Syndromes. Front Neurosci 2016;10:18.

[5] Dworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP, Kerns RD, Stucki G, Allen RR, Bellamy N, Carr DB, Chandler J, Cowan P, Dionne R, Galer BS, Hertz S, Jadad AR, Kramer LD, Manning DC, Martin S, McCormick CG, McDermott MP, McGrath P, Quessy S, Rappaport BA, Robbins W, Robinson JP, Rothman M, Royal MA, Simon L, Stauffer JW, Stein W, Tollett J, Wernicke J, Witter J. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain 2005;113:9–19.

[6] Fernandes C, Pidal-Miranda M, Samartin-Veiga N, Carrillo-De-La-Peña MT. Conditioned pain modulation as a biomarker of chronic pain: A systematic review of its concurrent validity. Pain 2019;160:2679–2690.

[7] Graff-Guerrero A, González-Olvera J, Fresán A, Gómez-Martín D, Méndez-Núñez JC, Pellicer F. Repetitive transcranial magnetic stimulation of dorsolateral prefrontal cortex increases tolerance to human experimental pain. Cognitive Brain Research 2005;25:153–160.

[8] Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, Yarnitsky D. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? Pain 2008;136:142–149.

[9] Granovsky Y, Nahman-Averbuch H, Khamaisi M, Granot M. Efficient conditioned pain modulation despite pain persistence in painful diabetic neuropathy. Pain Rep 2017;2:1–7.

[10] Granovsky Y, Shafran Topaz L, Laycock H, Zubiedat R, Crystal S, Buxbaum C, Bosak N, Hadad R, Domany E, Khamaisi M, Sprecher E, Bennett DL, Rice A, Yarnitsky D. Conditioned pain modulation is more efficient in patients with painful diabetic polyneuropathy than those with nonpainful diabetic polyneuropathy. Pain 2022;163:827–833. doi:10.1097/J.PAIN.0000000000002434.

[11] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–206.

[12] Jiang X, Yan W, Wan R, Lin Y, Zhu X, Song G, Zheng K, Wang Y, Wang X. Effects of repetitive transcranial magnetic stimulation on neuropathic pain: A systematic review and meta-analysis. Neurosci Biobehav Rev 2022;132:130–141.

[13] Klírová M, Hejzlar M, Kostýlková L, Mohr P, Rokyta R, Novák T. Prolonged Continuous Theta Burst Stimulation of the Motor Cortex Modulates Cortical Excitability But not Pain Perception. Front Syst Neurosci 2020;14:1–9.

[14] di Lazzaro V, Pilato F, Dileone M, Ranieri F, Ricci V, Profice P, Bria P, Tonali PA, Ziemann U. GABAA receptor subtype specific enhancement of inhibition in human motor cortex. Journal of Physiology 2006;575:721–726.

[15] Lefaucheur JP. The use of repetitive transcranial magnetic stimulation (rTMS) in chronic neuropathic pain. Neurophysiologie Clinique 2006;36:117–124.

[16] Lefaucheur JP, Ayache SS, Sorel M, Farhat WH, Zouari HG, Ciampi De Andrade D, Ahdab R, Ménard-Lefaucheur I, Brugières P, Goujon C. Analgesic effects of repetitive transcranial magnetic stimulation of the motor cortex in neuropathic pain: Influence of theta burst stimulation priming. European Journal of Pain (United Kingdom) 2012;16:1403–1413.

[17] Lefaucheur JP, Drouot X, Ménard-Lefaucheur I, Keravel Y, Nguyen JP. Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. Neurology 2006;67:1568–1574.

[18] Lefaucheur JP, Drouot X, Nguyen JP. Interventional neurophysiology for pain control: duration of pain relief following repetitive transcranial magnetic stimulation of the motor cortex. 2001 Available: www.elsevier.fr/direct/nc-cn.

[19] Lewis GN, Heales L, Rice DA, Rome K, McNair PJ. Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. Pain Res Manag 2012;17:98–102.

[20] Li CT, Huang YZ, Bai YM, Tsai SJ, Su TP, Cheng CM. Critical role of glutamatergic and GABAergic neurotransmission in the central mechanisms of theta-burst stimulation. Hum Brain Mapp 2019;40:2001–2009.

[21] Li C, Zhang N, Han Q, Zhang L, Xu S, Tu S, Xie Y, Wang Z. Prolonged Continuous Theta Burst Stimulation Can Regulate Sensitivity on Aβ Fibers: An Functional Near-Infrared Spectroscopy Study. Front Mol Neurosci 2022;15.

[22] Malcangio M. GABAB receptors and pain. Neuropharmacology 2018;136:102–105.

[23] de Martino E, Fernandes AM, Galhardoni R, de Oliveira Souza C, Ciampi De Andrade D, Graven-Nielsen T. Sessions of Prolonged Continuous Theta Burst Stimulation or High-frequency 10 Hz Stimulation to Left Dorsolateral Prefrontal Cortex for 3 Days Decreased Pain Sensitivity by Modulation of the Efficacy of Conditioned Pain Modulation. Journal of Pain 2019;20:1459–1469.

[24] Moisset X, de Andrade DC, Bouhassira D. From pulses to pain relief: An update on the mechanisms of rTMS-induced analgesic effects. European Journal of Pain (United Kingdom) 2016;20:689–700.

[25] Moisset X, Goudeau S, Poindessous-Jazat F, Baudic S, Clavelou P, Bouhassira D. Prolonged continuous theta-burst stimulation is more analgesic than “classical” high frequency repetitive transcranial magnetic stimulation. Brain Stimul 2015;8:135–141. doi:10.1016/j.brs.2014.10.006.

[26] Mylius V, Borckardt JJ, Lefaucheur JP. Noninvasive cortical modulation of experimental pain. Pain 2012;153:1350–1363.

[27] Nahman-Averbuch H, Yarnitsky D, Granovsky Y, Gerber E, Dagul P, Granot M. The role of stimulation parameters on the conditioned pain modulation response. Scand J Pain 2013;4:10–14. doi:10.1016/j.sjpain.2012.08.001.

[28] Oberman L, Edwards D, Eldaief M, Pascual-Leone A. Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. J Clin Neurophysiol 2011;28:67–74.

[29] Parker RS, Lewis GN, Rice DA, Mcnair PJ. Is Motor Cortical Excitability Altered in People with Chronic Pain? A Systematic Review and Meta-Analysis. Brain Stimul 2016;9:488–500.

[30] Pud D, Granovsky Y, Yarnitsky D. The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. Pain 2009;144:16–19.

[31] Rossi S, Antal A, Bestmann S, Bikson M, Brewer C, Brockmöller J, Carpenter LL, Cincotta M, Chen R, Daskalakis JD, di Lazzaro V, Fox MD, George MS, Gilbert D, Kimiskidis VK, Koch G, Ilmoniemi RJ, Pascal Lefaucheur J, Leocani L, Lisanby SH, Miniussi C, Padberg F, Pascual-Leone A, Paulus W, Peterchev A v., Quartarone A, Rotenberg A, Rothwell J, Rossini PM, Santarnecchi E, Shafi MM, Siebner HR, Ugawa Y, Wassermann EM, Zangen A, Ziemann U, Hallett M. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. Clinical Neurophysiology 2021;132:269–306.

[32] Rounis E, Huang YZ. Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease. Exp Brain Res 2020;238:1707–1714.

[33] Seminowicz DA, Moayedi M. The Dorsolateral Prefrontal Cortex in Acute and Chronic Pain. Journal of Pain 2017;18:1027–1035. doi:10.1016/j.jpain.2017.03.008.

[34] Taylor JJ, Borckardt JJ, George MS. Endogenous opioids mediate left dorsolateral prefrontal cortex rTMS-induced analgesia. Pain 2012;153:1219–1225. doi:https://doi.org/10.1016/j.pain.2012.02.030.

[35] Thibaut A, Zeng D, Caumo W, Liu J, Fregni F. Corticospinal excitability as a biomarker of myofascial pain syndrome. Pain Rep 2017;2:1–8.

[36] Turk DC, Dworkin RH, Allen RR, Bellamy N, Brandenburg N, Carr DB, Cleeland C, Dionne R, Farrar JT, Galer BS, Hewitt DJ, Jadad AR, Katz NP, Kramer LD, Manning DC, McCormick CG, McDermott MP, McGrath P, Quessy S, Rappaport BA, Robinson JP, Royal MA, Simon L, Stauffer JW, Stein W, Tollett J, Witter J. Core outcome domains for chronic pain clinical trials: IMMPACT recommendations. Pain 2003;106:337–345. doi:10.1016/J.PAIN.2003.08.001.

[37] Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y. Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 2012;153:1193–1198.

REFERENCES

Alles, S. R. A., Odem, M. A., Lu, V. B., Cassidy, R. M., & Smith, P. A. (2021). Chronic BDNF simultaneously inhibits and unmasks superficial dorsal horn neuronal activity. *Scientific Reports*, *11*(1), 1–14. https://doi.org/10.1038/s41598-021-81269-6

Amadio, P., Sandrini, L., Ieraci, A., Tremoli, E., & Barbieri, S. S. (2017). Effect of clotting duration and temperature on BDNF measurement in human serum. *International Journal of Molecular Sciences*, *18*(9). https://doi.org/10.3390/ijms18091987

Andero, R., Choi, D. C., & Ressler, K. J. (2014). BDNF–TrkB Receptor Regulation of Distributed Adult Neural Plasticity, Memory Formation, and Psychiatric Disorders. *Progress in Molecular Biology and Translational Science*, *122*, 169–192. https://doi.org/10.1016/B978-0-12-420170-5.00006-4

Attal, N., Lanteri-Minet, M., Laurent, B., Fermanian, J., & Bouhassira, D. (2011). The specific disease burden of neuropathic pain: Results of a French nationwide survey. *PAIN*, *152*(12), 2836–2843. https://doi.org/10.1016/J.PAIN.2011.09.014

Autry, A. E., & Monteggia, L. M. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. In *Pharmacological Reviews* (Vol. 64, Issue 2, pp. 238–258). https://doi.org/10.1124/pr.111.005108

Bannister, K., Sachau, J., Baron, R., & Dickenson, A. H. (2020). Neuropathic Pain: Mechanism-Based Therapeutics. *Https://Doi.Org/10.1146/Annurev-Pharmtox-010818-021524*, *60*, 257–274. https://doi.org/10.1146/ANNUREV-PHARMTOX-010818-021524

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *The EMBO Journal*, *1*(5), 549–553. https://doi.org/10.1002/J.1460-2075.1982.TB01207.X

Baumeister, D., Eich, W., Saft, S., Geisel, O., Hellweg, R., Finn, A., Svensson, C. I., & Tesarz, J. (n.d.). *no evidence for altered plasma nGf and BDNF levels in fibromyalgia patients Methods*. https://doi.org/10.1038/s41598-019-49403-7

Begliuomini, S., Casarosa, E., Pluchino, N., Lenzi, E., Centofanti, M., Freschi, L., Pieri, M., Genazzani, A. D., Luisi, S., & Genazzani, A. R. (2007). Influence of endogenous and exogenous sex hormones on plasma brain-derived neurotrophic factor. *Human Reproduction*, *22*(4), 995–1002. https://doi.org/10.1093/humrep/del479

Begliuomini, S., Lenzi, E., Ninni, F., Casarosa, E., Merlini, S., Pluchino, N., Valentino, V., Luisi, S., Luisi, M., & Genazzani, A. R. (2008). Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. *Journal of Endocrinology*, *197*(2), 429–435. https://doi.org/10.1677/JOE-07-0376

Bélanger, J.-C., Bouchard, V., le Blanc, J., Starnino, L., Welman, M., Chabot-Blanchet, M., Busseuil, D., Chertkow, H., D'Antono, B., & Lordkipanidzé, M. (2021). Brain-Derived Neurotrophic Factor Mitigates the Association Between Platelet Dysfunction and Cognitive Impairment. *Frontiers in Cardiovascular Medicine*, *0*, 1060. https://doi.org/10.3389/FCVM.2021.739045

Biggs, J. E., Lu, V. B., Stebbing, M. J., Balasubramanyan, S., & Smith, P. A. (2010). Is BDNF sufficient for information transfer between microglia and dorsal horn neurons during the onset of central sensitization? *Molecular Pain*, *6*, 1–14. https://doi.org/10.1186/1744-8069-6-44

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth Factors*, *22*(3), 123–131. https://doi.org/10.1080/08977190410001723308

Bliss, T. V. P., & Cooke, S. F. (2011). Long-term potentiation and long-term depression: A clinical perspective. *Clinics*, *66*(SUPPL.1), 3–17. https://doi.org/10.1590/S1807-59322011001300002

Bocchio-Chiavetto, L., Bagnardi, V., Zanardini, R., Molteni, R., Gabriela Nielsen, M., Placentino, A., Giovannini, C., Rillosi, L., Ventriglia, M., Riva, M. A., & Gennarelli, M. (2010). Serum and plasma BDNF levels in major depression: a replication study and meta-analyses. *The World Journal of Biological Psychiatry : The Official Journal of the World Federation of Societies of Biological Psychiatry*, *11*(6), 763–773. https://doi.org/10.3109/15622971003611319

Bönhof, G. J., Herder, C., Strom, A., Papanas, N., Roden, M., & Ziegler, D. (2018). Emerging biomarkers, tools, and treatments for diabetic polyneuropathy. In *Endocrine Reviews* (Vol. 40, Issue 1). https://doi.org/10.1210/er.2018-00107

Bouhassira, D. (2019). Neuropathic pain: Definition, assessment and epidemiology. *Revue Neurologique*, *175*(1–2), 16–25. https://doi.org/10.1016/j.neurol.2018.09.016

Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B., & Touboul, C. (2008). Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain*, *136*(3), 380–387. https://doi.org/10.1016/j.pain.2007.08.013

Brigadski, T., & Leßmann, V. (n.d.). *The physiology of regulated BDNF release*. https://doi.org/10.1007/s00441-020-03253-2/Published

Bus, B. A. A., Molendijk, M. L., Penninx, B. J. W. H., Buitelaar, J. K., Kenis, G., Prickaerts, J., Elzinga, B. M., & Voshaar, R. C. O. (2011). Determinants of serum brain-derived neurotrophic factor. *Psychoneuroendocrinology*, *36*(2), 228–239. https://doi.org/10.1016/J.PSYNEUEN.2010.07.013

Cain, S. W., Chang, A. M., Vlasac, I., Tare, A., Anderson, C., Czeisler, C. A., & Saxena, R. (2017). Circadian rhythms in plasma brain-derived neurotrophic factor differ in men and women. *Journal of Biological Rhythms*, *32*(1), 75–82. https://doi.org/10.1177/0748730417693124

Cao, T., Matyas, J. J., Renn, C. L., Faden, A. I., Dorsey, S. G., & Wu, J. (2020). Function and Mechanisms of Truncated BDNF Receptor TrkB.T1 in Neuropathic Pain. *Cells*, *9*(5). https://doi.org/10.3390/cells9051194

Cash, R. F. H., Udupa, K., Gunraj, C. A., Mazzella, F., Daskalakis, Z. J., Wong, A. H. C., Kennedy, J. L., & Chen, R. (2021). Influence of BDNF Val66Met polymorphism on excitatory-inhibitory balance and plasticity in human motor cortex. *Clinical Neurophysiology*, *132*(11), 2827–2839. https://doi.org/10.1016/j.clinph.2021.07.029

Caumo, W., Deitos, A., Carvalho, S., Leite, J., Carvalho, F., Dussán-Sarria, J. A., Tarragó, M. da G. L., Souza, A., Torres, I. L. da S., & Fregni, F. (2016). Motor cortex excitability and BDNF levels in chronic musculoskeletal pain according to structural pathology. *Frontiers in Human Neuroscience*, *10*(July), 1–15. https://doi.org/10.3389/fnhum.2016.00357

Cavalli, E., Mammana, S., Nicoletti, F., Bramanti, P., & Mazzon, E. (2019). The neuropathic pain: An overview of the current treatment and future therapeutic approaches: *Https://Doi.Org/10.1177/2058738419838383*, *33*. https://doi.org/10.1177/2058738419838383

Chao, M. v., & Hempstead, B. L. (1995). p75 and Trk: A two-receptor system. *Trends in Neurosciences*, *18*(7), 321–326. https://doi.org/10.1016/0166-2236(95)93922-K

Chen, W., Walwyn, W., Ennes, H. S., Kim, H., McRoberts, J. A., & Marvizón, J. C. G. (2014). BDNF released during neuropathic pain potentiates NMDA receptors in primary afferent terminals. *European Journal of Neuroscience*, *39*(9), 1439–1454. https://doi.org/https://doi.org/10.1111/ejn.12516

Cho, H. C., Kim, J., Kim, S., Son, Y. H., Lee, N., & Jung, S. H. (2012). The concentrations of serum, plasma and platelet BDNF are all increased by treadmill VO 2max performance in healthy college men. *Neuroscience Letters*, *519*(1), 78–83. https://doi.org/10.1016/j.neulet.2012.05.025

Coghill, R. C., & Eisenach, J. (2003). Individual differences in pain sensitivity: implications for treatment decisions. In *Anesthesiology* (Vol. 98, Issue 6, pp. 1312–1314). https://doi.org/10.1097/00000542-200306000-00003

Colloca, L., Ludman, T., Bouhassira, D., Baron, R., Dickenson, A. H., Yarnitsky, D., Freeman, R., Truini, A., Attal, N., Finnerup, N. B., Eccleston, C., Kalso, E., Bennett, D. L., Dworkin, R. H., & Raja, S. N. (2017). Neuropathic pain. *Nature Reviews Disease Primers*, *3*(1), 1–19. https://doi.org/10.1038/nrdp.2017.2

Costigan, M., Scholz, J., & Woolf, C. J. (2009). Neuropathic pain: A maladaptive response of the nervous system to damage. *Annual Review of Neuroscience*, *32*, 1–32. https://doi.org/10.1146/ANNUREV.NEURO.051508.135531

Coull, J. A. M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M. W., & de Koninck, Y. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature*, *438*(7070), 1017–1021. https://doi.org/10.1038/nature04223

Dahlhamer, J. M., Lucas, J., Zelaya, C., Nahin, R., Mackey, S., Debar, L., Kerns, R., von Korff, M., Porter, L., & Helmick, C. (2018). Prevalence of chronic pain and high-impact chronic pain among adults — United States, 2016. *Morbidity and Mortality Weekly Report*, *67*(36), 1001–1006. https://doi.org/10.15585/mmwr.mm6736a2

Dai, S., & Ma, Z. (2014). BDNF-trkB-KCC2-GABA pathway may be related to chronic stress-induced hyperalgesia at both the spinal and supraspinal level. *Medical Hypotheses*, *83*(6), 772–774. https://doi.org/10.1016/j.mehy.2014.10.008

Deitos, A., Dussán-Sarria, J. A., de Souza, A., Medeiros, L., da Graça Tarragô, M., Sehn, F., Chassot, M., Zanette, S., Schwertner, A., Fregni, F., Torres, I. L. S., & Caumo, W. (2015). Clinical value of serum neuroplasticity mediators in identifying the central sensitivity syndrome in patients with chronic pain with and without structural pathology. *Clinical Journal of Pain*, *31*(11), 959–967. https://doi.org/10.1097/AJP.0000000000000194

Ding, X., Cai, J., Li, S., Liu, X. D., Wan, Y., & Xing, G. G. (2015). BDNF contributes to the development of neuropathic pain by induction of spinal long-term potentiation via SHP2 associated GluN2B-containing NMDA receptors activation in rats with spinal nerve ligation. *Neurobiology of Disease*, *73*, 428–451. https://doi.org/10.1016/j.nbd.2014.10.025

Dinoff, A., Herrmann, N., Swardfager, W., & Lanctôt, K. L. (2017). The effect of acute exercise on blood concentrations of brain-derived neurotrophic factor in healthy adults: a meta-analysis. *European Journal of Neuroscience*, *46*(1), 1635–1646. https://doi.org/10.1111/EJN.13603

Doth, A. H., Hansson, P. T., Jensen, M. P., & Taylor, R. S. (2010). The burden of neuropathic pain: A systematic review and meta-analysis of health utilities. *Pain*, *149*(2), 338–344. https://doi.org/10.1016/J.PAIN.2010.02.034

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF val66met Polymorphism Affects Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function and its val/met polymorphism in human memory and hippocampal function and suggest val/met exerts these effects by impacting intracellular trafficking and activity-dependent secretion of BDNF. In *Cell* (Vol. 112).

Elfving, B., Plougmann, P. H., & Wegener, G. (2010). Detection of brain-derived neurotrophic factor (BDNF) in rat blood and brain preparations using ELISA: Pitfalls and solutions. *Journal of Neuroscience Methods*, *187*(1), 73–77. https://doi.org/10.1016/j.jneumeth.2009.12.017

FDA-NIH Biomarker Working Group. (2016). *BEST (Biomarkers , EndpointS , and other Tools) Resource*. Food and Drug Administration (US)/ Co-published by National Institutes of Health (US), Bethesda (MD). https://www.ncbi.nlm.nih.gov/books/NBK326791/

Finnerup, N. B., Kuner, R., & Jensen, T. S. (2020). Neuropathic Pain: From Mechanisms to Treatment. *Https://Doi.Org/10.1152/Physrev.00045.2019*, *101*(1), 259–301. https://doi.org/10.1152/PHYSREV.00045.2019

Fujimura, H., Altar, C. A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J. I., Sun, B., & Tandon, N. N. (2002). Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thrombosis and Haemostasis*, *87*(4), 728–734.

Gálvez, R., Marsal, C., Vidal, J., Ruiz, M., & Rejas, J. (2007). Cross-sectional evaluation of patient functioning and health-related quality of life in patients with neuropathic pain under standard care conditions. *European Journal of Pain*, *11*(3), 244–255. https://doi.org/10.1016/J.EJPAIN.2006.02.002

Gao, J., Wang, H., Liu, Y., Li, Y., Chen, C., Liu, L., Wu, Y., Li, S., & Yang, C. (2014). Glutamate and GABA imbalance promotes neuronal apoptosis in hippocampus after stress. *Medical Science Monitor*, *20*, 499–512. https://doi.org/10.12659/MSM.890589

Gasparin, A., Zortea, M., dos Santos, V. S., Carvalho, F., Torres, I. L. S., de Souza, A., Fregni, F., & Caumo, W. (2020). Brain-Derived Neurotrophic Factor Modulates the Effect of Sex on the Descending Pain Modulatory System in Healthy Volunteers. *Pain Medicine*, *0*(0), 1–9. https://doi.org/10.1093/pm/pnaa027

Gejl, A. K., Enevold, C., Bugge, A., Andersen, M. S., Nielsen, C. H., & Andersen, L. B. (2019). Associations between serum and plasma brain-derived neurotrophic factor and influence of storage time and centrifugation strategy. *Scientific Reports 2019 9:1*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-45976-5

Gomes, W. F., Lacerda, A. C. R., Mendonça, V. A., Arrieiro, A. N., Fonseca, S. F., Amorim, M. R., Teixeira, A. L., Teixeira, M. M., Miranda, A. S., Coimbra, C. C., & Brito-Melo, G. E. A. (2014). Effect of exercise on the plasma BDNF levels in elderly women with knee osteoarthritis. *Rheumatology International*, *34*(6), 841–846. https://doi.org/10.1007/s00296-013-2786-0

Gunn, J., Hill, M. M., Cotten, B. M., & Deer, T. R. (2020). Observational Study An Analysis of Biomarkers in Patients with Chronic Pain. *Pain Physician*, *23*(1), E41–E49. www.painphysicianjournal.com

Haas, L., Portela, L. V. C., Böhmer, A. E., Oses, J. P., & Lara, D. R. (2010). Increased plasma levels of brain derived neurotrophic factor (BDNF) in patients with fibromyalgia. *Neurochemical Research*, *35*(5), 830–834. https://doi.org/10.1007/s11064-010-0129-z

Hanoglu, L., Velioglu, H. A., Hanoglu, T., & Yulug, B. (n.d.). Neuroimaging-Guided Transcranial Magnetic and Direct Current Stimulation in MCI: Toward an Individual, Effective and Disease-Modifying Treatment. *Clinical EEG and Neuroscience*, *0*(0), 15500594211052816. https://doi.org/10.1177/15500594211052815

Ishikawa, T., Yasuda, S., Minoda, S., Ibuki, T., Fukuhara, K., Iwanaga, Y., Ariyoshi, T., & Sasaki, H. (2015). Neurotropin® Ameliorates Chronic Pain via Induction of Brain-Derived Neurotrophic Factor. *Cellular and Molecular Neurobiology*, *35*(2), 231–241. https://doi.org/10.1007/s10571-014-0118-x

Jasim, H., Ghafouri, B., Carlsson, A., Hedenberg-Magnusson, B., & Ernberg, M. (2020). Daytime changes of salivary biomarkers involved in pain. *Journal of Oral Rehabilitation*, *47*(7), 843–850. https://doi.org/10.1111/joor.12977

Jasim, H., Ghafouri, B., Gerdle, B., Hedenberg-Magnusson, B., & Ernberg, M. (n.d.). *Altered levels of salivary and plasma pain related markers in temporomandibular disorders*. https://doi.org/10.1186/s10194-020-01160-z

Jensen, M. P., Chodroff, M. J., & Dworkin, R. H. (2007). The impact of neuropathic pain on health-related quality of life: Review and implications. *Neurology*, *68*(15), 1178–1182. https://doi.org/10.1212/01.WNL.0000259085.61898.9E

Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J. M., & Bertschy, G. (2005). Low Brain-Derived Neurotrophic Factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biological Psychiatry*, *57*(9), 1068–1072. https://doi.org/10.1016/J.BIOPSYCH.2005.01.008

Kerr, B. J., Bradbury, E. J., Bennett, D. L. H., Trivedi, P. M., Dassan, P., French, J., Shelton, D. B., McMahon, S. B., & Thompson, S. W. N. (1999). Brain-Derived Neurotrophic Factor Modulates Nociceptive Sensory Inputs and NMDA-Evoked Responses in the Rat Spinal Cord. *The Journal of Neuroscience*, *19*(12), 5138. https://doi.org/10.1523/JNEUROSCI.19-12-05138.1999

Klein, A. B., Williamson, R., Santini, M. A., Clemmensen, C., Ettrup, A., Rios, M., Knudsen, G. M., & Aznar, S. (2011). Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *International Journal of Neuropsychopharmacology*, *14*(3), 347–353. https://doi.org/10.1017/S1461145710000738

Kohno, K., & Tsuda, M. (2021). Role of microglia and P2X4 receptors in chronic pain. *PAIN Reports*, *6*(1), e864. https://doi.org/10.1097/PR9.0000000000000864

Lang, U. E., Hellweg, R., Seifert, F., Schubert, F., & Gallinat, J. (2007). Correlation Between Serum Brain-Derived Neurotrophic Factor Level and An In Vivo Marker of Cortical Integrity. *Biological Psychiatry*, *62*(5), 530–535. https://doi.org/10.1016/J.BIOPSYCH.2007.01.002

Latremoliere, A., & Woolf, C. J. (2009). Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *The Journal of Pain*, *10*(9), 895–926. https://doi.org/http://dx.doi.org/10.1016/j.jpain.2009.06.012

Lee, B. H., & Kim, Y. K. (2009). Reduced platelet BDNF level in patients with major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(5), 849–853. https://doi.org/10.1016/J.PNPBP.2009.04.002

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H., & Barde, Y.-A. (1989). Molecular cloning and expression of brain-derived neurotrophic factor. *Nature 1989 341:6238*, *341*(6238), 149–152. https://doi.org/10.1038/341149a0

Leyhe, T., Stransky, E., Eschweiler, G. W., Buchkremer, G., & Laske, C. (2008). Increase of BDNF serum concentration during donepezil treatment of patients with early Alzheimer's disease. *European Archives of Psychiatry and Clinical Neuroscience*, *258*(2), 124–128. https://doi.org/10.1007/s00406-007-0764-9

Licien Karege, F., le Schwald, M., & Cisse, M. (n.d.). *Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets*. www.elsevier.com/locate/neulet

Linz, R., Puhlmann, L. M. C., Apostolakou, F., Mantzou, E., Papassotiriou, I., Chrousos, G. P., Engert, V., & Singer, T. (2019). Acute psychosocial stress increases serum BDNF levels: an antagonistic relation to cortisol but no group differences after mental training. *Neuropsychopharmacology*, *44*(10), 1797–1804. https://doi.org/10.1038/s41386-019-0391-y

Loeser, J. D., & Treede, R.-D. (2008). The Kyoto protocol of IASP Basic Pain Terminology. *Pain*, *137*(3), 473–477. https://doi.org/10.1016/j.pain.2008.04.025

Lommatzsch, M., Zingler, D., Schuhbaeck, K., Schloetcke, K., Zingler, C., Schuff-Werner, P., & Virchow, J. C. (2005). The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiology of Aging*, *26*(1), 115–123. https://doi.org/10.1016/j.neurobiolaging.2004.03.002

Lu, V. B., Ballanyi, K., Colmers, W. F., & Smith, P. A. (2007). Neuron type-specific effects of brain-derived neurotrophic factor in rat superficial dorsal horn and their relevance to “central sensitization.” *Journal of Physiology*, *584*(2), 543–563. https://doi.org/10.1113/jphysiol.2007.141267

Lu, V. B., Colmers, W. F., & Smith, P. A. (2009). Long-term effects of brain-derived neurotrophic factor on the frequency of inhibitory synaptic events in the rat superficial dorsal horn. *Neuroscience*, *161*(4), 1135–1143. https://doi.org/10.1016/j.neuroscience.2009.04.030

Maher, D. P., Wong, C. H., Siah, K. W., & Lo, A. W. (2022). Estimates of Probabilities of Successful Development of Pain Medications: An Analysis of Pharmaceutical Clinical Development Programs 2000-2020. *Anesthesiology*. https://doi.org/10.1097/ALN.0000000000004265

Malcangio, M. (2018). GABAB receptors and pain. *Neuropharmacology*, *136*, 102–105. https://doi.org/10.1016/j.neuropharm.2017.05.012

Meng, D., Wu, T. C., Rao, U., North, C. S., Xiao, H., Javors, M. A., & Adinoff, B. (2011). Serum NPY and BNDF response to a behavioral stressor in alcohol-dependent and healthy control participants. *Psychopharmacology*, *218*(1), 59–67. https://doi.org/10.1007/s00213-011-2414-1

Merighi, A., Salio, C., Ghirri, A., Lossi, L., Ferrini, F., Betelli, C., & Bardoni, R. (2008). BDNF as a pain modulator. *Progress in Neurobiology*, *85*(3), 297–317. https://doi.org/10.1016/J.PNEUROBIO.2008.04.004

Miesen, M. M. van der, Lindquist, M. A., & Wager, T. D. (2019). Neuroimaging-based biomarkers for pain: state of the field and current directions. *Pain Reports*, *4*(4). https://doi.org/10.1097/PR9.0000000000000751

Mitsi, V., & Zachariou, V. (2016). Modulation of pain, nociception, and analgesia by the brain reward center. *Neuroscience*, *338*, 81–92. https://doi.org/10.1016/J.NEUROSCIENCE.2016.05.017

Mulvey, M. R., Bennett, M. I., Liwowsky, I., & Freynhagen, R. (2014). The role of screening tools in diagnosing neuropathic pain. *Pain Management*, *4*(3), 233–243. https://doi.org/10.2217/pmt.14.8

Naegelin, Y., Dingsdale, H., Säuberli, K., Schädelin, S., Kappos, L., & Barde, Y.-A. (2018). Measuring and Validating the Levels of Brain-Derived Neurotrophic Factor in Human Serum. *ENeuro*, *5*(2). https://doi.org/10.1523/ENEURO.0419-17.2018

Navratilova, E., Xie, J. Y., Okun, A., Qu, C., Eyde, N., Ci, S., Ossipov, M. H., King, T., Fields, H. L., & Porreca, F. (2012). Pain relief produces negative reinforcement through activation of mesolimbic reward–valuation circuitry. *Proceedings of the National Academy of Sciences*, *109*(50), 20709–20713. https://doi.org/10.1073/PNAS.1214605109

Ohira, K., & Hayashi, M. (2009). A New Aspect of the TrkB Signaling Pathway in Neural Plasticity. *Current Neuropharmacology*, *7*(4), 276–285. https://doi.org/10.2174/157015909790031210

Panja, D., & Bramham, C. R. (2014). BDNF mechanisms in late LTP formation: A synthesis and breakdown. *Neuropharmacology*, *76*(PART C), 664–676. https://doi.org/10.1016/j.neuropharm.2013.06.024

Pan, W., Banks, W. A., Fasold, M. B., Bluth, J., & Kastin, A. J. (1998). Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology*, *37*(12), 1553–1561. https://doi.org/10.1016/S0028-3908(98)00141-5

Park, H., & Poo, M. (2012). Neurotrophin regulation of neural circuit development and function. *Nature Reviews Neuroscience 2013 14:1*, *14*(1), 7–23. https://doi.org/10.1038/nrn3379

Pezet, S., Malcangio, M., & Mcmahon, S. B. (2002). BDNF: a neuromodulator in nociceptive pathways? In *Brain Research Reviews* (Vol. 40). www.elsevier.com/locate/brainresrev

Pezet, S., & McMahon, S. B. (2006). Neurotrophins: Mediators and modulators of pain. *Annual Review of Neuroscience*, *29*, 507–538. https://doi.org/10.1146/annurev.neuro.29.051605.112929

Piccinni, A., Marazziti, D., del Debbio, A., Bianchi, C., Roncaglia, I., Mannari, C., Origlia, N., Catena Dell'Osso, M., Massimetti, G., Domenici, L., & Dell'Osso, L. (2008). Diurnal variation of plasma brain-derived neurotrophic factor (BDNF) in humans: An analysis of sex differences. *Chronobiology International*, *25*(5), 819–826. https://doi.org/10.1080/07420520802387773

Pillai, A., Kale, A., Joshi, S., Naphade, N., Raju, M. S. V. K., Nasrallah, H., & Mahadik, S. P. (2010). Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology. *International Journal of Neuropsychopharmacology*, *13*(4), 535–539. https://doi.org/10.1017/S1461145709991015

Pluchino, N., Cubeddu, A., Begliuomini, S., Merlini, S., Giannini, A., Bucci, F., Casarosa, E., Luisi, M., Cela, V., & Genazzani, A. R. (2009). Daily variation of brain-derived neurotrophic factor and cortisol in women with normal menstrual cycles, undergoing oral contraception and in postmenopause. *Human Reproduction*, *24*(9), 2303–2309. https://doi.org/10.1093/HUMREP/DEP119

Polacchini, A., Metelli, G., Francavilla, R., Baj, G., Florean, M., Mascaretti, L. G., & Tongiorgi, E. (2015). A method for reproducible measurements of serum BDNF: Comparison of the performance of six commercial assays. *Scientific Reports*, *5*(December), 1–10. https://doi.org/10.1038/srep17989

Polyakova, M., Stuke, K., Schuemberg, K., Mueller, K., Schoenknecht, P., & Schroeter, M. L. (2015). BDNF as a biomarker for successful treatment of mood disorders: A systematic & quantitative meta-analysis. *Journal of Affective Disorders*, *174*, 432–440. https://doi.org/10.1016/J.JAD.2014.11.044

Price, T. J., & Gold, M. S. (2018). From mechanism to cure: Renewing the goal to eliminate the disease of pain. *Pain Medicine (United States)*, *19*(8), 1525–1549. https://doi.org/10.1093/pm/pnx108

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M. v., Leick, L., Hart, E., Secher, N. H., Pedersen, B. K., & Pilegaard, H. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology*, *94*(10), 1062–1069. https://doi.org/10.1113/expphysiol.2009.048512

Reycraft, J. T., Islam, H., Townsend, L. K., Hayward, G. C., Hazell, T. O. M. J., & MacPherson, R. E. K. (2020). Exercise Intensity and Recovery on Circulating Brain-derived Neurotrophic Factor. *Medicine and Science in Sports and Exercise*, *52*(5), 1210–1217. https://doi.org/10.1249/MSS.0000000000002242

Rocha, A. L., Vieira, E. L., Ferreira, M. C., Maia, L. M., Teixeira, A. L., & Reis, F. M. (2017). Plasma brain-derived neurotrophic factor in women with pelvic pain: a potential biomarker for endometriosis? *Biomarkers in Medicine*, *11*(4), 313–317. https://doi.org/10.2217/bmm-2016-0327

Sandkühler, J. (2009). Models and Mechanisms of Hyperalgesia and Allodynia. *Https://Doi.Org/10.1152/Physrev.00025.2008*, *89*(2), 707–758. https://doi.org/10.1152/PHYSREV.00025.2008

Serra-Millàs, M. (2016). Are the changes in the peripheral brain-derived neurotrophic factor levels due to platelet activation? *World Journal of Psychiatry*, *6*(1), 84. https://doi.org/10.5498/wjp.v6.i1.84

Sikandar, S., Minett, M. S., Millet, Q., Santana-Varela, S., Lau, J., Wood, J. N., & Zhao, J. (2018). Brain-derived neurotrophic factor derived from sensory neurons plays a critical role in chronic pain. *Brain*, *141*(4), 1028–1039. https://doi.org/10.1093/brain/awy009

Sisignano, M., Lötsch, J., Parnham, M. J., & Geisslinger, G. (2019). Potential biomarkers for persistent and neuropathic pain therapy. *Pharmacology and Therapeutics*, *199*, 16–29. https://doi.org/10.1016/j.pharmthera.2019.02.004

Slusher, A. L., Patterson, V. T., Schwartz, C. S., & Acevedo, E. O. (2018). Impact of high intensity interval exercise on executive function and brain derived neurotrophic factor in healthy college aged males. *Physiology and Behavior*, *191*(March), 116–122. https://doi.org/10.1016/j.physbeh.2018.04.018

Smith, B. H., Torrance, N., Bennett, M. I., & Lee, A. J. (2007). Health and quality of life associated with chronic pain of predominantly neuropathic origin in the community. *Clinical Journal of Pain*, *23*(2), 143–149. https://doi.org/10.1097/01.AJP.0000210956.31997.89

Smith, P. A. (2014). BDNF: No gain without pain? *Neuroscience*, *283*, 107–123. https://doi.org/10.1016/j.neuroscience.2014.05.044

Smith, S. M., Amtmann, D., Askew, R. L., Gewandter, J. S., Hunsinger, M., Jensen, M. P., Mcdermott, M. P., Patel, K. V., Williams, M., Bacci, E. D., Burke, L. B., Chambers, C. T., Cooper, S. A., Cowan, P., Desjardins, P., Etropolski, M., Farrar, J. T., Gilron, I., Huang, I. Z., … Dworkin, R. H. (2016). Pain intensity rating training: Results from an exploratory study of the ACTTION PROTECCT system. *Pain*, *157*(5), 1056–1064. https://doi.org/10.1097/J.PAIN.0000000000000502

Smith, S. M., Dworkin, R. H., Turk, D. C., Baron, R., Polydefkis, M., Tracey, I., Borsook, D., Edwards, R. R., Harris, R. E., Wager, T. D., Arendt-Nielsen, L., Burke, L. B., Carr, D. B., Chappell, A., Farrar, J. T., Freeman, R., Gilron, I., Goli, V., Haeussler, J., … Witter, J. (2017). The Potential Role of Sensory Testing, Skin Biopsy, and Functional Brain Imaging as Biomarkers in Chronic Pain Clinical Trials: IMMPACT Considerations. *Journal of Pain*, *18*(7), 757–777. https://doi.org/10.1016/j.jpain.2017.02.429

Soril, L. J. J., Ramer, L. M., McPhail, L. T., Kaan, T. K. Y., & Ramer, M. S. (2008). Spinal brain-derived neurotrophic factor governs neuroplasticity and recovery from cold-hypersensitivity following dorsal rhizotomy. *Pain*, *138*(1), 98–110. https://doi.org/10.1016/j.pain.2007.11.014

Stefani, L. C., Leite, F. M., da Graça L. Tarragó, M., Zanette, S. A., de Souza, A., Castro, S. M., & Caumo, W. (2019). BDNF and serum S100B levels according the spectrum of structural pathology in chronic pain patients. *Neuroscience Letters*, *706*, 105–109. https://doi.org/https://doi.org/10.1016/j.neulet.2019.05.021

Szok, D., Tajti, J., Nyári, A., & Vécsei, L. (2019). Therapeutic Approaches for Peripheral and Central Neuropathic Pain. *Behavioural Neurology*, *2019*. https://doi.org/10.1155/2019/8685954

Taneja, A., Della Pasqua, O., & Danhof, M. (2017). Challenges in translational drug research in neuropathic and inflammatory pain: the prerequisites for a new paradigm. *European Journal of Clinical Pharmacology*, *73*(10), 1219–1236. https://doi.org/10.1007/s00228-017-2301-8

Tateiwa, H., Kawano, T., Nishigaki, A., Yamanaka, D., Aoyama, B., Shigematsu-Locatelli, M., Eguchi, S., Locatelli, F. M., & Yokoyama, M. (2018). The role of hippocampal brain-derived neurotrophic factor in age-related differences in neuropathic pain behavior in rats. *Life Sciences*, *197*(February), 56–66. https://doi.org/10.1016/j.lfs.2018.01.030

Terracciano, A., Lobina, M., Piras, M. G., Mulas, A., Cannas, A., Meirelles, O., Sutin, A. R., Zonderman, A. B., Uda, M., Crisponi, L., & Schlessinger, D. (2011). Neuroticism, depressive symptoms, and serum BDNF. *Psychosomatic Medicine*, *73*(8), 638–642. https://doi.org/10.1097/PSY.0B013E3182306A4F

Terracciano, A., Piras, M. G., Lobina, M., Mulas, A., Meirelles, O., Sutin, A. R., Chan, W., Sanna, S., Uda, M., Crisponi, L., & Schlessinger, D. (2013). Genetics of serum BDNF: Meta-analysis of the Val66Met and genome-wide association study. *World Journal of Biological Psychiatry*, *14*(8), 583–589. https://doi.org/10.3109/15622975.2011.616533

Thibault, K., Lin, W. K., Rancillac, A., Fan, M., Snollaerts, T., Sordoillet, V., Hamon, M., Smith, G. M., Lenkei, Z., & Pezet, S. (2014). *Development/Plasticity/Repair BDNF-Dependent Plasticity Induced by Peripheral Inflammation in the Primary Sensory and the Cingulate Cortex Triggers Cold Allodynia and Reveals a*

Major Role for Endogenous BDNF As a Tuner of the Affective Aspect of Pain. https://doi.org/10.1523/JNEUROSCI.0860-14.2014

Torrance, N., Smith, B. H., Bennett, M. I., & Lee, A. J. (2006). The Epidemiology of Chronic Pain of Predominantly Neuropathic Origin. Results From a General Population Survey. *Journal of Pain*, *7*(4), 281–289. https://doi.org/10.1016/j.jpain.2005.11.008

Trajkovska, V., Marcussen, A. B., Vinberg, M., Hartvig, P., Aznar, S., & Knudsen, G. M. (2007a). Measurements of brain-derived neurotrophic factor: Methodological aspects and demographical data. *Brain Research Bulletin*, *73*(1–3), 143–149. https://doi.org/10.1016/j.brainresbull.2007.03.009

Trajkovska, V., Marcussen, A. B., Vinberg, M., Hartvig, P., Aznar, S., & Knudsen, G. M. (2007b). Measurements of brain-derived neurotrophic factor: Methodological aspects and demographical data. *Brain Research Bulletin*, *73*(1–3), 143–149. https://doi.org/10.1016/j.brainresbull.2007.03.009

Trang, T., Beggs, S., & Salter, M. W. (2011). Brain-derived neurotrophic factor from microglia: a molecular substrate for neuropathic pain. *Neuron Glia Biology*, *7*(1), 99–108. https://doi.org/10.1017/S1740925X12000087

Trang, T., Beggs, S., & Salter, M. W. (2012). Brain-derived neurotrophic factor from microglia: A molecular substrate for neuropathic pain. *Neuron Glia Biology*, *7*(1), 99–108. https://doi.org/10.1017/S1740925X12000087

Tsuchimine, S., Sugawara, N., Ishioka, M., & Yasui-Furukori, N. (2014). Preanalysis storage conditions influence the measurement of brain-derived neurotrophic factor levels in peripheral blood. *Neuropsychobiology*, *69*(2), 83–88. https://doi.org/10.1159/000358061

Tyler, W. J., Alonso, M., Bramham, C. R., & Pozzo-Miller, L. D. (2002). From Acquisition to Consolidation: On the Role of Brain-Derived Neurotrophic Factor Signaling in Hippocampal-Dependent Learning. *Learning & Memory*, *9*(5), 224–237. https://doi.org/10.1101/LM.51202

Vanelderen, P., Rouwette, T., Kozicz, T., Roubos, E., Zundert, J. Van, Heylen, R., & Vissers, K. (2010). The role of brain-derived neurotrophic factor in different animal models of neuropathic pain. *European Journal of Pain*, *14*(5), 473.e1-473.e9. https://doi.org/10.1016/j.ejpain.2009.09.006

van Hecke, O., Austin, S. K., Khan, R. A., Smith, B. H., & Torrance, N. (2014). Neuropathic pain in the general population: A systematic review of epidemiological studies. *Pain*, *155*(4), 654–662. https://doi.org/10.1016/J.PAIN.2013.11.013

Ventriglia, M., Zanardini, R., Bonomini, C., Zanetti, O., Volpe, D., Pasqualetti, P., Gennarelli, M., & Bocchio-Chiavetto, L. (2013). Serum Brain-Derived Neurotrophic Factor Levels in Different Neurological Diseases. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/901082

Wang, X., Ratnam, J., Zou, B., England, P. M., & Basbaum, A. I. (2009). TrkB signaling is required for both the induction and maintenance of tissue and nerve injury-induced persistent pain. *Journal of Neuroscience*, *29*(17), 5508–5515. https://doi.org/10.1523/JNEUROSCI.4288-08.2009

Wessels, J. M., Agarwal, R. K., Somani, A., Verschoor, C. P., Agarwal, S. K., & Foster, W. G. (2020). Factors affecting stability of plasma brain-derived neurotrophic factor. *Scientific Reports 2020 10:1*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-77046-6

Woolf, C. J. (2007). Central sensitization: uncovering the relation between pain and plasticity. *Anesthesiology*, *106*(4), 864.

Woolf, C. J., & Thompson, S. W. N. (1991). The induction and maintenance of central sensitization is dependent on N-methyl-d-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain*, *44*(3), 293–299. https://doi.org/10.1016/0304-3959(91)90100-C

Wu, Y., Shen, Z., Xu, H., Zhang, K., Guo, M., Wang, F., & Li, J. (2021). BDNF Participates in Chronic Constriction Injury-Induced Neuropathic Pain via Transcriptionally Activating P2X7 in Primary Sensory Neurons. *Molecular Neurobiology*, *58*(9), 4226–4236. https://doi.org/10.1007/s12035-021-02410-0

Zafra, F., Castrén, E., Thoenen, H., & Lindholm, D. (1991). Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. *Proceedings of the National Academy of Sciences*, *88*(22), 10037–10041. https://doi.org/10.1073/PNAS.88.22.10037

Zhang, H., Qian, Y. L., Li, C., Liu, D., Wang, L., Wang, X. Y., Liu, M. J., Liu, H., Zhang, S., Guo, X. Y., Yang, J. X., Ding, H. L., Koo, J. W., Mouzon, E., Deisseroth, K., Nestler, E. J., Zachariou, V., Han, M. H., & Cao, J. L. (2017). Brain-Derived Neurotrophic Factor in the Mesolimbic Reward Circuitry Mediates Nociception in Chronic Neuropathic Pain. *Biological Psychiatry*, *82*(8), 608–618. https://doi.org/10.1016/j.biopsych.2017.02.1180

Zhang, X., Wang, J., Zhou, Q., Xu, Y., Pu, S., Wu, J., Xue, Y., Tian, Y., Lu, J., Jiang, W., & Du, D. (2011). Brain-derived neurotrophic factor-activated astrocytes produce mechanical allodynia in neuropathic pain. *Neuroscience*, *199*, 452–460. https://doi.org/10.1016/j.neuroscience.2011.10.017

Zhang, Z., Wang, X., Wang, W., Lu, Y. G., & Pan, Z. Z. (2013). Brain-derived neurotrophic factor-mediated downregulation of brainstem K+-Cl- cotransporter and cell-type-specific GABA impairment for activation of descending pain facilitation. *Molecular Pharmacology*, *84*(4), 511–520. https://doi.org/10.1124/mol.113.086496

Zhao, H., Alam, A., San, C.-Y., Eguchi, S., Chen, Q., Lian, Q., & Ma, D. (2017). Molecular mechanisms of brain-derived neurotrophic factor in neuro-protection: Recent developments. *Brain Research*, *1665*, 1–21. https://doi.org/10.1016/j.brainres.2017.03.029

Zhao, J., Seereeram, A., Nassar, M. A., Levato, A., Pezet, S., Hathaway, G., Morenilla-Palao, C., Stirling, C., Fitzgerald, M., McMahon, S. B., Rios, M., & Wood, J. N. (2006). Nociceptor-derived brain-derived neurotrophic factor regulates acute and inflammatory but not neuropathic pain. *Molecular and Cellular Neuroscience*, *31*(3), 539–548. https://doi.org/10.1016/j.mcn.2005.11.008

Zhou, L. J., Yang, T., Wei, X., Liu, Y., Xin, W. J., Chen, Y., Pang, R. P., Zang, Y., Li, Y. Y., & Liu, X. G. (2011). Brain-derived neurotrophic factor contributes to spinal long-term potentiation and mechanical hypersensitivity by activation of spinal microglia in rat. *Brain, Behavior, and Immunity*, *25*(2), 322–334. https://doi.org/10.1016/j.bbi.2010.09.025

Zhou, W., Xie, Z., Li, C., Xing, Z., Xie, S., Li, M., & Yao, J. (2021). Driving effect of BDNF in the spinal dorsal horn on neuropathic pain. In *Neuroscience Letters* (Vol. 756). Elsevier Ireland Ltd. https://doi.org/10.1016/j.neulet.2021.135965

Abdelkader, A. A., Gohary, A. M. E., Mourad, H. S., & Salmawy, D. A. E. (2019). Repetitive tms in treatment of resistant diabetic neuropathic pain. *Egyptian Journal of Neurology, Psychiatry and Neurosurgery*, *55*(1), 1–9. https://doi.org/10.1186/s41983-019-0075-x

Alam, U., Sloan, G., & Tesfaye, S. (2020). Treating Pain in Diabetic Neuropathy: Current and Developmental Drugs. *Drugs*, *80*(4), 363–384. https://doi.org/10.1007/s40265-020-01259-2

Antony, M. M., Cox, B. J., Enns, M. W., Bieling, P. J., & Swinson, R. P. (1998). Psychometric properties of the 42-item and 21-item versions of the Depression Anxiety Stress Scales in clinical groups and a community sample. *Psychological Assessment*, *10*(2), 176–181. https://doi.org/10.1037/1040-3590.10.2.176

Auvray, M., Myin, E., & Spence, C. (2010). The sensory-discriminative and affective-motivational aspects of pain. In *Neuroscience and Biobehavioral Reviews* (Vol. 34, Issue 2, pp. 214–223). https://doi.org/10.1016/j.neubiorev.2008.07.008

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet (London, England)*, *1*(8437), 1106–1107. https://doi.org/10.1016/S0140-6736(85)92413-4

Beam, W., Borckardt, J. J., Reeves, S. T., & George, M. S. (2009). An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. *Brain Stimulation*, *2*(1), 50–54. https://doi.org/10.1016/j.brs.2008.09.006

Berlim, M. T., Broadbent, H. J., & van den Eynde, F. (2013). Blinding integrity in randomized sham-controlled trials of repetitive transcranial magnetic stimulation for major depression: A systematic review and meta-analysis. *International Journal of Neuropsychopharmacology*, *16*(5), 1173–1181. https://doi.org/10.1017/S1461145712001691

Bliss, T. V. P., & Cooke, S. F. (2011). Long-term potentiation and long-term depression: A clinical perspective. *Clinics*, *66*(SUPPL.1), 3–17. https://doi.org/10.1590/S1807-59322011001300002

Blumberger, D. M., Vila-Rodriguez, F., Thorpe, K. E., Feffer, K., Noda, Y., Giacobbe, P., Knyahnytska, Y., Kennedy, S. H., Lam, R. W., Daskalakis, Z. J., & Downar, J. (2018). Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial. *The Lancet*, *391*(10131), 1683–1692. https://doi.org/https://doi.org/10.1016/S0140-6736(18)30295-2

Borckardt, J. J., Nahas, Z., Koola, J., & George, M. S. (2006). Estimating resting motor thresholds in transcranial magnetic stimulation research and practice: A computer simulation evaluation of best methods. *Journal of ECT*, *22*(3), 169–175. https://doi.org/10.1097/01.yct.0000235923.52741.72

Bouhassira, D. (2019). Neuropathic pain: Definition, assessment and epidemiology. *Revue Neurologique*, *175*(1–2), 16–25. https://doi.org/10.1016/j.neurol.2018.09.016

Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B., & Touboul, C. (2008). Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain*, *136*(3), 380–387. https://doi.org/10.1016/j.pain.2007.08.013

Bredfeldt, C., Altschuler, A., Adams, A. S., Portz, J. D., & Bayliss, E. A. (2015). Patient reported outcomes for diabetic peripheral neuropathy. *Journal of Diabetes and Its Complications*, *29*(8), 1112–1118. https://doi.org/10.1016/J.JDIACOMP.2015.08.015

Broadbent, H. J., van den Eynde, F., Guillaume, S., Hanif, E. L., Stahl, D., David, A. S., Campbell, I. C., & Schmidt, U. (2011). Blinding success of rTMS applied to the dorsolateral prefrontal cortex in randomised sham-controlled trials: A systematic review. *World Journal of Biological Psychiatry*, *12*(4), 240–248. https://doi.org/10.3109/15622975.2010.541281

Brown, T. A., Chorpita, B. F., Korotitsch, W., & Barlow, D. H. (1997). Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples. *Behaviour Research and Therapy*, *35*(1), 79–89. https://doi.org/10.1016/S0005-7967(96)00068-X

Cárdenas-Morales, L., Nowak, D. A., Kammer, T., Wolf, R. C., & Schönfeldt-Lecuona, C. (2010). Mechanisms and applications of theta-burst rTMS on the human motor cortex. In *Brain Topography* (Vol. 22, Issue 4, pp. 294–306). https://doi.org/10.1007/s10548-009-0084-7

Chang, M. C., Yang, S., Kwak, S. G., & Choi, G.-S. (n.d.). *Short-term Effect of Repetitive Transcranial Magnetic Stimulation on Diabetic Peripheral Neuropathic Pain*. www.painphysicianjournal.comwww.painphysicianjournal.com

Che, X., Cash, R. F. H., Luo, X., Luo, H., Lu, X., Xu, F., Zang, Y. F., Fitzgerald, P. B., & Fitzgibbon, B. M. (2021). High-frequency rTMS over the dorsolateral prefrontal cortex on chronic and provoked pain: A

systematic review and meta-analysis. In *Brain Stimulation* (Vol. 14, Issue 5, pp. 1135–1146). Elsevier Inc. https://doi.org/10.1016/j.brs.2021.07.004

Chung, S. W., Hill, A. T., Rogasch, N. C., Hoy, K. E., & Fitzgerald, P. B. (2016). Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis. *Neuroscience and Biobehavioral Reviews*, *63*, 43–64. https://doi.org/10.1016/j.neubiorev.2016.01.008

Ciampi De Andrade, D., Mhalla, A., Adam, F., Texeira, M. J., & Bouhassira, D. (2014). Repetitive transcranial magnetic stimulation induced analgesia depends on N-methyl-d-aspartate glutamate receptors. *Pain*, *155*(3), 598–605. https://doi.org/10.1016/j.pain.2013.12.022

Cleeland, C. S., & Ryan, K. M. (1994). Pain assessment: global use of the Brief Pain Inventory. *Annals of the Academy of Medicine, Singapore*, *23*(2), 129–138.

Cohen, S. L., Bikson, M., Badran, B. W., & George, M. S. (2022). A visual and narrative timeline of US FDA milestones for Transcranial Magnetic Stimulation (TMS) devices. *Brain Stimulation*, *15*(1), 73–75. https://doi.org/10.1016/J.BRS.2021.11.010

de Martino, E., Fernandes, A. M., Galhardoni, R., de Oliveira Souza, C., Ciampi De Andrade, D., & Graven-Nielsen, T. (2019). Sessions of Prolonged Continuous Theta Burst Stimulation or High-frequency 10 Hz Stimulation to Left Dorsolateral Prefrontal Cortex for 3 Days Decreased Pain Sensitivity by Modulation of the Efficacy of Conditioned Pain Modulation. *Journal of Pain*, *20*(12), 1459–1469. https://doi.org/10.1016/j.jpain.2019.05.010

Dworkin, R. H., Turk, D. C., Farrar, J. T., Haythornthwaite, J. A., Jensen, M. P., Katz, N. P., Kerns, R. D., Stucki, G., Allen, R. R., Bellamy, N., Carr, D. B., Chandler, J., Cowan, P., Dionne, R., Galer, B. S., Hertz, S., Jadad, A. R., Kramer, L. D., Manning, D. C., … Witter, J. (2005). Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. *Pain*, *113*(1–2), 9–19. https://doi.org/10.1016/j.pain.2004.09.012

Dworkin, R. H., Turk, D. C., Wyrwich, K. W., Beaton, D., Cleeland, C. S., Farrar, J. T., Haythornthwaite, J. A., Jensen, M. P., Kerns, R. D., Ader, D. N., Brandenburg, N., Burke, L. B., Cella, D., Chandler, J., Cowan, P., Dimitrova, R., Dionne, R., Hertz, S., Jadad, A. R., … Zavisic, S. (2008). Interpreting the Clinical Importance of Treatment Outcomes in Chronic Pain Clinical Trials: IMMPACT Recommendations. *The Journal of Pain*, *9*(2), 105–121. https://doi.org/10.1016/J.JPAIN.2007.09.005

Edwards, R. R., Dworkin, R. H., Sullivan, M. D., Turk, D. C., & Wasan, A. D. (2016). The Role of Psychosocial Processes in the Development and Maintenance of Chronic Pain. *Journal of Pain*, *17*(9), T70–T92. https://doi.org/10.1016/j.jpain.2016.01.001

Feldman, E. L., Callaghan, B. C., Pop-Busui, R., Zochodne, D. W., Wright, D. E., Bennett, D. L., Bril, V., Russell, J. W., & Viswanathan, V. (2019). Diabetic neuropathy. *Nature Reviews Disease Primers*, *5*(1). https://doi.org/10.1038/s41572-019-0092-1

Fillingim, R. B., Loeser, J. D., Baron, R., & Edwards, R. R. (2016). Assessment of Chronic Pain: Domains, Methods, and Mechanisms. *Journal of Pain*, *17*(9), T10–T20. https://doi.org/10.1016/j.jpain.2015.08.010

Fitzgerald, P. B., Brown, T. L., Marston, N. A. U., Daskalakis, Z. J., de Castella, A., & Kulkarni, J. (2003). Transcranial Magnetic Stimulation in the Treatment of Depression: A Double-blind, Placebo-Controlled Trial. *Archives of General Psychiatry*, *60*(10), 1002–1008. https://doi.org/10.1001/archpsyc.60.9.1002

Garcia-Larrea, L., & Peyron, R. (2013). Pain matrices and neuropathic pain matrices: A review. *Pain*, *154*(SUPPL. 1). https://doi.org/10.1016/j.pain.2013.09.001

Gatchel, R. J., Peng, Y. B., Peters, M. L., Fuchs, P. N., & Turk, D. C. (2007). The Biopsychosocial Approach to Chronic Pain: Scientific Advances and Future Directions. *Psychological Bulletin*, *133*(4), 581–624. https://doi.org/10.1037/0033-2909.133.4.581

Gatzinsky, K., Bergh, C., Liljegren, A., Silander, H., Samuelsson, J., Svanberg, T., & Samuelsson, O. (2021). Repetitive transcranial magnetic stimulation of the primary motor cortex in management of chronic neuropathic pain: A systematic review. In *Scandinavian Journal of Pain* (Vol. 21, Issue 1, pp. 8–21). De Gruyter Open Ltd. https://doi.org/10.1515/sjpain-2020-0054

Girach, A., Julian, T. H., Varrassi, G., Paladini, A., Vadalouka, A., & Zis, P. (2019). Quality of life in painful peripheral neuropathies: A systematic review. *Pain Research and Management*, *2019*. https://doi.org/10.1155/2019/2091960

Goldsworthy, M. R., Vallence, A. M., Hodyl, N. A., Semmler, J. G., Pitcher, J. B., & Ridding, M. C. (2016). Probing changes in corticospinal excitability following theta burst stimulation of the human primary motor cortex. *Clinical Neurophysiology*, *127*(1), 740–747. https://doi.org/10.1016/j.clinph.2015.06.014

Granovsky, Y., Sprecher, E., & Sinai, A. (2019). Motor corticospinal excitability: A novel facet of pain modulation? *Pain Reports*, *4*(2), 1–7. https://doi.org/10.1097/PR9.0000000000000725

Hallett, M. (2007). Transcranial Magnetic Stimulation: A Primer. *Neuron*, *55*(2), 187–199. https://doi.org/10.1016/j.neuron.2007.06.026

Harris, P. A., Taylor, R., Minor, B. L., Elliott, V., Fernandez, M., O'Neal, L., McLeod, L., Delacqua, G., Delacqua, F., Kirby, J., & Duda, S. N. (2019). The REDCap consortium: Building an international community of software platform partners. *Journal of Biomedical Informatics*, *95*. https://doi.org/10.1016/j.jbi.2019.103208

Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J. G. (2009). Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, *42*(2), 377–381. https://doi.org/10.1016/j.jbi.2008.08.010

Henry, J. D., & Crawford, J. R. (2005). The short-form version of the Depression anxiety stress scales (DASS-21): Construct validity and normative data in a large non-clinical sample. *British Journal of Clinical Psychology*, *44*(2), 227–239. https://doi.org/10.1348/014466505X29657

Henschke, N., Kamper, S. J., & Maher, C. G. (2015). The epidemiology and economic consequences of pain. *Mayo Clinic Proceedings*, *90*(1), 139–147. https://doi.org/10.1016/j.mayocp.2014.09.010

He, W., Fong, P. Y., Leung, T. W. H., & Huang, Y. Z. (2020). Protocols of non-invasive brain stimulation for neuroplasticity induction. *Neuroscience Letters*, *719*. https://doi.org/10.1016/j.neulet.2018.02.045

Hicks, C. W., & Selvin, E. (2019). Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. *Current Diabetes Reports 2019 19:10*, *19*(10), 1–8. https://doi.org/10.1007/S11892-019-1212-8

Huang, Y. Z., Chen, R. S., Rothwell, J. C., & Wen, H. Y. (2007). The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clinical Neurophysiology*, *118*(5), 1028–1032. https://doi.org/10.1016/J.CLINPH.2007.01.021

Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron*, *45*(2), 201–206. https://doi.org/10.1016/j.neuron.2004.12.033

Huang, Y. Z., Lu, M. K., Antal, A., Classen, J., Nitsche, M., Ziemann, U., Ridding, M., Hamada, M., Ugawa, Y., Jaberzadeh, S., Suppa, A., Paulus, W., & Rothwell, J. (2017). Plasticity induced by non-invasive transcranial brain stimulation: A position paper. *Clinical Neurophysiology*, *128*(11), 2318–2329. https://doi.org/10.1016/j.clinph.2017.09.007

Huang, Y. Z., Rothwell, J. C., Chen, R. S., Lu, C. S., & Chuang, W. L. (2011). The theoretical model of theta burst form of repetitive transcranial magnetic stimulation. *Clinical Neurophysiology*, *122*(5), 1011–1018. https://doi.org/10.1016/j.clinph.2010.08.016

Jiang, X., Yan, W., Wan, R., Lin, Y., Zhu, X., Song, G., Zheng, K., Wang, Y., & Wang, X. (2022). Effects of repetitive transcranial magnetic stimulation on neuropathic pain: A systematic review and meta-analysis. *Neuroscience and Biobehavioral Reviews*, *132*, 130–141. https://doi.org/10.1016/J.NEUBIOREV.2021.11.037

Kassebaum, N. J. (2022). Global, regional, and national burden of diseases and injuries for adults 70 years and older: Systematic analysis for the Global Burden of Disease 2019 Study. *The BMJ*, *376*. https://doi.org/10.1136/bmj-2021-068208

Kawai, K., Kawai, A. T., Wollan, P., & Yawn, B. P. (2017). Adverse impacts of chronic pain on health-related quality of life, work productivity, depression and anxiety in a community-based study. *Family Practice*, *34*(6), 656–661. https://doi.org/10.1093/fampra/cmx034

Klírová, M., Hejzlar, M., Kostýlková, L., Mohr, P., Rokyta, R., & Novák, T. (2020). Prolonged Continuous Theta Burst Stimulation of the Motor Cortex Modulates Cortical Excitability But not Pain Perception. *Frontiers in Systems Neuroscience*, *14*(May), 1–9. https://doi.org/10.3389/fnsys.2020.00027

Klomjai, W., Katz, R., & Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Annals of Physical and Rehabilitation Medicine*, *58*(4), 208–213. https://doi.org/10.1016/j.rehab.2015.05.005

Lefaucheur, J. P. (2016). Cortical neurostimulation for neuropathic pain: State of the art and perspectives. *Pain*, *157*(2), S81–S89. https://doi.org/10.1097/j.pain.0000000000000401

Lefaucheur, J.-P., Aleman, A., Baeken, C., Benninger, D. H., Brunelin, J., di Lazzaro, V., Filipović, S. R., Grefkes, C., Hasan, A., Hummel, F. C., Jääskeläinen, S. K., Langguth, B., Leocani, L., Londero, A., Nardone, R., Nguyen, J.-P., Nyffeler, T., Oliveira-Maia, A. J., Oliviero, A., … Ziemann, U. (2020). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). *Clinical Neurophysiology*, *131*(2), 474–528. https://doi.org/https://doi.org/10.1016/j.clinph.2019.11.002

Lefaucheur, J. P., Drouot, X., & Nguyen, J. P. (2001). *Interventional neurophysiology for pain control: duration of pain relief following repetitive transcranial magnetic stimulation of the motor cortex*. www.elsevier.fr/direct/nc-cn

Leung, A., Shirvalkar, P., Chen, R., Kuluva, J., Vaninetti, M., Bermudes, R., Poree, L., Wassermann, E., Kopell, B., & Levy, R. (2020). Transcranial Magnetic Stimulation for Pain, Headache, and Comorbid Depression: INS-NANS Expert Consensus Panel Review and Recommendation. *Neuromodulation*, *2019*, 267–290. https://doi.org/10.1111/ner.13094

Li, C. T., Huang, Y. Z., Bai, Y. M., Tsai, S. J., Su, T. P., & Cheng, C. M. (2019). Critical role of glutamatergic and GABAergic neurotransmission in the central mechanisms of theta-burst stimulation. *Human Brain Mapping*, *40*(6), 2001–2009. https://doi.org/10.1002/hbm.24485

Li, C., Zhang, N., Han, Q., Zhang, L., Xu, S., Tu, S., Xie, Y., & Wang, Z. (2022). Prolonged Continuous Theta Burst Stimulation Can Regulate Sensitivity on Aβ Fibers: An Functional Near-Infrared Spectroscopy Study. *Frontiers in Molecular Neuroscience*, *15*. https://doi.org/10.3389/fnmol.2022.887426

Lindsay, N. M., Chen, C., Gilam, G., Mackey, S., & Scherrer, G. (2021). Brain circuits for pain and its treatment. In *Science Translational Medicine* (Vol. 13, Issue 619). American Association for the Advancement of Science. https://doi.org/10.1126/scitranslmed.abj7360

Lipton, R. B., Dodick, D. W., Silberstein, S. D., Saper, J. R., Aurora, S. K., Pearlman, S. H., Fischell, R. E., Ruppel, P. L., & Goadsby, P. J. (2010). Single-pulse transcranial magnetic stimulation for acute treatment of migraine with aura: a randomised, double-blind, parallel-group, sham-controlled trial. *The Lancet Neurology*, *9*(4), 373–380. https://doi.org/10.1016/S1474-4422(10)70054-5

Lovibond, S.H. & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales.* (2nd ed.). Sydney: Psychology Foundation.

Malfliet, A., Coppieters, I., van Wilgen, P., Kregel, J., de Pauw, R., Dolphens, M., & Ickmans, K. (2017). Brain changes associated with cognitive and emotional factors in chronic pain: A systematic review. In *European Journal of Pain (United Kingdom)* (Vol. 21, Issue 5, pp. 769–786). Blackwell Publishing Ltd. https://doi.org/10.1002/ejp.1003

Mhalla, A., Baudic, S., de Andrade, D. C., Gautron, M., Perrot, S., Teixeira, M. J., Attal, N., & Bouhassira, D. (2011). Long-term maintenance of the analgesic effects of transcranial magnetic stimulation in fibromyalgia. *Pain*, *152*(7), 1478–1485. https://doi.org/10.1016/j.pain.2011.01.034

Mir-Moghtadaei, A., Caballero, R., Fried, P., Fox, M. D., Lee, K., Giacobbe, P., Daskalakis, Z. J., Blumberger, D. M., & Downar, J. (2015). Concordance between BeamF3 and MRI-neuronavigated target sites for repetitive transcranial magnetic stimulation of the left dorsolateral prefrontal cortex. *Brain Stimulation*, *8*(5), 965–973. https://doi.org/10.1016/j.brs.2015.05.008

Moisset, X., Bouhassira, D., Avez Couturier, J., Alchaar, H., Conradi, S., Delmotte, M. H., Lanteri-Minet, M., Lefaucheur, J. P., Mick, G., Piano, V., Pickering, G., Piquet, E., Regis, C., Salvat, E., & Attal, N. (2020). Pharmacological and non-pharmacological treatments for neuropathic pain: Systematic review and French recommendations. *Revue Neurologique*, *176*(5), 325–352. https://doi.org/10.1016/J.NEUROL.2020.01.361

Moisset, X., de Andrade, D. C., & Bouhassira, D. (2016). From pulses to pain relief: An update on the mechanisms of rTMS-induced analgesic effects. *European Journal of Pain (United Kingdom)*, *20*(5), 689–700. https://doi.org/10.1002/EJP.811

Moisset, X., Goudeau, S., Poindessous-Jazat, F., Baudic, S., Clavelou, P., & Bouhassira, D. (2015). Prolonged continuous theta-burst stimulation is more analgesic than “classical” high frequency repetitive transcranial magnetic stimulation. *Brain Stimulation*, *8*(1), 135–141. https://doi.org/10.1016/j.brs.2014.10.006

Moisset, X., & Lefaucheur, J. P. (2019). Non pharmacological treatment for neuropathic pain: Invasive and non-invasive cortical stimulation. *Revue Neurologique*, *175*(1–2), 51–58. https://doi.org/10.1016/J.NEUROL.2018.09.014

Murray, C. J. L., Mokdad, A. H., Ballestros, K., Echko, M., Glenn, S., Olsen, H. E., Mullany, E., Lee, A., Khan, A. R., Ahmadi, A., Ferrari, A. J., Kasaeian, A., Werdecker, A., Carter, A., Zipkin, B., Sartorius, B., Serdar, B., Sykes, B. L., Troeger, C., … Murray, C. J. L. (2018). The state of US health, 1990-2016: Burden of diseases, injuries, and risk factors among US states. *JAMA - Journal of the American Medical Association*, *319*(14), 1444–1472. https://doi.org/10.1001/jama.2018.0158

Nardone, R., Höller, Y., Langthaler, P. B., Lochner, P., Golaszewski, S., Schwenker, K., Brigo, F., & Trinka, E. (2016). *rTMS of the prefrontal cortex has analgesic effects on neuropathic pain in subjects with spinal cord injury*. https://doi.org/10.1038/sc.2016.87

Noda, Y., Silverstein, W. K., Barr, M. S., Vila-Rodriguez, F., Downar, J., Rajji, T. K., Fitzgerald, P. B., Mulsant, B. H., Vigod, S. N., Daskalakis, Z. J., & Blumberger, D. M. (2015). Neurobiological mechanisms of repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex in depression: A systematic review. *Psychological Medicine*, *45*(16), 3411–3432. https://doi.org/10.1017/S0033291715001609

Oberman, L., Edwards, D., Eldaief, M., & Pascual-Leone, A. (2011). Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. *Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society*, *28*(1), 67–74. https://doi.org/10.1097/WNP.0b013e318205135f

O'Connell, N. E., Marston, L., Spencer, S., DeSouza, L. H., & Wand, B. M. (2018). Non-invasive brain stimulation techniques for chronic pain. *The Cochrane Database of Systematic Reviews*, *4*, CD008208. https://doi.org/10.1002/14651858.CD008208.pub5

Onesti, E., Gabriele, M., Cambieri, C., Ceccanti, M., Raccah, R., di Stefano, G., Biasiotta, A., Truini, A., Zangen, A., & Inghilleri, M. (2013). H-coil repetitive transcranial magnetic stimulation for pain relief in patients with diabetic neuropathy. *European Journal of Pain (United Kingdom)*, *17*(9), 1347–1356. https://doi.org/10.1002/j.1532-2149.2013.00320.x

Ong, W.-Y., Stohler, C. S., & Herr, D. R. (2019). Role of the Prefrontal Cortex in Pain Processing. *Molecular Neurobiology*, *56*(2), 1137–1166. https://doi.org/10.1007/s12035-018-1130-9

Pop-Busui, R., Boulton, A. J. M., Feldman, E. L., Bril, V., Freeman, R., Malik, R. A., Sosenko, J. M., & Ziegler, D. (2017). Diabetic neuropathy: A position statement by the American diabetes association. *Diabetes Care*, *40*(1), 136–154. https://doi.org/10.2337/dc16-2042

Preti, A., Stocchino, S., Pinna, F., Deidda, M. C., Musu, M., Sancassiani, F., Romano, F., Machado, S., Finco, G., & Carta, M. G. (2019). BEEP—Bodily and Emotional Perception of Pain. A Questionnaire to Measure Reaction to Pain in Chronic Pain Disorders . In *Frontiers in Psychology* (Vol. 10, p. 480). https://www.frontiersin.org/articles/10.3389/fpsyg.2019.00480

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., di Iorio, R., di Lazzaro, V., Ferreri, F., Fitzgerald, P. B., George, M. S., Hallett, M., Lefaucheur, J. P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M. A., Pascual-Leone, A., Paulus, W., Rossi, S., … Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology*, *126*(6), 1071–1107. https://doi.org/10.1016/j.clinph.2015.02.001

Rossi, S., Antal, A., Bestmann, S., Bikson, M., Brewer, C., Brockmöller, J., Carpenter, L. L., Cincotta, M., Chen, R., Daskalakis, J. D., di Lazzaro, V., Fox, M. D., George, M. S., Gilbert, D., Kimiskidis, V. K., Koch, G., Ilmoniemi, R. J., Pascal Lefaucheur, J., Leocani, L., … Hallett, M. (2021). Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. In *Clinical Neurophysiology* (Vol. 132, Issue 1, pp. 269–306). Elsevier Ireland Ltd. https://doi.org/10.1016/j.clinph.2020.10.003

Rounis, E., & Huang, Y. Z. (2020). Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease. In *Experimental Brain Research* (Vol. 238, Issues 7–8, pp. 1707–1714). Springer. https://doi.org/10.1007/s00221-020-05880-1

Seminowicz, D. A., & Moayedi, M. (2017). The Dorsolateral Prefrontal Cortex in Acute and Chronic Pain. *Journal of Pain*, *18*(9), 1027–1035. https://doi.org/10.1016/j.jpain.2017.03.008

Short, E. B., Borckardt, J. J., Anderson, B. S., Frohman, H., Beam, W., Reeves, S. T., & George, M. S. (2011). Ten sessions of adjunctive left prefrontal rTMS significantly reduces fibromyalgia pain: A randomized, controlled pilot study. *Pain*, *152*(11), 2477–2484. https://doi.org/10.1016/j.pain.2011.05.033

Smith, S. M., Dworkin, R. H., Turk, D. C., McDermott, M. P., Eccleston, C., Farrar, J. T., Rowbotham, M. C., Bhagwagar, Z., Burke, L. B., Cowan, P., Ellenberg, S. S., Evans, S. R., Freeman, R. L., Garrison, L. P., Iyengar, S., Jadad, A., Jensen, M. P., Junor, R., Kamp, C., … Wilson, H. D. (2020). Interpretation of chronic pain clinical trial outcomes: IMMPACT recommended considerations. *Pain*, *161*(11), 2446–2461. https://doi.org/10.1097/j.pain.0000000000001952

Suppa, A., Huang, Y. Z., Funke, K., Ridding, M. C., Cheeran, B., di Lazzaro, V., Ziemann, U., & Rothwell, J. C. (2016). Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. *Brain Stimulation*, *9*(3), 323–335. https://doi.org/10.1016/j.brs.2016.01.006

Tan, G., Jensen, M. P., Thornby, J. I., & Shanti, B. F. (2004). Validation of the brief pain inventory for chronic nonmalignant pain. *Journal of Pain*, *5*(2), 133–137. https://doi.org/10.1016/j.jpain.2003.12.005

Taylor, J. J., Borckardt, J. J., Canterberry, M., Li, X., Hanlon, C. A., Brown, T. R., & George, M. S. (2013). Naloxone-Reversible Modulation of Pain Circuitry by Left Prefrontal rTMS. *Neuropsychopharmacology*, *38*, 1189–1197. https://doi.org/10.1038/npp.2013.13

Taylor, J. J., Borckardt, J. J., & George, M. S. (2012). Endogenous opioids mediate left dorsolateral prefrontal cortex rTMS-induced analgesia. *PAIN*, *153*(6), 1219–1225. https://doi.org/https://doi.org/10.1016/j.pain.2012.02.030

Tesfaye, S., Vileikyte, L., Rayman, G., Sindrup, S. H., Perkins, B. A., Baconja, M., Vinik, A. I., Boulton, A. J. M., & on behalf of The Toronto Expert Panel on Diabetic Neuropathy. (2011). Painful diabetic peripheral neuropathy: consensus recommendations on diagnosis, assessment and management. *Diabetes/Metabolism Research and Reviews*, *27*(7), 629–638. https://doi.org/https://doi.org/10.1002/dmrr.1225

Trapp, N. T., Bruss, J., King Johnson, M., Uitermarkt, B. D., Garrett, L., Heinzerling, A., Wu, C., Koscik, T. R., ten Eyck, P., & Boes, A. D. (2020). Reliability of targeting methods in TMS for depression: Beam F3 vs. 5.5 cm. *Brain Stimulation*, *13*(3), 578–581. https://doi.org/10.1016/j.brs.2020.01.010

Turk, D. C., Fillingim, R. B., Ohrbach, R., & Patel, K. v. (2016). Assessment of Psychosocial and Functional Impact of Chronic Pain. *Journal of Pain*, *17*(9), T21–T49. https://doi.org/10.1016/j.jpain.2016.02.006

Vinik, E. J., Hayes, R. P., Oglesby, A., Bastyr, E., Barlow, P., Ford-Molvik, S. L., & Vinik, A. I. (2005). The development and validation of the Norfolk QOL-DN, a new measure of patients' perception of the effects of diabetes and diabetic neuropathy. *Diabetes Technology and Therapeutics*, *7*(3), 497–508. https://doi.org/10.1089/dia.2005.7.497

Williams, R., Karuranga, S., Malanda, B., Saeedi, P., Basit, A., Besançon, S., Bommer, C., Esteghamati, A., Ogurtsova, K., Zhang, P., & Colagiuri, S. (2020). Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *162*, 108072. https://doi.org/10.1016/j.diabres.2020.108072

Wischnewski, M., & Schutter, D. J. L. G. (2015). Efficacy and time course of theta burst stimulation in healthy humans. In *Brain Stimulation* (Vol. 8, Issue 4, pp. 685–692). Elsevier Inc. https://doi.org/10.1016/j.brs.2015.03.004

Yang, S., & Chang, M. C. (2020). Effect of Repetitive Transcranial Magnetic Stimulation on Pain Management: A Systematic Narrative Review. *Frontiers in Neurology*, *11*(February). https://doi.org/10.3389/fneur.2020.00114

Yong, R. J., Mullins, P. M., & Bhattacharyya, N. (2021). *Prevalence of chronic pain among adults in the United States*. https://doi.org/10.1097/j.pain.0000000000002291

Zelaya, C. E., Ph, D., Dahlhamer, J. M., Ph, D., Lucas, J. W., & Connor, E. M. (2020). *Chronic pain and high-impact chronic pain among U.S. adults, 2019* (Issue 390). https://www.cdc.gov/nchs/products/index.htm.

Zelman, D. C., Gore, M., Dukes, E., Tai, K. S., & Brandenburg, N. (2005). Validation of a Modified Version of the Brief Pain Inventory for Painful Diabetic Peripheral Neuropathy. *Journal of Pain and Symptom Management*, *29*(4), 401–410. https://doi.org/10.1016/J.JPAINSYMMAN.2004.06.018

Appendix A

IRB Approval and Study Consent Form

VCU
Office of the Vice President
for Research and Innovation

Office of the Vice President for Research and Innovation
Human Research Protection Program
BioTechnology Research Park
800 East Leigh Street, Suite 3000
Box 980568
Richmond, Virginia 23298-0568

(804) 828-0868
Fax: (804) 827-1448

TO: Edmund Acevedo

CC: Edmund Acevedo
Carrie Peterson
Bhushan Thakkar

FROM: VCU IRB Panel A

RE: Edmund Acevedo; IRB HM20021531 Theta Burst Brain Stimulation in Diabetic Neuropathy Patients with Neuropathic Pain: Investigating Neural Mechanisms

On 6/15/2021, this study involving the research use of human subjects was ***approved*** according to 45 CFR 46.108(b) by VCU IRB Panel A.

The information found in the electronic version of this study's smart form and uploaded documents now represents the currently approved study, documents, informed consent process, and HIPAA pathway (if applicable). You may access this information by clicking the Study Number above.

COVID-19 Notice

In the context of the COVID-19 pandemic, the IRB expects the research will proceed in accordance with other institutional policies and as outlined in this submission and if applicable, in the study's COVID-19 Contingency Protocol. IRB approval does not necessarily mean that your research may proceed. For more information on investigator responsibilities and institutional requirements, please see https://research.vcu.edu/covid-19.htm

The Principal Investigator is also reminded of their responsibility to ensure that there are adequate resources to carry out the research safely. This includes, but is not limited to, sufficient investigator time, appropriately qualified research team members, equipment, and space. See WPP #: IX-1 Principal Investigator Eligibility and Statement of Responsibilities

This approval expires on 6/14/2022. Federal Regulations/VCU Policy and Procedures require continuing review prior to continuation of approval past that date. Continuing Review notices will be sent to you prior to the scheduled review.

If you have any questions, please contact the Office of Research Subjects Protection (ORSP) or the IRB reviewer(s) assigned to this study.

The reviewer(s) assigned to your study will be listed in the History tab and on the study workspace. Click on their name to see their contact information.

Attachment – *Conditions of Approval*

Conditions of Approval for Expedited and Full Board Studies *(version 1/21/2019):*

In order to comply with federal regulations, industry standards, and the terms of this approval, the investigator must (*as applicable*):

1. Conduct the research as described in and required by the IRB-approved protocol/smartform.
2. Obtain approval from the VCU IRB before implementing any changes in the approved research unless such changes are necessary to protect the safety of human research participants.
    - Report any departure from the approved protocol/smartform or documents to the VCU IRB immediately through a report submission.
    - Obtain approval from the VCU IRB before use of any advertisement or other material (print or electronic) for recruitment of research participants.
    - Obtain approval from the VCU IRB before implementing any changes related to the future sharing of individual-level research data.
3. Obtain informed consent from all prospective participants or the participant's legally authorized representative without coercion or undue influence, and provide the potential participant sufficient opportunity to consider whether or not to participate (unless a Waiver of Consent was specifically approved).
    - Obtain informed consent using only the most recently approved consent document (unless a Waiver of Consent was specifically approved).
    - Provide non-English speaking participants with a written translation of the approved consent document (or a translated version of the Short Form Consent document) in language understandable to the research participant. The IRB must approve the translated version and/or the use of a short form consent process prior to use.
4. Monitor all problems (anticipated and unanticipated) associated with risk to research participants or others.
5. Report all Unanticipated Problems (UPs) involving risk to participants or others following the VCU IRB requirements and timelines detailed in WPP VII-6.
6. Respond promptly to all inquiries from the VCU IRB and Office of Research Subjects Protection concerning the conduct of the approved research.

The VCU IRBs operate under the regulatory authorities as described within:

- *U.S. Department of Health and Human Services Title 45 CFR 46, Subparts A, B, C, and D (for all research, regardless of source of funding) and related guidance documents.*
- *U.S. Food and Drug Administration Chapter I of Title 21 CFR 50 and 56 (for FDA regulated research only) and related guidance documents.*
- *Commonwealth of Virginia Code of Virginia 32.1 Chapter 5.1 Human Research (for all research).*

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE: Theta Burst Brain Stimulation in Diabetic Neuropathy Patients with Neuropathic Pain: Investigating Neural Mechanisms

VCU INVESTIGATOR: Dr. Edmund O. Acevedo, Ph.D., FACSM, College of Humanities and Sciences, (804) 827-0948.

ABOUT THIS CONSENT FORM

You are being invited to participate in a research study. **It is important that you carefully think about whether being in this study is right for you and your situation.**

This consent form is meant to assist you in thinking about whether or not you want to be in this study. **Please ask the study staff to explain any information in this consent document that is not clear to you.** You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

Your participation is voluntary. You may decide not to participate in this study. If you do participate, you may withdraw from the study at any time. Your decision not to take part or to withdraw will involve no penalty or loss of benefits to which you are otherwise entitled.

AN OVERVIEW OF THE STUDY AND KEY INFORMATION

We are performing a research study using a newer form of non-invasive brain stimulation (called transcranial magnetic stimulation or TMS) placed just above your head as a treatment in patients with painful diabetic neuropathy to examine its effects on your understanding of your pain experience. The proposed brain stimulation device and technique in this study is an investigational device that has not been approved by the U.S. FDA for treating pain linked to diabetic neuropathy, but it has been approved to treat depression. We will be using surveys and monitoring how your body changes as your understanding of your pain experience changes. You will then be randomized (like the flip of a coin) to either receive brain stimulation at one of two brain regions which are involved in the processing and understanding of your pain experience.

Why is this study being done?

The purpose of the study is to determine the effects of transcranial magnetic stimulation (TMS) on your understanding of your pain experience. You are being asked to participate in this study because you have been diagnosed with diabetic neuropathy and meet the study entry requirements.

We plan to enroll 60 subjects who will participate in one experimental session that could take 2-2.5 hours. The results of this study will improve our understanding of the effects of this treatment as a therapeutic intervention in patients with painful diabetic neuropathy.

What will happen if I participate?

Your participation in this study consists of one session lasting up to 2-2.5 hours. You will be compensated for participating in this study and be provided free parking.

There are three components in this study:

1. **Completing surveys to determine your current pain status and its impact on your daily activities.**

When you come into the lab on the day of your study, you will be asked to complete paperwork that involves reading and signing the consent form, if you are willing to participate in the study, and it will also involve completing three surveys on an iPad to evaluate the quality of pain that you experience, its impact on your daily life, and lastly, your quality of life. You will then be randomized (like the flip of a coin) to either receive brain stimulation at one of two brain regions; the primary motor cortex (top middle of the scalp) or dorsolateral prefrontal cortex (front left side of the scalp). Both these regions are involved in your processing and understanding of your pain experience. You have an equal chance to be in one group or the other. After the study session is completed, you will be asked to complete the three surveys again that assess pain perception electronically 24 hours after the study session was completed. The study team will email you one link which contains all the three surveys. You will be provided with e-mail and phone reminders to complete the measures for four consecutive days after the study session was completed.

2. **Using non-invasive brain stimulation.**

You will then be asked to sit in a comfortable chair and place your right arm in a cast in the chair to support it and keep it stable. Surface electrodes will be placed on your hand muscles. These electrodes record the activity of your muscles. A linen cap will be secured tightly on your head to mark the region of stimulation. Next, we will use non-invasive brain stimulation to record muscle activity (from electrodes on your arm) in response to stimulation. The non-invasive brain stimulation we use, called transcranial magnetic stimulation or TMS, consists of a stimulating coil being held over your head. When this coil delivers a painless magnetic pulse, it feels like a quick, light tap on your head. We will use two different coils to perform TMS. One coil will be used to determine stimulation parameters for the longer continuous theta burst stimulation (prolonged continuous TBS; pcTBS) protocol. The second coil will be used to implement pcTBS, which is a repetitive non-invasive brain stimulation protocol which feels like many quick, light taps on your head. You may be asked to contract your muscles to a moderate level (20% effort) during the pcTBS protocol. Next, pcTBS will be applied for 3 minutes. Following that, response to the stimulation will be recorded. Everyone in the study will receive both forms of stimulation (active and inactive) using the second coil but will not

know which one they receive first. Inactive refers to where the coil will be “on” but you won’t receive brain stimulation.

3. **Performing tests to determine your** understanding of your pain experience**.** You will undergo different tests to evaluate your understanding of your pain experience each time you receive the brain stimulation. To examine your understanding of your pain experience, we will use devices that apply brief amounts of slight pressure and mild heat to different locations on your forearm, near the shoulder, and leg area depending on presence or absence of pain at these locations. We will choose only areas where you currently do not have pain. We will steadily keep applying slight pressure using a handheld pressure device on this location and determine the pressure at which your understanding of your pain experience becomes painful. We will then immerse your opposite hand in cold water and perform the same test again to assess your understanding of your pain experience to increasing pressure. We will then apply mild heat using a probe near the location of your pressure test and determine the temperature at which your understanding of your pain experience becomes painful. We will then change the temperature and assess if your understanding of your pain experience was painful. Lastly, we will provide a steady series of heat stimuli at different temperatures every few seconds to see whether your understanding of your pain experience becomes painful. **AT NO POINT DURING THE TESTING OF YOUR UNDERSTANDING OF YOUR PAIN EXPERIENCE WILL WE PROVIDE ANY PRESSURE OR HEAT STIMULUS THAT EXCEEDS YOUR PAIN TOLERANCE. WE WILL STOP THE PAIN TESTING IF YOU FEEL IT IS UNCOMFORTABLE OR INCREASES YOUR PAIN BEYOND WHAT YOU CAN TOLERATE.**

The study team will email you a safety questionnaire 24 hours after the study session is completed to evaluate for any side effects associated with the non-invasive brain stimulation procedure. This questionnaire will be sent with the surveys that you will be emailed electronically.

What alternative treatments or procedures are available?
Your alternative is not to participate in this study. If you decide not to participate in this study, you can receive the usual care that you would receive at VCU Health even if you were not in the study. You do not have to participate in this study to be treated for painful diabetic neuropathy.

What are the risks and benefits of participating?
Benefits to You and Others
There is no guarantee that you will receive any medical benefits from being in this study. However, possible benefits include a decrease in your understanding of your pain experience for a few days after your study visit. We hope the information learned from

this study will provide more information about this decrease in your understanding of your pain experience so we can use it in future studies to provide this treatment for multiple sessions and not just one study visit.

Risks and Discomforts
Possible side effects associated with non-invasive brain stimulation include:
• Local pain, headache, discomfort
• Rare risk of seizure or syncope
• Change in hearing (transient increases in auditory thresholds)
• Mild toothache

Typically, local pain or headache or tooth ache if observed during the pcTBS session which will resolve after the session is over. These side effects also can also be observed after the session is over, but they typically resolve within 24 hours. If these side effects persist, please contact the study staff for further guidance.

This research involves an investigational device, the Magstim Rapid²
and the procedure may involve risks to the participant which are currently unforeseeable. There is an unknown risk associated with pregnancy. Magnetic fields attenuate rapidly with distance, so it seems unlikely that the embryo or fetus might be directly affected by TMS.As the study procedures might injure an unborn child, pregnant women may not participate. We would strongly urge you to not participate in this study if you think that you might be pregnant. We are not recruiting pregnant or attempting to become pregnant women in this study.

Non-Physical Risks
Participation in research might involve some loss of privacy. There is a small risk that someone outside the research study could see and misuse information about you.

HOW WILL INFORMATION ABOUT ME BE PROTECTED?
VCU and the VCU Health System have established secure research databases and computer systems to store information and to help with monitoring and oversight of research. Your information may be kept in these databases according to VCU's policies (i.e. for a minimum of 5 years after the study is completed). It is only accessible to individuals working on this study or authorized individuals who have access for specific research related tasks.

Your data will be identified by ID numbers, not names, and stored electronically on REDCap (a secure web application for building and managing online surveys and databases). All personal identifying information will be stored electronically and kept in password protected files and these files will be deleted 5 years after the completion of

this study. Other records, including the link between your ID and name, will be stored electronically on REDCap and will be destroyed after the study ends. Access to research data will be limited to study personnel. The paper documents used in the study including this consent form will be stored in a locked cabinet inside a locked room. Only study staff with VCU ID access will be able to enter the lab. A data and safety monitoring plan is established for this study.

Identifiable information in these databases is not released outside VCU unless stated in this consent or required by law. Although results of this research may be presented at meetings or in publications, identifiable personal information about participants will not be disclosed.

Personal information about you might be shared with or copied by authorized representatives from the following organizations for the purposes of managing, monitoring and overseeing this study:

- Representatives of VCU and the VCU Health System
- Officials of the Department of Health and Human Services or the Federal Food and Drug Administration

This is a clinical trial but it is being performed for research purposes only and your participation in this study will not be entered in to your medical record or electronic health record at VCU Health. You or your insurance will be not be billed for this study. All the information that you provide us is protected as any of your health records are protected.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Web site at any time.

In the future, identifiers might be removed from the information you provide in this study, and after that removal, the information could be used for other research studies by this study team or another researcher without asking you for additional consent.

In general, we will not give you any individual results from the study. We will email you a one-page summary of the study results after the study is completed and all the data has been analyzed. If we find something of medical importance to you, the Study PI will call you and inform you, although we expect that this will be a very rare occurrence.

WHAT ARE THE COSTS?

There are no costs to you associated with participating in the study.

WILL I BE PAID TO PARTICIPATE IN THE STUDY?
You will be mailed a $50 check for your study participation. Total payments within one calendar year that exceed $600 will require the University to report these payments annually to the IRS and you. This may require you to claim the compensation you receive for participation in this study as taxable income. VCU is required by federal law to collect your social security number. Your social security number will be kept confidential and will only be used to process payment. If you withdraw before the end of the study, you will not be paid any compensation for your study visit.

WHAT HAPPENS IF I AM INJURED OR BECOME SICK BECAUSE I TOOK PART IN THE STUDY?
If you are injured by, or become ill from, participating in this study, please contact your study doctor immediately. Medical treatment is available at the Virginia Commonwealth University Health System (VCU Health System). Your study doctor will arrange for short-term emergency care at the VCU Health System or for a referral if it is needed.

Fees for such treatment may be billed to you or to appropriate third-party insurance. Your health insurance company may or may not pay for treatment of injuries or illness as a result of your participation in this study.

To help avoid research-related injury or illness, it is very important to follow all study directions.

CAN I STOP BEING IN THE STUDY?
You can stop being in this research study at any time. Leaving the study will not affect your medical care at VCU Health. Tell the study staff if you are thinking about stopping or decide to stop. If you withdraw from the study, data that has already been collected about you will remain part of the study database and may not be removed.

Your participation in this study may be stopped at any time by the study doctor without your consent. The reasons might include:

- the study staff thinks it necessary for your health or safety
- you have not followed study instructions
- administrative reasons require your withdrawal

OPTIONAL STORAGE FOR FUTURE RESEARCH STUDIES
To advance science, it is helpful for researchers to share information. They do this by putting data or samples into one or more scientific databases (called registries or repositories), where it is stored along with information from other studies. Researchers

can then study the information in other ways and combine information from many studies to learn even more about health and disease.

As part of this study, we would like to keep your name, email, phone number, diagnosis of your medical condition and your pain history that you provide in a registry/repository to be available for other research studies in the future. Your information would be stored electronically on REDCap by the study PI and could be used for other research studies that are investigating the pain experience in patients with diabetic neuropathy. Your data will be protected, but there is always a possibility that information could be accessed by individuals without authorization. There is no limit on the length of time we will store your information.

In the future, if you decide that you don't want to be part of this registry, you can request that your information be removed and destroyed by contacting Dr. Carrie L Peterson at (804) 827-5270. However, information that has already been shared with other researchers will continue to be used.

PERMISSION TO STORE DATA FOR FUTURE RESEARCH STUDIES

Please circle your answer:

I agree that my data may be stored and used for future research as described above.

YES NO

WHOM SHOULD I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY?

The investigator and study staff named below are the best person(s) to contact if you have any questions, complaints, or concerns about your participation in this research: **Dr. Edmund O. Acevedo, Ph.D., FACSM at eoacevedo@vcu.edu or (804) 828-1948.**

If you have general questions about your rights as a participant in this or any other research, or if you wish to discuss problems, concerns or questions, to obtain information, or to offer input about research, you may contact:

Virginia Commonwealth University Office of Research
800 East Leigh Street, Suite 3000, Box 980568, Richmond, VA 23298
(804) 827-2157; https://research.vcu.edu/human-research/

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all your questions.

STATEMENT OF CONSENT

I have been provided with an opportunity to read this consent form carefully. All the questions that I wish to raise concerning this study have been answered. By signing this consent form, I have not waived any of the legal rights or benefits to which I otherwise would be entitled. My signature indicates that I freely consent to participate in this research study. I will receive a copy of the consent form for my records.

Signature Block for Enrolling Adult Participants	
_____ Adult Participant Name (Printed)	
_____ Adult Participant's Signature	_____ Date
_____ Name of Person Conducting Consent Discussion (Printed)	
_____ Signature of Person Conducting Consent Discussion	_____ Date
_____ Principal Investigator Signature (if different from above)	_____ Date

Appendix B

Screening Forms and Self-report Measures of Pain

Screening form

Please complete the survey below.

Thank you!

1)	Have you ever been diagnosed with Diabetic neuropathy or Distal symmetric polyneuropathy	☐ Diabetic neuropathy ☐ Distal symmetric polyneuropathy ☐ both
2)	Do you have Type II Diabetes Mellitus?	◯ Yes ◯ No
3)	Do you have any pain in your feet, legs or hands	◯ Yes ◯ No
4)	If yes: where? since when (months)?	_____
5)	On a 0-10 scale where 0 is no pain and 10 is the worst possible pain which takes you to the ER, what is your current pain.	_____
6)	How old are you?	_____
7)	Do you have any skull abnormalities/fractures?	◯ Yes ◯ No
8)	Have you suffered a concussion within the last 6 months?	◯ Yes ◯ No
9)	Have you ever had surgery of your head?	◯ Yes ◯ No
10)	Do you have unexplained, recurring headaches?	◯ Yes ◯ No
11)	Do you use a pacemaker?	◯ Yes ◯ No
12)	Do you use Insulin pumps?	◯ Yes ◯ No
13)	Do you have any other implanted devices, e.g., a cochlear implant?	◯ Yes ◯ No
14)	Do you have metal implants in your head or face?	◯ Yes ◯ No
15)	Do you have epilepsy or have you ever had a seizure?	◯ Yes ◯ No
16)	Has anyone in your family had epilepsy?	◯ Yes ◯ No

17) Are you, or could you be pregnant?

- ◯ Yes
- ◯ No

18) Do you take any medications?

- ◯ Yes
- ◯ No

Pain Detect Questionnaire

Please complete the survey below.

Thank you!

Screening ID _____

Study ID _____

How would you assess your pain now, at this moment? (with 0 being 'no pain at all' and 10 being the 'pain as bad as it could be')

0 5 10

(Place a mark on the scale above)

How strong was the strongest pain during the past 4 weeks? (with 0 being 'no pain at all' and 10 being the 'pain as bad as it could be')

0 5 10

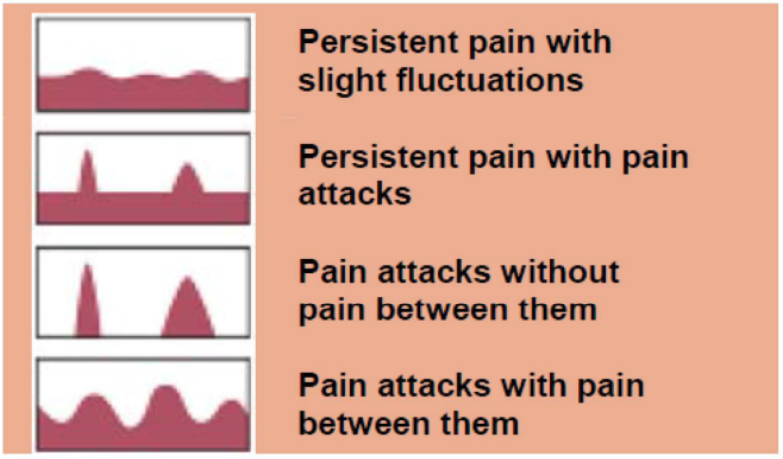
(Place a mark on the scale above)

How strong was the pain during the past 4 weeks on average? (with 0 being 'no pain at all' and 10 being the 'pain as bad as it could be')

0 5 10

(Place a mark on the scale above)

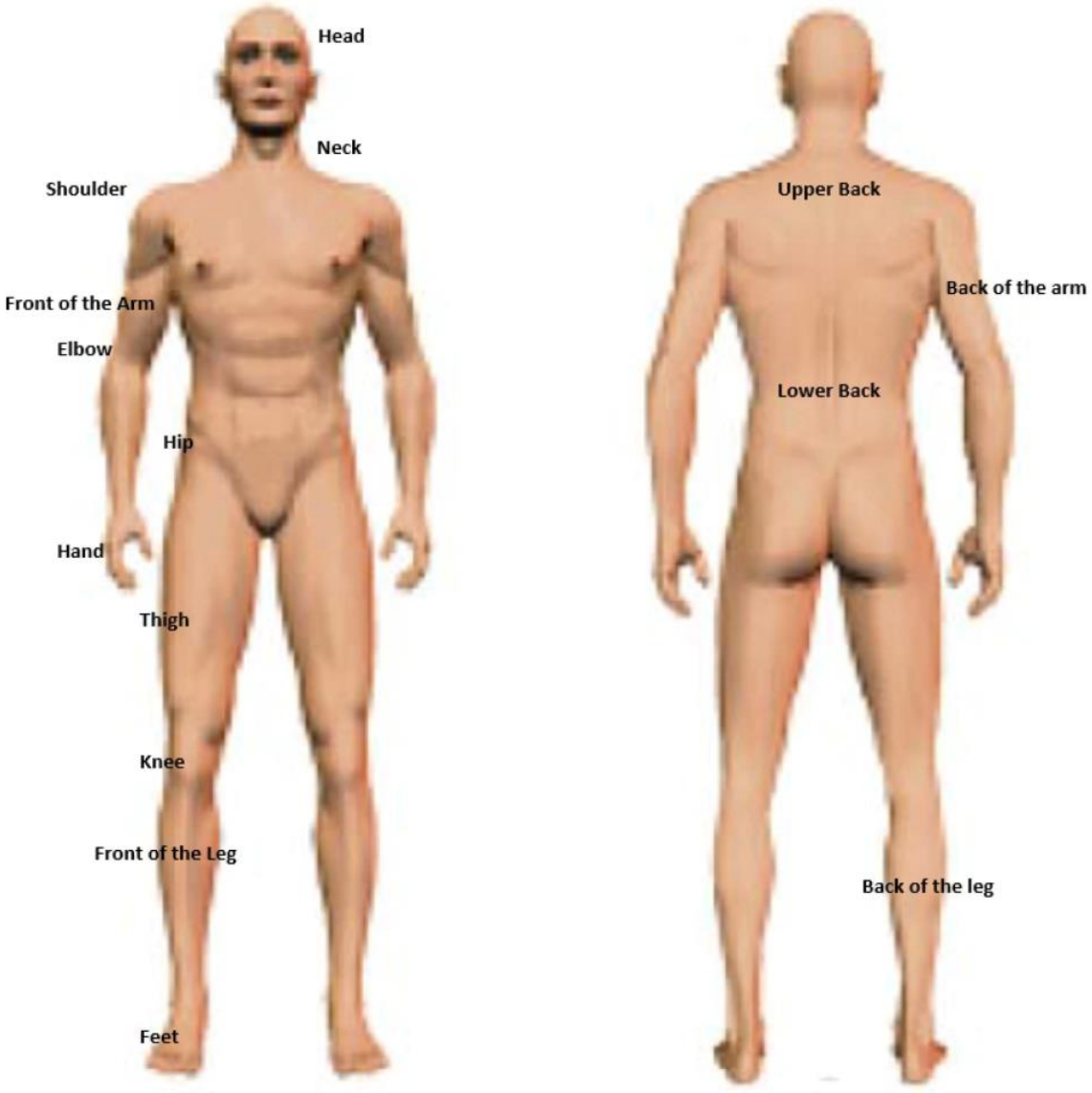
Please use this picture to answer the next question.



Please pick the description that best describes the course of your pain

- ◯ Persistent pain with slight fluctuations
- ◯ Persistent pain with pain attacks
- ◯ Pain attacks without pain between them
- ◯ Pain attacks with pain between them

Please look at the picture and answer the next question



Looking at the picture, Please write in the box your main area of pain (Front or Back and Right or Left)

_____

If the pain location isn't described above, please describe it here.

_____

Does your pain radiate to other regions of your body ?

◯ Yes
◯ No

If yes, please describe where the pain radiates.

Do you suffer from a burning sensation (e.g., stinging nettles) in the areas you marked?

◯ never ◯ hardly noticed ◯ slightly ◯ moderately ◯ strongly ◯ very strongly

Do you have a tingling or prickling sensation in the area of your pain (like crawling ants or electrical tingling)?

◯ never ◯ hardly noticed ◯ slightly ◯ moderately ◯ strongly ◯ very strongly

Is light touching (clothing, a blanket) in this area painful?

◯ never ◯ hardly noticed ◯ slightly ◯ moderately ◯ strongly ◯ very strongly

Do you have sudden pain attacks in the area of your pain, like electric shocks?

◯ never ◯ hardly noticed ◯ slightly ◯ moderately ◯ strongly ◯ very strongly

Is cold or heat (bath water) in this area occasionally painful?

◯ never ◯ hardly noticed ◯ slightly ◯ moderately ◯ strongly ◯ very strongly

Do you suffer from a sensation of numbness in the areas you marked?

◯ never ◯ hardly noticed ◯ slightly ◯ moderately ◯ strongly ◯ very strongly

Does slight pressure in this area, e.g., with a finger, trigger pain?

◯ never ◯ hardly noticed ◯ slightly ◯ moderately ◯ strongly ◯ very strongly

PDQ score (Research Use only)

Demographic Questionnaire

Please complete the survey below.

Thank you!

Today's Date	_____
Age	_____
Weight in lbs	_____
Height in INCHES	_____
BMI	_____
What race do you consider yourself?	◯ White ◯ Black or African American ◯ Asian ◯ American Indian or Alaskan Native ◯ Middle Eastern or North African ◯ Native Hawaiian or Other Pacific Islander ◯ Other race or ethnicity ◯ Hispanic or Latino or Spanish ◯ Prefer not to say
Please describe other race or ethnicity:	_____
What is the highest level of education that you have completed? (choose one best option)	◯ Less than high school ◯ High school graduate or GED ◯ Some college ◯ Associate's degree ◯ Bachelor's degree ◯ Graduate degree ◯ Prefer not to say
What is your current employment status? (choose one best option)	◯ Employed/self-employed ◯ A homemaker ◯ A student ◯ Retired ◯ Unemployed ◯ Temporarily laid off, on sick leave or maternity leave ◯ On disability or unable to work ◯ Prefer not to say
Please report any current medications if you can recall them	
Please specifically report any pain medications if you can recall them if you haven't included them above	

Other Medical Conditions (Please select multiple answers if applicable)				
	yes	no	I don't know	receiving treatment
Diabetes	☐	☐	☐	☐
Hypertension	☐	☐	☐	☐
Heart failure	☐	☐	☐	☐
Pulmonary disease	☐	☐	☐	☐
Other	☐	☐	☐	☐

Please describe "Other" medical conditions _____

Brief Pain Inventory (Short Form)- DPN

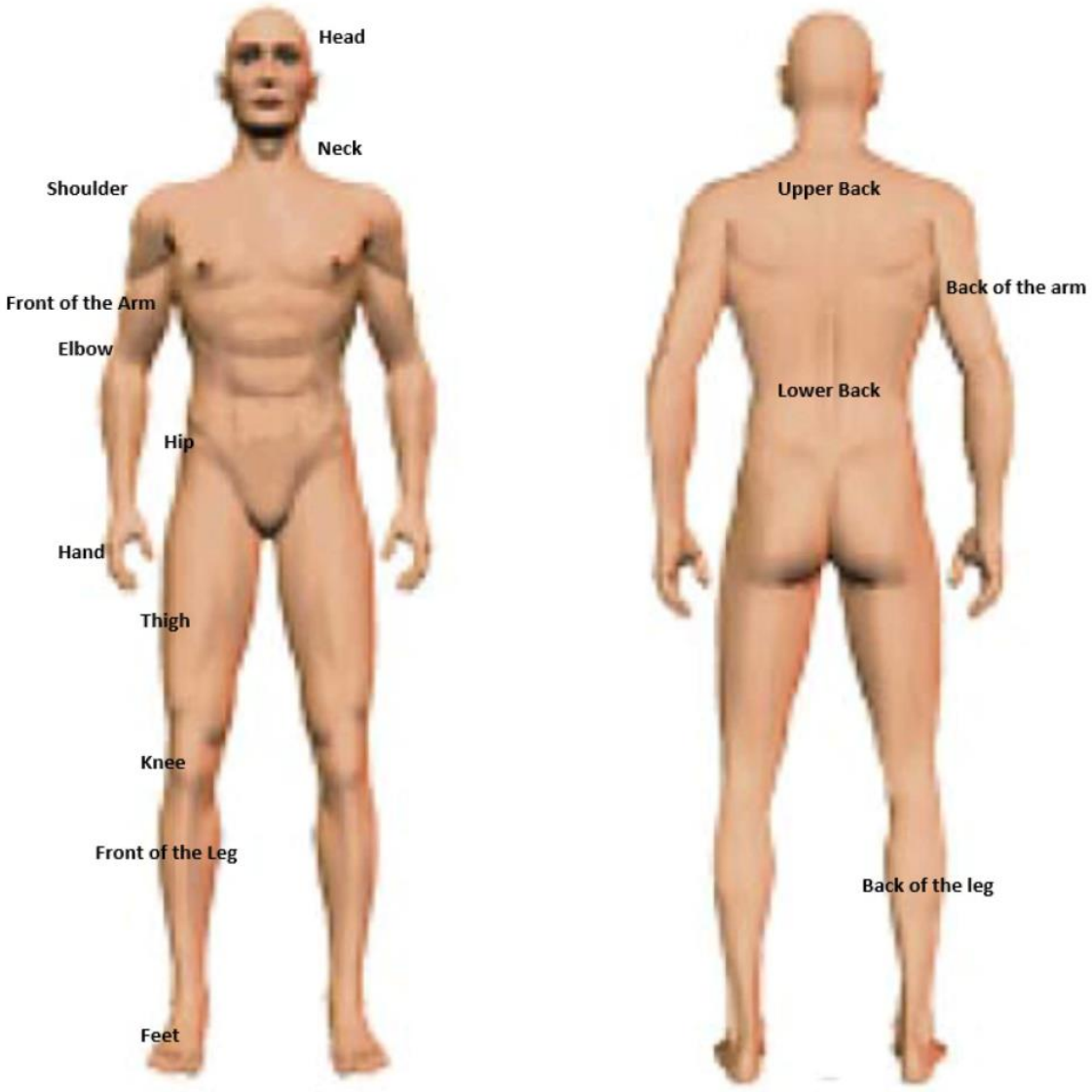
Please complete the survey below.

Thank you!

1) Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?

- ◯ Yes
- ◯ No

Please look at the picture and answer the next question



2) Looking at the picture, Please write in the box your main area of pain (Front or Back and Right or Left)

_____

3) Please rate your pain due to your diabetes by sliding to the one number that best describes your pain at its worst in the last 24 hours.

0 is No Pain

10 is Pain as bad as you can imagine

No Pain — Pain as bad as you can imagine

(Place a mark on the scale above)

4) Please rate your pain due to your diabetes by sliding to the one number that best describes your pain at its least in the last 24 hours.

0 is No Pain

10 is Pain as bad as you can imagine

0 — 10

(Place a mark on the scale above)

5) Please rate your pain due to your diabetes by sliding to the one number that best describes your pain on the average

0 is No Pain

10 is Pain as bad as you can imagine

0 — 10

(Place a mark on the scale above)

6) Please rate your pain due to your diabetes by sliding to the one number that tells how much pain you have right now.

0 is No Pain

10 is Pain as bad as you can imagine

0 — 10

(Place a mark on the scale above)

7) In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0% No Relief

100% Complete Relief

0 — 50 — 100

(Place a mark on the scale above)

Indicate number that describes how, during the past 24 hours, pain due to your diabetes has interfered with your:

0 Does not Interfere
10 Completely Interferes

		0	1	2	3	4	5	6	7	8	9	10
8)	General Activity	○	○	○	○	○	○	○	○	○	○	○
9)	Mood	○	○	○	○	○	○	○	○	○	○	○
10)	Walking Ability	○	○	○	○	○	○	○	○	○	○	○
11)	Normal Work (includes both work outside the home and housework)	○	○	○	○	○	○	○	○	○	○	○
12)	Relations with other people	○	○	○	○	○	○	○	○	○	○	○
13)	Sleep	○	○	○	○	○	○	○	○	○	○	○
14)	Enjoyment of life	○	○	○	○	○	○	○	○	○	○	○

Bodily and Emotional Perception of Pain (BEEP)

Please complete the survey below.

Thank you!

Please specify how intense were the following moods in the occasion you felt the strongest pain:

	never	hardly noticed	slightly	moderately	strongly	very strongly
Irritability (I lose my patience at the slightest thing)	○	○	○	○	○	○
Feeling powerless	○	○	○	○	○	○
Depression (deep sadness with loss of any interest)	○	○	○	○	○	○
Feeling of injustice (why me?)	○	○	○	○	○	○
Pessimism (a negative vision of the future)	○	○	○	○	○	○
Anxiety	○	○	○	○	○	○
Feeling guilty (for example: I feel I am a burden for my family)	○	○	○	○	○	○
Frustration (I can do nothing about it and I am angry at this)	○	○	○	○	○	○
I lack confidence in my abilities and skills	○	○	○	○	○	○
I'm afraid I will not recover	○	○	○	○	○	○
Confusion (I feel my mind is less clear)	○	○	○	○	○	○
I do not recognize myself	○	○	○	○	○	○
I feel I have become older	○	○	○	○	○	○
I feel impaired	○	○	○	○	○	○
I don't feel independent	○	○	○	○	○	○

Please specify how intense are the following moods "at this moment (right now)"

	never	hardly noticed	slightly	moderately	strongly	very strongly
Irritability (I lose my patience at the slightest thing)	○	○	○	○	○	○
Feeling powerless	○	○	○	○	○	○
Depression (deep sadness with loss of any interest)	○	○	○	○	○	○
Feeling of injustice (why me?)	○	○	○	○	○	○
Pessimism (a negative vision of the future)	○	○	○	○	○	○
Anxiety	○	○	○	○	○	○

Feeling guilty (for example: I feel I am a burden for my family)	○	○	○	○	○	○
Frustration (I can do nothing about it and I am angry at this)	○	○	○	○	○	○
I lack confidence in my abilities and skills	○	○	○	○	○	○
I'm afraid I will not recover	○	○	○	○	○	○
Confusion (I feel my mind is less clear)	○	○	○	○	○	○
I do not recognize myself	○	○	○	○	○	○
I feel I have become older	○	○	○	○	○	○
I feel impaired	○	○	○	○	○	○
I don't feel independent	○	○	○	○	○	○

During your lifetime, how seriously did having this pain limit

	never	hardly noticed	slightly	moderately	strongly	very strongly
Your working performance	○	○	○	○	○	○
Your capability to move	○	○	○	○	○	○
Your social role	○	○	○	○	○	○
Your sports activity	○	○	○	○	○	○

During your lifetime, how seriously did having this pain Interfere with

	never	hardly noticed	slightly	moderately	strongly	very strongly
Mood (inner affective-emotional tone, e.g.: I'm more often sad, more often cheerful)	○	○	○	○	○	○
Interpersonal relationships	○	○	○	○	○	○
Sleep	○	○	○	○	○	○
The pleasure of living	○	○	○	○	○	○

Please select the numeric response on the scale that best describes your pain

On average in the past 24 hours (with 0 being 'no pain at all' and 10 being the 'pain as bad as it could be')

0 5 10

(Place a mark on the scale above)

In this moment (with 0 being 'no pain at all' and 10 being the 'pain as bad as it could be')

0 5 10

(Place a mark on the scale above)

Norfolk QOL-DN

Please complete the survey below.

Thank you!

Record ID _____

Please describe your symptoms over the past 4 weeks. Please check all that apply

	Feet	Legs	Hands	Arms
Numbness	☐	☐	☐	☐
Tingling, pins, and needles	☐	☐	☐	☐
Electric shocks	☐	☐	☐	☐
Unusual sensations	☐	☐	☐	☐
Superficial pain	☐	☐	☐	☐
Deep pain	☐	☐	☐	☐
Weakness	☐	☐	☐	☐

Duration and nature of symptoms

How long have you had any symptoms of neuropathy ?
Please Insert your response in Months.
For example two and half years would be 30 months _____

Are the symptoms the same on the right as on the left?
◯ Yes
◯ No

Which is worse ?
◯ Right
◯ Left

Are the symptoms worse at night?
◯ Yes
◯ No

How many medications have been used for these symptoms?
Please write the number if you can recall _____

Diagnosis/existence of complications

Please indicate "yes" or "no" to indicate a presence diagnosis of neuropathy or the existence of complications

	Yes	No
Told that you have neuropathy?	◯	◯
Ulcers on your feet?	◯	◯
Gangrene?	◯	◯

Toes or fingers amputated?	○	○

In the past 4 weeks, have you had a problem with involuntary urinating when laughing or coughing?

○ Yes ○ No

(FEMALES ONLY) In the past 4 weeks, have you had a problem with vaginal dryness during intercourse?

○ Yes ○ No ○ Not Applicable

(MALES ONLY) In the past 4 weeks, have you had a problem with obtaining or maintaining erections?

○ Yes ○ No ○ Not Applicable

Please indicate the extent to which physical problems related to neuropathy have presented a problem when performing Activities of Daily Living (example: bathing) Please check one box only

In the past 4 weeks,

	not a problem	very mild problem	mild problem	moderate problem	severe problem.
Has the pain kept you awake or woken you at night ?	○	○	○	○	○
Have you been unable to tell hot from cold water with your hands?	○	○	○	○	○
Have you been unable to tell hot from cold water with your feet?	○	○	○	○	○
Have you had a problem with vomiting, particularly after meals (but not due to flu or other illness)?	○	○	○	○	○
Have you had a problem with diarrhea and/or loss of bowel control?	○	○	○	○	○
Have you had a problem with fainting or dizziness when you stand?	○	○	○	○	○
How much difficulty have you had while bathing/showering?	○	○	○	○	○
How much difficulty have you had while dressing?	○	○	○	○	○
How much difficulty have you had during walking?	○	○	○	○	○

How much difficulty have you had getting on or off the toilet?	○	○	○	○	○
How much difficulty have you had using eating utensils?	○	○	○	○	○
Have you experienced hyperalgesia to touch? Hyperalgesia refers to an increased sensitivity to feeling pain and an extreme response to pain	○	○	○	○	○
Have you burned or injured self and unable to feel it?	○	○	○	○	○
Have any symptoms kept you from doing your usual activities during the day?	○	○	○	○	○
Have you had difficulty doing fine movements with your fingers, like buttoning your clothes, turning pages in a book, picking up coins from a table?	○	○	○	○	○
Have you felt unsteady on your feet when you walk?	○	○	○	○	○
Have you had any problem getting out of a chair without pushing with your hands?	○	○	○	○	○
Have you had a problem walking down stairs?	○	○	○	○	○
Have you been unable to feel your feet when walking?	○	○	○	○	○

Please describe your Generic Health Status over the last 4 weeks. Please check one box only

In the past 4 weeks have you

	Not at all	A little	Somewhat	Moderately	Severely
Cut down the time you spent on work/other activities?	○	○	○	○	○
Accomplished less than you would like?	○	○	○	○	○
Were limited in the kind of work or other activities you could perform?	○	○	○	○	○

Had difficulty performing the work/other activities (it took extra effort)?	◯	◯	◯	◯	◯

In general, would you say your health now is:

◯ Excellent ◯ Very Good ◯ Good ◯ Fair ◯ Poor

Compared with 3 months ago, how would you rate your health in general now?

◯ Much Better ◯ Somewhat Better ◯ About the Same ◯ Somewhat Worse ◯ Much Worse

In the past 4 weeks, to what extent has your physical health interfered with your normal social activities with family, friends, neighbors, or groups?

◯ Not at all ◯ A little ◯ Somewhat ◯ Moderately ◯ Severely

In the past 4 weeks, how much did pain interfere with your normal work (including your work responsibilities while working from home/at work and housework)?

◯ Not at all ◯ A little ◯ Somewhat ◯ Moderately ◯ Severely

In the past 4 weeks, how much did weakness or shakiness interfere with your normal work (including your work responsibilities while working from home/at work and housework)?

◯ Not at all ◯ A little ◯ Somewhat ◯ Moderately ◯ Severely

DASS-21

Please complete the survey below.

Thank you!

Record ID _____

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you WHEN YOU FELT YOUR STRONGEST PAIN. There are no right or wrong answers. Do not spend too much time on any statement.

	Did not apply to me at all	Applied to me to some degree, or some of the time	Applied to me to a considerable degree, or a good part of time	Applied to me very much, or most of the time
I found it hard to wind down	○	○	○	○
I was aware of dryness of my mouth	○	○	○	○
I couldn't seem to experience any positive feeling at all	○	○	○	○
I experienced breathing difficulty (e.g. excessively rapid breathing, breathlessness in the absence of physical exertion)	○	○	○	○
I found it difficult to work up the initiative to do things	○	○	○	○
I tended to over-react to situations	○	○	○	○
I experienced trembling (e.g. in the hands)	○	○	○	○
I felt that I was using a lot of nervous energy	○	○	○	○
I was worried about situations in which I might panic and make a fool of myself	○	○	○	○
I felt that I had nothing to look forward to	○	○	○	○
I found myself getting agitated	○	○	○	○
I found it difficult to relax	○	○	○	○
I felt down-hearted and blue	○	○	○	○
I was intolerant of anything that kept me from getting on with what I was doing	○	○	○	○

I felt I was close to panic	○	○	○	○
I was unable to become enthusiastic about anything	○	○	○	○
I felt I wasn't worth much as a person	○	○	○	○
I felt that I was rather touchy	○	○	○	○
I was aware of the action of my heart in the absence of physical exertion (e.g. sense of heart rate increase, heart missing a beat)	○	○	○	○
I felt scared without any good reason	○	○	○	○
I felt that life was meaningless	○	○	○	○

pcTBS 24hrs post questionnaire

Please complete the survey below.

Thank you!

Please indicate below if you experienced any side effects after your participation in the study.	Yes	No
Headache	○	○
Neck Pain	○	○
Seizure	○	○
Hearing Impairment	○	○
Any Discomfort	○	○
Any toothache	○	○
Increase in pain	○	○
Other	○	○

If "Other" Please specify _____

If you experienced any side effects please indicate the severity of these symptoms using the severity ratings given below	absent	mild	moderate	severe
Headache	○	○	○	○
Neck Pain	○	○	○	○
Seizure	○	○	○	○
Hearing Impairment	○	○	○	○
Any Discomfort	○	○	○	○
Any toothache	○	○	○	○
Increase in pain	○	○	○	○
other	○	○	○	○

If you experienced any side effects please describe how likely do you think these symptoms are linked to your participation in the study.	none	remote	possible	probable	definite
Headache	○	○	○	○	○
Neck Pain	○	○	○	○	○
Seizure	○	○	○	○	○
Hearing Impairment	○	○	○	○	○
Any Discomfort	○	○	○	○	○
Any toothache	○	○	○	○	○
Increase in pain	○	○	○	○	○

other	○	○	○	○	○

Patient Blinding questionnaire

Please complete the survey below.

Thank you!

1) "You received two forms of pcTBS treatment during this study, active and inactive, which one do you think you received first?

- ◯ Active pcTBS
- ◯ Inactive pcTBS

2) How certain are you in your ability to correctly guess the treatment that you received first ?

Active — Inactive

(Place a mark on the scale above)

Appendix C

Curriculum Vita

Bhushan Thakkar PT, MS

1101 East Marshall Street, 11th Floor, Room 11-027, Richmond, VA 23298. 804-300-5578.
thakkarbs2@vcu.edu. Twitter: @ThakkarBhushan. ORCID: 0000-0001-6711-975X

ACADEMIC EDUCATION:

- **Doctor of Philosophy**, Rehabilitation and Movement Science **Expected September 2022**
  Virginia Commonwealth University. Richmond, VA.
  Advisor: Edmund Acevedo, Ph.D., FACSM
  Thesis: Theta Burst Brain Stimulation in Painful Diabetic Neuropathy Patients: Investigating Neural Mechanisms

- **Master of Science in Physical Therapy,** Orthopedic Specialization **May 2011**
  MGH Institute of Health Professions. Boston, MA.
  Advisor: K. Douglas Gross, MPT, DPT, ScD, FAAOMPT, CPed
  Thesis: The relationship between pelvic drop during walking and medial knee osteoarthritis: The MOST study.

- **Bachelor of Physiotherapy** **February 2009**
  Manipal University. Manipal, India.
  Advisor: Kavitha Raja MS, PT, PhD, PGDR
  Independent Research Project: Effects of patella taping and elastocrepe bandage on knee joint proprioception in normal individuals.

CURRENT RESEARCH INTERESTS:

- Evaluation of neural mechanisms that govern the presence of co-morbid chronic pain and psychiatric disorders in older adults to identify biomarkers and therapeutic interventions using pharmacological and neuromodulation based approaches.
- Assessment of sex and gender differences in mechanisms of chronic pain, pain assessment and treatment especially in people with neuropathic pain and substance use disorders

RESEARCH EXPERIENCE:

InRecovery lab (IVY Lab) **December 2019 – Current**

Position: Research Coordinator

Principal Investigator: Dr. Caitlin E. Martin MD, MPH

The overall aim of the IVY Lab is to elucidate how sex and gender intersect with both biological factors, such as pregnancy and the postpartum state, and psychosocial contexts to impact recovery trajectories of people receiving SUD treatment.

- Ensure the smooth and efficient day-to-day operation of research and data collection activities; acts as the primary administrative point of contact for current and prospective lab members
- Plan and coordinate the initiation of research study protocol, and the establishment of operating policies and procedures
- Assist research personnel in the implementation of research studies to ensure successful completion of study goals
- Coordinate, train, and mentor research assistants including medical, graduate, and undergraduate students
- Collaborate across interdisciplinary groups of colleagues in the writing and submission of study findings for poster presentations, publication in peer-reviewed journals, grant writing and funding opportunities for lab members
- Organize the agenda, emailing the invites, presenting lab updates for the Monthly Lab meetings
- Plans, implements, and maintains data collection and analysis systems in support of research protocol and coordinate the collection and analysis of research data
- Participate in internal/external research related conferences and investigator meetings

- Prepare and maintains all Institutional Review Board (IRB) and regulatory paperwork to comply with institutional regulatory requirements
- Formulate yearly progress reports and funding reports for IVY Lab documenting research productivity and educational objectives.
- Set up projects and surveys in REDCap, perform sample size calculations using GPower 3.1, analyze qualitative data using ATLAS.ti.8 and statistical analysis using SAS 9.4, SPSS 28.1 and GraphPad Prism.
- Created the IVY Lab Twitter and Youtube channels and responsible for regularly maintaining it.

Rehabilitation Engineering to Advance Ability Lab (REALab) December 2019 – July 2021
Position: Graduate Research Assistant
Principal Investigator: Carrie L. Peterson, Ph.D.
The mission of the Rehabilitation Engineering to Advance Ability Lab (REALab) is to improve health, mobility and independence for individuals with physical disabilities. Towards this mission, we take an interdisciplinary approach by combining techniques and knowledge from engineering, physiology, anatomy, and clinical practice.

- Assist with data collection for research studies involving Transcranial Magnetic stimulation in healthy participants, patients with spinal cord injury and painful diabetic neuropathy
- Coordinate, train, and mentor undergraduate students to apply for funding opportunities, research presentations and getting them up to speed with research studies when they join the lab
- Collecting data using Magstim BiStim^2 monophasic stimulator and Rapid^2 Family biphasic stimulators combined with Delsys Electromyography sensors.
- Analyzing data using MATLAB and CED Spike 2 software.
- Collaborating on abstract submissions to scientific meetings and publications to peer reviewed journals.
- Attend Weekly Lab meetings and present during journal clubs and research presentations
- Mentored three undergraduate students

VCU RUNLAB August 2014 – August 2018
Position: Graduate Research Assistant
Principal Investigator: D.S. Blaise Williams, PT, Ph.D.

- Prepared order lists, purchased lab supplies, organized schedule of research studies and coordinating with other lab members and students
- Performed 3D gait analysis on research subjects, as well as VCU athletes using Qualisys motion capture system on a Treadmetrix instrumented treadmill with force plates for running analysis. Using Vicon motion capture to perform walking and jumping data collection. Integrating Delsys Electromyography software to capture muscle activity in combination with Qualisys and Vicon motion capture systems.
- Analysis of motion capture data using C-Motion software and MATLAB.
- Developed research projects related to walking biomechanics, running injury and performance.
- Supervised abstracts submissions to scientific meetings and reviewed manuscripts.
- Mentored six undergraduate and two graduate students.

PUBLICATIONS:
† Mentored Graduate Student. *Mentored Undergraduate Student.

1. Kathryn Harrison, **Bhushan Thakkar**, Yong Un Kwon, Gregory Crosswell, Jacqueline Morgan, DS Blaise Williams 3rd. Kinematic predictors of loading during running differ by demographic group. *Physical Therapy in Sport.* 2018; 32:221-226. https://doi.org/10.1016/j.ptsp.2018.05.019

2. **Bhushan Thakkar**, John D Willson, Kathryn Harrison, Robert Tickes, DS Blaise Williams 3rd. Tibiofemoral Joint Forces in Female Recreational Runners Vary with Step Frequency. *Medicine*

Science in Sports and Exercise. 2019; Jul,51(7):1444-1450. doi: 10.1249/MSS.0000000000001915

3. Kathryn Harrison, Yong Un Kwon, Adam Sima, **Bhushan Thakkar**, Gregory Crosswell, Jacqueline Morgan, DS Blaise Williams 3rd. Inter-joint coordination patterns differ between younger and older runners. *Human Movement Science.* 2019; 64:164-170. https://doi.org/10.1016/j.humov.2019.01.014

4. Caitlin E. Martin, Caroline Shadowen, **Bhushan Thakkar**, Travis Oakes, Tamas Gal, Gerard F Moeller. Buprenorphine dosing through the perinatal period. *Current Treatment Options in Psychiatry.* 2020; 7:375–399. https://doi.org/10.1007/s40501-020-00221-z

5. Caitlin E. Martin, Tawany Almeida, **Bhushan Thakkar**, Tiffany Kimbrough. Patient reported challenging and promoting factors for recovery through the postpartum transition among women in treatment for opioid used disorder: A qualitative study. Substance Abuse. 2021; 43:1, 389-396, DOI: 10.1080/08897077.2021.1944954

6. Kara Hostetter, **Bhushan Thakkar**, Cherie Edwards, Caitlin E. Martin. Addiction Curriculum Design in Medical Students. *Clinical Teacher. 2022; 19(1): 29– 35.* https://doi.org/10.1111/tct.13438

7. Geetika Reichmann, Anna Beth Parlier-Ahmad, Lori Beck, **Bhushan Thakkar**, Meryl Alappattu, Jeff Boissoneault, Caitlin E. Martin. Chronic Pelvic Pain and Sexual Dysfunction among Females and Males Receiving Treatment for Opioid use Disorder. Frontiers in pain research (Lausanne, Switzerland) vol. 2 787559. 11 Jan. 2022, doi:10.3389/fpain.2021.787559

8. Caitlin E. Martin, **Bhushan Thakkar,** DaShaunda D H Taylor, Derek A. Chapman. Disparities by Sex in COVID-19 Risk and Related Harms among People with Opioid Use Disorder. *Journal of Women's Health*. 2022; 31:5, 640-647. https://doi.org/10.1089/jwh.2021.0457

9. Neil Mittal, **Bhushan Thakkar**, Cooper B. Hodges, Yeajin Cho, Connor Lewis, Ravi L. Hadimani, Carrie Peterson. Effect of neuroanatomy on corticomotor excitability during and after transcranial magnetic stimulation and intermittent theta burst stimulation. Human Brain Mapping, 1– 16. https://doi.org/10.1002/hbm.25968

10. *Elizabeth Grist, **Bhushan Thakkar,** Phoebe Dacha, Madison Maxwell, Erika Lutins, Caitlin E. Martin. Medication Safety Practices of Pregnant and Parenting People Receiving Outpatient Opioid Use Disorder Treatment. In press with *Journal of Addiction Medicine*.

11. Caitlin E. Martin, **Bhushan Thakkar**, Lauren Cox, Elisabeth Johnson, Hendree E Jones, Anna Marie Connolly. Beyond Opioid Prescribing: An Evaluation of a Pilot Curriculum for OBGYN Residents on Substance Use Disorder Assessment and Treatment. PLOS ONE 17(9): e0274563. https://doi.org/10.1371/journal.pone.0274563.

SUBMITTED FOR PEER REVIEW:

1. **Bhushan Thakkar,** Edmund Acevedo. Role of BDNF in patients with chronic pain. Update on mechanisms of action and assessment. In review with *Brain and Behavior*.

2. Andrea Nguyen, Hannah Shadowen, Caroline Shadowen, **Bhushan Thakkar,** Andrea Knittel, Caitlin E. Martin. Incarceration status at start of medication treatment for opioid use disorder during pregnancy. In review *with American Journal of Preventive Medicine.*

3. Thibault Roumengous, **Bhushan Thakkar**, Carrie L. Peterson. Paired-pulse TMS in the Assessment of Voluntary Activation in Individuals with Tetraplegia and Non-impaired Individuals. In review with *Frontiers in Human Neuroscience*.

4. Sumaya Smarony, Annabeth Parlier-Ahmad, Hannah Shadowen, **Bhushan Thakkar,** Marjorie Scheikl, Caitlin E. Martin. Impact of COVID-19 driven changes in an integrated OBGYN addiction treatment clinic on substance use disorder outcomes. In review with *Journal of Addiction Medicine*.

IN PREPARATION:

1. **Bhushan Thakkar,** Carrie L. Peterson, Edmund O. Acevedo. Changes in Neural Mechanisms following Prolonged Continuous Theta Burst Stimulation Targeted at M1 and DLPFC in Patients with Painful Diabetic Neuropathy. To be submitted to *Journal of Pain.*

2. **Bhushan Thakkar,** Carrie L. Peterson, Edmund O. Acevedo. Short Term Effects of Prolonged Continuous Theta Burst Stimulation Targeted at M1 and DLPFC in Patients with Painful Diabetic Neuropathy. To be submitted to *PAIN*.

3. **Bhushan Thakkar,** Annabeth Parlier-Ahmad, Taylor Crouch, Caitlin E. Martin. Gender Differences in Chronic Pain among an Urban Opioid Use Disorder Outpatient Buprenorphine Treatment Population. To be submitted to *Addiction*.

4. **Bhushan Thakkar,** Kara Hostetter, Ashley Wilson, Caitlin E. Martin**.** Implementation and Evaluation of a Curriculum to Include Substance Use Disorders during Pregnancy. *To be submitted to American Journal of Obstetrics & Gynecology.*

5. **Bhushan Thakkar,** Morgan Meyer, Kathryn Harrison, Gregory Crosswell, D.S. Blaise Williams III. Influence of Gluteus Medius Strength on Interlimb Asymmetry in Female Recreational Runners. To be submitted to *Medicine & Science in Sports & Exercise.*

PLATFORM PRESENTATIONS:

1. **Bhushan Thakkar**, Morgan Meyer, Gregory Crosswell, Jacqueline Morgan, Kathryn Harrison. Influence of Age and Gender on Interlimb Asymmetry in Recreational Runners. Proceedings of the *20th Biennial Meeting of the Canadian Society for Biomechanics.* Halifax, Nova Scotia, Canada. August 14-17, 2018.

2. **Bhushan Thakkar,** Kathryn Harrison, Jacqueline Morgan, Gregory Crosswell, Robert Tickes, D.S Blaise Williams III. Influence of Stride Frequency on Knee Joint Stiffness and Anterior Tibial Shear Forces in Female Runners. Proceedings of the *Annual Meeting of the American Society of Biomechanics.* Boulder, Colorado. August 8 - 11, 2017.

3. **Bhushan Thakkar**, Jacqueline Morgan, D. S. Blaise Williams III. Effect of Training on Knee Torsional Stiffness and Its Relationship to Tibial Compressive and Anterior Shear Forces in Recreational Female Runners. Proceedings of the *American Physical Therapy Combined Sections Meeting 2016.* Anaheim, California, February 17 – 20, 2016.

4. **Bhushan Thakkar**, Jacqueline Morgan, D. S. Blaise Williams III. Effect of Training on Knee Torsional Stiffness and Its Relationship to Tibial Compressive and Anterior Shear Forces in Recreational Female Runners. Proceedings of the *American College of Rheumatology/ Association*

of Rheumatology Health Professionals Annual Meeting. San Francisco, California; November 6 – 11, 2015.

POSTER PRESENTATIONS:

1. Sajanee Chithranjan, Michelle Eglovitch, **Bhushan Thakkar**, Stephanie Violante, Caitlin E. Martin. Social determinants of health and insomnia in women receiving buprenorphine for OUD. Abstract submitted to The Virginia Society of Addiction Medicine, October 2022.

2. Brenna Cook, Michelle Eglovitch, **Bhushan Thakkar**, Stephanie Violante, Caitlin E. Martin. Factors Promoting Buprenorphine Consistency in Women in Outpatient Treatment for Opioid Use Disorder. Abstract submitted to The Virginia Society of Addiction Medicine, October 2022.

3. Phoebe Dacha, Michelle Eglovitch, **Bhushan Thakkar**, Stephanie Violante, Caitlin E. Martin. Examination of Patient Reported Buprenorphine Dosing Regimens in patients with Opioid Use Disorder. Abstract submitted to The Virginia Society of Addiction Medicine, October 2022.

4. *Catherine Legge, Michelle Eglovitch, **Bhushan Thakkar**, Stephanie Violante, Caitlin E. Martin. Role of Nicotine use on OUD Treatment Outcomes and Sleep Dysfunction in Women in Treatment for OUD. Abstract submitted to The Virginia Society of Addiction Medicine, October 2022.

5. *Saisriya Kolli, Michelle Eglovitch, **Bhushan Thakkar**, Stephanie Violante, Caitlin E. Martin. Role of Cannabis use on Clinical and Psychosocial outcomes in Women in Treatment for OUD. Abstract submitted to The Virginia Society of Addiction Medicine, October 2022.

6. Caitlin E. Martin, Hostetter K, Edwards C, **Bhushan Thakkar**. Implementation and Evaluation of a Curriculum to Include Substance Use Disorders during Pregnancy. *Proceedings of the Society for Academic Specialists in General Obstetrics and Gynecology Meeting.* San Diego, California. *May 5, 2022.*

7. *Elizabeth B. Grist, **Bhushan Thakkar**, Phoebe Dacha, Madison Maxwell, Erika Lutins, Caitlin E. Martin. A Harm Reduction Approach to Medication Storage for Pregnant and Parenting People with Substance Use Disorders. *Proceedings of the Society for Academic Specialists in General Obstetrics and Gynecology Meeting*. San Diego, California*. May 5, 2022.*

8. Andrea Nguyen, Hannah Shadowen, Caroline Shadowen, **Bhushan Thakkar**, Caitlin E. Martin. Incarceration status at start of medication treatment for opioid use disorder during pregnancy. *Proceedings of the Society for Academic Specialists in General Obstetrics and Gynecology Meeting*. San Diego, California*. May 5, 2022.*

9. Andrea Gataric A, Hannah Shadowen H, Stephanie Violante, **Bhushan Thakkar,** Caitlin E. Martin. Effect of Psychiatric Medications on Buprenorphine Use in Postpartum People with Opioid Use Disorder. *Proceedings of the Society for Academic Specialists in General Obstetrics and Gynecology Meeting*. San Diego, California. *May 5, 2022.*

10. *Brent Nevadomski, **Bhushan Thakkar**, Abigail Andrade, Peter Baek, Lavie Ngo, Deanna Skrivanek, Edmund Acevedo, Carrie Peterson. Effectiveness of Theta Burst Stimulation on Corticospinal Excitability and Cortical Inhibition in Painful Diabetic Neuropathy Patients. *2022 Annual Poster Symposium for Undergraduate Research and Creativity. Virginia Commonwealth University.* Richmond, Virginia. *April 20, 2022.*

11. **Bhushan Thakkar**, Carrie Peterson, Edmund Acevedo. Examining the Efficacy of Theta Burst Stimulation on Different Brain Regions Using Self-Report Measures in Patients with Painful

Diabetic Neuropathy. *Proceedings of the Graduate Student Association's 25th Annual Research Symposium & Exhibit. Virginia Commonwealth University.* Richmond, Virginia. *April 19, 2022.*

12. Hannah Shadowen, Andrea Gataric, Stephanie Violante, **Bhushan Thakkar,** Caitlin E. Martin. Effect of psychiatric co-morbidities and their treatments on buprenorphine continuation in postpartum people with opioid use disorder. *Proceedings of the American Society of Addiction Medicine 53rd Annual Conference.* Hollywood, Florida. *March 31-April 3, 2022.*

13. *Deanna Skrivanek, **Bhushan Thakkar,** Abigail Andrade, Brent Nevadomski, Edmund Acevedo, Carrie Peterson. Effectiveness of Theta Burst Stimulation on Different Brain Regions in Patients with Painful Diabetic Neuropathy. *Proceedings of the Virginia Commonwealth University College of Engineering Dean's Undergraduate Research Initiative.* Richmond, Virginia. *November 17, 2021.*

14. Sumaya Smarony, Anna Parlier-Ahmad, Hannah Shadowen, **Bhushan Thakkar**, Caitlin E. Martin. Assessment of COVID-19 Driven Changes in an Integrated OBGYN Addiction Treatment Clinic. *Proceedings of the American Society of Addiction Medicine 53rd Annual Conference.* Hollywood, Florida. *March 31-April 3, 2022.*

15. **Bhushan Thakkar**, Neil Mittal, Cooper Hodges, Yeajin Cho, Connor Lewis, Abigail Andrade, Brent Nevadomski, Keith Li, Ravi L Hadimani, Carrie Peterson. Effect of Neuroanatomy on Transcranial Magnetic Stimulation Resting Motor Thresholds. *Proceedings of the 4th International Brain Stimulation Conference*. Charleston, South Carolina*. December 6-9, 2021.*

16. Neil Mittal, **Bhushan Thakkar**, Cooper B. Hodges, Yeajin Cho, Connor Lewis, Abigail Andrade, Brent Nevadomski, Keith Li, Ravi L. Hadimani, Carrie Peterson. Effect of Neuroanatomy on Intermittent Theta Burst Stimulation Motor Evoked Potentials. *Proceedings of the 4th International Brain Stimulation Conference*. Charleston, South Carolina. *December 6-9, 2021.*

17. Cooper B. Hodges, Neil Mittal, **Bhushan Thakkar**, Yeajin Cho, Connor Lewis, Ravi L Hadimani, Carrie Peterson. Effect of Neuroanatomy on Intermittent Theta Burst Stimulation Motor Evoked Potentials. Presented virtually at the *American Congress of Rehabilitation Medicine 98th Annual Virtual Conference, September 26-29,2021.*

18. Connor Lewis, Neil Mittal, Cooper Hodges, **Bhushan Thakkar**, Yeajin Cho, Abigail Andrade, Brent Nevadomski, Keith Li, Carrie Peterson, Ravi L Hadimani. Effect of Fiber Tract Surface Area on Resting Motor Thresholds during Transcranial Magnetic Stimulation. *Proceedings of the Biomedical Engineering Society Annual Meeting,* Orlando, Florida. *October 6-9, 2021.*

19. Yeajin Cho, Neil Mittal, **Bhushan Thakkar**, Cooper Hodges, Connor Lewis, Abigail Andrade, Brent Nevadomski, Keith Li, Ravi L Hadimani, Carrie Peterson. Effect of Neuroanatomy on the Response to Neuromodulation in the Biceps Brachii. *Proceedings of the Biomedical Engineering Society Annual Meeting.* Orlando, Florida. *October 6-9, 2021.*

20. **Bhushan Thakkar**, Neil Mittal, Cooper Hodges, Yeajin Cho, Connor Lewis, Ravi L Hadimani, Carrie Peterson. Effect of Neuroanatomy on Transcranial Magnetic Stimulation Resting Motor Thresholds. *Proceedings of the American Congress of Rehabilitation Medicine 98th Annual Virtual Conference, September 26-29, 2021.*

21. **Bhushan Thakkar**, Edmund Acevedo. Theta Burst Brain Stimulation in Diabetic Neuropathy Patients with Neuropathic Pain: Investigating Neural Mechanisms (Protocol). *Proceedings of the FRESCO@CNAP Workshop 2021.*

22. Caitlin E. Martin, **Bhushan Thakkar**, DaShaunda D H Taylor, Derek A. Chapman. Disparities by Sex in COVID-19 Risk and Related Harms among People with Opioid Use Disorder. *Proceedings of The College on Problems of Drug Dependence Annual Meeting June, 21, 2021.*

23. Caitlin E. Martin, **Bhushan Thakkar**, Lauren Cox, Elisabeth Johnson, Hendree E Jones, Anna Marie Connolly. Evaluation of an OBGYN Resident Curriculum on Substance Use Disorder Assessment and Treatment. *Proceedings of The Association of Professors of Gynecology and Obstetrics Annual Meeting 2021.*

24. Kara Hostetter, **Bhushan Thakkar**, Cherie Edwards, Caitlin E. Martin. Expanding Medical Education to include Substance Use Disorders during Pregnancy and Postpartum: A Clinical Curriculum for Medical Students. *Proceedings of The Virginia Society of Addiction Medicine, Virtual, October 24, 2020.*

25. Caroline Shadowen, **Bhushan Thakkar**, Travis Oakes, Tamas Gal, Gerard F Moeller, Caitlin E. Martin. Buprenorphine Dosing for the Treatment of Opioid Use Disorder Through Pregnancy and Postpartum. Presented virtually at *The Virginia Society of Addiction Medicine, Virtual, October 24, 2020.*

26. Lauren Cox, **Bhushan Thakkar**, Elisabeth Johnson, Hendree E Jones, Anna Marie Connolly, Caitlin E. Martin. Beyond Opioid Prescribing: An Evaluation of a Pilot Curriculum for OBGYN Residents on Substance Use Disorder Assessment and Treatment. *Proceedings of The Virginia Society of Addiction Medicine, Virtual, October 24, 2020.*

27. **Bhushan Thakkar**, Tawany Almeida, Tiffany Kimbrough, Caitlin E. Martin. Factors that Promote and Challenge Recovery through the Postpartum Transition for Women in Treatment for Opioid Use Disorders as Reported by Health Care Providers. *Proceedings of The Virginia Society of Addiction Medicine, Virtual, October 24, 2020.*

28. Tawany Almeida, **Bhushan Thakkar**, Tiffany Kimbrough, Caitlin E. Martin. Neonatal Opioid Withdrawal Syndrome (NOWS) knowledge among women in treatment for opioid use disorder. *Proceedings of The 12th International Women's and Children's Health and Gender (InWomen's) Group conference, Virtual, June 19, 2020.*

29. Tawany Almeida, **Bhushan Thakkar**, Tiffany Kimbrough, Caitlin E. Martin. Patient reported challenging and promoting factors for recovery through the postpartum transition among women in treatment for opioid used disorder. *Proceedings of The 12th International Women's and Children's Health and Gender (InWomen's) Group conference, Virtual, June 19, 2020.*

30. Kathryn Harrison, Adam Sima, **Bhushan Thakkar,** Sheryl Finucane, D.S. Blaise Williams III. Kinematic differences between experienced and non-runners. *Proceedings of the International Society of Biomechanics/ Annual Meeting of the American Society of Biomechanics 2019.* Calgary, Alberta, Canada. July 31-August 4, 2019.

31. **Bhushan Thakkar,** Gregory Golladay, Rebecca Etz, Daniel Riddle. Interpreting Patient's Ratings Obtained from The Forgotten Joint Score Following Total Knee Replacement Surgery: A Pilot Study. *Proceedings of the Graduate Student Association's 22nd Annual Research Symposium & Exhibit. Virginia Commonwealth University.* Richmond, Virginia. *April 22, 2019.*

32. **Bhushan Thakkar** Kathryn Harrison, D. S. Blaise Williams III. Effects of Running at Two Different Speeds on Interlimb Asymmetry in Female Recreational Runners. *Proceedings of the 2019 Human Movement Science Research Symposium.* Chapel Hill, North Carolina. *March 22, 2019.*

33. *Lauren Beshada, Kathryn Harrison, **Bhushan Thakkar**, Sheryl Finucane. Effect of Running Speed on Knee Joint Biomechanics in Male and Female Novice Runners. *Proceedings of the 2019 Annual Meeting of the Southeast Chapter of the American College of Sports Medicine*. Greenville, South Carolina. February 14-16, 2019.

34. †David Pumphrey, Kathryn Harrison, **Bhushan Thakkar**, Robert Tickes, D.S. Blaise Williams III. The Effect of Increased Running Speed on Frontal Plane Kinetics in Female Runners with Strong and Weak Hip Abductors. *Proceedings of the 2019 American Physical Therapy Combined Sections Meeting*. Washington, DC. January 23-26, 2019.

35. **Bhushan Thakkar**, Jenna Kostiuk, Kathryn Harrison, Jacqueline Morgan, Gregory Crosswell, D.S. Blaise Williams III. Relationship Between Knee Valgus Asymmetry During Running and Side-Step Cutting Mechanics in Female Lacrosse Players. *Proceedings of the 2018 American College of Sports Medicine.* Minnesota, Minneapolis. May 29 - June 2, 2018.

36. Kathryn Harrison, **Bhushan Thakkar**, David Pumphrey, Robert Tickes, Gregory Crosswell, D.S. Blaise Williams III, FACSM. Association of Isometric Hip and Ankle Strength with Frontal Plane Kinetics in Females During Running. *Proceedings of the 2018 American College of Sports Medicine.* Minnesota, Minneapolis. May 29 - June 2, 2018.

37. *Morgan Meyer, Olivia Moody, Kathryn Harrison, Gregory Crosswell**, Bhushan Thakkar.*** Influence of Gluteus Medius Strength on Interlimb Asymmetry in Female Recreational Runners*. 2018 Annual Poster Symposium for Undergraduate Research and Creativity. Virginia Commonwealth University.* Richmond, Virginia. April 25, 2018.

38. *Olivia Moody, Morgan Meyer, Kathryn Harrison, Gregory Crosswell**, Bhushan Thakkar.*** Differences in Lowerlimb Kinematics During Running in Female Recreational Runners with History of Medial Tibial Stress Syndrome. *2018 Annual Poster Symposium for Undergraduate Research and Creativity. Virginia Commonwealth University.* Richmond, Virginia. April 25, 2018.

39. Zayd Abdul-Ali, **Bhushan Thakkar**, Jacqueline Morgan, Kathryn Harrison. Gender comparison of ankle kinematics during single leg landings.*2018 Annual Poster Symposium for Undergraduate Research and Creativity. Virginia Commonwealth University*. Richmond, Virginia. April 25, 2018.

40. **Bhushan Thakkar**, Kathryn Harrison, Jacqueline Morgan, Jenna Kostiuk, Zayd Abdul-Ali, Lauren Beshada, Ali Lodhi, Gregory Crosswell, D.S. Blaise Williams III. Relationship between knee valgus asymmetry during running and knee loading during single leg landing in female basketball athletes. *Graduate Student Association's 21st Annual Research Symposium & Exhibit. Virginia Commonwealth University.* Richmond, Virginia. April 24, 2018.

41. Jacqueline Morgan, Gregory Crosswell, **Bhushan Thakkar**, Kathryn Harrison, D.S. Blaise Williams III. Ankle and Knee Torsional Stiffness across the Female Lifespan. *Proceedings of the 2018 American Physical Therapy Combined Sections Meeting*. New Orleans, Louisiana. February 21 - 24, 2018.

42. Gregory Crosswell, **Bhushan Thakkar**, Kathryn Harrison, Jacqueline Morgan, D.S. Blaise Williams III. Effect of Age and Pace and Ground Reaction Force Asymmetry in Recreational Male Runners. *Proceedings of the 2018 American Physical Therapy Combined Sections Meeting.* New Orleans, Louisiana. *February 21 - 24, 2018.*

43. **Bhushan Thakkar**, Kathryn Harrison, Jacqueline Morgan, Jenna Kostiuk, Zayd Abdul-Ali, Lauren Beshada, Ali Lodhi, Gregory Crosswell1, D.S. Blaise Williams III. Relationship between knee valgus asymmetry during running and knee loading during single leg landing in female basketball

athletes. *Proceedings of the 2018 Annual Meeting of the Southeast Chapter of the American College of Sports Medicine*. Chattanooga, Tennessee. February 15 - 17, 2018.

44. *Jenna Kostiuk, **Bhushan Thakkar**, Kathryn Harrison, Jacqueline Morgan, Gregory Crosswell, D.S. Blaise Williams III. Differences in neuromuscular strategies between two tasks in female lacrosse athletes. *Proceedings of the 2018 Annual Meeting of the Southeast Chapter of the American College of Sports Medicine*. Chattanooga, Tennessee. February 15 - 17, 2018.

45. Rondy Michael Lazaro, Jacqueline Morgan, **Bhushan Thakkar**, Katherine Dec, D.S. Blaise Williams III. The Effect of Modified Arm Swing Patterns on Hip Adduction and Internal Rotation During Running. *Proceedings of the 2018 Association of Academic Physiatrists Annual Meeting.* Atlanta, Georgia. February 13-17, 2018.

46. Jacqueline Morgan, **Bhushan Thakkar**, Kathryn Harrison, Gregory Crosswell, Robert Tickes, D.S. Blaise Williams III. Knee joint stiffness as a neuromuscular component of preferred speed. *Proceedings of the 2017 Annual Meeting of the American Society of Biomechanics.* Boulder, Colorado. *August 8 - 11, 2017.*

47. Kathryn Harrison, Jacqueline Morgan, Gregory Crosswell, Yongung Kwon, **Bhushan Thakkar**, DS Blaise Williams III. Differences in coordination between young and old runners. *Proceedings of the 2017 Annual Meeting of the American Society of Biomechanics.* Boulder, Colorado. August 8 - 11, 2017.

48. Kathryn Harrison, **Bhushan Thakkar**, Gregory Crosswell, Jacqueline Morgan, D.S. Blaise Williams III. The ability of sagittal plane kinematic variables to predict loading in different populations of runners. *Proceedings of the 2017 American College of Sports Medicine.* Denver, Colorado. May 30 - June 3, 2017.

49. Jacqueline Morgan, **Bhushan Thakkar**, Kathryn Harrison, Gregory Crosswell, D.S. Blaise Williams III. Sport specific comparisons of joint work and contributions during running. *Proceedings of the 2017 American College of Sports Medicine*. Denver, Colorado. May 30 - June 3, 2017.

50. Kristen Renner, **Bhushan Thakkar**, DS Blaise Williams III, Robin Queen. Validation of Single Sensor Wireless In-shoe Pressure Insoles during Running. *Proceedings of the 2017 American College of Sports Medicine*. Denver, Colorado. May 30 - June 3, 2017.

51. **Bhushan Thakkar**, Courtney Holleran, James Jones, D.S. Blaise Williams III. Calculation of Gait Asymmetry During Walking Using Different Symmetry Measures. *The 20th Annual Graduate Research Symposium and Exhibit, Virginia Commonwealth University.* Richmond, Virginia. April 18, 2017.

52. †Robert Tickes, Kathryn Harrison**, Bhushan Thakkar**, Jackie Morgan, Gregory Crosswell, D.S. Blaise Williams III. Does Running Experience Correlate with Risk Factors for Overuse Injuries? *Medical Student Research Day, Virginia Commonwealth University*. Richmond, April 2017.

53. **Bhushan Thakkar**, D.S. Blaise Williams III, Kathryn Harrison, Jacqueline Morgan, Robin Queen. Vertical Ground Reaction Force Asymmetry during uphill, level and downhill running. *Proceedings of the 2016 Annual Meeting of the American Society of Biomechanics*. Raleigh, North Carolina. August 2 - 5, 2016.

54. Kathryn Harrison, **Bhushan Thakkar**, Jacqueline Morgan, Robin Queen, D.S. Blaise Williams III. Intralimb co-ordination in level and uphill running. *Proceedings of the 2016 Annual Meeting of the American Society of Biomechanics.* Raleigh, North Carolina. August 2 - 5, 2016

55. Jacqueline Morgan, **Bhushan Thakkar,** Kathryn Harrison, Robin Queen, D.S. Williams III. Lower extremity stiffness during uphill and level running. *Proceedings of the 2016 Annual Meeting of the American Society of Biomechanics.* Raleigh, North Carolina. August 2 - 5, 2016.

56. **Bhushan Thakkar**, Jacqueline Morgan, D. S. Blaise Williams III. Influence of Stride Frequency on Knee Joint Stiffness and Anterior Shear Force in Female Runners. *The 19th Annual Graduate Research Symposium and Exhibit, Virginia Commonwealth University.* Richmond, Virginia. April 19, 2016.

57. Kevin Douglas Gross, **Bhushan Thakkar**, Jingbo Nui, Howard Hillstrom, Neil Segal, Michael Nevitt, Cora Lewis, David Felson. The relationship between medial knee osteoarthritis and pelvic drop during walking: The MOST Study. *Proceedings of the 2012 American Physical Therapy Combined Sections Meeting.* Chicago, Illinois. February 8 - 11, 2012.

58. **Bhushan Thakkar**, Divya Adhia. 'Contralateral Neural mobilization and Testing'. *All India Student Physical Therapy Conference.* Mangalore, India 2007.

TEACHING EXPERIENCE:

Department of Kinesiology and Health Sciences
Virginia Commonwealth University, Richmond, VA.

Lab Instructor: HPEX 374(Musculoskeletal Structure and Movement) (4 credits)

- **Fall 2019 -** Four sections: 25 students each.
- **Fall 2016 -** Two sections: 25 students each.
- Provided an understanding of the mechanical aspects of human motion with specific emphasis on application of anatomical structure, terminology and biomechanics in the analysis of physical activity. Laboratory learning allowed students to acquire practical knowledge and skills in Anatomy, biomechanical analysis and instrumentation.

PROFESSIONAL EXPERIENCE:

Home Care Physical Therapist **December 2012 – January 2014**
Mumbai, India.

- Provided in-home hands on physical therapy to patients with a range of disabilities.
- Evaluated patients for physical therapy needs and designed physical therapy programs.

Physical Therapist on Temporary Permit **November 2011 – November 2012**
American Medical, Inc. Brooklyn, NY.

- Evaluated, planned treatment, and administered care to individual patients through collaboration with a Physical Therapist.
- Updated and maintained charts to reflect procedures completed and patient progress.

International Student Liaison **May 2010 to April 2011**
Office of Student Affairs, MGH Institute of Health Professions, Boston, MA.

- Liaised between the incoming international students and the Office of Student Affairs by organizing events and communicating via email.

Graduate Assistant **March 2011 to April 2011**
Information and Technology Department, MGH Institute of Health Professions, Boston, MA.

- Worked as an afterhours help desk guide providing personalized consultation services to support student research needs, assist in issues with wireless networks and connectivity.

Volunteer **April 2010 to October 2011**
Physical Therapy Aide, Mount Auburn Hospital Physical Therapy Outpatient Clinic, Cambridge, MA.

Volunteer **April 2010 to July 2011**

Bhushan Thakkar PT, MS

Patient Escort, Massachusetts General Hospital, Boston, MA.

SERVICE:

- **Co-Chair at Scientific Meetings:**
  a) Thematic Posters Session I (Think Tank)
  Biomechanics, Gait, and Balance
  Southeast Chapter of American College of Sports Medicine.
  Greenville, SC. February 16 - 18, 2017.
  b) Podium Presentations
  Walking Biomechanics
  American College of Sports Medicine.
  Denver, CO. May 31-June 3, 2017.

- **University and Community Volunteering:**

a) Organize VCU RUNLAB Tours for Undergraduate students, Richmond Public School students for National Biomechanics Day and STEM in Sports events.
b) Assisting Physical Therapists at the VCU Neuroscience, Orthopaedic and Wellness Center to set up the Motion Performance and Gait Lab and training them about 3D gait analysis, walking gait data collection and data analysis.

GRANT SUBMISSIONS:

- **Student Investigator**, "Theta Burst Brain Stimulation in Painful Diabetic Neuropathy Patients: Investigating Neural Mechanisms". SigmaXi Grants in Aid of Research ($1,000 - Funded)
- **Student Investigator**, "Theta Burst Brain Stimulation in Patients with Neuropathic Pain: Investigating Neural Mechanisms". American College of Sports Medicine Foundation ($5,000 - Unfunded)
- **Student Investigator,** "Sagittal Plane Laxity, Gait Mechanics and Outcomes in Total Knee Arthroplasty" Orthopaedic Research and Education Foundation Goldberg Arthritis Research Grant. ($50,000 - Unfunded)
- **Principle Investigator,** "Changes in Knee Loading Mechanics in Subjects 6 Months Post Total Knee Arthroplasty Using Kinematic Alignment." The Force and Motion Foundation/ORS Young Scientist Scholarship. ($10,000 -Unfunded)
- **Co-Principle Investigator**, "Joint kinetics, neuromuscular control and strength in female runners." The Force and Motion Foundation. ($10,000 - Unfunded)

MENTORSHIP EXPERIENCE:

- Courtney Holleran (Spring 2017): Performed data analysis and contributed to a Poster presentation
- Erin Austin (Summer 2017): Performed data collection and data analysis
- Jenna Kostiuk (Fall 2017): Performed data analysis and presented a first author Poster
- Morgan Meyer (Spring 2018): Performed data analysis and presented a first author Poster
- Olivia Moody (Spring 2018): Performed data analysis and presented a first author Poster
- David Pumphrey (Fall 2018): Performed data analysis and presented a first author Poster
- Lauren Beshada (Fall 2018): Performed data analysis and presented a first author Poster
- Elizabeth B. Grist (Spring 2021- Current): Performed data collection, presented a first author Poster and published a first author paper
- Abigail Andrade (Summer 2021- Spring 2022): Performed data collection and contributed to a Poster presentation
- Brent Nevamdowski (Summer 2021- Spring 2022): Performed data collection and presented a first author Poster
- Deanna Skrivanek (Summer 2021- Fall 2021): Performed data collection and presented a first author Poster
- Peter Baek (Spring 2022- Current): Performed data collection and contributed to a Poster presentation

- Lavie Ngo (Spring 2022): Performed data collection and contributed to a Poster presentation

PROFESSIONAL AFFILIATIONS:

- Current Student Member at the International Association for the Study of Pain.
- Current Student member at the American College of Sports Medicine.
- Past Student member at the American Society of Biomechanics.
  a) Member of the Professional Development sub-committee of the Student Advisory Committee of the American Society of Biomechanics 2016-2018.
- Past Student member at the Southeast Chapter of American College of Sports Medicine.
- Past Student member at the Canadian Society of Biomechanics.
- Past Student member at the American Physical Therapy Association.

HONORS:

- **Dissertation Assistantship Award** **Spring and Summer 2022**
  Graduate School, Virginia Commonwealth University

- **Toronto Pain Institute Fellow** **Fall 2021**
  Online Program, University of Toronto, Toronto, Canada
  This program combines learning and intentional networking opportunities with experts in the pain research, education, and clinical community. The goals of the program are to cultivate research survival skills, foster networking among future leaders, and promote personal goal setting and growth.

- **Pain Research Forum Virtual Correspondent** **Summer-Fall 2021**
  Pain Research Forum, International Association for the Study of Pain
  The purpose of the PRF Correspondents program is to provide early-career pain investigators with knowledge, skills and best practices needed to communicate science effectively to a wide range of pain researchers and to patients and the broader public by conducting interviews, writing news stories or summaries of talks; and by creating blog posts.
  Link to blogs: https://www.painresearchforum.org/forums/discussion/175635-prf-virtual-correspondents-blog-%E2%80%93-cycle-4
  Link to Interviews: https://www.painresearchforum.org/forums/interview/196213-engaging-our-patient-partners-meaningful-way-enhance-chronic-pain-research

- **Tied for the 1st place at the 21st Annual Graduate Student Research Symposium**, Virginia Commonwealth University **Spring 2018**

- **Travel Scholarship** **Summer 2016 and Fall 2018**
  Force and Motion Foundation, AMTI

- **Graduate School Travel Grant** **Fall 2015, Summer 2016, Summer 2017,**
  Virginia Commonwealth University **Fall 2018 and Fall 2021**

- **Post-Professional Adams Fellow** **Spring 2011**
  MGH Institute of Health Professions, Boston
  This award is presented to students for showing evidence of leadership abilities, service to the Physical Therapy profession, and the potential to make a significant contribution as a clinical scholar.