

THE EFFECT OF EXPERIMENTAL PAIN ON NEURAL FUNCTION AND MOTOR
LEARNING

By

Erin Dancey

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Certificate of Approval

Statement of Contributions

This thesis presents the research of Erin Dancey in collaboration with her thesis supervisors Dr. Bernadette Murphy and Dr. Paul Yelder. The sum of this work resulted in the following contributions to the literature.

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Abstract:

This thesis investigated whether acute experimental pain interacts with motor learning acquisition to create adaptive and maladaptive changes in neural function. The first study consisted of two experiments where we determined the interactive effects of acute pain versus control (*Experiment 1*) and local versus remote acute pain (*Experiment 2*) on motor learning and sensorimotor processing and provided supportive evidence for early somatosensory evoked potential (SEP) peaks as markers for sensorimotor integration (SMI) and acute pain. Motor performance was better in the presence of pain pre-motor learning and motor learning retention improved in the presence of local pain. A limitation of this first study was that performance saturation occurred and therefore we used a more complex tracing task for the subsequent studies. Our second SEP study *The interactive effect of acute pain and motor learning acquisition on sensorimotor integration and motor learning outcomes* provides corroboration for the enhancement of motor learning while in acute pain. In addition, the changes in the amplitudes of SEP peaks suggests that SEP peak alterations reflect neurophysiological alterations accompanying both motor learning acquisition and mild acute pain. Improved motor learning acquisition during acute pain may be the result of increased attention or increased arousal, and therefore we concluded that it was important to compare the effects of local versus remote versus contralateral acute pain in conjunction with a complex motor learning task which was the focus of the third study. Our third study found that motor learning occurred in the presence of mild acute pain and there were no significant differences in motor learning acquisition or retention between three groups that had capsaicin applied at different locations. We hypothesized that improved motor learning acquisition during acute pain may have been caused through increased arousal. For the fourth study we explored the effect of acute pain on

neuroplasticity of the motor cortex (MI) by using input-output curves elicited via transcranial magnetic stimulation (TMS). The acute pain in this study was shown to negate the increase in slope that was observed for the control group despite having a positive impact on motor learning acquisition.

Keywords: acute pain, motor learning, somatosensory evoked potentials (SEPs), transcranial magnetic stimulation (TMS)

Table of Contents

1 OVERVIEW	4
2 LITERATURE REVIEW – BACKGROUND INFORMATION	7
2.1 The somatosensory system	7
2.2 Motor Control.....	11
2.2.1 Motor Control and the human hand.....	13
2.3 Pain	13
2.4 Capsaicin.....	14
2.5 Somatosensory evoked potentials (SEPs).....	15
2.5.1 Neural Generators	16
2.6 Single and paired pulse transcranial magnetic stimulation	27
2.6.1 Transcranial Electrical Stimulation (TES)	27
2.6.2 Transcranial Magnetic Stimulation (TMS).....	27
2.6.3 Motor Evoked Potentials (MEPs)	29
2.6.4 Paired-pulse TMS:.....	30
2.7 Cortical neuroplasticity	33
3 THE SIGNIFICANCE OF THE RESEARCH	35
3.1 Chronic pain conditions:	35
3.2 Rehabilitation	36
4 LITERATURE REVIEW - ALTERED AFFERENT INPUT	38
4.1 Deafferentation and somatosensory processing	38
4.2 Deafferentation and motor output.....	40
4.3 Repetitive movement.....	40
5 LITERATURE REVIEW – PAIN	42
5.1 Ascending Pathways.....	42
5.1.1 The spinothalamic tract	42
5.1.2 Spinoreticular tract	43
5.1.3 Spinomesencephalic tract.....	43
5.1.4 Cervicothalamic tract.....	43
5.2 Pain: Current view	44
5.3 Pain: Brainstem	45

5.4 Pain: Changes in excitability at the supraspinal level	45
5.5 Pain and somatosensory processing.....	47
5.5.1 Chronic Pain and somatosensory processing:.....	47
5.5.2 Acute Pain and somatosensory processing:.....	48
5.5.3 Research Gaps:	49
5.6 Pain and the motor system	50
5.6.1 Chronic Pain and the motor system:	50
5.6.2 Acute pain and the motor system:.....	51
5.6.3 Remote versus local pain and motor output:	52
5.7 Research Gaps:	53
5.8 Pain: Mechanisms of nociceptive plasticity	53
6 LITERATURE REVIEW - MOTOR LEARNING	56
6.1 Motor learning.....	56
6.2 Motor learning acquisition and neuroplasticity	59
6.3 Motor learning acquisition tasks	60
6.3.1 Motor learning: typing task.....	61
6.3.2 Motor learning: tracing.....	61
6.4 Motor learning acquisition and the motor system	63
6.4.1 Motor learning acquisition and the MI:	63
6.4.2 Motor learning acquisition and the cerebellum:	64
6.5 Motor learning acquisition and SEPs.....	65
6.6 Motor learning and attention:.....	66
6.7 Motor learning acquisition versus retention:	67
7 LITERATURE REVIEW – MOTOR LEARNING AND PAIN.....	70
7.1 Motor learning and Pain.....	70
7.2 Motor learning and Pain: mechanism	72
7.3 Remote versus local pain and motor learning:	73
7.4 Research Gaps:	73
8 GENERAL METHODS	74
8.1 Participants:.....	74
8.2 SEPs:.....	74

8.2.1 Stimulation of median nerve.....	74
8.2.2 SEP recording parameters.....	75
8.2.3 Data collection.....	75
8.3 TMS:.....	76
8.3.1 Input-output curves.....	76
8.4 Motor acquisition tasks.....	77
8.4.1 Motor sequence typing task:.....	77
8.4.2 Motor tracing task:.....	77
8.5 NPRS:	80
8.6 Statistical Analysis.....	80
8.6.1 SEP Peaks:.....	80
8.6.2 Behavioural data.....	81
8.6.3 NPRS.....	81
9 MANUSCRIPTS	82
9.1 Manuscript 1: The effect of local versus remote experimental pain on motor learning and sensorimotor integration using a complex typing task.....	82
9.1.1 Abstract	84
9.1.2 Introduction	85
9.1.3 Methods	87
9.1.3 Results:	92
9.1.4 Discussion	110
9.1.5 Conclusion	115
9.2 Manuscript 2: The interactive effect of acute pain and motor learning acquisition on sensorimotor integration and motor learning outcomes.	118
9.2.1 Abstract	119
9.2.2 Introduction	121
9.2.3 Methods	124
9.2.4 Results.....	129
9.2.5 Discussion	138
9.2.6 Conclusion	144
9.3 Manuscript 3: The effect of local, remote, and contralateral tonic pain on motor learning and sensorimotor integration using a motor tracing task	147

9.3.1 Abstract	148
9.3.2 Introduction	150
9.3.3 Methods	152
9.3.4 Results:	156
9.3.5 Discussion	163
9.3.6 Conclusion	168
9.4: Manuscript 4: The effect of tonic pain and motor learning on corticospinal excitability	171
9.4.1 Abstract	172
9.4.2 Introduction:	174
9.4.3 Methods:	176
9.4.4 Results.....	180
9.4.5 Discussion	185
9.4.6 Conclusion	188
10 – GENERAL DISCUSSION AND CONCLUSIONS	189
Appendix:.....	195
A.1 Pain: from the periphery to the cortex.....	195
A.1.1 Nociceptors	195
A.1.2 Classes of nociceptors	198
A.1.4 <i>A-d fibers</i> :.....	198
A.1.5A- <i>b fibers</i> :.....	199
A.1.6 Transducer molecules:	199
A.1.7 Mediators:	200
A.1.8 Sensitization in the periphery:.....	201
A.1.9 The spinal cord:	202
A.1.10 The spinal cord: Central sensitization	203
A.1.11LTP:	204
A.1.12 Changes in excitability at the supraspinal level:.....	205
A.1.13 Brainstem:	206
A.1.14 Pain inhibition.....	207
A.1.14 The historical record of pain research: The two major main pain models.....	210

A.1.15 The specificity theory	210
A.1.16 The gate control theory	210
A.1.17 The counterirritant theory:	211
A.1.18 Current view of pain:	212
A.1.19 Ascending Pathways	214
A.1.20 The spinothalamic tract	214
A.1.21 Spinoreticular tract	215
A.1.22 Spinomesencephalic tract.....	215
A.1.23 Cervicothalamic tract.....	215
A.1.24 The pain matrix:	215
A.1.25 Thalamus:	217
A.1.26 The limbic system	219
A.1.27 Anterior Cingulate Cortex (ACC):	219
A.1.28 Insula:	220
A.1.29 Amygdala:	222
A.1.30 Cerebellum:	222
A.1.31 The Cerebellum and Basal ganglia (BG)	224
A.1.32 Hypothalamus:	225
A.1.34 SI:	226
A.1.35 SII:.....	227
A.1.36 The prefrontal cortex:	227
A.1.37 mPFC	228
A.1.38 DLPFC	228
A.1.39 OFC.....	229
A.1.40 Anticipation, empathy, attention: prefrontal cortex and the limbic system	229
A.2 Consent form	231
A.3 Pain questionnaire.....	236
A.4 TMS safety checklist	239
A.5 Copyright permission Letters	241

List of Figures/Tables:

Figure 1: The Dorsal Column Medial Lemniscal system (left) and the Anterolateral system (right) pathways. Permission from [25]. 8

Figure 2: The sensory and motor homunculi. The location of limb representation within the cortex is seen here. The amount of cortical area dedicated to a certain region is represented by the size of the image, reflecting their degrees of innervations. Requested permission from [25] on page 343... 10

Figure 3: An illustration showing the different layers that compose the MI is displayed. Each layer is composed of different projects that have different roles in the modification and refinement of motor output, as well as communication with neighbouring structures through horizontal connections. Adapted from [29]...... 12

Figure 4: Schematic Illustrating the Main Factors that Influence Nociceptive Inputs to Affect Pain Perception [36] 14

Figure 5: Example of a SEP peak. Adapted from[52] 17

Figure 6: Examples of SEP peaks. Adapted from [53]...... 17

Figure 7: Overview of SEP peaks and their neural generators. Adapted from [25]...... 18

Figure 8: N9 SEP peak and it's neural generator. Adapted from [25] 19

Figure 9: N11 SEP peak and it's neural generator Adapted from [25]...... 20

Figure 10: N13 SEP peak and it's neural generator. Adapted from [25]...... 21

Figure 11: N18 SEP peak and it's neural generator. Adapted from [25]...... 22

Figure 12: P14 SEP peak and it's neural generator. Adapted from [25] 23

Figure 13: N20 SEP peak and it's neural generator. Adapted from [25]...... 24

Figure 14: P25 SEP peak and it's neural generator. Adapted from [25] 25

Figure 15: N30 SEP peak and it's neural generator. Adapted from [25]...... 26

Figure 16: Example of an electromyography trace showing a motor evoked potential [107]...... 30

Figure 17. Example EMG trace showing SICL. The MEP evoked by the test stimulus alone is inhibited when preceded by a smaller stimulus [107]...... 31

Figure 18: Example EMG trace showing SICF (or IwF). The MEP from the test stimulus (S1) alone is facilitated when followed with a smaller stimulus (S2) [107]. 32

Figure 19: An illustration of the motor learning task which was performed by each participant.... 78

Figure 20: Photograph of individual performing the motor tracing task on the touchpad 79

Figure 21: An illustration of the order of the 4 different task variations is shown. The block order and learning task version was identical for all participants during acquisition and retention. 79

Figure 22: the rexed laminae system of the dorsal horn grey matter (Reproduced from <http://www.thr brain.mcgill.ca>) 195

Figure 23: Anatomy of nociceptors. 197

Figure 24: Activated regions during nociceptive input 206

Figure 25: pro and anti-nociceptive influences respectively. [36]...... 209

Abbreviations:

ADM: Abductor digiti minimi

AMH: A δ mechano-heat nociceptors ()

ANOVA: Analysis of variance

APB: Abductor pollicis brevis

ARAS: ascending reticular activating system

BDNF: brain-derived neurotrophic factor

CaMK II: calmodulin-dependent kinase II

CMH: C mechano-heat nociceptors

CNS: Central nervous system

CSP: Cortical silent period

DCC: dorsal column

EEG: Electroencephalography

EIP: Extensor indices proprius

EMG: Electromyography

EP: Evoked potential

EPSPs: Excitatory postsynaptic potentials

FDI: First dorsal interosseous

GABA: γ -aminobutyric acid

HTM : high-threshold mechanoreceptor

ICF: Intracortical facilitation

ICI: Intracortical inhibition

ISI: Inter-stimulus interval

IwF: I-wave facilitation

LEP: laser evoked potentials

LICI: Long interval intracortical inhibition

LBP: Lower back pain

M1: Primary motor cortex area

MEG: Magnetoencephalography

MEP: Motor evoked potential

MI: Primary somatosensory cortex

NMDA: N-methyl D-aspartate

NT-3: Neurotrophin

NT-4: Neurotrophin

PAG: periaqueductal gray

PFC: prefrontal cortex

PMN: Polymodal nociceptors

RA: rheumatoid arthritis

SEP: Somatosensory evoked potential

SICF: Short interval intracortical facilitation

SICI: Short interval intracortical inhibition
SMA: Supplementary motor area
SMI: Primary somatosensory area
SMII: Secondary somatosensory system
SP: Substance P
STT: Spinothalamic tract
TENS: transcutaneous nerve stimulation
TMS: Transcranial magnetic stimulation
UDP: Use-dependent plasticity

1 OVERVIEW

Cortical neuroplasticity is a functional or morphological change in neurons that can include cortical reorganization, alterations in the strength of connections, or modified representational areas [1, 2]. Cortical neuroplasticity is associated with altered motor output following motor learning acquisition [3, 4] and occurs with acute and chronic pain [5-8]. With motor learning acquisition cortical neuroplasticity is accompanied by improved motor performance, while with pain, cortical neuroplasticity is often accompanied by a decrease in performance [9]. Motor learning acquisition leads to changes in corticomotor control and requires sensorimotor integration (SMI) which is the processing of somatosensory information and integrating this with the motor output from the primary motor cortex (MI), in order to fine tune and improve motor task performance. Peripheral somatosensory information is necessary for motor-skill acquisition. The cerebellum and the MI are both involved in SMI, with the cerebellum receiving information from the MI with respect to motor output and integrating this with sensory input [10, 11]. Many individuals participating in rehabilitation have pain and motor learning deficits. Motor learning deficits are usually regarded as a consequence of movement related pain and there is confirmation that pain affects motor control and has the ability to negatively influence the neuroplasticity associated with motor output [12-14] and can interfere with motor skill acquisition [9, 15, 16]. However, recent work demonstrated that local and remote acute pain did not negatively impact motor skill acquisition [17, 18] and can improve motor learning acquisition [19]. The acute pain used in these studies [17, 19] induced cutaneous pain that did not impact movement which may help to explain why there wasn't an adverse effect of pain on motor learning. A limitation of previous work is that motor performance saturation occurred and

therefore it is important to verify these results using a more complex task [19]. Another limitation is that the majority of previous studies utilizing acute cutaneous pain have not measured retention [9, 20]. Two previous studies that measured retention used an experimental tonic pain model combined with a locomotor adaptation task [18] or an upper limb reaching task [21] and found that pain throughout training impacted retention [18, 21] with no impact at baseline [21] or on motor learning acquisition [18]. It is hypothesized that improved performance with acute pain may have been caused by increased attention or arousal [19]. The inconsistencies in the literature underscore the need for research investigating pain unrelated to motor control, and the impact of remote versus local pain, as the impact of pain on motor learning (acquisition and retention) may vary according to these factors. The effects of a control versus remote and local pain on SMI, motor learning acquisition, and retention are unknown. It is fundamental to understand the neurophysiological and behavioural consequences of motor learning acquisition in conjunction with pain.

The overall goal of this thesis was to understand how acute experimental pain interacts with motor learning to create both adaptive and maladaptive changes in neural function. Cutaneous pain, induced via application of capsaicin, provides a model to study the consequences of acute experimental pain on movement induced plasticity. This was explored through four separate studies. These results will further our understanding of the impact of pain on motor learning and motor learning associated plasticity.

This thesis takes the form of a thesis by publication, with an extended literature (chapters 2 to 7) followed by a general methods section (chapter 8), and then four linked experimental studies, each presented as a separate published or submitted research article (chapter 9), followed by a general discussion (chapter 10). The literature review is divided into 6 chapters, the first chapter,

chapter 2, covers background neuroanatomy and neurophysiology relevant to the thesis. Chapter 3 covers the practical significance of the research. This is important as this research has possible applications to rehabilitation and injury prevention strategies as many people participating in rehabilitation programs have motor deficits and pain. Chapter 4 covers altered afferent input which is relevant to the study of pain and sensory processing as it is hypothesized that pain also results in central plastic changes that outlast the period of altered input. Chapter 5 covers pain and reviews the ascending nociceptive tracts and describes the changes in excitability in the spinal cord, brainstem, and supraspinal levels in response to pain and the mechanisms behind nociceptive plasticity. This is followed by chapter 6 that covers motor learning which is important as we are investigating the neurophysiological and behavioural responses to motor learning acquisition and retention. Chapter 7 covers motor learning and pain and discusses how pain impacts sensorimotor integration and motor learning acquisition and retention and will describe some possible mechanisms for how pain affects motor learning. This is important as the studies included in this thesis investigate the interactive effect of motor learning and pain on neurophysiological and behavioural responses.

2 LITERATURE REVIEW – BACKGROUND INFORMATION

This chapter covers background information and terminology related to the somatosensory system, motor control, pain, capsaicin cream, cortisol, SEPs, TMS, and cortical plasticity.

2.1 The somatosensory system

The somatosensory system enables the perception of sensory information (i.e. temperature, pain, touch, pressure, and proprioception) from the skin, the muscles, and the viscera, by conveying information to the cortex [22]. Sensory transduction is the process by which stimuli from the external environment are converted into electrical signals and transmitted through the central nervous system (CNS) [23]. The somatosensory system has been investigated to study information processing and the functional organization of the nervous system [23] and can be divided into two systems which carry ascending information to the contralateral cortex: the dorsal column system (DCCs) and the spinothalamic system (STT) (See Figure 1) [24].

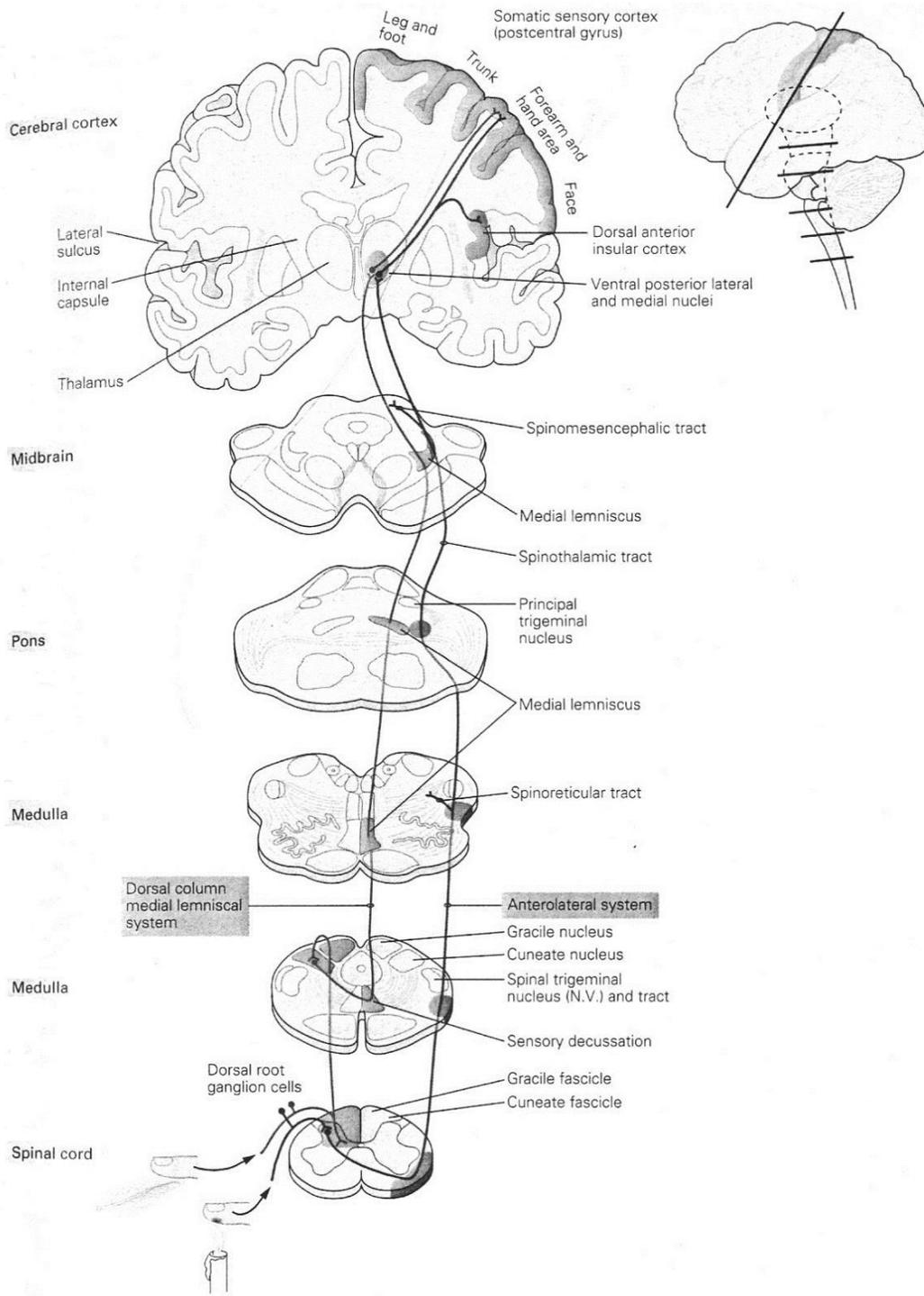


Figure 1: The Dorsal Column Medial Lemniscal system (left) and the Anterolateral system (right) pathways. Permission from [25].

The dorsal column pathway transmits touch, pressure, vibration and proprioception as discrete neural impulses. The spinothalamic pathway transmits neural impulses of thermoreception (temperature), and nociception (pain) [24]. Both transmission pathways include three neurons which ascend to the cortex. The specialized sensory receptor, afferent axon, and cell body are known as the primary afferent. This first order neuron is located in the dorsal root ganglia and connects receptors of the limbs, trunk, neck, or posterior head with the spinal cord. For the dorsal column pathway, the first order neuron synapses with the second order neuron at the medulla oblongata and the axons of the second neuron cross the midline (decussates) and ascends to the thalamus (See Figure 1). For the spinothalamic pathway, the first neuron synapses with the second neuron at the level of the spinal cord and decussates. For both pathways, the third order neurons ascend from the thalamus into the somatosensory areas. These areas include the SI, secondary somatosensory area (SII), posterior parietal cortex, posterior and mid-insula and the mid-cingulate cortex [24].

The SI is situated in the postcentral gyrus of the parietal lobe of the cerebral cortex and is subdivided into four different areas (classified as Brodmann's area 3a, 3b, 1 and 2). Brodmann's classification defines the cortical territories of interest and is a region of the cerebral cortex that is defined based on the structure and organization of cells. Brodmann areas 1, 2, and 3 represent the SI, and area 4 represents the motor cortex (MI) [26, 27]. The SI plays a critical role in processing somatosensory input and is important in somatosensory acuity, detection, and discrimination [28]. Specific areas of the SI receive somatic sensory information from different parts of the body with each area containing a topographic somatotopic representation of the contralateral body with the tongue represented laterally and the feet medially (See Figure 2). Somatotopic arrangement is the maintenance of spatial organization within the CNS. Within the

SI, densely innervated areas of the body such as the hands and face occupy larger regions of the cortex [28]. The SII receives projections from the SI and projects to the association areas: the posterior parietal cortex (Brodmann's area 39, 40) the prefrontal cortex (Brodmann's area 9-12 and 44-47) and the temporal cortex (Brodmann's areas 21, 22, 37, and 41-43) which are implicated in the higher order processing of sensory input involved in perception and the initiation of movement. The association areas then project to the motor and limbic systems [24, 27].

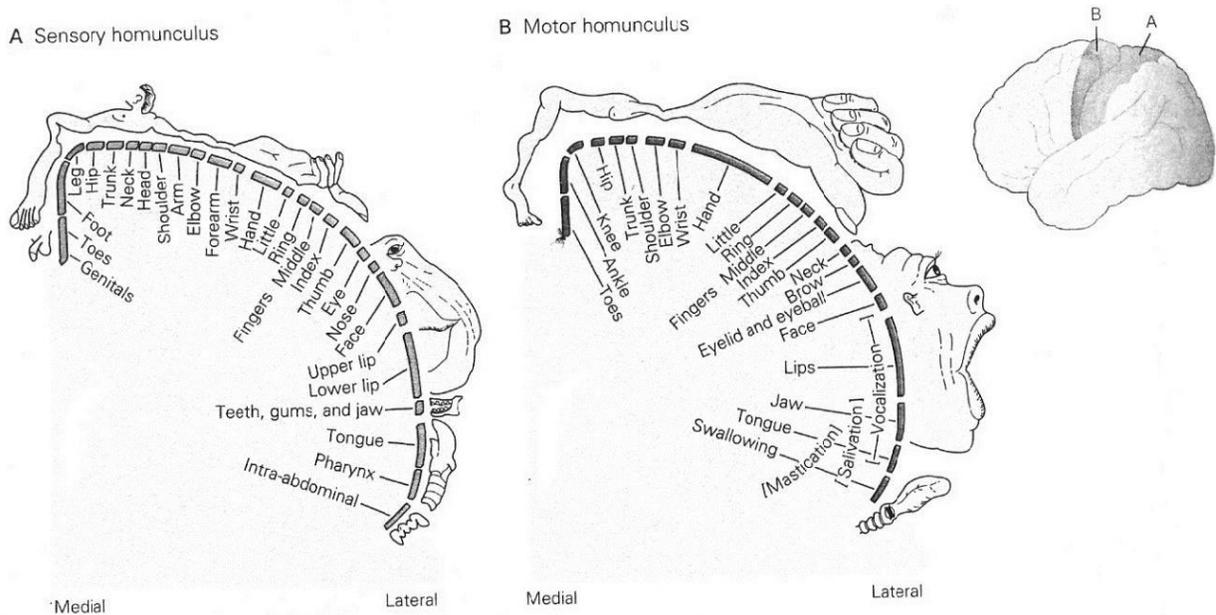


Figure 2: The sensory and motor homunculi. The location of limb representation within the cortex is seen here. The amount of cortical area dedicated to a certain region is represented by the size of the image, reflecting their degrees of innervations. Requested permission from [25] on page 343.

2.2 Motor Control

The somatosensory pathway has evolved in association with the corticospinal tract and functions to control fine movement. The corticospinal tract consists of two neurons with the first motor neuron descending from the cerebral cortex to the second motor neuron which innervates a muscle [26]. The MI is situated in the precentral gyrus of the frontal lobe and is crucial in SMI, motor control, and motor learning [28]. Similar to the SI, the MI contains a somatotopic representation of the contralateral body with the tongue represented laterally and the feet medially. The MI is composed of six different layers that consist of different microstructures (See Figure 3) [29] that differ in composition and function. Layer I is composed of dendrites and is responsible for collecting incoming motor signals and transferring them to the cell bodies for processing. Layers II and III are the first networks of intracortical communication, as well as the location of the most superficial pyramidal cells. These connections are responsible for connecting several structures allowing for orchestration of motor commands. Pyramidal cells are abundant within the cortex and are responsible for the transmission of information between structures [29]. Layer IV is mainly involved with afferent sensory input, and motor areas including the MI and supplementary motor area (SMA) have significantly smaller layer IV's than the SI. Layer V is composed of the largest pyramidal cells and has the greatest horizontal dendritic connections resulting in the greatest between-structure communication of neural macrostructures. Layer VI is a mixed composition layer that begins the transition of cortical to subcortical areas and is comprised of both grey and white matter.

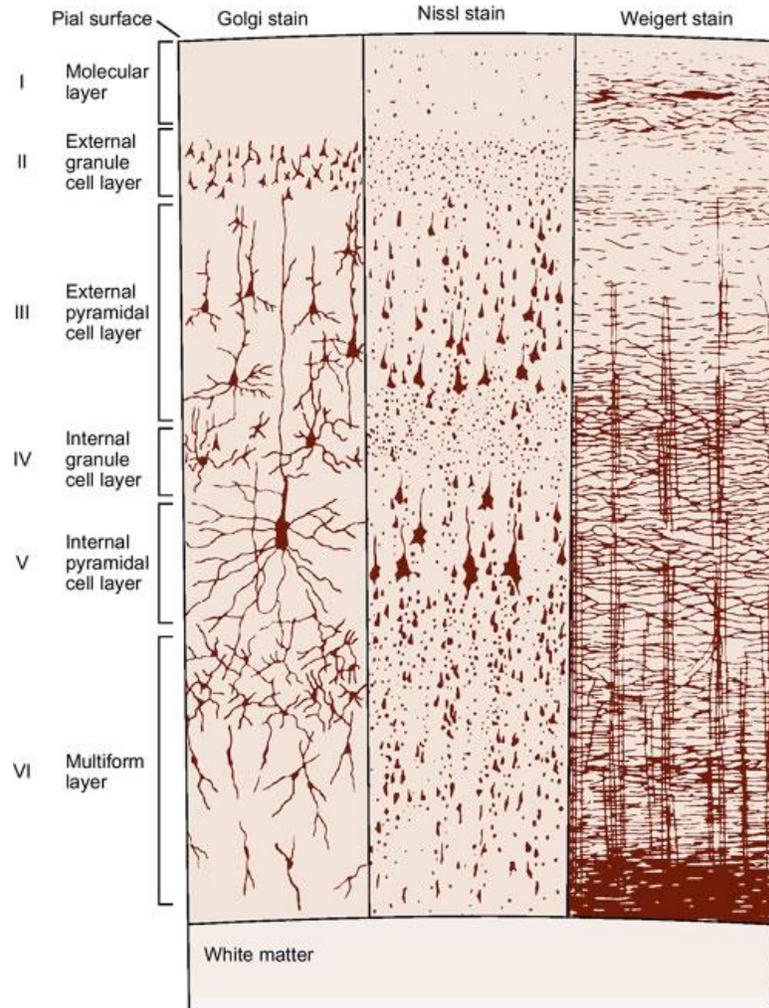


Figure 3: An illustration showing the different layers that compose the MI is displayed. Each layer is composed of different projects that have different roles in the modification and refinement of motor output, as well as communication with neighbouring structures through horizontal connections. Adapted from [29].

A range of other motor cortical areas play a role in motor control including the SMA (lateral part of Brodmann's area 6), the premotor cortex (PMC) (medial part of Brodmann's area 6), and a number of motor areas centered on the anterior cingulate cortex (ACC) on the medial aspect of the frontal lobe, the frontal eye fields, and the posterior parietal cortex (Brodmann's area 7).

Research indicates that the SMA and the PMC are separate motor areas and have distinct functions in motor control [30]. The SMA receives input from the parietal cortex and sensory

areas and the output of the SMA targets the MI and the spinal cord [27]. The PMC has a direct input to the spinal motor neurons via the corticospinal or pyramidal tract [30].

2.2.1 Motor Control and the human hand

The human hand can perform diverse functions and has enormous dexterity. Research demonstrates that humans have extensive cortical systems utilized for the control of hand muscles [31-33]. The corticospinal tract originates from the MI, PMC, SMA as well as cingulate motor areas and plays a critical role in controlling movement [34]. Therefore, the cerebral cortex controls spinal motor neurons which make direct connections with the muscles in the hand. In addition to the MI, the hand is also influenced by other cortical areas and has input from subcortical structures including the basal ganglia (BG) and the cerebellum [31].

2.3 Pain

When nociception is considered as a spinal reflex, it is a uncomplicated system [35]. However, at the level of the cortex, the perception of pain is much more complicated and includes sensory-discriminative, affective-motivational, motor output-control, and immune components [36]. Pain perception is not related linearly to the nociceptive stimulus and is individual and subjective [37] (See Figure 4). Pain perception is affected by memories, emotions, genetic, and cognitive factors and has different qualities depending on the circumstances [38]. The subjective and individual nature of pain makes it difficult to define and treat clinically [24]. There are three types of pain: nociceptive, inflammatory, and neuropathic. Nociceptive pain is the processing of brief nociceptive input, prolonged nociceptive input leads to inflammatory pain and neuropathic pain is the result of damage to the somatosensory nervous system. Neuropathic pain may include peripheral neuropathies and central sensitization [36].

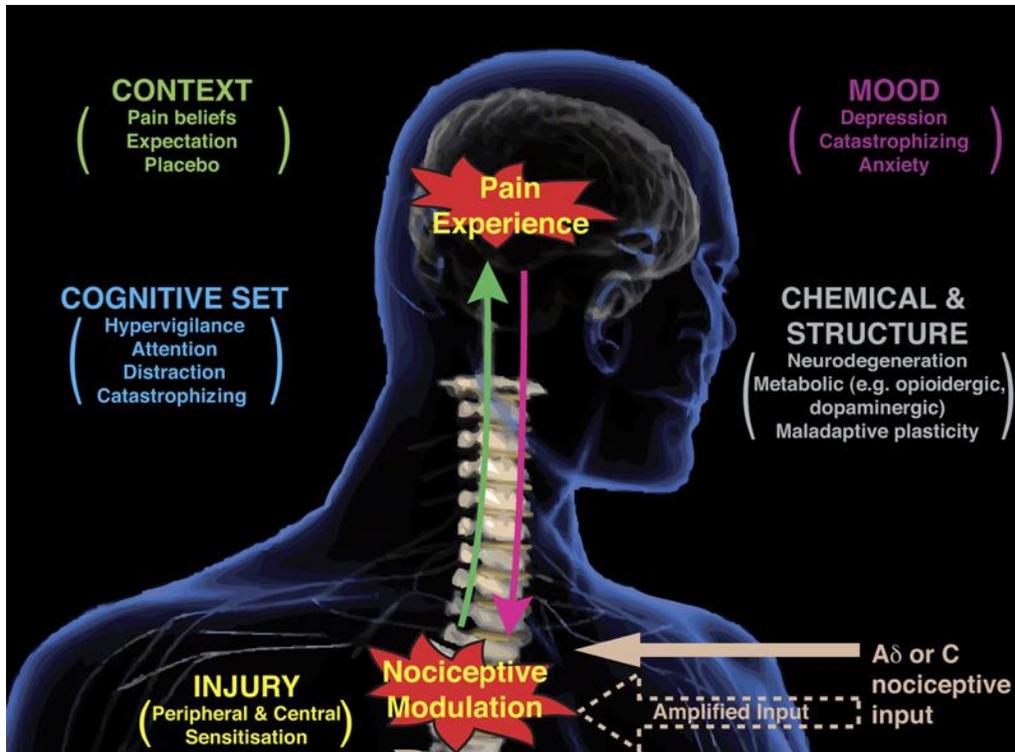


Figure 4: Schematic Illustrating the Main Factors that Influence Nociceptive Inputs to Affect Pain Perception [36]

2.4 Capsaicin

An acute experimental tonic pain model (capsaicin cream) that is not augmented in response to motor movements was selected for this thesis [39]. Capsaicin can be applied topically and is a suitable alternative to painful hot thermal stimuli as it avoids the possibility of tissue damage and provides nociceptive input with negligible contributions from other somatosensory modalities [40]. Capsaicin binds to the TRPV1 receptor (a heat activated protein channel on the membranes of nociceptive and heat neurons) that opens between 37 and 45 °C. When capsaicin binds to TRPV1, the channel opens below 37 °C, which explains why capsaicin is accompanied by heat [41].

Capsaicin leads to a sensitization of C-fiber nociceptors by triggering cation influx, through the release of inflammatory substances including vasoactive peptides (substance P) and by inhibiting

the reuptake of substance P from C fibers [42-44]. Capsaicin induces peripheral sensitization due to excitability changes of the nociceptor and central sensitization through the ongoing activation of the nociceptor [45]. Capsaicin transiently induces sensory abnormalities that are associated with tissue inflammation including hyperalgesia (increased pain sensation to painful stimulation) and allodynia (increased pain to non-painful stimulation) [40, 43].

2.5 Somatosensory evoked potentials (SEPs)

Evoked potentials (EPs) are electrical responses of the nervous system to sensory stimulation and can be evoked in the visual pathway, auditory pathway, or peripheral nerves in the arms or legs (somatosensory evoked potential, SEPs) [24]. EPs involve stimulating the peripheral nerve (eye, ear, or median/ulnar/peroneal/tibial nerve) and measuring the cortical response. This gives a measure of conduction along the pathway that has a peripheral and central component [46]. SEPs are evoked by transcutaneous bipolar electrical stimulation applied over the selected nerve and are an objective and direct method of assessing the integrity of the sensory pathways of the central and peripheral nervous systems [24]. Following peripheral stimulation, the resulting afferent fiber activity leads to excitatory postsynaptic potentials (EPSPs) in connecting neurons [47]. This afferent activation, when recorded from the scalp, generates wave-like EPSPs. A unique feature of SEPs is the ability to bypass peripheral sensory receptors and directly stimulate nerves of interest. The most commonly stimulated nerves in the upper limb are the median, ulnar, and radial nerves. The most commonly stimulated in the lower limb are the peroneal and tibial nerves. As long as the stimulation intensity is not too high this stimulation depolarizes large diameter myelinated afferents, but not the small myelinated A δ or unmyelinated C afferents that convey pain and temperature [48].

SEPs are recorded at various locations along the pathway from the peripheral nerve to the cortex. As it is a non-invasive technique the generated waveforms are recorded at some distance from the neural generators, which may attenuate the evoked potentials [47].

2.5.1 Neural Generators

Along the conduction pathway, various locations provide optimal sites for detection of potentials from different neural generators. SEP peaks measure the activity in the underlying neural structures that are referred to as neuronal generators (See Figure 5). Waveform peaks are greater when recording electrodes are close to their neuronal generators [49] and the amplitude of the peak reflects the degree of activity of each neural structure that the peaks represent. Therefore alterations in the amplitude of the peaks following an intervention are believed to be alterations in the amount of activity of the same neural structures. The latency represents the transmission time between the point of stimulation of the nerve and the neural structures responsible for generating the peaks [49]. Various waveforms are discussed according to their deflection direction and title latency. The International Federation of Clinical Neurophysiologists (IFC) [50] and the American Clinical Neurophysiology Society [51] utilizes the convention of labeling upwards deflections negative. This convention will be used throughout this thesis (See Figure 3). The labelled latency of a peak is the conventional latency and not the actual latency recorded. The variation in latency depends on factors such as participant height and age [50]. For the purpose of this thesis, the peak-to-peak amplitude (μV) of the following SEP peaks will be measured in the SEP experiments: the peripheral N9, the spinal N11 and N13, the far-field N18, the parietal N20 and P25, and the frontal N24 and N30 (See Figure 6).

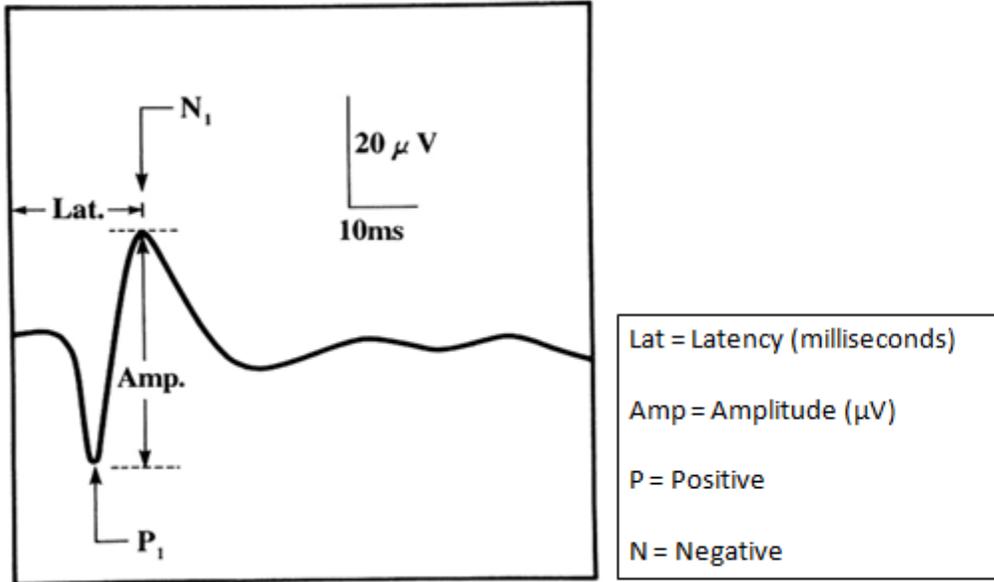


Figure 5: Example of a SEP peak. Adapted from[52]

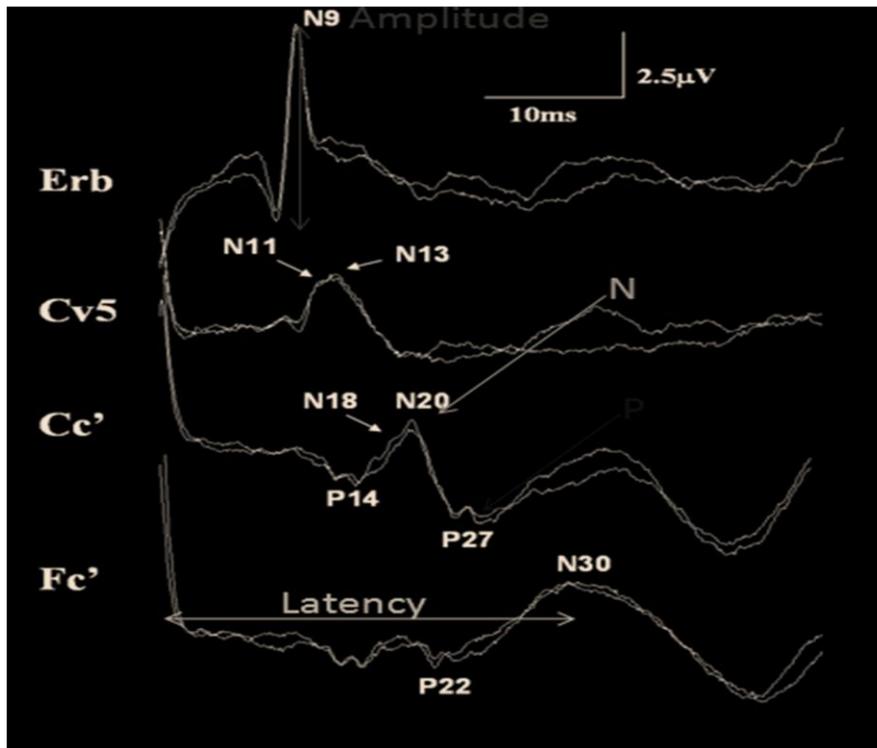


Figure 6: Examples of SEP peaks. Adapted from [53].

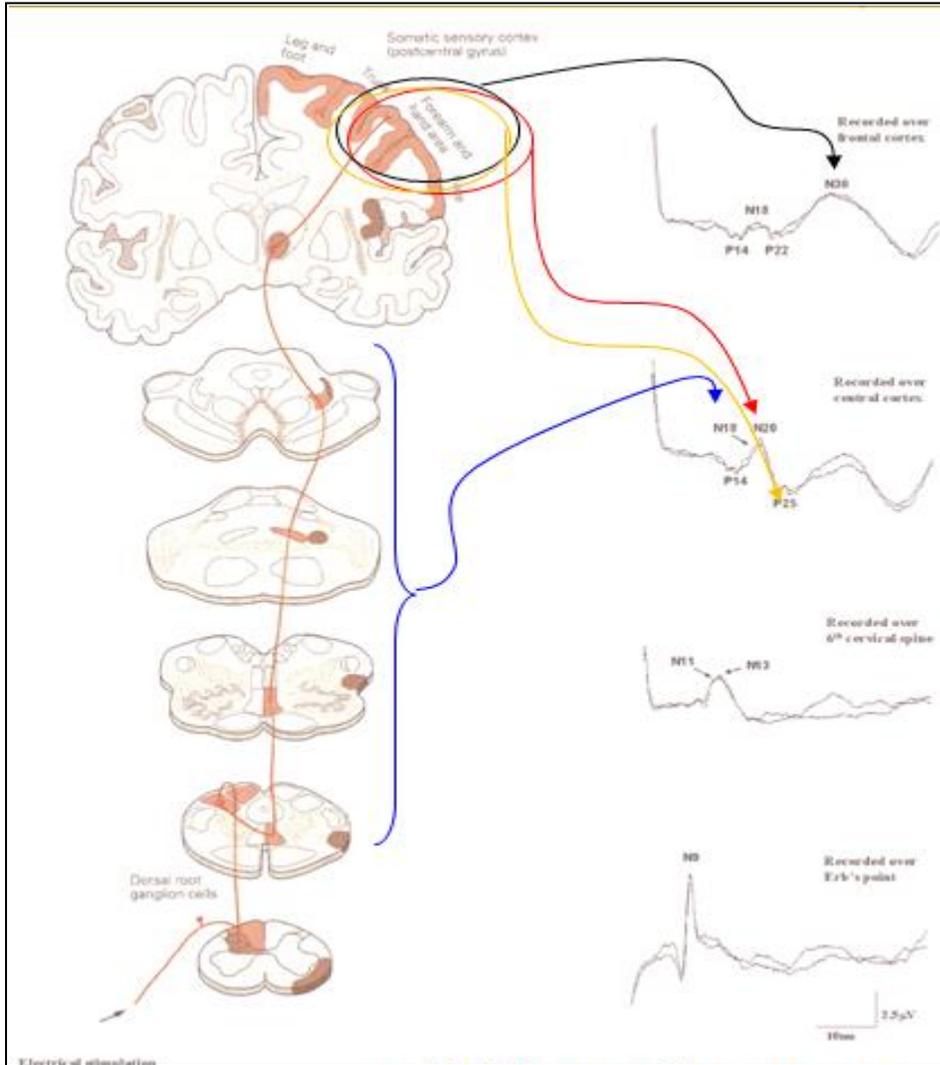


Figure 7: Overview of SEP peaks and their neural generators. Adapted from [25]

N9

The N9 peak is recorded at Erb's point over the brachial plexus (located on the shoulder above the proximal clavicle). Erb's point is abnormal when there is a lesion from the peripheral median nerve indicating that the neural generator of the N9 peak is in the peripheral nerve pathway [54, 55].

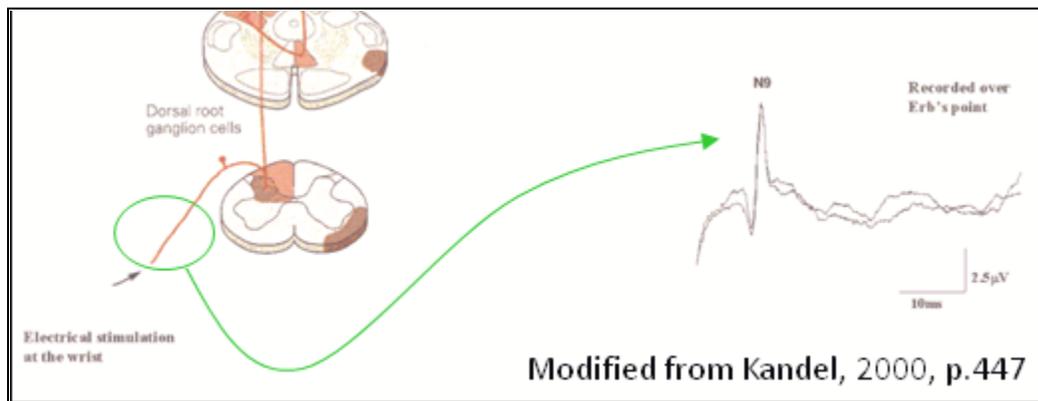


Figure 8: N9 SEP peak and it's neural generator. Adapted from [25]

N11

The N11 peak is recorded over the 5th cervical spinous process [50] and signifies the afferent volley entering the spinal cord as it starts to ascend towards the cuneate nucleus [56]. Evidence to support this comes from patients with nerve root avulsions who lack N11 and N13 peaks although the N9 SEP peak is preserved [55].

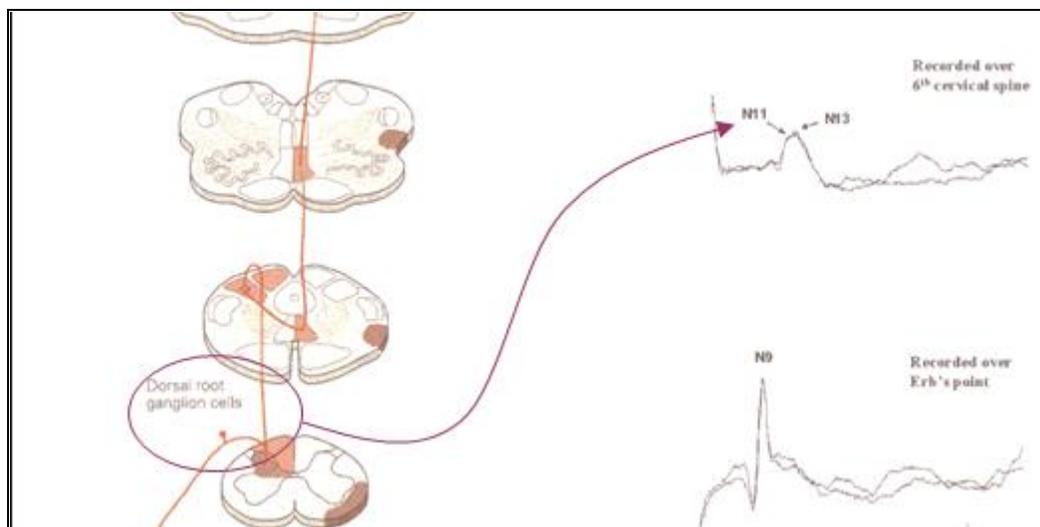


Figure 9: N11 SEP peak and it's neural generator Adapted from [25]

N13

The N13 SEP peak begins as an inflection upon N11, and is also recorded over the 5th spinous process [50] and is thought to demonstrate the activity of inhibitory interneurons in the dorsal horn and is generated near the first synaptic relay of the spinothalamic tract [26, 56]. The theory that the N13 reflects activity in dorsal horn interneurons is corroborated by patients with cervical dorsal column lesions that lack the N13 SEP peak [57].

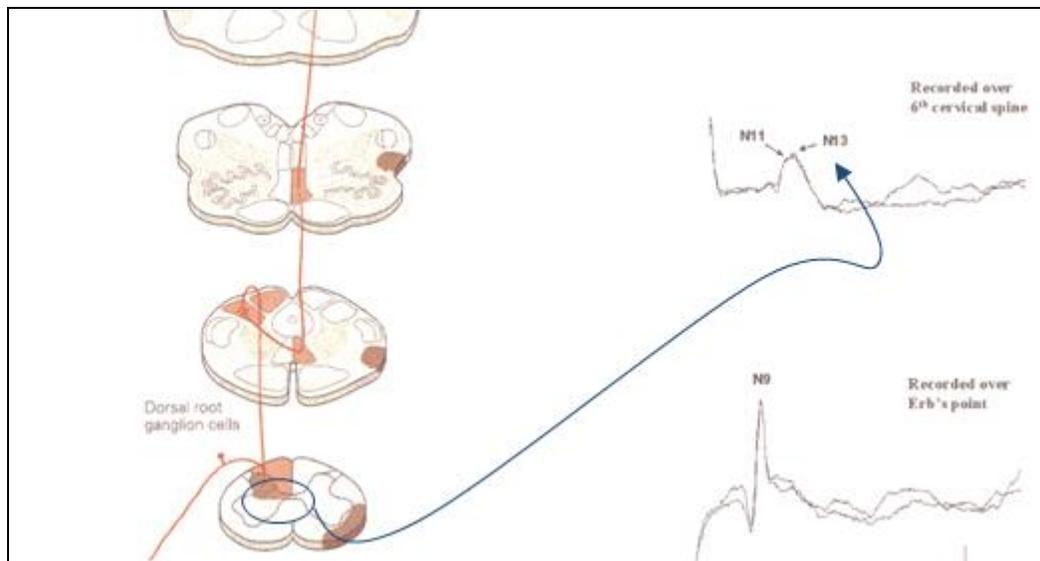


Figure 10: N13 SEP peak and its neural generator. Adapted from [25]

N18

The N18 peak is the broadest elevation following the P14 peak [58]. The N18 peak was originally thought to be generated by the thalamus, however this was disproven as the N18 peak is preserved following lesions of the thalamus [59]. Noel, Ozaki and Desmedt [60] demonstrated that the N18 peak was preserved in patients with lesions of the medial lemniscus and therefore concluded that the generator for the N18 SEP peak is the lower medulla. This finding is supported by Manzano et al. [61] who found that the N18 SEP peak was the only SEP peak resistant to vibratory changes.

Therefore, N18 originates in the brain stem, between the lower medulla and midbrain-pontine region (the dorsal column and the inferior olives) and reflects activity in the olivo-cerebellar pathways. Thus the N18 peak thus originates above the spinal cord but below the cortex [50, 62] and can show alterations in cerebellar activity [63].

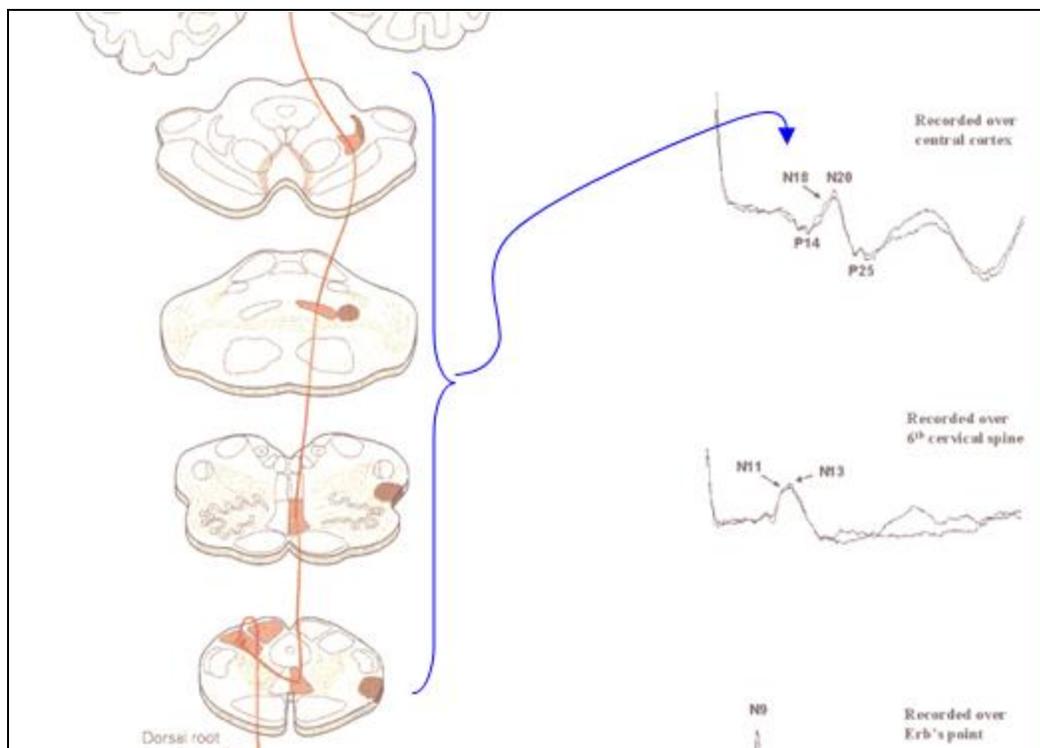


Figure 11: N18 SEP peak and its neural generator. Adapted from [25]

P14

The P14 is generated by the afferent volley in the medial lemniscus and is measured at the onset of the N18 peak. It is generated at or near the foramen magnum (originates above the spinal cord, but below the cortex) [26, 62]. This is supported by an absence of the P14 in patients with cervicomedullary lesions [64] and in brain dead patients [65]. In addition, patients with brainstem lesions at the level of the midbrain or upper medulla [63] or thalamic lesions [66] have a P14 peak but lack all of the cortical SEP peaks.

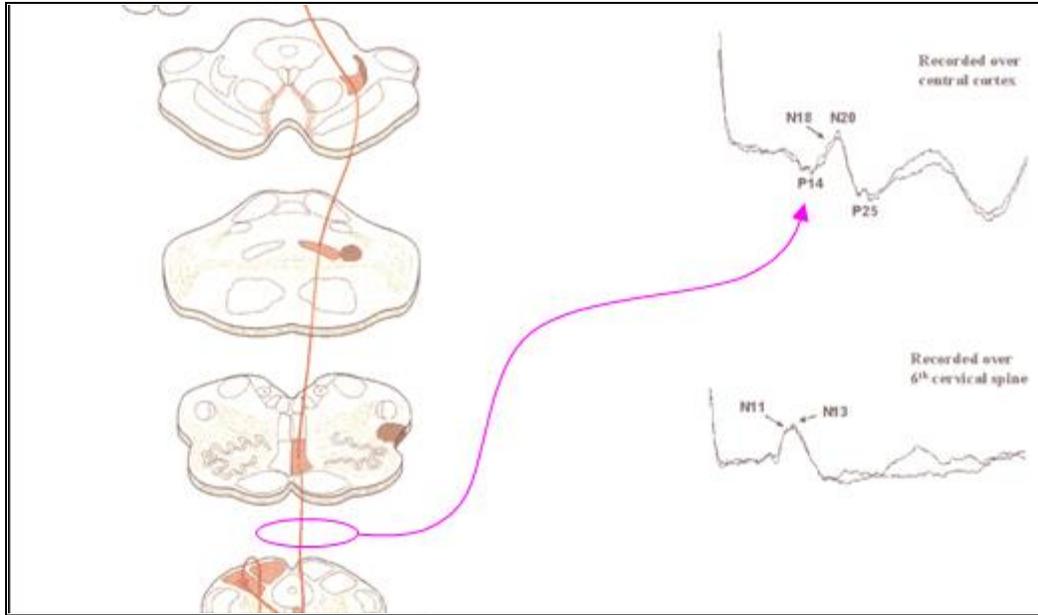


Figure 12: P14 SEP peak and it's neural generator. Adapted from [25]

N20

The P14 SEP peak is followed by the N20 SEP peak which is known to reflect the earliest cortical processing or activity in the SI, specifically in Brodmann's area 3b [49]. The parietal N20 SEP peak occurs contralateral to the site of stimulation [49] and responds to contralateral tactile stimuli [67]. Brodmann's area 3b (SI) is activated with cutaneous input, but not joint movement. Desmedt and Ozaki [68] found that the N20 SEP peak is activated in response to cutaneous stimulation, and not joint movement.

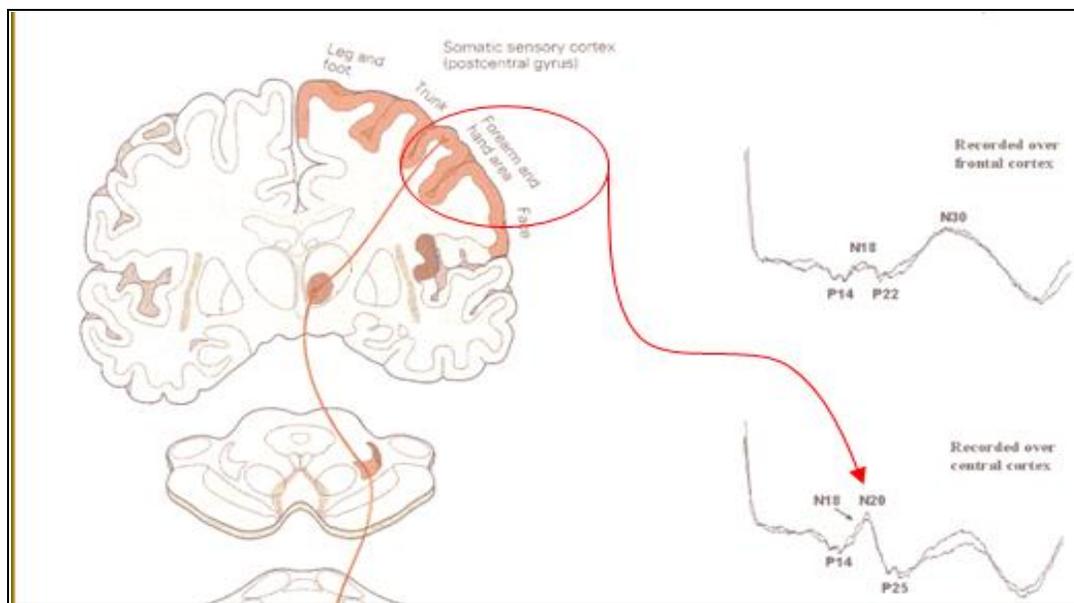


Figure 13: N20 SEP peak and its neural generator. Adapted from [25]

N24

The N24 SEP peak emerges on the ascending slope of the N30 SEP peak and is located close to the N20 SEP peak. García Larrea et al. [69] found that N24 SEP peak can be seen at higher stimulus rates (greater than 3 Hz) that decreases the N30 peak. There is some variability in its latency and therefore the N24 SEP is also referred to as the N23 [70], or the N25 SEP peak [71]. Waberski et al. [71] utilized source localization and identified the posterior wall of the central sulcus in area 3b of the SI as the neural generator of the N24 SEP peak. The input to the SI travels through the cerebellum [72]. Evidence for this comes from patients with lesions in the cerebellum resulting in a N24 SEP peak that is reduced or absent, but with the preceding SEP peaks present [73]. This confirms that the N24 SEP peak is directly linked to the integrity of the cerebellum.

P25

The P25 peak is recorded from the contralateral parietal region, and originates in Brodmann's area 1 of the SI, (posterior to Brodmann's area 3b) [47].

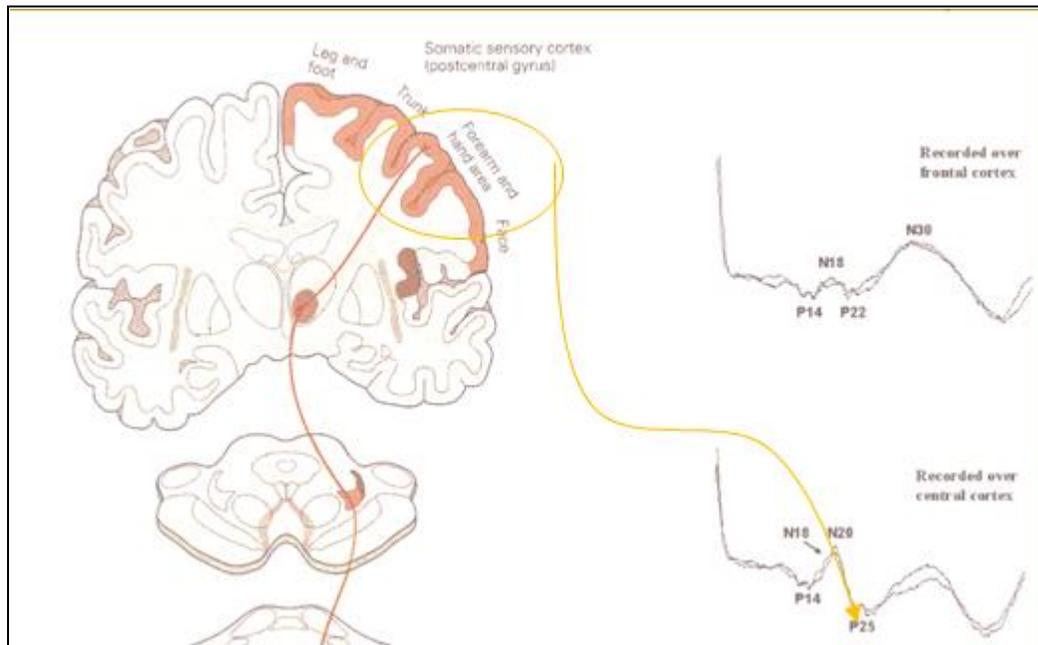


Figure 14: P25 SEP peak and its neural generator. Adapted from [25]

N30

The N30 SEP peak reflects SMI [74] and is a complex subcortical and cortical loop connecting the thalamus, BG, premotor areas and the MI [75, 76]. Originally, the N30 peak was assumed to reflect activity in the SMA as it reaches maximal amplitude when recorded over the SMA [77] and was absent in a patient who suffered from a lesion of the SMA [76]. Additional evidence for the SMA as the neural generator of the N30 SEP peak is that the N30 SEP peak is reduced by muscle movement. Chéron et al. [70] found that finger movements attenuated the N30 SEP peak. As regional cerebral blood flow (rCBF) is increased solely in the SMA during mental training of finger movements [78] the attenuation of N30 during imagined movement supported the SMA being its neural generator. However, a study using intracortical electrodes demonstrated no early

SEP peak is generated in pre-SMA or SMA [79]. There is also research that points to the BG as a neural generator for the N30 peak as Parkinson's disease (PD) involves the BG and patient's with PD demonstrate a decreased N30 peak as compared to healthy participants [80]. In addition, BG stimulation increases the amplitude of the N30 SEP peak [80]. Additional evidence points to the MI as the neural generator of the N30 SEP peak [71, 81]. Intracortical electrodes have shown that afferent input following stimulation of the median nerve reaches the MI [81, 82]. In addition, Waberski et al. [71] utilized source localization and determined that the MI is the N30 SEP peak generator. Cebolla et al. [83] utilized swLORETA (standardized weighted Low Resolution Brain Electromagnetic Tomography) and found that the N30 SEP peak is produced by activation in the premotor, motor, and prefrontal areas as opposed to having a singular generator. The N30 SEP peak has numerous inputs with separate thalamo-cortical pathways and is a marker of somatosensory processing pertinent to SMI [81].

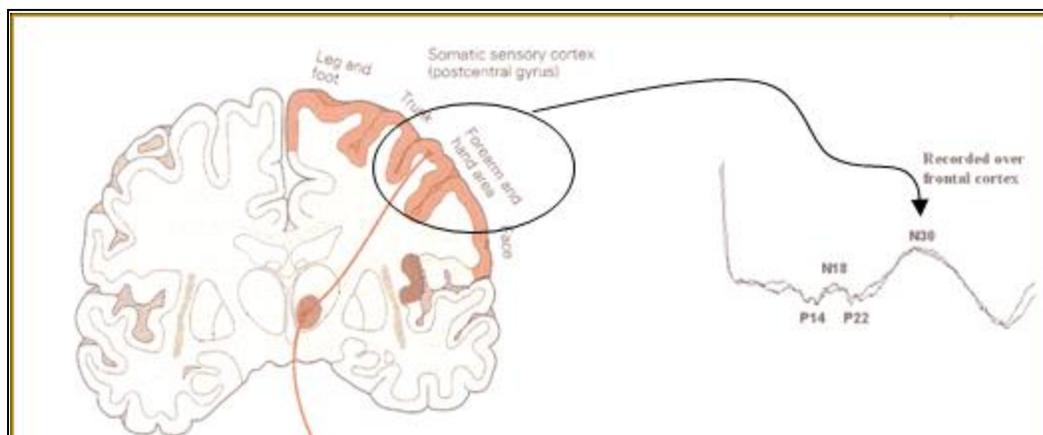


Figure 15: N30 SEP peak and it's neural generator. Adapted from [25]

2.6 Single and paired pulse transcranial magnetic stimulation

Transcranial stimulation (TCS) is a technique used to investigate the excitability of the MI. Initially, stimulation of the MI was only possible by direct cortical stimulation, which was invasive and was limited to the evaluation of patients for surgery [84, 85]. Over time, non-invasive techniques have been developed for the activation of the MI. Merton et al. [86] developed transcranial electrical stimulation (TES) and found that stimulation over the MI can lead to contraction of contralateral muscles. In the 1980's the modern magnetic stimulator was developed [87]. There has been the development of increasingly focal stimulation techniques which have allowed transcranial magnetic stimulation (TMS) to be applied in studies of the organization of corticomotor representations [88].

2.6.1 Transcranial Electrical Stimulation (TES)

With Transcranial Electrical Stimulation (TES) anodal stimulation with high voltage transient electric shocks are used to activate the MI. The stimuli delivered to the MI elicits descending waves in the pyramidal tract [89]. The earliest of these waves is the D wave which reflects direct activations of the corticospinal tract [90]. The following waves are termed I waves which are considered to be the result of indirect depolarization of the corticospinal tract [90]. TES most likely activates the corticospinal tract at or near the cerebral cortex [91]

2.6.2 Transcranial Magnetic Stimulation (TMS)

TMS is a safe way to painlessly stimulate the motor areas that control movement. This occurs due to a rapid discharge of current through a coil being placed over the scalp, which induces a magnetic field that is oriented perpendicular to the coil, and can reach values of up to 2 Tesla [92]. This rapidly changing magnetic field induces an electric field which in turn activates the neural tissue, specifically the interneurons that synapse onto the neurons of the MI. The magnetic

field diminishes significantly with distance from the coil surface, and therefore deeper cortical structures in the brain are not activated [93]. Currents produced by TMS are much less than those produced by TES [93]. TES and TMS stimulate the same axonal population [94], however TMS is thought to activate the MI indirectly [90]. This is explained by the orientation of corticospinal neurons in the MI. The electric currents produced by the magnetic field flow parallel to the surface of the cortex. Corticospinal neurons are oriented perpendicular to the surface and they will therefore be activated by TES but will not be activated directly by the horizontal current induced by TMS. However pyramidal cells in layers II and III are activated by TMS which then activate layer V corticospinal cells.

There are many different types of TMS coils that can be used in research studies including round, figure-eight, and double cone coils. Round coils affect a large region of the brain and are sensitive to the radius of the circle [95]. Larger coils do not produce a very local stimulation, but are able to penetrate the MI more deeply and can therefore activate deeper muscles [96]. The figure-eight shape coil allows for the most localized current under the intersection of both wings of the magnetic coil [95, 97] and thus will be used for this thesis. TMS allows for the study of plastic changes in cortical areas that function in motor and sensory mechanisms [98], and mechanisms of neuroplasticity [99]. The corticospinal pathway is the main pathway activated through TMS. However, activation of corticostriate, corticothalamic, corticocortical, corticopontine, corticobulbar, and corticoreticular pathways may also be activated by TMS [100].

2.6.3 Motor Evoked Potentials (MEPs)

Both electrical and magnetic stimulation evokes electromyographic (EMG) responses in contralateral muscles [93]. Once the TMS coil stimulates the MI it will then induce neural activity which discharges an action potential down the lateral corticospinal tract to the muscle [93, 101]. Magnetic stimulation of the MI evokes EMG responses in contralateral and distal muscle [93] which is known as a motor evoked potential (MEP) and is thought to reflect the excitability of the corticospinal tract to the muscle [93]. In order to identify the area of MI which corresponds to the target muscle a trial and error TMS mapping technique occurs, where the participant is stimulated along the MI region until there is activation of the muscle [102]. Once the area of the brain is identified, progressively decreasing the intensity of the stimulation while recording EMG will allow for the development of a threshold level, which has previously been defined as the probability of evoking a MEP of at least 0.05 mV 5 out of every 10 stimulations [102]. Inter-participant variability of the optimal coil position for evoking a response in a muscle may vary up to 2 cm [103]. A coil orientation with handle pointed backwards and rotated 45 degrees away from the mid-sagittal line has been shown to allow for optimal activation of corticospinal neurons [104, 105]. When performing trials, an average of 8-16 MEP's is usually taken for each stimulus parameter and a tight fitting cap is used in order to accurately place the coil.

The MEP is usually larger in the hand and forearm region in the axial skeleton when compared to the leg, foot and pelvis regions due to the positioning and the orientation of the MI [102]. The somatotopic position of the hand region on the MI is located near the most superior and superficial part of the skull, and has the largest representation [106].

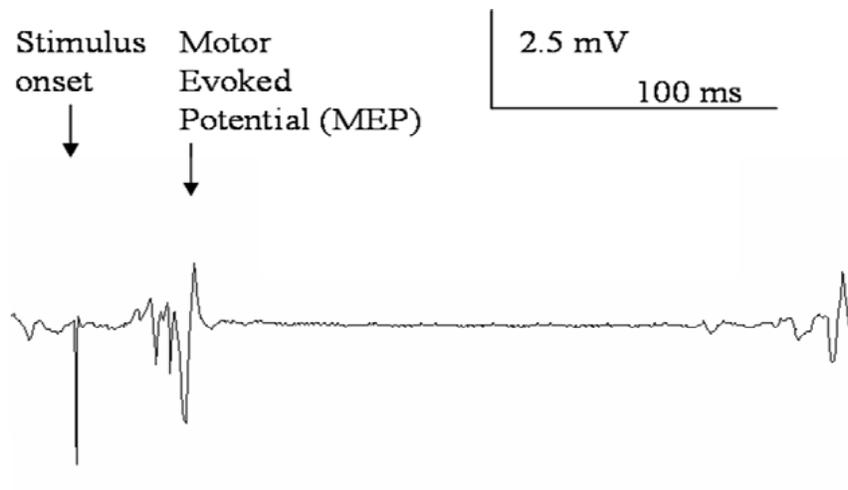


Figure 16: Example of an electromyography trace showing a motor evoked potential [107].

2.6.4 Paired-pulse TMS:

The paired-pulse technique is used to non-invasively investigate the excitability of inhibitory [108, 109] and excitatory [110, 111] neuronal networks at the cortex. Two separate stimuli are delivered to the MI through the same stimulation coil [112]. In order to investigate inhibitory neuronal networks, a subthreshold pulse precedes a test pulse by 1 to 6 milliseconds and recruits inhibitory interneurons. This leads to a MEP response that is inhibited. When a suprathreshold pulse precedes a second pulse which is subthreshold (interstimulus interval 1.5 - 3 milliseconds) the MEP response is facilitated.

Short Interval Intracortical Inhibition:

Short-interval intracortical inhibition (SICI) occurs when a subthreshold CS is followed by a suprathreshold TS at an interstimulus interval (ISI) of 1 to 6 milliseconds [113]. The response in the MEP of the target muscle is inhibited during this phenomenon. There are two distinct phases of SICI, with one occurring at approximately at an ISI of 1 milliseconds, while the other occurs at an ISI of ~2.5-4.5 milliseconds [114-116]. Studies have shown that the first phase of SICI is due to refractoriness of the neural structures that are accountable for corticospinal neuron

activation, whereas the second phase is a synaptic inhibition facilitated by gamma-aminobutyric acid A (GABA_A) [113, 117-119]. A reduction of SICI occurs prior to and during voluntary motor output [120, 121], following repetitive contraction tasks [122, 123] and enhances the neuroplasticity associated with motor output [124]. Research has shown that SICI is mediated by GABA_A receptors since there is an increase in SICI with drugs that enhance GABA_A transmission [109] and this suppresses neuroplasticity in the MI [125]. However, it is unlikely that TMS activates GABAergic neurons directly, as these neurons have limited horizontal connections [126] and therefore TMS likely activates cortico-cortical neurons that project onto GABAergic neurons [127].

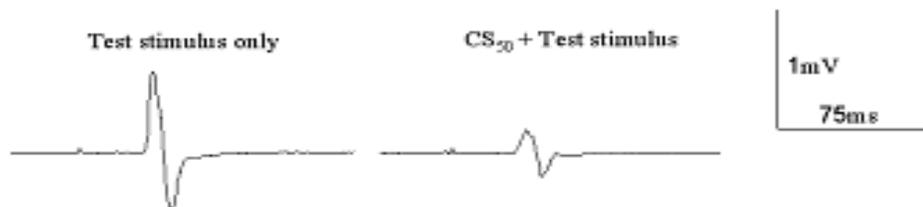


Figure 17. Example EMG trace showing SICI. The MEP evoked by the test stimulus alone is inhibited when preceded by a smaller stimulus [107].

Short Interval Intracortical Facilitation/ I wave Facilitation

Short-interval intracortical facilitation (SICF) or I-wave facilitation (IwF) occurs when the first stimulus (S1) is above the MEP threshold and the second stimulus (S2) is below or at the level of the MEP threshold [119, 128, 129]. When this occurs, the EMG response of the target muscles is larger than responses to S1 alone. This has been shown to occur at three distinct phases of ISI at: 1.0-1.5; 2.5-3.0; and 4.0-4.5 [130, 131]. There are two types of corticospinal waves following the stimulation of the MI: D and I waves, and SICF has been shown to be related to I-wave generation [132]. D-waves are due to the activation of the axon of corticospinal neurons, while I-waves are due to the trans-synaptic activation of these neurons [132]. SICF is thought to occur

because the second stimulus acts on the neuronal tissue around the motor neuron that have been partially facilitated, but have not yet reached threshold [133]. I waves occur at 1.5 milliseconds intervals, and since the three phases of SICF also occur at intervals of 1.5 milliseconds, it is thought that SICF is due to the interaction of I waves generated by the two stimuli (S1 and S2) [129].

Through epidural recordings of the corticospinal pathway at the spinal cord [134] it has been verified that the interactions between S1 and S2 occurs at the MI. SICF is thought to be mediated by different neuronal circuits than SICI [108] although studies have demonstrated that SICF is reduced by drugs that increase GABAergic function [135]

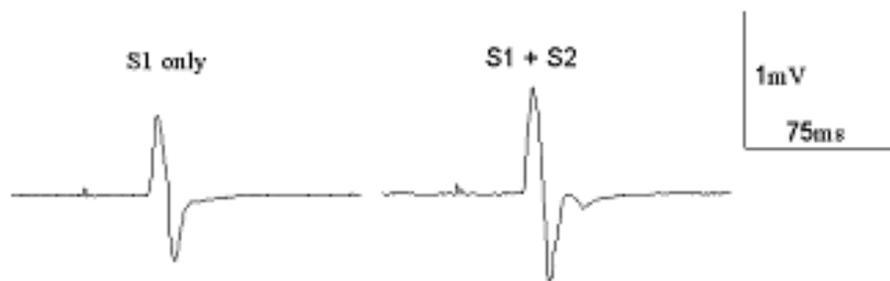


Figure 18: Example EMG trace showing SICF (or IwF). The MEP from the test stimulus (S1) alone is facilitated when followed with a smaller stimulus (S2) [107].

2.6.5 Input-output curves

The literature demonstrates that an input/output curve is a robust measure of cortical excitability [136]. The slope of the linear aspect of the sigmoid shaped curve represents cortical excitability [137] and since the input-output curve is comprised of TMS stimulation at different intensity levels, it is a reliable way to determine overall cortical excitability. The slope of the input/output curve represents the rate at which cortical excitability increases and provides a measure of the alterations in cortical excitability. This is less vulnerable to the fluctuations in MEP amplitude

that occurs when stimulating at a single intensity and therefore it is a robust and useful method of measuring alterations in excitability that occurs with motor learning acquisition and pain.

2.7 Cortical neuroplasticity

Plasticity means the capacity for pliancy and malleability [138] and cortical neuroplasticity is any enduring morphological or functional alterations in neurons through changes in the strength of connections, reorganization, or altered representational patterns [1, 2]. Cortical neuroplasticity includes the potential for change and the mechanisms of self-repair or reorganization of neural connections. Cortical maps are modified by sensory input, experience, and learning, and change in response to motor learning and cognitive tasks [138]. Cortical neuroplasticity has been demonstrated in response to experience [139-141], motor learning acquisition [4, 142-146], and pain [5, 13, 14, 147]. These alterations can be transient, reflecting the ability of the system to respond to external demands and can transpire over short time periods [3, 148]. For example, the enlargement of cortical representation areas has been shown after a few days of repeating a skilled movement pattern, such as the learning of a new piano sequence [139]. Over time, these changes become stable as exemplified by the permanent enlargement of cortical representation areas in lifelong musicians [149]. Research indicates that chronic pain is correlated with changes in cortical organization [15, 150]. Reorganization and altered excitability of cortical maps in response to nociceptive input is hypothesized to be a contributing factor in chronic pain and may also help to determine the level of recovery of function following an injury [151]. Changes in cortical reorganization are also seen in individuals suffering from phantom limb pain as the severity of phantom limb pain is correlated with reorganization of the SI [152]. In addition, Flor et al. [150] found that activation in the SI was correlated with pain perception among individuals with phantom limb pain. In a study conducted on individuals suffering from phantom pain,

stimulation of the skin of the forearm produced sensations on parts of the phantom hand [152].

As the hand and forearm are somatotopically close within the SI, it was hypothesized that the pain associated with this condition contributed to cortical neuroplastic changes in representations of the hand and forearm within the SI. Similar cortical reorganization is thought to occur in other chronic pain conditions and it is hypothesized that there is a link between cortical organization and chronic pain, although the causality of this relationship is currently unknown.

Neuroplasticity can be expressed in different ways including cellular alterations. The anatomy of a neural network is much larger than the area of its functional influence and there are multiple representations of each muscle and joint area in the cortex. Modifications of the synapse underlie learning and memory, and this also occurs in response to deafferentation and pain [1, 153, 154].

Cortical organization depends on excitation and inhibition. Some areas are silent through active GABA inhibition which can be altered or removed (unmasking), which can cause a rapid change in size or distribution of the network [138]. While modulation is reversible, modification involves alterations in receptors or in the structure and connectivity of neurons [41].

3 THE SIGNIFICANCE OF THE RESEARCH

This section will discuss the practical significance of this thesis.

3.1 Chronic pain conditions:

Following injury, pain is one of the most disabling and frequently described symptoms. Extended periods of repetitive activity can lead to occupational overuse injuries (OOS) and repetitive strain injuries (RSIs) [155] which are significant public health problems [15]. The mechanism as to how these overuse injuries develop is unknown. In addition, chronic pain, fibromyalgia, dystonia and phantom limb pain are all conditions that occur in the absence of a peripheral pathology or are disproportionate to the peripheral injury. Studies of patients with chronic pain have indicated that there is a poor correlation between peripheral injuries and pain [156]. Peripheral [157] and central [158] neuroplastic changes in cortical organization are emerging as a contributing factor for chronic pain [159]. Cortical reorganization occurs as a result of repetitive muscular activation [159-161]. Specifically, dedifferentiation of the SI has been documented in primates following repetitive motor activity [159] and in individuals with dystonia [160]. Additionally, altered SMI has been documented in patients with musician's cramp [162] and in patients with dystonia [163-165]. The transmission of somatosensory input is attenuated with repetitive motor activity [166-168] and this could lead to long term changes in SMI leading to the initiation of overuse injuries and chronic pain. Processing of continuous peripheral input may cause abnormal control of specific muscles, leading to pain and altered motor control. SMI conflict generates pain in healthy volunteers [169] and SMI conflict is a potential reason that pain occurs in the absence of nociceptive input or when it is disproportionate to nociceptive input as in the instance of fibromyalgia. The pain associated with fibromyalgia is difficult to comprehend as there is a lack of pathology. Investigating SMI in

response to pain may help to explain the mechanisms involved in the initiation of RSIs and chronic pain.

3.2 Rehabilitation

Many individuals undergoing rehabilitation present with pain and deficits in motor control.

Typically, motor deficits are regarded as a consequence of movement related pain, however, there is confirmation that pain impacts motor control and has the ability to negatively influence the neuroplasticity associated with motor output [12-14]. Cortical neuroplastic alterations correlate with recovery following cortical, spinal or peripheral injuries [88, 170-172].

Manipulation of these neuroplastic alterations with rehabilitation programs is effective for individuals experiencing motor deficits or weakness (for example following a stroke) [173-175] and can also be utilized for individuals suffering from lower back pain (LBP) [176] and neck pain [177]. As chronic pain may be influenced by altered motor control, rehabilitation programs which help to establish healthy motor control are instrumental for effective treatment. LBP patients who participated in motor- learning based rehabilitation demonstrated a reduction in pain and a reversal in the location of the centre of gravity (CoG) [178]. Neck pain patients demonstrated improvements in the activation of the neck muscles and this occurred solely with isolated learning of these muscles [179] suggesting that the improvements are specific to motor learning. Pleger et al. [180] demonstrated that for patients suffering from complex regional pain syndrome (CRP) there was a decrease in pain ratings and increased representation of the affected limb following motor learning. A reduction in pain and restoration of sensorimotor maps is demonstrated following sensory discrimination learning in patients suffering from phantom limb pain [181]. This research demonstrates that there is altered representations of muscles affected by pain, and that the degree of neuroplastic alterations is associated with maladaptive motor control,

and these alterations can be reversed by motor learning. The sensory and motor systems are functionally linked and research has highlighted how the neuroplasticity of MI and SI can change in a use-dependent manner [28]. The effects of motor learning on pain perception may be due to cortico-thalamic loops, inhibiting the nociceptive sensory input. However, a detailed understanding of this process is unknown.

By understanding the role of somatosensory processing in response to pain, and through the understanding the differences in the somatosensory processing of acute pain versus chronic pain, future research might eventually lead to practical applications for the rehabilitation of diseases that occur without a discernible peripheral causality such as dystonia and fibromyalgia. Although it is hypothesized that pain interferes with learning-induced neuroplasticity, less is understood regarding the neurophysiological consequences of pain on motor learning induced neuroplasticity. The results of this research may provide insight in to how acute experimental pain contributes to plasticity (adaptive or maladaptive) during motor learning acquisition, and provides insight as to how well the motor skill has been retained when acquired during acute pain. Gaining a better grasp of the influence of pain on motor learning is vital in order to deliver effective rehabilitation programs.

4 LITERATURE REVIEW - ALTERED AFFERENT INPUT

This chapter reviews how altered afferent input in the form of deafferentation and repetitive movement affects plasticity in the CNS. This is relevant to the study of pain and sensory processing as it is hypothesized that pain also results in central plastic changes that outlast the period of altered input.

4.1 Deafferentation and somatosensory processing

Deafferentation is a partial or total loss of sensation resulting from the interruption of sensory neurons [182]. Somatosensory input is critical for the learning of new skills; the hand area of the MI receives cutaneous and proprioceptive input from the hand and the arm [183]. Interruption of this input results in motor control deficits [184]. Several studies have examined plasticity in the CNS in response to deafferentation [56, 182, 185]. These studies [56, 182, 185] demonstrate plasticity in the CNS in response to deafferentation which is relevant to my thesis as it is proposed that pain is a form of altered afferent input that also leads to cortical plasticity in the CNS. A few studies have explored the effect of deafferentation on MI plasticity and sensory processing. Most experimental research has utilized ischemic deafferentation [186-191] although several studies have utilized a local anaesthetic [122, 149, 192, 193]. Ischemic deafferentation leads to the elimination or interruption of afferent sensory input [182] and brings about an increase in cortical SEPs [56, 182, 194]. However, there have been contradictory findings on the effect of deafferentation on subcortical versus cortical structures of the CNS [182, 185]. Tinazzi et al. [185] determined that the N20, P27 and N30 cortical peaks showed increases in amplitude with no significant differences in any of the subcortical peaks during temporary anaesthesia of the ulnar nerve. N20, P20, and P27 are generated from the SI [49] and the N30 is thought to originate from the frontal lobe and the posterior wall of the central sulcus and reflects SMI [56].

In contrast, Tinazzi et al. [182] found increased amplitudes of the cortical and subcortical SEP peaks evoked by ulnar nerve stimulation that was ipsilateral to the deafferented median nerve. However, Tinazzi et al. [182] determined that the differences in subcortical amplitudes were not as pronounced as the cortical SEPs. These findings are in contrast to Tinazzi et al. [185] who didn't find changes at the subcortical level. However, Tinazzi et al. [182] was studying individuals with chronic deafferentation while Tinazzi et al. [185] was studying individuals with acute deafferentation. These results [182, 185] suggest that chronic exposure to altered afferent input may result in long term cortical modulation which then modifies subcortical excitability. These results added to the body of literature that the somatosensory system of humans is capable of undergoing reorganization in response to altered input.

Tinazzi et al. [182] found increases in subcortical structures involved sensory processing, while Tinazzi et al. [195] found that the N13 SEP peak and P14 SEP peak did not change during deafferentation study in healthy participants. This study helped to change thinking in the field as it indicated that deafferentation can induce cortical plasticity even in somatic structures not directly undergoing deafferentation [195]. Other studies have investigated deafferentation, cortical neuroplasticity, and the roles played by cortical and subcortical structures. Murphy et al. [196] investigated the effect of deafferentation of the radial nerve and found that the N30 median nerve SEP peak was significantly increased. Weiss et al. [192] established that after deafferentation of the radial and median nerves, the cortical representation of the finger and the lip moved closer together. The hand and lip are somatotopically close within the SI, and therefore this study provides evidence that plasticity in response to deafferentation is occurring at the cortical level.

These experimental results added to the evidence that the somatosensory system of humans is capable of undergoing reorganization and the primary importance of cortical structures in sensory processing [182, 185]. Pain is also a form of altered afferent input and it is hypothesized that there might be a similar mechanism in the progression of chronic pain. Long term exposure to pain (a form of altered afferent input) may result in cortical modulation which then modifies the subcortical structures. Chronic pain is ongoing pain that occurs from injury to the nervous system [197].

4.2 Deafferentation and motor output

In terms of the effect of deafferentation on motor output, research demonstrates that ischemic deafferentation increases the excitability of the contralateral hand as well as the upper arm [186]. Though ischemic deafferentation generally leads to increased MEP amplitudes, results from studies using nerve blocks provide inconsistent results. For example, a study found decreased motor output from a muscle within the anesthetized area but increased or unchanged output from muscles adjacent to this region [149]. Research demonstrates a synergistic effect of deafferentation and motor training as deafferentation of the hand enhances the effect of motor training and increases corticospinal excitability much more than either deafferentation or motor practice in isolation [190].

4.3 Repetitive movement

In contrast to deafferentation, repetitive activity is an increase in afferent input. Studies have demonstrated that the CNS reorganizes itself in response to motor performance [3, 122] and that this outlasts the period of altered input [3, 198]. It has been shown that repetitive hand contractions results in a degradation of the hand representation of the SI and reduced motor control [159]. Murphy et al. [198] added to the literature in support of the role of cortical

structures in somatosensory processing by demonstrating that repetitive activity leads to attenuations in the amplitudes of subcortical and cortical SEP peaks. These results support the growing body of evidence that decreased and increased afferent inputs can lead to neuroplastic alterations in the corticomotor and somatosensory systems. The mechanisms responsible for use-dependent neuroplasticity are not well understood. Findings from recent research suggest that intracortical inhibition plays an essential role [127, 199] and that afferent input alters cortical inhibitory circuits [200]. These inhibitory circuits help to maintain the boundaries of the cortical maps [122, 127]. Liepert et al. [123] found that following repetitive thumb abduction there was a decrease in SICI in the APB, and an increase in the FDI, which was relaxed throughout the study and they concluded that the plastic changes observed following motor learning are task dependent [123].

Research demonstrates that both cortical and subcortical components of the CNS increase after deafferentation and decrease after increased afferent input (repetitive movement) demonstrating that altered afferent input induces cortical neuroplasticity [182, 198, 201]. The literature demonstrates that the cortical structures play a primary role in somatosensory processing as it has been shown that acute altered afferent input leads to rapid cortical modulation and chronic altered afferent input results in cortical changes which may subsequently modulate subcortical structures [182, 185, 198, 201]. These studies established that the CNS has the capacity to reorganize in response to afferent input and that by affecting the CNS these plastic changes outlast the period of afferent input. This is relevant to the study of pain and sensorimotor processing as it is hypothesized that pain also results in central plastic changes that outlast the period of altered input which over time may result in syndromes which lack a discernible peripheral pathology (chronic pain, dystonia, fibromyalgia, phantom limb pain).

5 LITERATURE REVIEW – PAIN

This chapter reviews the ascending nociceptive tracts and describes the changes in excitability in the spinal cord, brainstem, and supraspinal structures in response to pain and the mechanisms behind nociceptive plasticity. For a more detailed description of how pain travels from the periphery to the cortex please see the Appendix A1.

5.1 Ascending Pathways

5.1.1 The spinothalamic tract

The spinothalamic tract is the major ascending nociceptive pathway and it consists of the axons of wide-dynamic-range and nociceptive neurons of the dorsal horn [44]. The spinothalamic tract ascends contralaterally in the anterolateral white matter to the ventroposterior and posterior thalamus and eventually reaching the SI, the SII, prefrontal cortex, posterior and mid-insula, posterior parietal cortex, and mid-cingulate cortex [202, 203]. The spinothalamic tract projects to the SI and is responsible for mediating the sensory discriminative components of a pain sensation (location, texture, and intensity) [24]. Nociceptive neurons in SI with input from the lateral system are mainly found in Brodmann area 1, but there is some evidence that Brodmann area 3a may also have some nociceptive input [24]. Historically, thermal and pain sensations had been considered to be sub-served by common pathways within both the peripheral and the CNS through the spinothalamic pathway. However, a segregation of thermal and nociceptive inputs has been demonstrated [44]. Injury to the spinothalamic tract can result in a severe pain termed central pain [25].

5.1.2 Spinoreticular tract

The spinoreticular tract consists of the axons of neurons in laminae VII and VIII and ascends in the white matter (close to the spinothalamic tract) terminating in the reticular formation and the thalamus [202, 203]. In contrast to the spinothalamic tract, a significant number of the axons of the spinoreticular tract do not decussate [25].

5.1.3 Spinomesencephalic tract

The spinomesencephalic tract consists of the axons from lamina I and V projecting in the white matter to the mesencephalic reticular formation and periaqueductal gray matter (PAG), and through the spinoparabrachial tract, it ascends to the parabrachial nuclei [204]. Neurons then project to the amygdala (a major component of the limbic system), which plays a role in the processing of emotions [205]. The spinomesencephalic tract contributes to the affective processing of pain. A significant portion of these axons ascend in the dorsal lateral funiculus [25].

5.1.4 Cervicothalamic tract

The cervicothalamic tract arises from neurons in the cervical nucleus [206]. The lateral cervical nucleus has contributions from nociceptors in laminae IV and III [207]. The majority of these axons decussate and project in the medial lemniscus to the midbrain and to the ventroposterior lateral and posteromedial nuclei of the thalamus.

5.2 Pain: Current view

Peripheral tissue damage that affects components of the peripheral nervous system (PNS) and CNS and increases pain sensitivity is referred to as central sensitization [208]. Central sensitization is associated with allodynia (pain perception in response to innocuous stimuli) and hyperalgesia (exaggerated response to nociceptive input) [209] and may result in persistent pain that leads to a decreased threshold and the amplification of subsequent input [210]. This becomes pathological as the perception of pain is maintained in the absence of nociceptive input and persists even after the injured body part is healed. This is a chronic pain that occurs from damage to the PNS or CNS and is referred to as neuropathic pain [210]. It is with central sensitization that syndromes like chronic LBP or phantom limb pain occur [211]. In contrast to chronic pain, acute pain is a response to peripheral input and is referred to as nociceptive pain.

A system modulating the transmission of pain at the dorsal horn was proposed by the gate control theory [25]. The dorsal horn synapse receives information from the periphery and from supraspinal sources and is the initial state of modulation whereby connections between nociceptive and non-nociceptive afferent neurons controls the transmission of nociceptive input [25]. Descending facilitatory and inhibitory influences from the brainstem as well as inhibitory and excitatory interneurons in the dorsal horn mediate the transmission of nociceptive input, impacting the perception of pain [38].

The endogenous pain control system modulates the excitability of spinal nociceptive second order neurons and this descending modulation utilizes the following neurochemical systems: serotonergic, noradrenergic, and opioidergic [212]. This system can exert inhibitory or facilitatory effects. Excitatory signals include peptides (substance P, somatostatin, bombesin, galnin, and vasoactive intestinal peptide), amino acids (glutamate, aspartate), nitric oxide, and

prostaglandins [212]. Inhibitory signals include endorphins, amino acids (GABA and glycine), serotonin, and adenosine [212].

5.3 Pain: Brainstem

The brainstem includes the medulla oblongata, pons, and midbrain and is continuous with the spinal cord [36]. The motor and sensory systems traveling to the brain pass through the brainstem. Neurons of the brainstem receive convergent inputs from nociceptive and non-nociceptive inputs and have large receptive fields [213]. The brainstem integrates nociceptive input with homeostatic, arousal, and autonomic processes and is part of the ascending system carrying pain to supraspinal structures [36]. Projections to the brainstem can impact spinal and supraspinal excitability, suggesting that these pathways directly affect pain perception [36].

The intensity and affective components of perceived pain is a consequence of the interaction between ascending nociceptive inputs and anti-nociceptive controls. An imbalance in these systems underlie chronic pain [214]. Three major areas of the brainstem make up the brainstem pain modulatory centers and mediate pain perception: the periaqueductal gray (PAG), the locus coeruleus (LC) and the rostral ventral medulla (RVM) [213]. These areas of the brainstem can inhibit or facilitate nociceptive processing within the dorsal horn and are influenced by the diencephalon, amygdala, hypothalamus, ACC, insula, and prefrontal cortex [36].

5.4 Pain: Changes in excitability at the supraspinal level

As with the deafferentation studies, there has been a debate as to whether subcortical or cortical components play a primary role in the somatosensory processing of nociceptive input. Changes in excitability in response to pain are found in diverse components of the somatosensory system: in the periphery, at the spinal cord, and in supraspinal structures [152, 215]. There is evidence of this in patients with neuropathic pain that display high spontaneous firing rates in the neurons of

the thalamus [216]. In addition, changes in the amygdala [217], and ACC [218] have been described in response to pain.

Wall et al. [215] suggested that injury alters neural components at subcortical and cortical locations. Peripheral injuries may cause rapid changes in peripheral, spinal, and brainstem components which are more widespread than cortical alterations [215]. The result is that injuries become embodied in the CNS, from the peripheral sensory neurons to the cortex. Despite differences in sensation, emotions, and motor output activated by different categories of pain, individuals can recognize each as being painful. Therefore, there is a shared construct of pain with a similar underlying network of brain activation. This network receives parallel inputs from diverse nociceptive pathways [214]. The presence of this network is corroborated by invasive and non-invasive electrophysiological research, utilizing electroencephalography (EEG), magnetoencephalography (MEG), subdural recordings, and in depth recordings [219]. If a stimulus activates nociceptors, diverse areas of the pain matrix respond with the response correlated to the pain intensity [220]. These brain regions encompass a number of distinct areas whose activation is correlated to pain intensity [197]. These areas include the SI, SII, posterior parietal cortex, thalamus, posterior and mid-insula and the mid-cingulate cortex [24, 27]. Other neural regions such as the prefrontal cortex, BG, amygdala, cerebellum, and hippocampus are activated by experimental pain in several studies [36]. Of particular interest is a study by Iadarola et al. [40] that utilized positron emission tomography (PET) to investigate brain activity in healthy participants during acute pain induced by the intra-dermal injection of capsaicin. Capsaicin produced activation in many brain regions which subserve four main functions: sensory (SI, insula, and thalamus); attention (ACC); descending control (PAG); and SMI (SMA, bilateral putamen and insula, the cerebellum and superior colliculus). Capsaicin pain did not

activate the SII whereas the cerebellum was strongly activated by capsaicin [40]. It is important to note that the capsaicin through an intra-dermal injection and may lead to differences when compared to topical capsaicin.

5.5 Pain and somatosensory processing

Neuroplasticity is observed following chronic and acute pain. The literature reveals that there are subcortical and cortical changes in excitability in response to pain [217, 221, 222]. Seminal studies reveal that pain in the absence of deafferentation induces plasticity at the cortical level [43, 56, 223, 224]. This research was conducted on healthy humans and thus this is in contrast to those studies that conducted their research on individuals suffering from chronic or recurrent pain. It is important to study SEP peaks in conjunction with acute pain as alterations in their amplitudes post-sensitization reflects the effect of pain on somatosensory processing.

5.5.1 Chronic Pain and somatosensory processing:

Maladaptive plastic changes are associated with the development of chronic pain [215].

Alterations in somatosensory processing have been identified in painful conditions [e.g. ankle sprain [225], shoulder pain [225] and LBP [226]] and can subsequently affect motor control.

These alterations encompass reduced sensory acuity [227] and increased errors [228]. Individuals have cortical reorganization of the somatosensory area representing the painful muscles. For example, individuals with LBP have a representational shift of the back muscles in the SI [5] and reduced cortical spinal drive [229]. Cortical neuroplasticity in the somatosensory system are a potential factor for maintaining chronic pain. In individuals suffering from CRP, pain perception is correlated to the change of the ulnar and median nerve dipole localizations of the SI [230] and an fMRI imaging study of individuals suffering from CRP showed a similar correlation [231].

There are a few studies that have examined the response of early SEP peak amplitudes in chronic pain models. Tinazzi et al. [224] stimulated the median nerve ipsilateral to facial pain in individuals with trigeminal neuralgia resulting in greater amplitudes of cortical potentials (N20, N30, and P27 SEP peaks) which was associated with the magnitude of pain. Tinazzi et al. [56] measured SEPs in individuals who were experiencing chronic pain in the thumb. Amplitudes of subcortical and cortical potentials (N13, P14, N20, and N30 SEP peaks) after stimulation of the painful thumb were significantly larger than when compared to the stimulation of the non-painful thumb and were correlated with pain perception.

Other studies in deafferentation and spinal manipulation have found a modulation of the cortical peaks and no change in subcortical peaks in response to altered input [185, 196, 232, 233].

Therefore, in response to chronic deafferentation and chronic pain, the cortex may modulate processing in subcortical areas.

Decreased cortical SEP peak amplitudes have been observed succeeding spinal manipulation reflecting a normalization of nociceptive-induced neuroplasticity [233, 234]. Current research suggests that pain (in the absence of deafferentation) plays a pivotal function in defining cortical somatosensory reorganization [43, 56, 223, 224]. However this work has been conducted on individuals suffering from chronic pain [56, 224] and the spinal manipulation research studies have been conducted on individuals with recurrent neck pain and stiffness [233, 234].

5.5.2 Acute Pain and somatosensory processing:

Seminal studies reveal that pain induces neuroplasticity at the level of the cortex [43, 56, 223, 224]. Sörös et al. [43] determined that acute pain in the hand caused a reorganization of the SI.

The size of the hand and the distance between the hand and the ipsilateral lip representations decreased at the cortex. The hand and the lip are represented somatotopically close at the level of

the cortex, suggesting that acute experimental pain induces rapid cortical neuroplasticity [43]. Knecht et al. [223] applied acute experimental pain to the hand followed by tactile input (innocuous) to the lip in healthy humans. Participants perceived phantom sensations in the hand synchronously to the non-noxious lip stimulation indicating that acute pain induces cortical neuroplasticity which is likely due to a disinhibition between the respective cortical regions. Two previous studies examined the effect of acute experimental pain (muscle pain) on SEP peak amplitudes in healthy participants [74, 235]. Rossi et al. [74] found a decrease in the N20-P25-N33 complex and an increase in the N18 SEP peak following muscle pain. Although the studies by Knecht et al. [223] and Sörös et al. [43] demonstrated that there were cortical rearrangements in response to acute experimental pain in healthy individuals, they did not measure individual SEP peaks. Two other studies found that electrical stimulation and acupuncture attenuated later SEP peak amplitudes when combined with pain [236, 237]. In contrast to these findings, our previous study [20] did not find any significant changes in SEP peaks following the application of capsaicin (an acute cutaneous pain model), however, there was an interaction effect of acute pain and motor skill acquisition as the amplitude of the N30 SEP peak was significantly increased following motor learning acquisition in the intervention group [20].

5.5.3 Research Gaps:

Knecht et al. [223] and Sörös et al. [43] demonstrated altered cortical organization following acute pain and Schabrun et al. [235] found significant differences in SEP peaks in healthy individuals with an acute muscle pain stimulus. In addition, although Tinazzi et al. [56] and Tinazzi et al. [224] found significant differences in SEP peaks in individuals who were suffering from chronic pain, there is still a gap in the literature in terms of the response of SEP peak

amplitudes to acute experimental cutaneous pain in healthy individuals, which this thesis will seek to address.

5.6 Pain and the motor system

Individuals have reorganization of the areas of the cortex for muscles affected by pain.

Individuals with LBP have an altered representation of the back muscles in the SI [5] and reduced cortical spinal drive [229]. In a TMS study, individuals with LBP demonstrated neuroplasticity as shown by increased representation of the transversus abdominus in the MI, and it is suggested that pain has altered the representation of muscles at the cortical level [238]. The patients also demonstrated a lag in the activation of the transversus abdominis muscle when an arm motor task was performed and this was associated with cortical reorganization. It is therefore hypothesized that pain alters cortical neuroplasticity which then impacts subsequent motor output. In patients affected by CRP, Pleger et al. [230] found that pain was correlated to the difference of the ulnar nerve and median dipole localizations within the SI. A TMS study demonstrated that with CRP there is decreased MI excitability associated with the muscles affected by pain [239]. Additionally, in the MI there is a smaller representation of the muscles of the arm affected by CRP [240].

In healthy participants, acute experimental pain of the neck muscles alters coordination [241].

Cortical neuroplasticity in the sensory and motor systems are emerging as a contributor to chronic pain, although less is known about the impact of acute experimental pain.

5.6.1 Chronic Pain and the motor system:

Neuroplasticity has been demonstrated in the sensory system in response to pain, and SMI at a reflex level in response to nociceptive input is well understood [242]. Grönroos et al. [243] determined the effect of cutaneous application of capsaicin on a nociceptive reflex and found that

capsaicin led to a decrease of the threshold suggesting that capsaicin facilitates the nociceptive flexion reflex [243].

Persistent pain usually inhibits movement, as individuals tend to limit movement in order to protect the painful region [242]. For example, with arthritis, the ability to perform skilled movements of the hand is negatively impacted [244] and dexterity declines as pain increases [244, 245]. Neuroplasticity, as reflected by changes in excitability of the MI, has been reported with peripheral nerve lesions [246, 247] and in association with chronic and phantom limb pain [239, 248].

5.6.2 Acute pain and the motor system:

In healthy participants, experimental pain can modulate motor control strategies and motor output [249]. Acute experimental muscle pain decreases the discharge [250, 251] and increases the twitch amplitude [252] of motor units throughout muscular contractions. Farina et al. [253] found that acute muscle pain reduces the rate of motor unit discharge during muscle contractions and Falla et al. [251] found that the discharge rate decreased following experimental muscle pain. The literature indicates that experimentally induced muscle pain modulates motor control by altering the coordination of muscle groups [251, 254-256]. There is a reorganization in muscle activity following experimental muscle pain in the shoulder [254], upper limb [255], and neck [241]. Following acute muscle pain, Falla et al. [257] found that the upper trapezius showed decreased EMG amplitude while the lower trapezius showed increased EMG amplitude. Madeleine et al. [254] found that during experimental muscle pain the EMG signal decreased and there was a shift of the CoG demonstrating that acute pain alters cortical organization and muscle activation. Sae-Lee et al. [256] found that the effects of acute muscle pain on EMG

activity varied with the task in which the muscle participated suggesting that the effects of acute pain on muscle activation are task dependent.

The literature suggests that there are inconsistent effects of experimental pain on MI excitability. In contrast to the neuroplastic alterations associated with motor learning, the modifications of the sensory and motor systems in response to acute pain differ between muscles. In healthy participants, decreased MI excitability has been demonstrated following cutaneous capsaicin pain and experimental muscle pain [7, 8, 258]. Cheong et al. [8] induced cutaneous pain by applying capsaicin cream on the skin over the flexor carpi radialis (FCR). Amplitudes of MEPs at FCR were decreased supporting the hypothesis that acute pain inhibits MI excitability by cortico-cortical circuits. In addition, Farina et al. [258] found a similar inhibition of MI excitability after the application of capsaicin.

5.6.3 Remote versus local pain and motor output:

The evidence suggests that there are differing responses to acute experimental pain and that the changes to the MI contributes to protective motor control. It has been shown using EMG that acute experimental pain produces a shift in the upper trapezius muscle activity during muscle contractions and that this occurs irrespective of the location of the pain [254]. In an acute experimental muscle pain model, Martin et al. [259] found that there was decreased excitability of triceps and biceps MEPs, but increased excitability of neurons at the cervicomedullary site. This finding suggests that following acute experimental muscle pain there may be opposite effects at cortical and spinal sites. The alterations in excitability that occur in conjunction with acute experimental pain are inconsistent. During local acute experimental pain, MEP amplitudes increase [260-262], decrease [6, 7, 258, 259, 263], or do not change [264]. During remote pain (e.g. pain induced at an anatomical site that is not anatomically close to the muscle being tested) MEP amplitudes also change variably: electrical nociceptive input to the finger leads to

decreased excitability of the hand MI but an increased excitability of the arm muscles [6]. Experimental LBP induced via hypertonic saline decreases MEP amplitude in the transversus abdominis, but increases MEP amplitude in the oblique externus abdominis and lumbar erector spinae [265]. In a topical capsaicin pain model, Fierro et al. [266] found that capsaicin-induced pain on the dorsal side of the hand significantly decreased SICI. In contrast, a recent study [267] investigated SICI during and after acute muscle pain induced of the right FDI in healthy individuals and found that compared to baseline SICI was increased following but not during nociceptive input for both the FDI and ADM (abductor digiti minimi) muscles.

5.7 Research Gaps:

There is a gap in the literature in terms of how acute experimental pain affects input-output curves. There have been variable findings in the response of input-output curves to experimental pain and therefore there is a gap in the understanding of how input-output curves will be affected by acute experimental cutaneous pain in healthy individuals, which will be examined by the fourth study of this thesis.

5.8 Pain: Mechanisms of nociceptive plasticity

There is neuroplasticity of the somatosensory system following motor learning, inflammation, and pain [268]. The perception of pain is a function of neurons in nociceptive pathways due to reduced inhibition, increased excitability, and increased synaptic efficacy [268]. Neuroplasticity in response to nociceptive input is an activity-dependent change in neurons. Damage to peripheral tissue and injury to neurons produces chronic pain [268]. Neuroplasticity occurs in the spinal cord and provides a mechanism for the CNS overreacting to normal input [268] and provides an explanation for chronic pain that persists after peripheral tissue damage has resolved.

Central sensitization is increased excitability of the neurons in nociceptive pathways which reduces the threshold and increases the response to nociceptive input [210].

The literature suggests that there are many mechanisms that can lead to central sensitization as there are a number of different forms of neuroplasticity that can sensitize the somatosensory system and lead to hypersensitivity to pain [210]. There are distinct changes in somatosensory processing which can increase excitability, increase connectivity between neurons, or decrease inhibition [210, 269]. Mechanisms include a decrease in the threshold and the activation of AMPA and NMDA receptors. NMDA and AMPA are both glutamate agonists (an excitatory neurotransmitter) and therefore increased amounts of glutamate leads to excitation [270].

Another mechanism that can produce central sensitization is the reduction of glycine and GABA neurotransmitters. GABA and glycine are inhibitory neurotransmitters and therefore reductions in these neurotransmitters lead to disinhibition leading to pain hypersensitivity [210]. GABA is a significant inhibitory neurotransmitter in the CNS, and the most likely mechanism for cortical neuroplasticity in response to pain is disinhibition of GABA, which is in alignment with the previously discussed deafferentation studies [187, 192, 271, 272]. GABA plays a significant role in determining rapid cortical reorganization in response to deafferentation [188, 192, 271, 272]. Levy et al. [271] demonstrated that GABA levels in the SI and MI are reduced following deafferentation and is associated with an expansion of motor representations. Ziemann et al. [188] found increased plasticity through a deafferentation-induced reduction of GABA inhibition. Marty et al. [272] demonstrated that activity dependent modulation affects GABA-containing interneurons. These findings help to verify the hypothesis that cortical neuroplasticity is a consequence of reduced GABA inhibition resulting in the release of latent thalamo-cortical

projections. These deafferentation studies [188, 192, 271, 272] suggest a common mechanism of GABA-mediated disinhibition in response to altered afferent input.

6 LITERATURE REVIEW - MOTOR LEARNING

This chapter will review the relevant literature on motor learning which is important to this thesis as we are investigating the neurophysiological and behavioural responses to motor learning acquisition and retention.

6.1 Motor learning

Learning and motor learning have been described in various ways. Four distinct characteristics are included: Learning results from experience or practice and is a process of acquiring the ability to produce skilled actions. Learning cannot be observed directly; one must infer that learning has occurred on the basis of changes in behavior, i.e. improved ability to produce a skilled action. It is thought that learning leads to permanent alterations in the ability to perform this skilled action [273]. Motor learning acquisition is the process by which movements are performed effortlessly after practice [274]. In humans and animals, motor learning acquisition is gaged by a reduction in response time and the error rates [275].

Stages of Motor learning:

Learners pass through separate learning stages as they practice a skill. A three stage view of learning was proposed by Fitts et al. [276] and later Anderson [277]. These three stages are: the cognitive, fixation, and autonomous stages.

Stage 1: Cognitive

When an individual first encounters a task, the initial challenge is determining what actions need to be carried out in order to achieve the goal of the task. Effective strategies are retained, and inappropriate ones are rejected. The performance improvements during this stage are dramatic and larger than at any other stage.

Stage 2: Fixation

The second stage begins when the individual has established the most efficient way of performing the task and starts to make more subtle changes. Motor performance is consistent and the improvement in motor performance is gradual.

Stage 3: Autonomous

After extended practice the individual moves into the autonomous stage, as the skill has become largely automatic. The automaticity theory of movement theorizes that through separate instances of exposure to a new task automatization occurs leading to the acquisition of a specific knowledge base [278, 279]. There is a benefit from previous exposure to a task and this is known as repetition priming [279-282] which is the first step on the way to automaticity [279].

Automatization is the effect of hundreds of exposures of a task on subsequent performance [279, 283] and is important to motor learning acquisition and includes daily tasks that can now be performed effortlessly [278]. It is hypothesized that motor learning acquisition and repetition priming rely on common underlying mechanisms as fMRI imaging studies have demonstrated that specific neural regions exhibit changes after motor learning acquisition and repetition priming [284]. There is a positive relationship between the number of repetitions or exposures and the amount and length of knowledge retention, indicating that exposure results in motor learning [283, 285]. Several studies have examined the time course of motor learning acquisition [286, 287] and demonstrate that motor learning acquisition occurs in two stages. Initially, fast learning occurs in which there is a within-session improvement induced by a few trials on a time scale of minutes [286]. Following fast learning, there is slow increase in performance gains and this is referred to as slow learning [286]. This phase in motor learning acquisition is a result of the consolidation of experience dependent changes in the cortex triggered by learning.

In the 1970s two seminal motor learning articles papers were published describing closed-loop theory and schema theory that had a significant impact on subsequent motor learning research [273].

Closed-Loop theory:

Adams [288] developed the closed loop theory of motor learning. Adams [288] hypothesized that a movement is performed by comparing the feedback from the arms to a perceptual trace.

However, evidence on deafferentation in animals [289] and humans [290] contradicts this theory.

Humans and animals deprived of sensory input can move well and can learn new skills. If skilled actions are dependent upon feedback, then animals and humans should not be able to produce skilled actions when deprived of sensory feedback [273].

Schema learning:

Schema theory hypothesizes that there are two memory states, a recall memory producing the movement and a recognition memory evaluating the movement. Schmidt [291] used the idea of the schema to form a theory of motor learning. Movements are completed through the selection of a generalized motor program (GMP) and then adding parameters that specify the program execution. Following the addition of parameters, additional information is stored in memory: the initial conditions prior to the movement, the parameters, feedback about the outcome of the movement, and the sensory consequences.

Transfer/Retention designs:

Transfer or retention designs involve two related components and are quite similar. First, the individuals are provided a retention interval of sufficient length. The second feature is that the experiment involves the same independent variable. In general, tests involving the same task as

practiced in the acquisition phase are called retention tests, as they evaluate the extent to which a given skill has been retained over the retention interval. Transfer tests typically involve new variations of the task practiced in acquisition or might involve essentially new tasks.

Retention learning sessions are important for determining consolidation effects with degrees of incremental learning between trials [292, 293]. Factors that may improve or negatively impact motor learning acquisition are not necessarily predictive of retention [294, 295] and from both a learning and practical perspective it is retention that indicates whether learning has been impacted positively or negatively.

6.2 Motor learning acquisition and neuroplasticity

The literature has demonstrated neuroplasticity with motor learning acquisition [145, 173, 275, 296-303]. In humans, cortical neuroplasticity has been reported with novel motor learning acquisition [304] in PET [305], fMRI [287], and TMS [139] studies. Imaging research indicates that the prefrontal cortex and the pre-SMA are activated during early stages of motor learning acquisition, whereas parietal areas are activated at later stages of learning [306]. Studies have demonstrated the involvement of the MI, the cerebellum and the BG depending of the stage of motor learning acquisition. Animal studies have demonstrated that motor learning acquisition is associated with increased number of synapses within the cerebellum [307-309] and the MI [310]. Motor learning acquisition leads to improvements in behavioural measures and increases the representation of the muscle in the cortex [139, 287, 311]. Research has demonstrated that there is increased excitability of the MI following one week of one-hour daily novel tongue-protrusion learning and with one-hour of novel tongue-protrusion learning in humans [304, 312]. Changes in the MI are associated with improved motor performance and motor control [313, 314]. It has been hypothesized that early neuroplasticity in the MI may be produced through the unmasking

of connections, while long-term neuroplasticity is mediated by increased cortical synaptic connections and synaptogenesis [315]. In addition, imaging research demonstrated that the cerebellum is activated with repetitive motor tasks [316-318], motor sequence tasks [275], and the learning of a new task [299]. In the cerebellum, early learning is mediated by the climbing fibers within the cerebellar cortex [319], while later learning involves neuroplastic alterations within the cerebellar nuclei and the cerebellar hemispheres [320-322]. Furthermore, in the BG, the anterior putamen is implicated in early learning, while the posterior region is involved in later learning [323, 324]. This research suggests that different components of the BG and cerebellum are responsible for differing stages of learning [323, 324] and that separate cortico-cerebellar and cortico-striatal systems play a role in different stages [275, 299] and different types of learning [299].

6.3 Motor learning acquisition tasks

Learning tasks can be implemented before, during, or after taking measures of cortical excitability. Using a learning task in a pre-post design in conjunction with SEPs or TMS can provide insight into changes in excitability that occur following a motor learning acquisition task. When identifying a task that is appropriate to include in a research design it is important that the task be novel so that the participants are naïve to the requirements of the task [1], as this is hypothesized to be imperative in causing significant changes in excitability to the MI.

Continuous motor traces involve tracing an object with undefined velocity and start/end points, such as drawing a circle, while discrete tracing tasks involve defined areas of a required increase in trace velocity with a ordered sequential movement profile [325]. Continuous and discrete motor tracing tasks have both been found to actively stimulate the SI, MI, premotor and parietal cortices, and the cerebellum [326, 327]. Habas et al. [325] found recruitment of the MI and SI with a continuous learning task and increased activation of the right prefrontal cortex. Varying

the amount of feedback participants receive is an important variable that can impact performance. Smyth et al. [328] used two groups that received varying levels of performance feedback during a skilled movement task to see changes in cortical excitability and found that feedback was an important factor leading to an increase in performance, with focus and attention being a possible variable. Most studies involve motor learning tasks that are either gross movements such as reaching tasks [329-331], or movements focusing on the fingers [332-334], in combination with studying varying characteristics of cortical refinement. Peg board tasks have been used in many studies as a measure of motor performance, as the number of pegs placed in a board during a 30s trial are associated with accuracy and efficiency in ability [329]. A few other studies used ballistic thumb abductions to study MI excitability [333, 335]. A drawback to most of the motor tasks used in motor learning studies is that they lack complexity. Few studies have targeted a novel approach to motor learning with discrete finger movements, with only one to our knowledge [336]. Novel tasks, such that the participant is naïve to the movement and skill required, will allow us to identify differences in cortical neuroplasticity measures.

6.3.1 Motor learning: typing task

Imaging research determined that there are differences in activity as the complexity of the task changes [337, 338]. In a PET study, Sadato et al. [338] found that the dorsal premotor cortex and the right precuneus showed an increase of rCBF as sequence complexity increased. In another PET study, Catalan et al. [337] found that the premotor area, posterior parietal areas and precuneus showed an increase in rCBF that was correlated to the sequence length.

6.3.2 Motor learning: tracing

A motor tracing task is a more complex task than a typing task that introduces a novel movement not typically required in day to day usage such as finger abduction/adduction. Recent work found

that both left and right hands had a significant decrease in performance error over both days of motor learning using a tracing task (40% and 41% decrease in error for right and left hands, respectively) [339]. Holland et al. [339] demonstrated that both hands had continued motor learning acquisition and there was a significant consolidation of motor performance between the two days of learning and Andrew et al. [340] found a decrease in the N24 SEP peak amplitude following this motor learning tracing task. This finding corroborates findings seen by [341], suggesting the cerebellum plays a vital function in the integration of somatosensory information. Most motor learning studies report changes within both cerebellar hemispheres, regardless of what hand performs the task [342, 343]. Studies using inhibitory and excitatory stimulation of the cerebellum have demonstrated impaired motor adaptation [344-346]. Studies demonstrate that the cerebellum is activated with the fast motor learning stage [323, 347-349] but that this activation diminishes once the movement is well learned [11, 298, 348]. In contrast, the BG, particularly the putamen is active throughout all of the learning stages. However, it is unclear how the cortico-striatal and cortico-cerebellar loops interact during the early stages of motor learning acquisition.

6.4 Motor learning acquisition and the motor system

6.4.1 Motor learning acquisition and the MI:

In healthy individuals, novel motor learning acquisition occurs with improved behavioural measures and enlarged representation of the muscle at the level of the cortex [139, 287, 304, 311]. Animal studies have shown changes in the MI during the acquisition of fine motor skills [145, 350, 351] and research indicates that motor learning acquisition can alter cortical representations within the MI [352]. Increased synaptic connections of the MI is correlated with improved task proficiency [353] through the strengthening of horizontal cortical connections [354] and increased synapses in layer V [308]. Motor learning acquisition involves the MI when kinematic variables are changed [3]. Imaging research has found that there is an increase in activation in areas involved in executive function when comparing simple movements to complex tasks [337, 338]. It is theorized that during early learning, rapid neuroplasticity within the MI is facilitated through the unmasking of connections, whereas during later learning there are long-term alterations produced by synaptogenesis and the strengthening of synapses [315]. In humans, PET, fMRI and TMS studies have demonstrated alterations in the MI following the acquisition of complex motor skills [139]. Several studies have shown that following motor learning acquisition, there is an expansion of representations corresponding to trained movements [4, 355]. Svensson et al. [304] demonstrated that short term motor learning acquisition increases corticomotor excitability in the tongue. This can happen very rapidly as improvements in behavioural measures and increased cortical excitability can occur after 15 min of tongue motor learning [9]. In addition, increased excitability has been found for the MI following 24 weeks of motor learning [334] and comparable results were demonstrated using a short learning interval [3]. Classen et al. [3] used TMS of the MI to evoke thumb movements.

Thumb movements were then practiced in the opposite direction. After the practice session, TMS evoked movements in the practiced direction suggesting that the learning rapidly established a change in the cortical map. These findings confirm that cortical neuroplastic alterations in the MI can occur over very short time periods [3, 9]. Imaging research has shown that a complex task results in increased activation of cortical areas as compared to a simple task [338] and with fine motor skill learning when compared to gross motor learning [311]. This research indicates that with the acquisition of skilled movements there is reorganization within the MI that depends on the complexity and attentional demands involved in motor learning. Other studies [144, 350] have demonstrated that repetition of movement is insufficient in producing reorganization within the MI. Plautz [144] found that repetitive finger movements did not produce reorganization within the MI. In addition, Remple et al. [350] found that the representation of the MI of rats that spent several weeks in running wheels was similar to that of inactive rats. This research demonstrates that repetition does not alter cortical neuroplasticity. Changes in neuronal networks of the MI alters motor performance and underlie motor learning [313, 314].

6.4.2 Motor learning acquisition and the cerebellum:

Marr [356] proposed that the cerebellum plays a central function in motor learning acquisition and hypothesized that the cerebellum is specifically responsible for the process whereby movements can be performed automatically. He theorized that learning involves synaptic changes in the cerebellum and that cerebellar inputs via the climbing fibers and mossy fibers are integrated through their connections to the Purkinje cells [356]. In the cerebellum, early learning is facilitated by the climbing fiber system of the cerebellar cortex [319], while later learning may involve neuroplastic alterations in the cerebellar nuclei and cerebellar hemispheres [320-322]. The cerebellum modifies extracerebellar output through inhibition from GABAergic neurons.

Evidence for the role of the cerebellum is provided by animal studies [319, 357-359]. In animals, lesions in the cerebellum impairs classically conditioned responses [357, 359] and affects the reflexes [358]. In humans, patients with cerebellar pathology demonstrate impaired motor learning [1] and imaging studies reveal that the cerebellum is active during motor sequence [275] and motor repetition [316-318] tasks. Friston et al. [360] examined the effect of simple repetitive motor tasks on rCBF changes using PET and demonstrated that during this task there were bilateral rCBF increases of the cerebellar cortex and cerebellar nuclei. Similarly, in another PET study, Jenkins et al. [347] found significant increases in activation found in the bilateral cerebellar hemispheres and the cerebellar nuclei following motor learning. These results [347, 360] indicate that the cerebellum plays a part in the automaticity of motor tasks. The cerebellum participates in motor adaption and in the behavioural learning of unfamiliar tasks [299] and it is suggested that once the skill becomes automatic, the representation of the movement becomes less dependent on the cerebellum and more dependent on the cortex [361, 362].

6.5 Motor learning acquisition and SEPs

Following repetitive movement, Murphy et al. [198] demonstrated that there are decreases in SEP peak amplitudes following a repetitive motor task. Haavik-Taylor & Murphy [363] demonstrated that there were significant increases for P25 and N30 SEP peaks following the typing of the numbers 7,8,9 while Haavik et al. [364] found that following a typing task the N24 SEP peak was increased and the N18 SEP peak was decreased. We recently demonstrated that the amplitude of the N24 SEP peak (reflecting activation in the pathway between the cerebellum and the SI) [73] was increased following a motor learning acquisition task that involved typing three numbers presented in random sequence [20]. Andrew et al. [340] subsequently

demonstrated significant changes in spinal (N13) and cortical (N20, N24, P25, N30) SEP peaks following both a complex tracing task and a typing task.

6.6 Motor learning and attention:

One important component of attention is the idea that attention is limited. Another feature is that attention is selective: we can shift attention our attention to different things depending on the circumstances [273].

Attention plays a key role in motor learning and retention [279]. Attention is required to encode events into memory and is also required to retrieve those events from memory [365].

Automaticity is defined as processing without attention. Novice performance is based on solving the initial problem that is presented by the task. In contrast, automatic performance is a direct retrieval of the solution from memory. Automatic processing is therefore effortless and fast [278, 279, 365]. Complex tasks are an example of retrieval interference. By presenting a key press sequence in a random order, the participant uses more attentional resources, and thus the response and response times will be longer and movement responses will not be automated. In addition, increased exposures results in a stronger memory because each individual experience creates a separate trace that can be subsequently retrieved. Research has provided evidence that learning acquisition under high interference results in improved retention and facilitation of transfer [366, 367]. It is hypothesized that practice under increased contextual interference can produce more elaborate processing and thus facilitate retention.

Yerkes et al. [368] studied discrimination learning in mice and discovered one of the most interesting aspects of the impact of arousal on performance. They found that increased intensity of electric shocks reduced the number of trials (improved performance) but only up to a certain point, producing an inverted-U phenomenon. Weinberg et al. [369] provided evidence for the

inverted-U phenomenon in movement behavior in humans. Therefore the relationship between stress and performance is complicated and not as simple as originally thought. The theory of perceptual narrowing has also been described by Kahneman [370]. With perceptual narrowing, increased arousal leads to a narrowing of attention. Easterbrook [371] used a notion similar to perceptual narrowing to account for the inverted-U relation. With low arousal, the selectivity for the cues is poor. With an increase in arousal to moderate levels there is a reduction in the number of cues used (because of the narrowing of focus) so that there is a shift to an area where relevant cues are more prevalent and irrelevant cues are less prevalent.

Research demonstrates that neuroplasticity accompanying motor learning acquisition is altered by changes in attention [372-375] as motor learning depends strongly on attentional resources [376, 377]. Focused attention to the task has been correlated with activation of prefrontal cortex and pre-SMA [378]. We hypothesize that improved motor learning acquisition for the acute pain group as observed in our previous research [20] is due to increased attention to the arm that is performing the motor learning acquisition task [372-375]. Growing evidence demonstrates that affective processing is modulated by attention and cognitive regulation [379] and that stress leads to a narrowing of attention [380, 381] resulting in decreased processing of irrelevant stimuli [382]. Cognitive load studies corroborate that with a high load there is decreased activation in brain regions associated with emotion and increased activation in executive control areas [383-385].

6.7 Motor learning acquisition versus retention:

Research has provided evidence that learning acquisition under high interference leads to improved retention and transfer [366, 367]. It is hypothesized that acquisition combined with increased contextual interference produces produce more distinctive and elaborate processing

and thus facilitates retention. In line with this theory, Shea et al. [386] demonstrated that retention was superior following high interference in comparison to low interference [386]. Low interference acquisition results in better performance during initial learning than high interference, but high interference results in better performance at retention [387].

Thus learning under high contextual interference results in multiple information processing, and this provides more elaborate representations. Lee et al. [388] alternatively hypothesized that with high interference, there is an increase in the decay of information while with low interference, movement information related to a given task variation is held in working memory. Thus, with high interference participants have to access long term memory frequently and low interference involves superficial processing resulting in comparatively poor performance on retention tests. Both theories highlight the role of the level of cognition on memory and the retention of learning [389, 390] and highlights the importance of measuring retention when conducting a motor learning study.

Previous work has found that the application of tactile-proprioceptive noise improved sensorimotor performance [391] and that one sensory modality (tactile noise) can increase the response of another sensory modality (visual evoked potentials) [392]. Additionally, extraneous stimuli may increase the ability to detect the target stimuli [393, 394] and it is hypothesized that mild acute pain may also improve motor learning under certain circumstances. Several studies have compared a target stimuli paired with a secondary stimulus which led to improved detection of the target stimuli [393, 394]. Zhang et al. [394] found that when heat was paired with a target stimulus tactile detection was enhanced and Verrillo et al. [393] demonstrated that submersion in water increased skin sensitivity. Another research study which corroborates this is a study by Passmore [395]. Passmore [395] had participants recreate Morse code patterns and demonstrated

that when paresthesia occurred concurrently there was an improvement in performance. These findings indicate that a secondary stimulus may increase attention toward discerning the meaningful stimulus. We hypothesize that a non-target stimuli (pain) may help to enhance motor learning acquisition and retention.

Motor learning acquisition: Mechanisms

Two mechanisms have been proposed for the changes induced in the cortex following motor learning acquisition: the disinhibition of previously existing connections between neurons, and the growth of new connections and synaptic connections. Disinhibition of previously existing connections between neurons can induce changes on a short time scale and underlies fast learning. This increase in excitability can come with as little as 5-15 minutes of rapid motor movement [9, 333] and can be more pronounced if the task is novel and the participants are naive to the required level of performance [1]. In contrast, the growth of new connections and synapses is responsible for slow learning [286]. For this research, motor learning acquisition is occurring on a short time scale and likely occurs through disinhibition of previously existing lateral connections. This is the same mechanism that underlies plasticity in response to deafferentation and pain [188, 192, 271, 272].

7 LITERATURE REVIEW – MOTOR LEARNING AND PAIN

This chapter will discuss how pain impacts SMI and motor learning acquisition and retention and will describe some possible mechanisms for how pain affects motor learning.

7.1 Motor learning and Pain

Motor learning deficits have been demonstrated with acute pain in animals [396, 397]. Hook et al. [396] administered shock to one hindleg when it is extended and found that the rats learned to maintain the leg in a flexed stance but that rats injected with capsaicin are unable to learn.

Similarly, Ferguson et al. [397] administered shock to the hind leg when the leg was extended and found that the rat rapidly learned to hold the leg in a flexed stance. However, if shock was independent of leg position the rats failed to learn and this compromised future learning [397].

Although there is a gap in the body of knowledge on the effects of pain on motor learning acquisition and retention in humans, it is well established that there is a negative impact of reduced sensory input on balance [398] and hand manipulation [31]. Boudreau et al. [399] demonstrated that somatosensory manipulations: capsaicin (pain) and lidocaine (sensory loss) to the tongue reduced motor performance. Similarly, Boudreau et al. [9] demonstrated that MI neuroplasticity occurred with successful performance in novel tongue-task learning, but that capsaicin had a negative impact on motor performance. While motor learning acquisition occurred for both groups, the participants in the capsaicin group did not learn the task as well as the control group [9]. This corroborates animal research demonstrating that acute pain interferes with the neuroplasticity that underlies learning [396, 397].

Pain can impede motor learning which is in line with the findings of individuals who are undergoing rehabilitation. The literature suggests that pain alters excitability at the level of the cortex [43, 56, 223, 224], modulates the neuroplasticity associated with motor learning [9], and

impairs motor learning [9]. In contrast to these findings, Dancey et al. [20] found improved performance in a motor learning acquisition task in the presence of capsaicin and it was hypothesized that the mild acute cutaneous pain (that was unrelated to the performance of the motor task) focused attention and increased motor learning acquisition. It was hypothesized that improved motor learning during acute pain may have been caused through increased attention or through increased arousal. Pain may have acted as a non-target stimulus and focused attention during skill acquisition [20].

In contrast to the work of others, Dancey et al. [20] studied the effects of acute pain to the arm on a simple learning task that involved the fingers in healthy individuals. These results indicate that the effects of pain on motor learning may depend on location as the site of experimental pain in the Dancey et al. [20] study was remote as compared to the work of others [9] who found that capsaicin applied to the tongue decreased tongue motor performance as compared to a control group. Although pain may affect motor learning [400], the Boudreau et al. [9] outcome can be explained by altered performance of the learning task with pain, in contrast to pain having an effect on the neuroplasticity associated with motor learning. If motor performance is maintained, the neuroplastic alterations associated with learning are conserved [401]. Therefore, it is hypothesized that pain has a negative impact on acquisition as it impacts the ability to perform the motor task. A few studies have studied the impact of capsaicin application on retention with the use of a motor adaptation [18] or reaching [21] task and found that pain throughout the acquisition phase impacted retention despite not having an impact at baseline [21] or motor learning acquisition [18]. Recently, Bilodeau et al. [17] studied the effect of heat pain on motor learning of a task involving the fingers and found that acquisition and retention were unaffected by acute pain. Motor performance may contribute to and be a consequence of pain, and therefore

motor rehabilitation that re-establishes motor control is fundamental for effective treatment. For example, LBP patients who took part in motor learning had reduced pain and a reversal of the location of the CoG towards that of healthy participants [178]. And in patients with neck pain, improvements in the activation of the neck muscles occurred with motor learning involving these same muscles [179]. In a study on CRP patients, Pleger et al. [180] found that following motor learning acquisition there was a decrease in pain perception and an increase in the area of the affected limb in the SI. This research suggests that motor learning tasks can be used in order to facilitate neuroplasticity and may potentially decrease pain perception in patients suffering from chronic pain.

7.2 Motor learning and Pain: mechanism

An understanding of how pain affects the MI is not currently known. Inhibition of the MI by pain could be through cortico-cortical, thalamo-cortical, cerebellar-cortical or striato-cortical circuits [242]. In addition, the antidromic activation of thalamocortical afferents by TMS may modulate nociceptive transmission. The thalamus is the relay for the cerebellum and BG to the MI [402]. Pain afferents to the BG from the spinal cord have been shown in animal work [403] and activation in the BG following pain has been reported in fMRI studies in humans [404]. Animal studies show that activation of the MI modulates nociception as activation of the MI inhibits spinothalamic neurons [405] and electrical stimulation to the MI inhibited the response of the dorsal horn neurons to nociceptive input [406].

Additional evidence comes from human studies utilizing repetitive transcranial magnetic stimulation (rTMS) [407] and by the application of MI stimulation electrodes [408] that have demonstrated improvements in chronic pain. In addition to chronic pain [409, 410] stimulation of the MI has also been used in patients suffering from a stroke [411, 412], and phantom limb pain

[410]. The effects of MI stimulation on pain perception may be due to cortico-thalamic connections, producing inhibition on the sensory pathway. There is currently a gap in the body of knowledge of how motor control is affected by pain, and how pain impacts motor control, motor learning acquisition, and retention.

7.3 Remote versus local pain and motor learning:

Experimental pain research demonstrates that remote pain can have a negative impact on motor learning acquisition and retention [401]. Ingham et al. [413] found that plastic change was observed after motor learning acquisition with local pain, but not during remote pain [413]. The findings of Ingham et al. [413] do not support direct effects of pain on the neuroplasticity associated with motor learning and it was hypothesized that remote pain may negatively impact learning as it may distract from the learning task. Therefore remote pain can impact neuroplasticity and can impact motor learning outcomes.

7.4 Research Gaps:

There is a gap in the research as to the effect of a novel motor learning task on sensorimotor processing and the interactive effect of novel motor learning and acute pain on sensorimotor processing and motor learning acquisition and retention. Additional studies are required to determine the neuroplastic alterations associated with motor learning acquisition and that which occurs in conjunction with acute cutaneous pain. There is a knowledge gap in the response of SEP peaks and TMS input-output curves following a motor learning acquisition task while in acute experimental pain which will be investigated in study 1 (typing SEPs), 2 (tracing SEPs) and 4 (tracing input output TMS) respectively. There is also a gap in the research as to the effect of remote versus local pain on SEPs an motor learning skill acquisition which will be investigated in study 1(typing task) and 3 (tracing task).

8 GENERAL METHODS

8.1 Participants:

Since the capacity for cortical plasticity declines with age [414, 415] healthy participants (between 18 – 50 years of age) were recruited for all of the studies included in this thesis.

Qualified participants filled out a health survey to identify and exclude any medical condition which may impact normal somatosensation including neurologic conditions, cervicothoracic injury or the use of medication. For the studies we tested healthy participants and aimed to recruit 12 participants (6 males, 6 females) for each group (for example intervention and control groups). Outcome measures included performance on a motor learning task (motor typing or motor tracing), Numeric pain rating scale (NPRS), SEPs, and TMS.

The protocols for each measure are described in detail below:

8.2 SEPs:

SEPs are evoked by bipolar transcutaneous electrical stimulation over the selected nerve and are an objective and direct method of assessing the integrity of the sensory pathways of the central and peripheral nervous systems [24] and this technique was utilized in study 1, 2, and 3.

8.2.1 Stimulation of median nerve

Ag/AgCl ECG conductive adhesive electrodes (MEDITRACE™ 130 by Ludlow Technical Products Canada Ltd., Mansfield, MA) (impedance <5 kΩ) were placed over the median nerve of the dominant hand, with anode distal. Stimuli 1 ms in duration were delivered at rates of 2.47 Hz and 4.98 Hz. These two rates were utilized as the slow rate 2.47 Hz does not attenuate the SEP peak amplitudes [46] and the fast rate 4.98 Hz attenuates the N30 SEP peak amplitude allowing the measurement of the N24 SEP peak amplitude [46]. The stimulus intensity was increased until

motor threshold was achieved for each participant. The lowest stimulation intensity evoking a visible muscle contraction of the APB muscle was defined as motor threshold.

8.2.2 SEP recording parameters

In accord with the recommendations of the International Federation of Clinical Neurophysiologists (IFCN) SEP recording electrodes (1.8288m Traditional Lead, 10mm disc, 2mm hole gold cup EEG electrodes, Grass Technologies, An Astro-Med, Inc. Subsidiary, Rockland, MA) (impedance <5 k Ω) were placed on the ipsilateral Erb's point, over the C5 spinous process, the anterior neck (trachea), 2cm posterior to contralateral central C3/4, denoted as Cc', and a frontal site (6cm anterior and 2cm contralateral to Cz), denoted as the Rossi site [74]. C5 was referenced to the trachea and the other electrodes were referenced to the ipsilateral earlobe. A ground electrode 1.8288m Traditional Lead, 10mm disc, 2mm hole gold cup EEG ground electrode was placed in the participants mouth.

8.2.3 Data collection

1000 sweeps were averaged per stimulation rate using a Signal[®] configuration (Cambridge Electronic Design, Cambridge, UK). The SEP signal was amplified (gain 10,000) and filtered (0.2-1000 Hz). The averaged waveform was displayed in an analysis window from which the amplitudes of the specific SEP peaks of interest were measured. SEP peak amplitudes were measured in accordance with IFCN guidelines [26]. We measured the following SEP components: the peripheral N9, the spinal N11 and N13, the N18, the parietal N20 and P25 and the frontal N24 and N30. The data was inspected during the collection of data as examination of the raw data can identify artifacts that would alter the SEP peak amplitudes [47].

8.3 TMS:

TMS over the MI has been utilized to investigate the excitability of the motor system and can be utilized to measure changes in excitability of the motor system with learning [190]. Surface EMG recordings were recorded from the APB muscles of the dominant arm (self-reported) and the reference electrode was positioned over the metacarpophalangeal joint. The figure-eight coil (butterfly coil) was used as it results in a more focal pattern of activation. MEPs at the lowest % TMS output that elicits a MEP over the MI were determined to be the ‘hot spot’ for the APB MI. In order to identify the area of MI which corresponds to the target muscle a “trial and error” TMS mapping technique must occur, where the participant is stimulated along the MI region of the brain until there is activation of the muscle [102]. In order to relocate the hot spot each participant was fitted with a cloth cap and the coil position and orientation that corresponded to the participant’s APB MI hotspot was marked on the cap. Once the area of the brain is identified, progressively decreasing the intensity of the stimulation while recording EMG allowed for the development of a resting threshold level (rMT), which has previously been defined as the probability of evoking a MEP of at least 0.05 mV 5 out of every 10 stimulations [102]. When performing trials, an average of 12 MEP’s were taken for each stimulus parameter.

8.3.1 Input-output curves

In study 4, input-output curves were performed and the intensities used to develop the TMS input-output curve were determined for each participant using their rMT attained at the beginning of the experiment. Magnetic stimuli were applied in 10 % increments between 90 and 140 % of rMT. Twelve stimuli were delivered at each stimulus intensity, and the order of different stimulation intensities was pseudo-randomized. Therefore, a single input-output curve block consisted of 72 stimuli. MEP amplitudes were measured for each TMS pulse to calculate the mean MEP amplitude for each intensity.

8.4 Motor acquisition tasks

8.4.1 Motor sequence typing task:

For study 1, participants typed randomized eight-letter combinations of the letters Z, P, D, and F with the right thumb (Z,D,P,Z,F,P,D,D). This typing task, programmed in E-Prime 2.0 software (Psychology Software Tools, Sharpsburg, Pennsylvania), took approximately 15 minutes to complete and was selected as similar tasks have been shown to activate the cerebellum and the MI in the early stages of motor learning acquisition [11, 298]. Participants completed ten randomized sequences of the eight letters at the start and end of the motor acquisition task to evaluate accuracy and response time.

8.4.2 Motor tracing task:

For studies 2-4, each participant completed a motor learning tracing task using their dominant hand. A custom Leap Motion software tool was utilized for the motor learning task (Leap Motion, Inc., San Francisco, CA) and participants were required to trace sequences of sinusoidal waves with varying amplitude and frequency using their thumb on a touchpad (Logitech, Inc., Fremont, CA) and included a pre-motor learning acquisition test, a motor learning acquisition phase, a post-motor learning acquisition test and a retention test 24-48 hours later. The pre-motor learning acquisition, post-motor learning acquisition, and retention tests were four minutes in duration while the motor learning acquisition phase (that occurred between the pre-motor learning acquisition and post-motor learning acquisition tests) was 15 minutes in duration. The traces consisted of a series of dots and each trial included 500 dots. The participants were instructed to trace a continuous vertical sinusoidal wave composed of coloured dots using only their thumb (Figure 19). The trace moved vertically down a monitor while the participants attempted to copy the trace, using only their thumb on a wireless tracking pad. Each tracing task

consisted of four sinusoidal patterns that varied in frequency and amplitude that were verified by a previous study [339] (See Figure 20 and 21).

The software determined motor error as the average distance of the participant's attempted trace from the sinusoidal wave that was presented. Motor error was measured as a percent that the participant's tracing cursor was from the 'perfect' trace. Pre-motor learning acquisition, post-motor learning acquisition, and at retention, each of the versions, 1-4, were performed once; while for the motor learning acquisition phase each version was performed three times totaling 12 traces. The participants swept their thumb from left to right using their APB muscle.

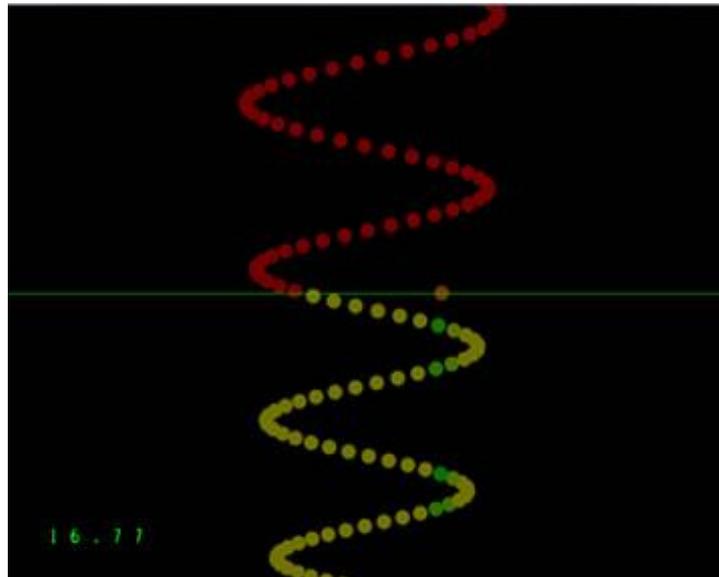


Figure 19: An illustration of the motor learning task which was performed by each participant.



Figure 20: Photograph of individual performing the motor tracing task on the touchpad

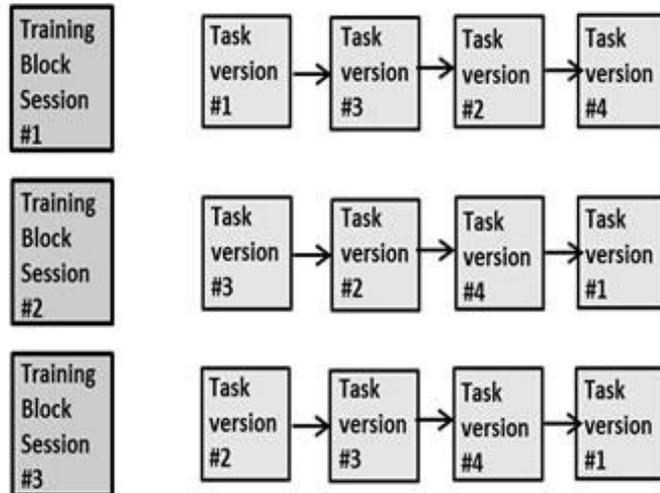


Figure 21: An illustration of the order of the 4 different task variations is shown. The block order and learning task version was identical for all participants during acquisition and retention.

8.5 NPRS:

For all of the studies, pain was rated by using an NPRS rating system, in which “0” corresponds to no pain and “10” to the worst painful sensation one may conceive.

8.6 Statistical Analysis

IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp) was utilized for statistical analysis. Statistical significance was set at $p < 0.05$.

8.6.1 SEP Peaks:

In order to account for variability between participants and allowing for between participant comparisons, SEP peak amplitudes were normalized to baseline (prior to cream application).

A mixed design repeated measures ANOVA (with time as the repeated measure and group (capsaicin, control etc.) as the grouping variable) was performed on the SEP peaks. If the overall ANOVA was significant, post-hoc tests were performed to ascertain differences.

Input-output curves:

MEP amplitudes were measured from peak-to-peak for each trial and averaged for each stimulus intensity. This file was exported to Microsoft Excel and the MEP amplitudes for every intensity were averaged and graphed. The slope of the linear aspect of the input-output curve was calculated and exported to IBM SPSS Statistics for statistical analysis. The plateau phase was excluded for those participants that had levelling off at the lower (90% rMT) pulse intensities, and therefore only the slope of the curve from the 100% intensity to 140% intensity was included in the analysis. MEP amplitudes were normalized to baseline values to account for variability between participants and to allow for comparisons between participants. To explore the interactive effect of pain and motor learning on the input-output slopes, a two-way repeated measures ANOVA with factors TIME (baseline, post-application, post-motor learning) and

GROUP (control versus intervention) was performed.

8.6.2 Behavioural data

Motor sequence task:

A mixed model repeated measures ANOVAs will be utilized to measure changes in response time with group (for example: intervention, control for study 1) as the grouping variable. For the accuracy data, a chi square test will be performed with post hoc chi square tests planned if there is a significant finding.

Motor tracing task:

The mean percent error in every trace attempt was averaged pre-motor learning, post-motor learning and at retention. A mixed-design ANOVA will be used to determine if there is a significant change in the motor learning effect between groups.

8.6.3 NPRS

A mixed-design repeated measures ANOVA will be performed using the dependent variable of NPRS value and independent variable of condition.

9 MANUSCRIPTS

9.1 Manuscript 1: The effect of local versus remote experimental pain on motor learning and sensorimotor integration using a complex typing task

Preface to Manuscript 1:

Previous work demonstrated that the amplitude of the N24 SEP peak, reflecting activation of the pathway between the cerebellum and the SI [73] was increased following simple motor learning of a task that involved typing three numbers presented in random sequence [20]. Andrew et al. [340] subsequently demonstrated significant changes in spinal (N13) and cortical (N20, N24, P25, N30) SEP peaks following both a complex tracing task and a typing task, with differential changes in SEP peaks following the complex task, indicating that a more complex task may be better suited to demonstrate the impact of acute pain on the neuroplasticity associated with motor learning. Our previous work demonstrated improved learning with the application of capsaicin cream [20], however motor performance saturation occurred and therefore it is important to verify these results using a more complex task. The purpose of *Experiment 1* was to determine whether motor learning acquisition in conjunction with acute experimental pain leads to significant differences in SEP peaks when compared with a control group. The purpose of *Experiment 2* was to determine how capsaicin cream applied over a local versus remote area alters sensory processing (as measured by SEPs), motor learning acquisition, and motor learning retention.

THE EFFECT OF LOCAL VERSUS REMOTE EXPERIMENTAL PAIN ON MOTOR LEARNING AND SENSORIMOTOR INTEGRATION USING A COMPLEX TYPING TASK

Author(s): Erin Dancey, Bernadette Murphy, Danielle Andrew, Paul Yelder

Affiliation(s): University of Ontario Institute of Technology
Faculty of Health Sciences
Oshawa, ON Canada
L1H 7K4

Corresponding Author Address: Bernadette Murphy, University of Ontario Institute of Technology,
2000 Simcoe Street North, Oshawa, Ontario, Canada.

Email: Bernadette.Murphy@uoit.ca

Telephone: (905) 721-8668 x 2768

Fax: (905) 721-3179

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9.1.1 Abstract

Recent work demonstrated that capsaicin induced acute pain and improved motor learning performance [20], however baseline accuracy was very high, making it impossible to discern the impact of acute pain on motor learning and retention. In addition, the effects of the spatial location of capsaicin application were not explored. Two experiments were conducted to determine the interactive effects of acute pain versus control (*Experiment 1*) and local versus remote acute pain (*Experiment 2*) on motor learning and sensorimotor processing. For both experiments, somatosensory evoked potential (SEP) amplitudes and motor learning acquisition and retention (accuracy and response time) data were collected at baseline, post-application and following motor learning. *Experiment 1*: N11 ($p < 0.05$), N13 ($p < 0.05$) and N30 ($p < 0.05$) SEP peak amplitudes increased following motor learning for both groups while the N20 SEP peak increased for the control group ($p < 0.05$). At baseline, the intervention group outperformed the control group in accuracy ($p < 0.001$). Response time improved following motor learning ($p < 0.001$) and at retention ($p < 0.001$). *Experiment 2*: The P25 SEP peak decreased for the local group following application of capsaicin cream ($p < 0.01$) while the N30 SEP peaks increased following motor learning for both groups ($p < 0.05$). Accuracy improved in the local group at retention ($p < 0.005$), and response time improved following motor learning ($p < 0.005$) and at retention ($p < 0.001$). This study suggests that acute pain may increase focal attention to the body part utilized in motor learning; contributing to our understanding of how the location of pain impacts somatosensory processing and the associated motor learning.

KEYWORDS

Somatosensory evoked potentials (SEP); motor learning; local pain; remote pain; sensorimotor integration (SMI)

9.1.2 Introduction

Motor learning leads to changes in sensorimotor integration (SMI) which is the processing of somatosensory information and the integration of this information with the motor command from the MI (primary motor cortex), in order to fine tune motor task performance. Neuroplasticity occurs following motor learning [3, 4] and with acute [6-8] and chronic pain [5]. Neuroplasticity can be investigated using somatosensory evoked potentials (SEPs) that measure the electrical field potentials generated by structures of the nervous system [49] enabling the exploration of the neuroplastic consequences of pain and motor learning. We recently demonstrated that the amplitude of the N24 SEP peak, which reflects activation of the pathway linking the cerebellum and the primary somatosensory cortex (SI) [73] was increased following a simple motor learning task that involved typing three numbers presented in random sequence [20]. Andrew et al. [340] subsequently demonstrated significant changes in spinal (N13) and cortical (N20, N24, P25, N30) SEP peaks following both a complex tracing task and a typing task, with differential changes in SEP peaks following the complex task, indicating that a more complex task may be more sensitive to demonstrating the impact of acute pain on motor learning. Capsaicin cream is applied topically and provides an acute pain stimulus that does not increase with specific movements [40]. Our previous work demonstrated improved performance in the presence of capsaicin [20], however motor performance saturation occurred and therefore it is important to verify these results using a more complex task. Another limitation is that the majority of previous studies of acute cutaneous pain have not measured retention [9, 20]. Two previous studies that measured retention used an experimental tonic pain model combined with a locomotor adaptation task [18] or an upper limb reaching task [21] and found that pain during training had an impact on retention [18, 21] despite not having an impact on baseline measures [21] or acquisition [18]. Factors improving or negatively impacting motor learning acquisition are not

necessarily predictive of motor retention [294, 295] and from both a learning and practical perspective it is retention that indicates whether learning has been impacted positively or negatively.

The presence of local acute pain during motor training has been shown to interfere with skill acquisition [9], which is in contrast to our findings of improvement in task performance in the presence of remote acute pain [20]. In addition, Ingham et al. [413] using an acute experimental muscle pain model, found that plastic changes were observed after motor learning during control and local pain, but not during remote pain. In order to address these conflicting findings, the effects of local versus remote acute pain in conjunction with a complex motor learning task needs to be investigated at both neurophysiological and behavioural levels.

The purpose of *Experiment 1* was to determine whether motor learning acquisition combined with acute pain leads to significant differences in SEP peaks when compared with a control group. The purpose of *Experiment 2* was to determine how capsaicin cream applied over a local versus remote area alters sensory processing (as measured by SEPs), motor learning acquisition, and motor learning retention. This has possible applications to rehabilitation and injury prevention strategies as many individuals in rehabilitation present with pain and motor deficits concurrently.

9.1.3 Methods

Methods Overview:

In total, 48 student volunteers at UOIT participated in two studies; *Experiment 1* (13 males, 11 females; aged 20 – 41 (M 23.9 SD 6.3) and *Experiment 2* (11 males, 13 females; aged 20 – 41 (M 21.8 SD 3.9). Each participant completed a confidential health history form in order to detect any medical conditions which could affect normal somatosensation. This encompassed neurologic conditions, recent cervicothoracic injury, or medication use; it was also required that participants were not to present with any chronic pain conditions that could affect the measurement of SEPs. This study received approval from the University of Ontario Institute of Technology Research Ethics Board (REB# 11-067) and informed consent was obtained for all participants. This study was performed in accordance with the principles set out by the Declaration of Helsinki for the use of humans in experimental studies. Participants in *Experiment 1* were assigned randomly to either an intervention or control group and participants in *Experiment 2* were assigned randomly to either a remote pain or local pain group and were counterbalanced for gender.

Experimental Design – Experiment 1 – Control versus Intervention (remote acute pain)

The effect of acute pain and motor learning was determined by examining alterations in the amplitude of SEPs from baseline, at 20 minutes post-application of the creams, and then following a motor learning typing task (45 minutes from baseline). Participants in the control group received a topical control skin lotion (Life Brand, Shopper's Drug Mart, Ontario, Canada) while those in the intervention group received a topical application of capsaicin (0.075% Zostrix, New York, USA) which was applied to a 50 cm² area of the skin on the lateral aspect of the dominant elbow.

Experimental Design – Experiment 2 – Remote acute pain versus local acute pain

The effect of remote versus local acute pain and motor learning was determined by examining alterations in the amplitude of SEPs from baseline, at 20 minutes post-application of the cream, and then following a motor learning typing task (45 minutes from baseline). Participants in both groups received a topical application of capsaicin (0.075% Zostrix, New York, USA) which was applied either to a 50 cm² area of the skin overlying the APB muscle (local pain group) or on the lateral aspect of the dominant elbow (remote pain group).

Outcome Measures

The outcome measures for both *Experiment 1* and *2* included, motor learning accuracy response time (msec), the amplitude (μ V) of the SEP peaks and pain (Numeric Pain Rating Score).

Motor learning task:

The motor learning task utilized in both experiments consisted of a typing task in which participants typed randomized eight-letter sequences of the letters Z, P, D, and F e.g. (Z,D,P,Z,F,P,D,D), with the dominant thumb for a total of approximately 15 minutes. This task occurred following the application of the creams and was selected as similar tasks have been shown to activate the cerebellum and MI in early stages of motor sequence learning [11, 298]. Custom E-Prime 2.0.10.242 (Psychology Software Tools, Inc., Pennsylvania, USA) software was utilized for the typing task while recording response time and accuracy. Participants in both experiments completed ten randomized sequences of the eight letters at the start and end of the motor acquisition task to evaluate motor learning acquisition (accuracy and response time). Participants completed ten randomized sequences of the eight letters 48 hours later to evaluate motor learning retention (as measured by accuracy and response time). Response time was recorded from the time of visual presentation of the numbers to the time of key press. Accuracy

was determined based on whether the key in the sequence was pressed correctly (1) or incorrectly (0).

Pain:

For *Experiment's 1* and 2, pain was quantified using a Numeric Pain Rating Scale (NPRS) in which participants graded their pain from 1–10 [416]. Participants in both groups were asked to rate their pain at baseline, 5 minutes post-application, 20 minutes post-application, post-motor learning (35 minutes post application) and following the last round of SEP measurements (45 minutes from baseline) in order to measure levels of acute pain.

Stimulation of median nerve to elicit SEPs

Ag/AgCl ECG conductive adhesive electrodes (MEDITRACE™ 130 by Ludlow Technical Products Canada Ltd., Massachusetts, USA) (impedance <5 kΩ) were situated 2-3 cm proximal to the distal crease of the wrist over the median nerve at the wrist of the right hand, with the cathode situated 2 cm proximal to the anode. Electrical stimuli 0.1ms in duration were delivered at frequencies of 2.47 Hz. Following the 2.47 Hz session, electrical stimuli were then delivered at a frequency of 4.98Hz for. SEPs were recorded at two different rates in order to record both the N24 and N30 SEP peaks. The use of the slower rate of 2.47 Hz does not attenuate the SEP peak amplitudes while the faster rate, 4.98 Hz attenuates the N30 SEP peak, allowing for the measurement of the N24 SEP peak [46, 364].

SEP recording parameters

In accordance with the recommendations of International Federation of Clinical Neurophysiologists (IFCN) SEP recoding electrodes (1.8m long Traditional Grass™ Lead, 10mm disc, 2mm hole gold cup EEG electrodes, Grass Technologies, An Astro-Med, Inc. Subsidiary, Massachusetts, USA) (impedance <5 kΩ) were placed using Grass Technologies

EEG adhesive conducting paste (Type TEN20™). Recording electrodes were placed on ipsilateral Erb's point, over the C5 spinous process, the anterior neck (trachea), 2cm posterior to contralateral central C3/4, referred to as Cc', and a frontal site (6cm anterior and 2cm contralateral to Cz), denoted as the Rossi site [74]. The C5 spinous process was referenced to the trachea while other electrodes were referenced to the ipsilateral earlobe. A ground electrode (1.8m long Traditional Grass™ Lead, 10mm disc, 2mm hole gold cup EEG electrodes, Grass Technologies, An Astro-Med, Inc. Subsidiary, Massachusetts, USA) was placed in the mouth of participants.

A total of 1000 sweeps were averaged per stimulation rate using a Signal® configuration (Cambridge Electronic Design, England, UK). The SEP signal was amplified (Gain 10,000), filtered (0.2-1000 Hz) and stored on a computer. The averaged waveform was used in order to measure the SEP peak amplitudes according to the IFCN guidelines [26]. We measured the peak-to-peak amplitude (μV) of the following SEP peaks: the peripheral N9, the spinal N11 and N13, the N18, the parietal N20 and P25 and the frontal N24 and N30. In accordance with international recommendations [417] and previous studies in this field [62, 167, 168]. SEP amplitudes were measured from the averaged traces from the peak of interest to the succeeding or preceding peak of opposite deflection.

Statistical Analysis

SEP peak amplitudes in both *Experiment 1* and *2* were normalized to baseline values to account for variability between participants and to allow for between participant comparisons. The Shapiro-Wilk test for normality was performed on each SEP peak.

Experiment 1:

To explore the effects of pain versus control application, a repeated measures ANOVA with factors TIME (baseline versus post-application) and GROUP (control versus intervention) was performed on each SEP peak separately to explore the effects of pain versus control application.

To explore the interactive effect of pain and motor learning a repeated measures ANOVA with factors TIME (baseline versus post-motor learning) and GROUP (control versus intervention) was performed.

For the response time data, a repeated measures ANOVA with factors TIME (baseline, post motor learning, retention) and GROUP (control versus intervention) was performed. For the accuracy data, a Friedman test was utilized with post hoc chi square tests planned if the Friedman test was significant. For the NPRS measurements, a repeated measures ANOVA with factors TIME [baseline, post-application (5 minutes), post-application (20 minutes), post motor learning (35 minutes), post motor learning (45 minutes)] and GROUP (control versus intervention) was performed. IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp) was used for the statistical analysis. Statistical significance was set at $p < 0.05$.

Experiment 2:

For the SEP peaks that were normally distributed, a repeated measures ANOVA with factors TIME (baseline versus post application) and GROUP (remote, local) was run on each SEP peak separately to explore the effects of remote pain versus local pain application. To explore the interactive effect of pain and motor learning a repeated measures ANOVA with factors TIME (baseline versus post motor learning) and GROUP (remote, local) was performed. For the peak that was not normally distributed, a Friedman's test was run post-application and post-motor learning.

For the response time data, a repeated measures ANOVA with factors TIME (baseline, post

motor learning, retention) and GROUP (remote versus local) was performed. For the accuracy data, a Friedman test was utilized with post hoc chi square tests planned if the Friedman test was significant. For the NPRS measurements, a repeated measures ANOVA with factors TIME [baseline, post-application (5 minutes), post-application (20 minutes), post motor learning (35 minutes), post motor learning (45 minutes)] and GROUP (remote versus local) was performed. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp). Statistical significance was set at $p < 0.05$.

9.1.3 Results:

Pain versus control

Experiment 1:

All 24 participants who took part in this experiment were included in the analysis of SEP peaks. There were no major differences in demographics (gender and age) between groups; intervention group (5 males, 7 females; aged 19 – 32 (M 20.8 SD 3.3) and control group (6 males, 6 females; aged 20 – 24 (M 22.8 SD 2.0)).

SEPs: electrophysiological measures

Following motor learning, the amplitudes of the N11, N13 and N30 SEP peaks differed significantly for both groups, and the amplitude of the N20 SEP peaks differed for the control group. There were no significant differences for any of the other SEP peaks (N9, N18, P25 and N24) post-application or following motor learning although the P25 SEP peak approached significance following motor learning ($p = 0.063$).

There were no significant changes in latency data for any SEP peak in either the control or intervention groups. Table 1 indicates the average amplitudes of all SEP peaks while Table 2 indicates the average latencies of all SEP peaks.

N11 SEP peak: For the N11 SEP peak amplitudes, the Shapiro-Wilk test for normality

demonstrated that all the categories were normally distributed except for post-application (intervention group); hence a repeated measures ANOVA was run on the N11 SEP peak amplitude data. Following the cream application, there was no main effect of TIME on N11 SEP amplitude ($p=0.89$), or a TIME by GROUP interaction effect ($p=0.89$). Following motor learning, the repeated measures ANOVA revealed a significant overall TIME effect [$F(2,23) = 9.10, p<0.05$], while the interaction effect of TIME by GROUP was not significant ($p = 0.88$). There was a 17.2 % increase in the N11 SEP peak for the control group and a 19.0 % increase in the N11 SEP peak for the intervention group.

N13 SEP peak: For the N13 SEP peak amplitudes, the Shapiro-Wilk test for normality demonstrated that all the categories were normally distributed except for post application (control group); hence a repeated measures ANOVA was run on the N13 SEP peak amplitude data. Following the cream application, there was no main effect of TIME on N13 SEP amplitude ($p = 0.56$) or a TIME by GROUP interaction effect ($p = 0.54$). Following motor learning, there was a significant TIME effect [$F(2,23) = 4.35, p<0.05$], while the interaction effect of TIME by GROUP was not significant ($p = 0.36$) with the N13 SEP peak increasing by 16% for the control group and by 8.7 % for the intervention group.

N30 SEP peak: For the N30 SEP peak amplitudes, the Shapiro-Wilk test for normality demonstrated that all the categories were normally distributed except for post-motor learning (intervention group); hence a repeated measures ANOVA was run on the N30 SEP peak amplitude data. Following the cream application, there was no main effect of TIME on N30 SEP amplitude ($p = 0.98$) or a TIME by GROUP interaction effect ($p= 0.85$). Following motor learning there was a significant TIME effect [$F(2,23) = 9.64, p<0.01$], while the interaction effect of TIME by GROUP was not significant ($p = 0.42$). The N30 SEP peak increased by

18.9% for the control group and by 32.4 % for the intervention group.

N20 SEP peak: For the N20 SEP peak amplitudes, the Shapiro-Wilk test for normality demonstrated that all the categories were normally distributed except for post-application (intervention group); hence a repeated measures ANOVA was run on the N20 SEP peak amplitude data. Following the cream application, there was no main effect of TIME on the N20 SEP peak amplitude ($p = 0.89$) or a TIME by GROUP interaction effect ($p = 0.78$). There was, however, a significant effect of motor learning on the N20 SEP peak amplitude [$F(2,23) = 15.90, p < 0.005$], with a 35.5 % increase in the N20 SEP peak following motor learning in the control group and an 11.2% increase in the N20 SEP peak was observed following motor learning in the intervention group. The interaction effect of TIME by GROUP was also significant [$F(2,23) = 4.32, p < 0.05$] with post hoc tests demonstrating that the control group N20 SEP peak was significantly increased following motor learning [$F(1, 11) = 1.35, p < 0.001$] while there was no significant difference for the intervention group ($p = 0.27$).

The N9, N18, N24, and P25 SEP peaks were normally distributed and therefore an ANOVA was performed on these peaks and no significant differences were seen.

The normalized averages for the post-application intervention versus control group SEP peaks are illustrated in Figure 1A and the post-motor learning intervention versus control group SEP peaks are illustrated in Figure 1B. Figure 2 illustrates the raw data from a representational control participant indicating SEP peaks and Figure 3 illustrates the raw data from a representational intervention participant indicating SEP peaks. Significant differences from baseline are indicated by asterisks.

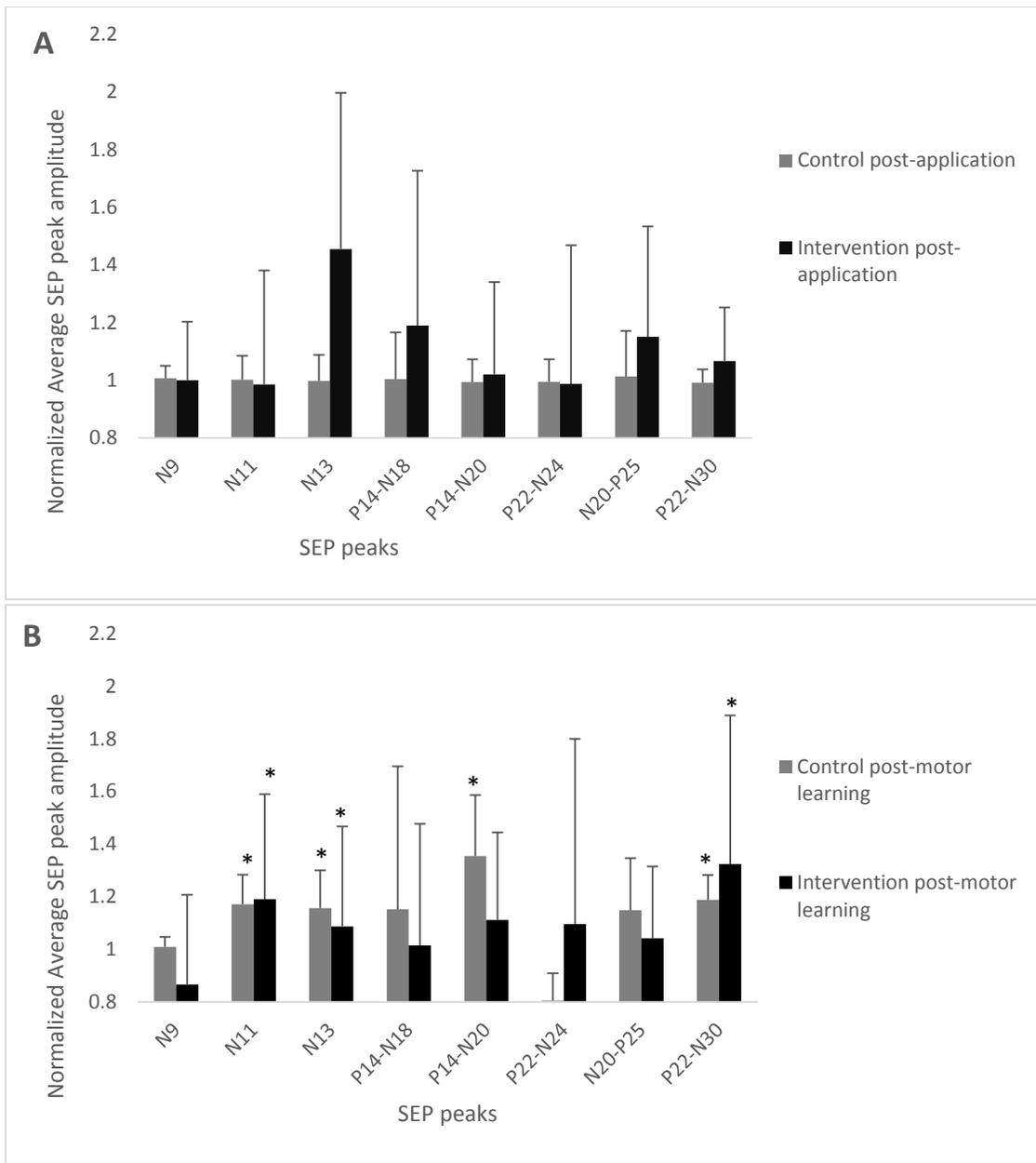
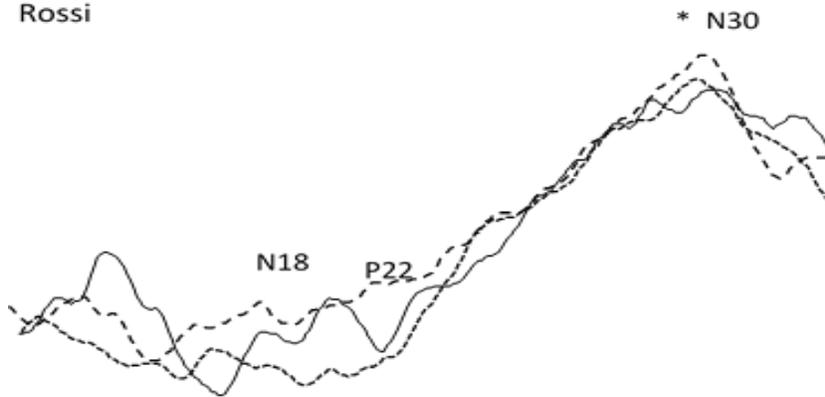


Figure 1: Bar-graph of averaged normalized SEP ratios from *Experiment 1* showing intervention versus control groups post-application (A), and post-motor learning (B). A: No significant differences for the control group or the intervention group post-application. B: Following motor learning, significantly different changes from baseline are indicated by asterisks for the N11, N13, N20, and N30 SEP peaks for the control group and significantly different changes from baseline are indicated by asterisks for the N11, N13, and N30 SEP peaks for the intervention group. Error bars represent the standard deviation.

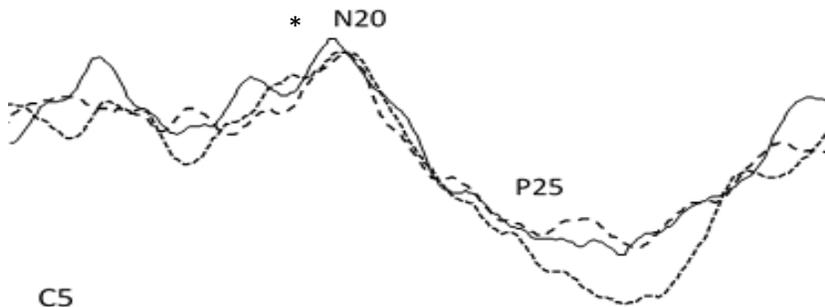
Intervention group (remote pain)

A

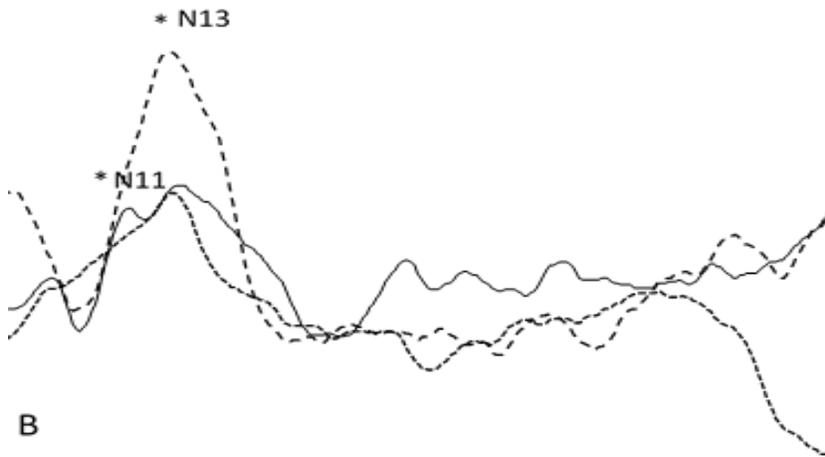
Rossi



Cc'



C5



B

Rossi

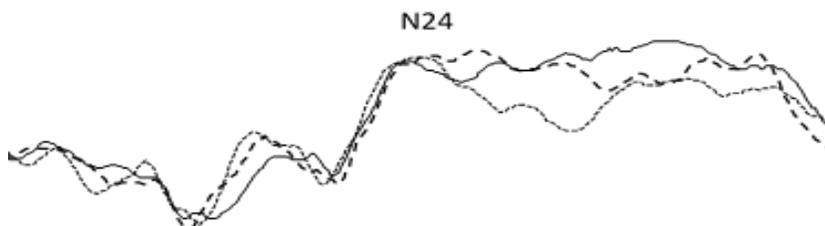
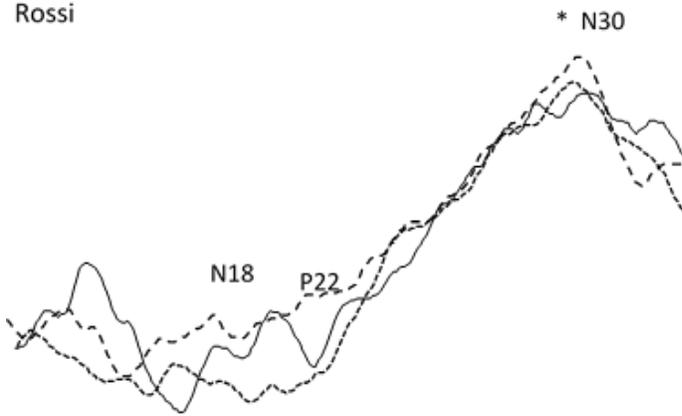


Figure 2: raw data from a representational control participant indicating SEP peaks. A: SEPs recorded using the rate of 2.47 Hz. B: SEPs recorded using the rate of 4.98 Hz. Note the significant differences for the N11, N13, N20 and N30 peaks post-motor learning as indicated by asterisks.

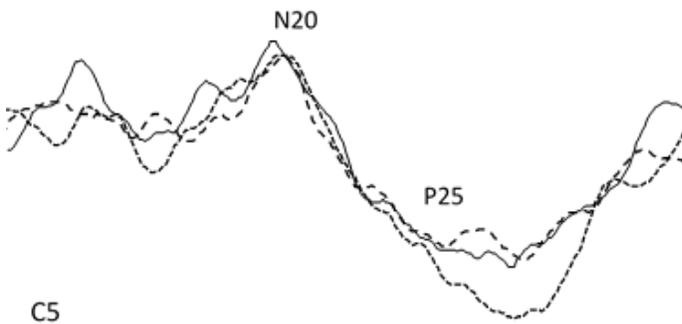
Intervention group (remote pain)

A

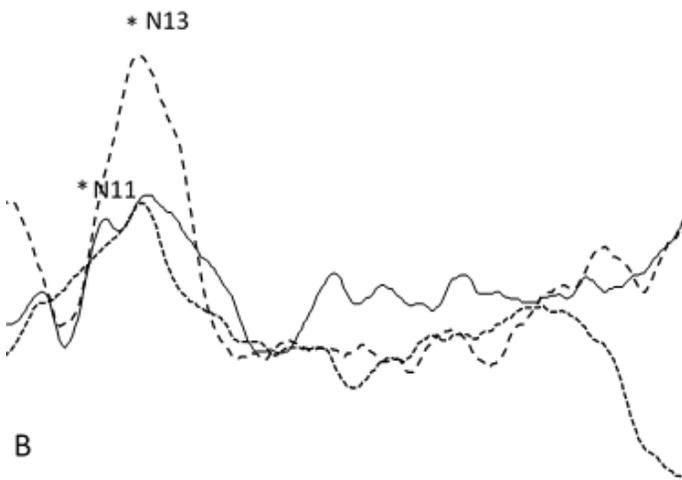
Rossi



Cc'



C5



B

Rossi



Figure 3: raw data from a representational intervention participant indicating cortical peaks. A: SEPs recorded using the rate of 2.47 Hz. B: SEPs recorded using the rate of 4.98 Hz. Note the significant differences for the N11, N13, and N30 peaks post-motor learning as indicated by asterisks.

SEP peak	Control		Intervention (remote pain)		Intervention (local pain)	
	Post-application	Post-motor learning	Post-application	Post-motor learning	Post-application	Post-motor learning
N9	1.01 ± 0.04	1.01 ± 0.04	1.00 ± 0.20	0.87 ± 0.34	0.95 ± 0.17	0.93 ± 0.20
N11	1.00 ± 0.08	1.17 ± 0.11	0.99 ± 0.40	1.19 ± 0.40	0.96 ± 0.51	1.04 ± 0.51
N13	1.00 ± 0.09	1.16 ± 0.14	1.10 ± 0.54	1.09 ± 0.38	0.83 ± 0.40	1.00 ± 0.33
P14-N18	1.00 ± 0.16	1.15 ± 0.54	1.00 ± 0.54	1.01 ± 0.46	0.99 ± 0.37	1.09 ± 0.39
P14-N20	0.99 ± 0.08	1.35 ± 0.23	1.02 ± 0.32	1.11 ± 0.33	1.06 ± 0.37	1.08 ± 0.38
P22-N24	1.00 ± 0.08	0.81 ± 0.10	0.99 ± 0.48	1.10 ± 0.70	0.93 ± 0.28	0.99 ± 0.22
N20-P25	1.01 ± 0.16	1.15 ± 0.20	1.15 ± 0.38	1.04 ± 0.27	0.78 ± 0.15	0.93 ± 0.18
P22-N30	0.99 ± 0.05	1.19 ± 0.09	1.07 ± 0.19	1.32 ± 0.56	1.11 ± 0.33	1.02 ± 0.89

Table 1: Normalized average amplitudes for all SEP peaks +/- standard deviation.

SEP peak	Intervention: control			Intervention: remote pain			Intervention: local pain		
	Baseline	Post application	Post motor learning	Baseline	Post application	Post motor learning	Baseline	Post application	Post motor learning
N9	10.2 ± 0.7	10.0 ± 0.8	10.2 ± 0.7	9.7 ± 0.6	9.7 ± 0.5	9.8 ± 0.6	10.0 ± 0.7	10.1 ± 0.7	9.9 ± 0.6
N11	12.0 ± 0.6	12.0 ± 0.7	12.0 ± 0.8	11.5 ± 0.7	11.5 ± 0.7	11.4 ± 0.6	11.8 ± 0.8	11.8 ± 0.7	11.8 ± 0.7
N13	13.3 ± 0.8	13.2 ± 0.8	13.2 ± 0.9	13.0 ± 0.6	13.1 ± 0.5	13.1 ± 0.5	13.3 ± 0.6	13.3 ± 0.5	13.3 ± 0.6
P14-N18	18.2 ± 0.5	18.2 ± 0.6	18.4 ± 0.4	17.6 ± 0.8	17.7 ± 1.1	17.3 ± 1.0	17.7 ± 0.8	17.8 ± 0.7	17.8 ± 0.6
P14-N20	20.0 ± 0.7	19.9 ± 0.8	20.3 ± 0.7	19.1 ± 0.9	19.2 ± 1.0	19.4 ± 1.1	19.3 ± 1.0	19.3 ± 0.9	19.4 ± 0.8
P22-N24	23.6 ± 0.7	23.5 ± 0.7	23.7 ± 0.8	24.0 ± 0.9	23.8 ± 1.0	23.7 ± 0.8	24.3 ± 0.8	24.3 ± 0.6	24.2 ± 0.5
N20-P25	24.7 ± 1.0	24.3 ± 1.4	25.1 ± 1.0	25.3 ± 0.9	25.7 ± 1.0	25.6 ± 0.9	24.9 ± 1.1	25.1 ± 0.9	25.0 ± 0.7
P22-N30	31.3 ± 0.7	31.5 ± 0.7	31.7 ± 1.0	30.2 ± 1.1	29.9 ± 1.2	30.2 ± 1.0	29.5 ± 1.2	29.4 ± 0.9	29.7 ± 0.6

Data are presented as mean ± SD

Table 2: Average latencies for all SEP peaks +/- standard deviations.

Pain ratings:

There were significant differences in subjective pain levels relative to baseline for the intervention group 5 minutes post-application [$F(3, 23) = 18.41, p = 0.01$], 20 minutes post-application [$F(3, 23) = 58.06, p < 0.01$], post-motor learning (35 minute mark) [$F(3, 23) = 69.87, p < 0.01$] and post-motor learning (45 minute mark) [$F(3, 23) = 29.54, p < 0.01$]. The average NPRS ratings are illustrated in Figure 4. None of the participants in the control group reported any pain.

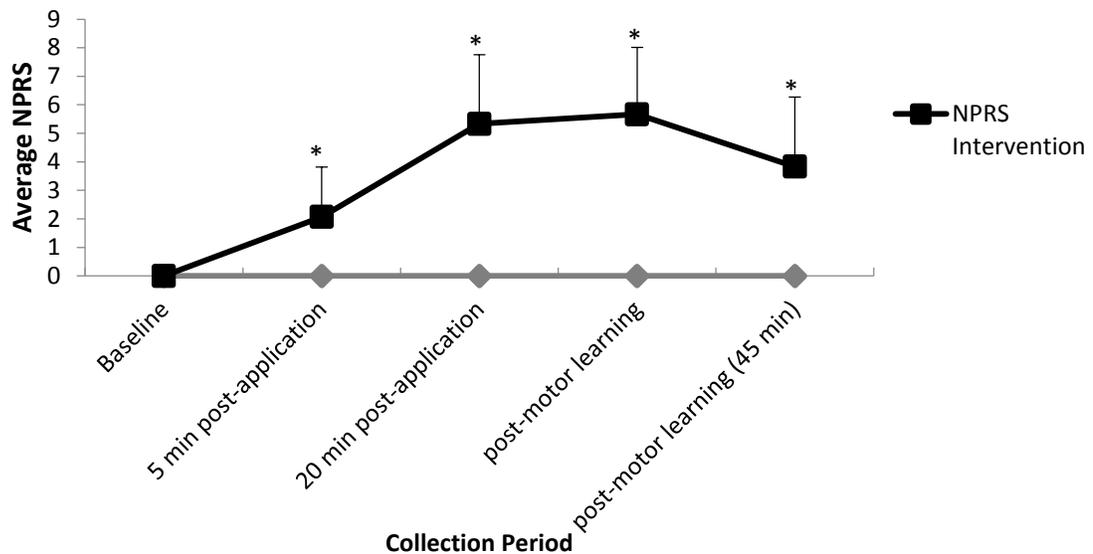


Figure 4: Line-graph depicting averaged NPRS ratings of subjects in the control and the intervention groups. Error bars represent the standard deviation. Significant differences post application for the intervention group are indicated by asterisks.

Local versus remote pain

Experiment 2:

A total of 24 participants were tested and none of the participants withdrew. There were no major differences in demographics (age and gender) between groups; remote pain group (5 males, 7 females; aged 19 – 28 (M 21.8 SD 3.3) and local pain group (5 males, 7 females; aged 19 – 36 (M 22.9 SD 4.3).

SEPs: electrophysiological measures

Following local capsaicin application, the P25 SEP peak differed significantly. Following motor learning, the amplitudes of the N30 SEP peak differed significantly for both groups. There were no significant differences for any of the other peaks (N9, N11, N13, P14, and N24) post-

application or following motor learning. There were no significant changes in latency data for any SEP peak in either the remote pain or local pain groups.

P25 SEP peak: For the P25 SEP peak normality was met in all conditions and therefore an ANOVA was used to assess these peak changes. The repeated measures ANOVA revealed a significant interaction effect of TIME by GROUP [F (2,23) = 9.55, $p < 0.01$]. Post hoc tests indicated that the local group differed post application [F (1,11) = 23.47, $p < 0.01$] decreasing by 21.8 % while the remote group did not change ($p = 0.20$), (increasing by 1.1%). Following motor learning, there was no main effect of TIME on P25 SEP peak amplitude ($p = 0.78$) or a TIME by GROUP interaction effect ($p = 0.26$).

N30 SEP peak: There was a violation of normality for the N30 SEP peak in *Experiment 2* post application (local group) and following motor learning for both groups. Therefore we ran a Friedman test on the overall data post-application and post-motor learning and found that there was no significant difference following the application of the creams ($p = 0.124$) but there was a significant difference in the N30 SEP peak following motor learning [χ^2 (df = 2, $p < 0.05$)], with a 32.4 % increase in the N30 SEP peak for the remote group compared to only a 2.1 % increase in the N30 SEP peak for the local group.

For the N9, N11, N13, N18, N20, N24 SEP peaks normality was met in all conditions and thus an ANOVA was used to assess these peak changes and no significant changes were seen.

The normalized averages for the post-application local pain versus remote pain SEP peaks are illustrated in Figure 5A and the normalized averages for the post-motor learning local pain remote pain SEP peaks are illustrated in Figure 5B. Figure 6 illustrates the raw data from a representational local pain participant indicating SEP peaks. Significant differences from baseline are indicated by asterisks.

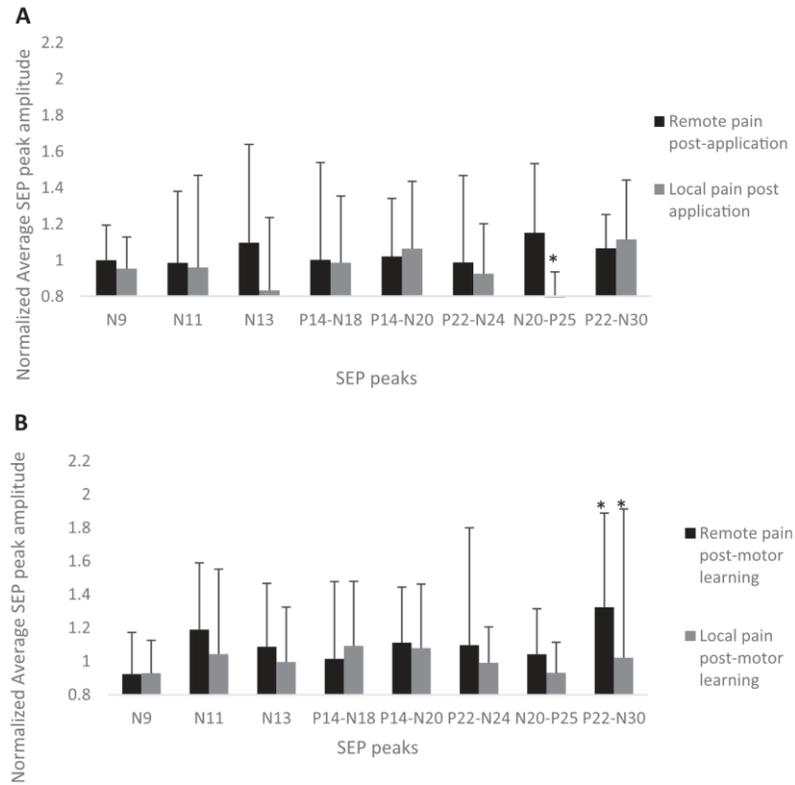


Figure 5: Bar-graph of averaged normalized SEP ratios from *Experiment 2* showing remote pain group versus local pain group, post-application (A), and post-motor learning (B). A: for the local pain group, significantly different changes post-application is indicated by an asterisk for the P25 peak. B: Following motor learning, significantly different changes from baseline are indicated by asterisks for the Error bars represent the standard deviation.

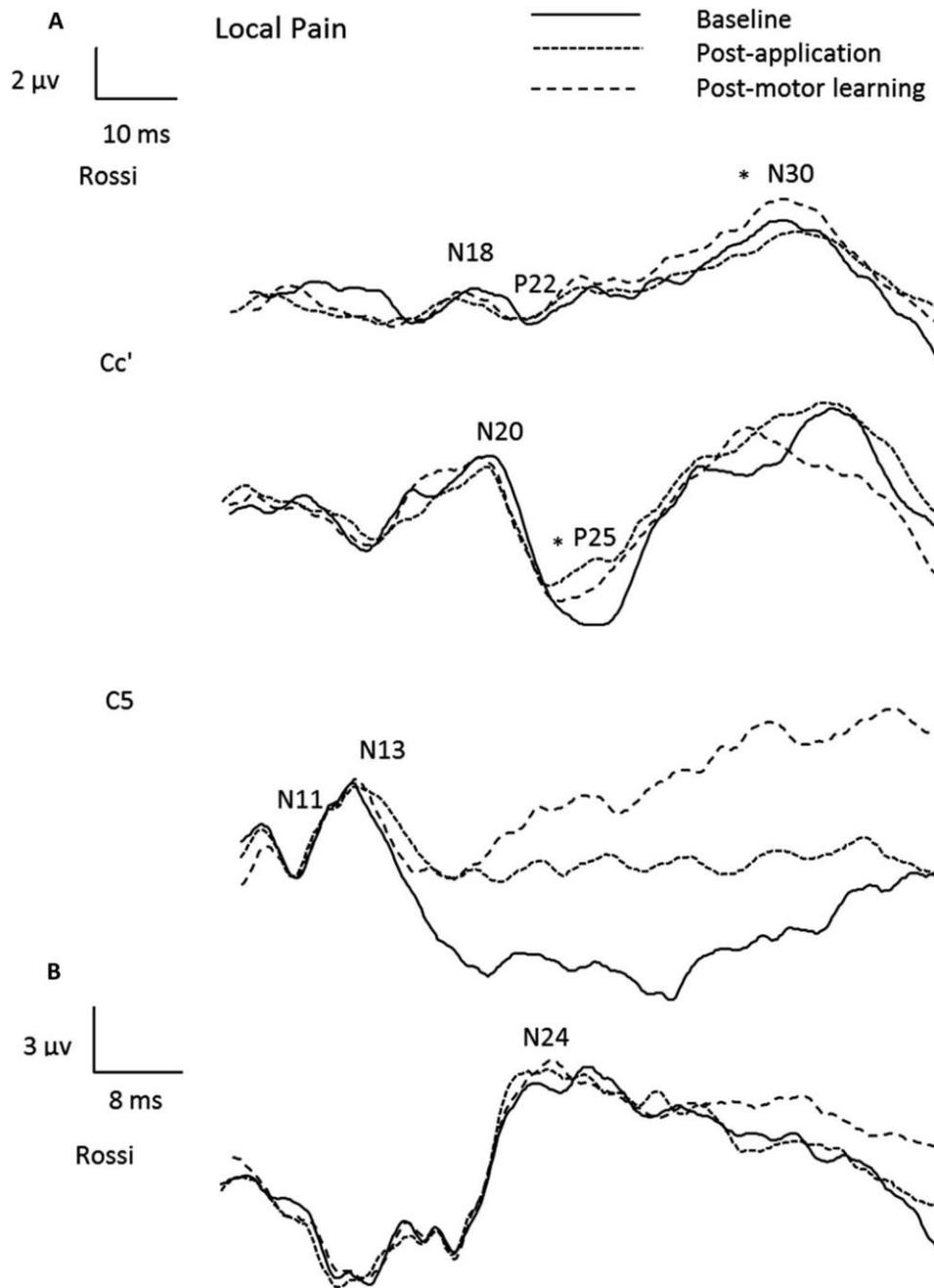


Figure 6: raw data from a representational local pain participant indicating cortical peaks. A: SEPs recorded using the rate of 2.47 Hz. B: SEPs recorded using the rate of 4.98 Hz. Note the significant differences for the P25 SEP peak following capsaicin application and the N30 peak post-motor learning as indicated by asterisks.

Pain ratings:

There were significant differences in subjective pain levels over time [$F(2,23) = 46.67$, $p < 0.001$], while the interaction effect of TIME by GROUP was not significant ($p = 0.17$). Post hoc tests indicate that there were significant differences in subjective pain levels relative to baseline 5 minutes post-application [$F(3, 23) = 4.80$, $p < 0.001$], 20 minutes post-application [$F(3,23) = 8.85$, $p < 0.001$], post-motor learning [$F(3, 23) = 10.10$, $p < 0.001$] and post-motor learning (45 minute mark) [$F(3,23) = 7.23$, $p < 0.001$]. The average NPRS ratings are illustrated in Figure 7.

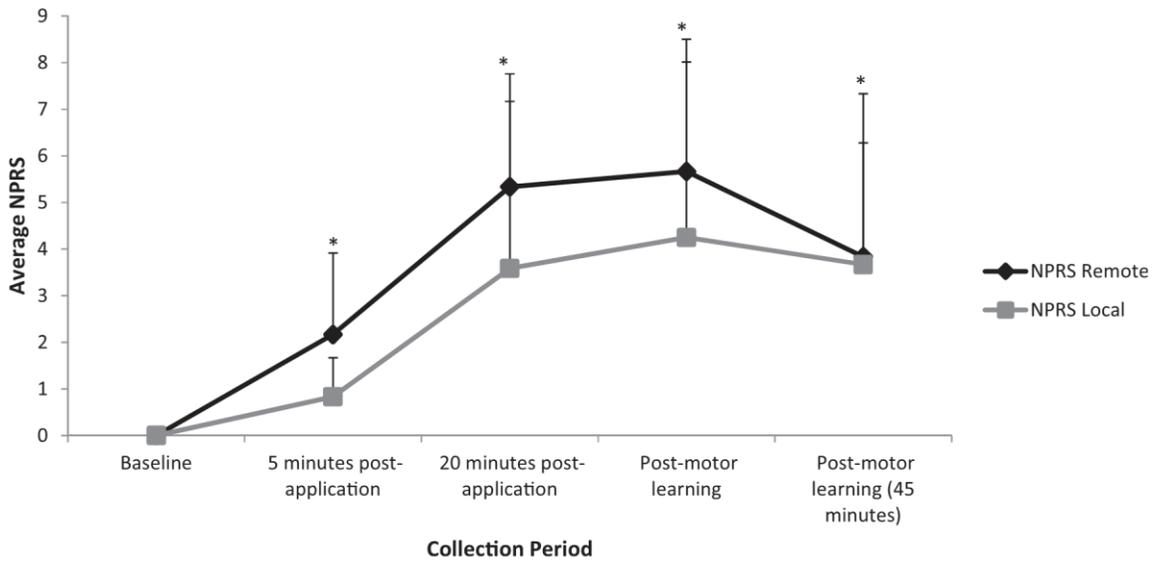


Figure 7: Line-graph depicting averaged NPRS ratings of subjects in the local and remote pain groups. Significant differences are indicated by asterisks. Error bars represent the standard deviation.

The interactive effect of pain on motor learning: behavioural measures

Experiment 1:

Accuracy

At baseline the control group had 132 incorrect responses out of 960, while the intervention group had 72 incorrect responses out of 960. Post motor learning the control group had 54 incorrect responses out of 960 while the intervention group had 58 incorrect responses out of 960. At retention the control group had 85 incorrect responses out of 960 while the intervention group had 71 incorrect responses. The accuracy data for the control and intervention groups are illustrated in Figure 8A. Friedman's test indicated a significant overall improvement following the motor learning acquisition task (χ^2 (df=2, $p < 0.0001$) = 17.02). Individual Friedman tests were run for intervention and control groups, and a significant effect was seen in the control group (χ^2 (df=2, $p < 0.0001$) = 16.04). Post hoc chi square tests demonstrated that the control group differed post-motor learning as compared to baseline (χ^2 (df=1, $p < 0.001$) = 36.22) and at retention as compared to baseline (χ^2 (df=1, $p < 0.001$) = 12.02), whereas the improvement in the intervention group was not significantly different post motor learning as compared to baseline ($p = 0.20$) or at retention as compared to baseline ($p = 0.93$). However, the intervention group started with higher baseline accuracy, with post hoc chi square tests indicating that the accuracy between the two groups differed significantly at baseline (χ^2 (df=1, $p < 0.001$) = 19.01) but not post-motor learning or at retention.

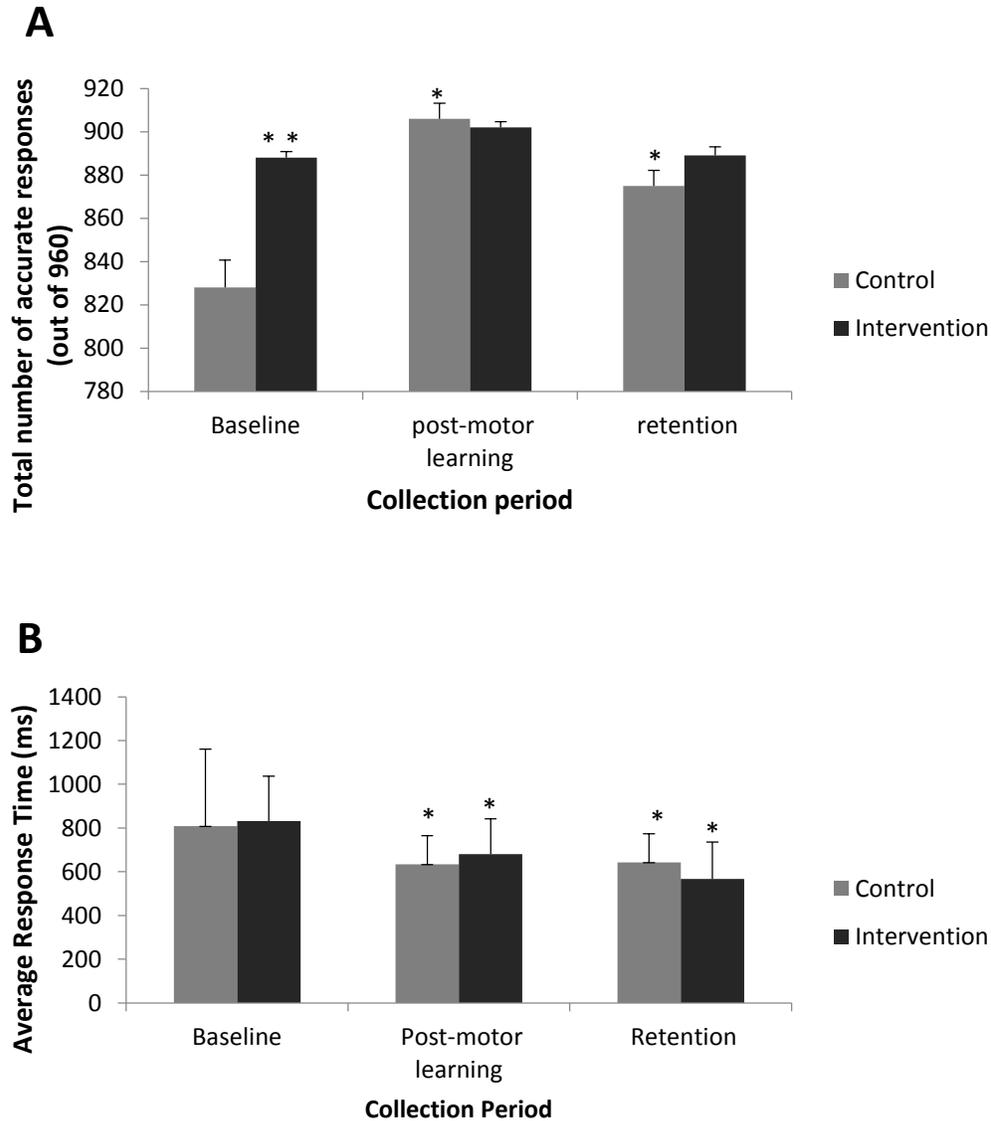


Figure 8: Behavioural measures for *Experiment 1*. A: bar-graph depicting the total number of accurate responses by group. Significant differences are indicated by asterisks demonstrating that following motor learning and at retention the control group had improved accuracy as compared to baseline. Significant differences between the groups at baseline are indicated by a double asterisk. B: bar-graph depicting the average response time pre and post-motor learning for the intervention and control groups. Significant differences for both groups are indicated by an asterisk. Error bars represent the standard deviation.

Response Time

There was a significant decrease in mean response time over time [$F(1, 23) = 16.88, p < 0.01$]. Participants in the control group demonstrated a decrease from to M 808.7 msec SD 204.8 to M 633.4 msec SD 160.6 following motor learning (a 21.7% decrease), and then increased slightly to M 642.0 msec SD 168.6 at retention (a 1.4% increase). Participants in the intervention group demonstrated a decrease from M 809.0 msec SD 351.6 to 617.3 msec SD 131.5 following motor learning (a 23.7% decrease), and then decreased further to M 599.5 msec SD 131.4 at retention (a 2.9% decrease) (see Figure 8B). Post hoc tests comparing post-motor learning and retention response time data to baseline values demonstrated that significant changes in response time occurred from baseline to post-motor learning ($p < 0.001$) and baseline to retention ($p < 0.001$), but did not differ significantly from post-motor learning to retention ($p = 0.82$). The interaction effect of TIME by GROUP was not significant ($p = 0.84$).

Experiment 2:

Accuracy

At baseline the local group had 67 incorrect responses out of 960, while the remote group had 72 incorrect responses out of 960. Following motor learning the local group had 52 incorrect responses out of 960 while the remote group had 58 incorrect responses out of 960. At retention the local group had 47 incorrect responses out of 960 while the remote group had 71 incorrect responses. The accuracy data for the local and remote and groups are illustrated in Figure 9A. Friedman's test indicated a significant overall improvement following the motor learning acquisition task ($\chi^2 (df=2, p < 0.01) = 10.41$). Individual Friedman tests were run for the remote and local groups, and a significant effect was seen in the local group ($\chi^2 (df=2, p < 0.05) = 8.33$) but not for the remote group ($p = 0.20$). Post hoc chi square tests demonstrated that the local group

approached significance post-motor learning as compared to baseline ($p = 0.083$) and was significant at retention as compared to baseline (χ^2 (df=1, $p < 0.005$) = 9.00).

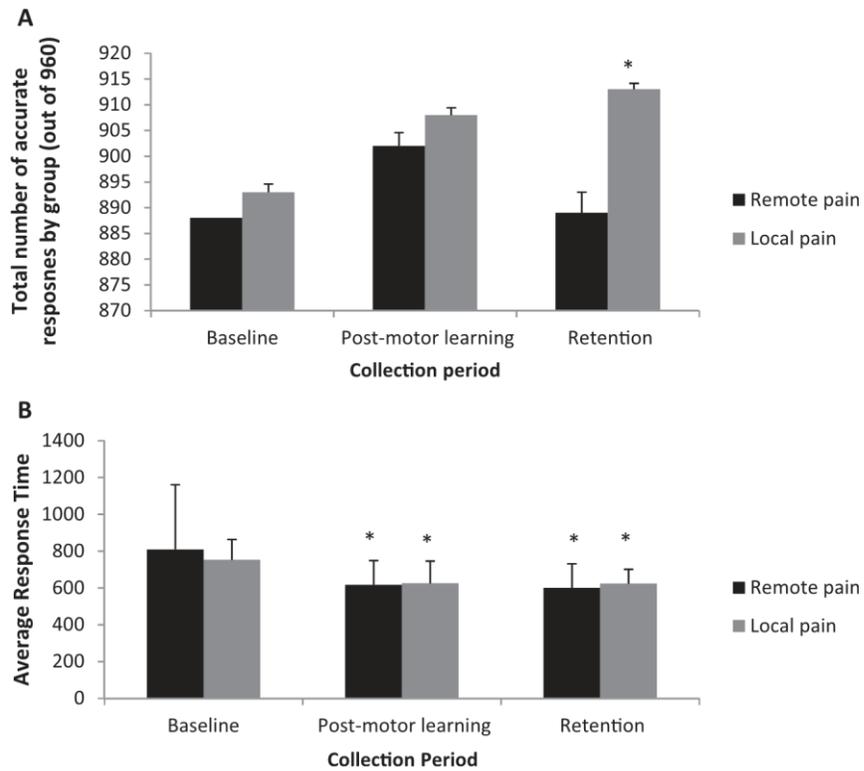


Figure 9: Behavioural measures for *Experiment 2*. A: bar-graph depicting the total number of accurate responses by group. Significant differences are indicated by asterisks demonstrating that the local pain group had improved accuracy at retention as compared to baseline. B: bar-graph depicting the average response time pre and post-motor learning for the local and remote groups. Significant differences for both groups are indicated by asterisks. Error bars represent the standard deviation.

Response Time

There was a significant decrease in mean response time over time [$F(1, 23) = 8.44, p < 0.005$].

Participants in the local group demonstrated a decrease from M 752.9 msec SD 110.7 to M 624.8 msec SD following motor learning (a 17.0 % decrease), and then further decreased to M 623.3 msec SD 121.0 at retention (a 0.24 % decrease). Participants in the remote group demonstrated a decrease from M 809.0 msec SD 351.6 to M 617.3 msec SD 131.5 following motor learning (a 23.7% decrease), and then decreased further to M 599.5 msec SD 131.4 at retention (a 2.9% decrease) (see Figure 9B). Post hoc tests comparing post-motor learning and retention response time data to baseline values demonstrated that significant changes in response time occurred from baseline to post-motor learning ($p < 0.005$) and from baseline to retention ($p < 0.001$), but did not differ significantly from post-motor learning to retention ($p = 0.63$). The interaction effect of TIME by GROUP was not significant ($p = 0.49$).

9.1.4 Discussion

Remote pain versus local pain: electrophysiological measures

SEP peaks: P25

The P25 peak was significantly decreased following the local capsaicin intervention in *Experiment 2*. It is hypothesized that this peak reflects activity in the pyramidal cells of area 3b [77] and it is thought that cerebellar-induced SEP peak changes are found within the 3b area of the SI [72]. The decrease observed following the local capsaicin intervention (*Experiment 2*) is indicative of the role that the SI and the cerebellum play in somatosensory processing. Pain fibers project to the SI and may produce inhibition of MI via thalamocortical or cortico-cortical inhibitory inputs.

While Knecht et al. [223] and Sörös et al. [43] demonstrated cortical reorganization with acute experimental pain in healthy participants, most of the literature on SEP peaks and pain have been

conducted on participants who are in chronic pain or have utilized an experimental muscle pain model. Tinazzi et al. [56] and Tinazzi et al. [224] found significantly increased SEP peaks in individuals who were in chronic pain. In contrast, studies using experimental muscle pain [74, 235] and the current study demonstrated decreases in early SEP peaks in healthy individuals suggesting that there may be opposing effects of acute versus chronic pain on SEP peak amplitudes.

Research demonstrates that the cerebellum responds to nociceptive input as fMRI studies show activation in the cerebellum in response to pain [214, 242]. Discriminating sensory information significantly increases cerebellar activation and therefore the cerebellum is hypothesized to play a role in somatosensory processing [418]. The pain-induced gating of the P25 demonstrated by the current study is in line with top down regulation of cortical systems implicated in pain processing [419].

The interactive effect of pain and motor learning: electrophysiological measures

SEP peaks:

SEP peaks: N11 and N13

The N11 peak represents the afferent input as it enters the spinal cord and starts to ascend towards the cuneate nucleus [56] while the N13 peak is thought to demonstrate the activity of inhibitory interneurons in the dorsal horn [26, 56]. Evidence to support this comes from patients with multiple nerve root avulsions who lack N11 and N13 SEP peaks although the N9 SEP peak is preserved [55]. Our finding of significant increases in the N11 and N13 SEP peaks following motor learning for *Experiment 1* is in line with other studies that hypothesize that SMI occurs directly at the spinal cord level with sensory input having a direct effect on motor output through bifurcations [69] and with the findings of Andrew et al. [340] who observed significant increases

in the N13 SEP peak following 10 minutes of tracing and 10 minutes of repetitive typing. In *Experiment 2*, for the remote group, the same trends were noted with the N11 increasing by 19.0 % and the N13 increasing by 8.7 %, however, because the overall ANOVA comparing the local and remote groups was not significant, further post-hoc tests were not performed. The reason that the overall ANOVA was not significant was because in the local group there were minimal changes in the N11 and N13 (4.3% and 0.4 % respectively), indicating that the presence of local pain appears to inhibit the spinal cord changes normally observed in response to motor learning.

SEP peaks: N20

A widely distributed P14 potential is followed by N20 which is generated in the SI and is known to reflect the earliest cortical processing [49]. Our finding of a significant increase in the N20 SEP peak for the control group in *Experiment 1* is in line with the recent study by Andrew et al. [340] who found a significant increase in the N20 SEP peak following 10 minutes of tracing and 10 minutes of typing. The tasks used for the Andrew et al. [340] and the current study are more complex than the repetitive motor tasks used in other studies [20, 341, 363] that haven't found significant differences in the N20 SEP peak. fMRI Research has shown that a complex task results in an increase in the areas of activation when compared to a simple task [338]. The increase in the N20 SEP peak was only significant for the control group (*Experiment 1*) and we hypothesize that the acute pain in the intervention group may have negated an increase in the N20 SEP peak that would have otherwise occurred. This corroborates previous research whereby a motor learning task increased pressure pain thresholds in healthy participants [420] and Ferguson et al. [421] who suggested that plasticity associated with pain and with motor training share neuronal mechanisms that might interact. In addition, previous studies report that cortical

regions inhibit limbic regions during cognitive processes [422] and performing an attention-demanding task mitigates the impact of negative stimuli [383, 423-425].

SEP peaks: N30

The amplitude of the N30 peak was significantly increased following motor learning in both groups in *Experiment 1* and *Experiment 2*. Waberski et al. [71] utilized source localization to suggest that the MI is the N30 peak generator. Primate [82, 183], and human [81] intracortical recordings support that the N30 SEP peak is generated at the MI. In contrast, there are topographic [426, 427] and intracerebral [79, 428] studies which support that this peak is generated in the SI. Cebolla et al. [83] utilized swLORETA (standardized weighted Low Resolution Brain Electromagnetic Tomography) and found that the N30 SEP peak is produced through activation in the prefrontal cortex, as well as motor and premotor areas. Thus the N30 SEP plays a role in somatosensory processing and this is relevant to SMI. Significant increases in the N30 SEP peak following motor learning in both *Experiment 1* and *2* is in line with previous studies that found significant alterations in the N30 peak following motor activity [198, 363], with our previous research [20], and that of Andrew et al. [340].

The effect of pain on motor learning: behavioural measures

Behavioural Data

Experiment 1 and *2* demonstrated that there were significant decreases in response time for all groups, and therefore motor learning acquisition occurred. For *Experiment 1*, post hoc testing demonstrated that the significant improvement occurred for the control group but not for the intervention group. However, accuracy differed by group at baseline with the intervention group outperforming the control group. Baseline accuracy performances were different with the intervention group having greater accuracy, and therefore the intervention group may have

achieved performance saturation. In order to assess the sensitivity of a learning task, you can utilize the challenge point framework [285]. Guadagnoli et al. [285] propose that as the difficulty of the learning task increases the success will decrease, and that when the difficulty of a task is appropriate, the optimal challenge point is determined. In the design of future motor learning tasks the optimal challenge point should be determined so that task difficulty may be set to ensure adequate sensitivity. If the challenge point is not met as has occurred in the current study a type 2 error may occur.

For *Experiment 2*, accuracy improved in the local pain group, with post hoc testing indicating that the local group improved significantly at retention as compared to baseline. Previous research has shown motor learning deficits with acute pain in humans [15, 16] and animals [396, 397]. Results from *Experiment's 1* and *2* imply that there may be differing results of local versus remote pain on motor learning and are in line with our previous research demonstrating improved accuracy following capsaicin application [20]. These findings contrast to those of Boudreau et al. [9] who found decreased tongue motor performance with topical capsaicin to the tongue in comparison with a control group. The Boudreau et al. [9] results can be explained by altered performance of the learning task throughout the period of painful input, as pain has been shown to have no effect if the quality of movement is maintained [413].

Plasticity of the MI can be facilitated through attention [372-375] and attention can also influence cortical plasticity changes in the SI [429]. Motor learning depends on attentional resources [376, 377]. Forebrain cholinergic function is involved in attention [372] and its disruption impairs motor learning [373]. Research has demonstrated that there are disruptions in neuroplasticity when participants focused attention to a limb that wasn't involved in the motor learning task [375], and increased attention to the limb undergoing training can increase

neuroplasticity [374]. We suggest that improved retention during local pain versus remote pain (*Experiment 2*) and remote pain versus control at baseline (*Experiment 1*) is due to attention to the limb undergoing learning.

9.1.5 Conclusion

This study provides evidence for early SEP peaks as markers for SMI and acute pain. Motor performance was better in the presence of pain at baseline (*Experiment 1*) and motor learning retention improved with local pain (*Experiment 2*). The development of a motor learning task with lower accuracy at baseline in order to prevent learning saturation is an important course for future experiments. The current study helps in the understanding of how acute pain affects motor learning and how local versus remote pain affects motor learning. This has important consequences for rehabilitation and exercise training.

Limitations:

The C5 spinous process was referenced to the trachea while all other electrodes were referenced to the ipsilateral earlobe. However, the far-field N18 SEP complex is best recorded referentially from scalp electrodes ipsilateral to the stimulated nerve, with a non-cephalic reference electrode [51]. Recording N18 from the contralateral scalp recording electrode with a cephalic reference electrode is likely to cancel out most of this signal, and therefore changes to the N18 SEP peak cannot be ruled out with the setup we utilized.

Acknowledgements:

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are listed and all have contributed substantially to the manuscript.

Preface to Manuscript 2:

The first study of this thesis consisted of two experiments where we determined the interactive effects of acute pain versus control (*Experiment 1*) and local versus remote acute pain (*Experiment 2*) on motor learning and sensorimotor processing. This study utilized a typing task and provided supportive evidence for early SEP peaks as markers for SMI and acute pain. A limitation of this first study was that accuracy was too high and therefore performance saturation occurred. In order to address this limitation a more complex tracing task was utilized for the subsequent studies. The second study investigated whether participants performing a motor learning acquisition task combined with acute pain would exhibit improved accuracy pre-motor learning acquisition, post-motor learning acquisition and at retention when compared to a control group and whether a novel motor learning acquisition task performed during acute experimental cutaneous pain (capsaicin group) as compared with a control group would show differential changes in SEP peak amplitudes.

9.2 Manuscript 2: The interactive effect of acute pain and motor learning acquisition on sensorimotor integration and motor learning outcomes.

The interactive effect of acute pain and motor learning acquisition on sensorimotor integration and motor learning outcomes

Author(s): Erin Dancey, Bernadette Murphy, Danielle Andrew, Paul Yelder

Affiliation(s): University of Ontario Institute of Technology
Faculty of Health Sciences
Oshawa, ON Canada
L1H 7K4

Corresponding Author Address: Bernadette Murphy, University of Ontario Institute of Technology,
2000 Simcoe Street North, Oshawa, Ontario, Canada.

Email: Bernadette.Murphy@uoit.ca

Telephone: (905) 721-8668 x 2768

Fax: (905) 721-3179

9.2.1 Abstract

Previous work has demonstrated alterations in early somatosensory evoked potentials (SEPs) when motor learning acquisition occurred in the presence of acute pain, however the learning task was insufficiently complex to determine how these underlying neurophysiological differences impacted learning acquisition and retention. To address this limitation, we have utilized a complex motor task in conjunction with SEPs. Two groups of twelve participants (N=24) were assigned randomly to either a capsaicin (capsaicin cream) or control (inert lotion) group. SEP peak amplitudes were obtained at baseline, post-application of the creams, and following motor learning acquisition. Participants completed a motor learning acquisition task followed by a pain-free retention task within 24-48 hours. Following motor learning acquisition, the amplitude of the N20 SEP peak significantly increased ($p<0.05$) and the N24 SEP peak significantly decreased ($p<0.001$) for the control group while the N18 SEP peak significantly decreased ($p<0.01$) for the capsaicin group. The N30 SEP peak was significantly increased ($p<0.001$) following motor learning acquisition for both groups. The P25 SEP peak decreased significantly ($p<0.05$) following the application of capsaicin cream. Both groups improved in accuracy following motor learning acquisition ($p<0.001$). The capsaicin group outperformed the control group pre-motor learning acquisition ($p<0.05$), following motor learning acquisition ($p<0.05$), and approached significance at retention ($p=0.06$). Improved motor learning in the presence of capsaicin supports the augmentation of motor learning while in acute pain. Furthermore, the changes in SEP peak amplitudes corroborates evidence that SEP amplitude alterations reflect neurophysiological alterations accompanying both motor learning and mild acute pain.

New and noteworthy:

Enhanced learning was found when motor skill acquisition took place in the presence of acute capsaicin-induced experimental pain, indicating that pain does not always have negative effects on motor learning, a finding relevant for rehabilitation and skill training. Differential changes in somatosensory evoked potentials (SEPs) were seen between those that performed the motor skill acquisition during pain vs control, indicating that SEPs may serve as markers for the early neuroplastic changes accompanying motor learning.

KEYWORDS

Somatosensory evoked potentials (SEP); motor learning; acute pain; sensorimotor integration (SMI)

9.2.2 Introduction

Within rehabilitation programs, the concurrent presentation of pain and motor deficits are ubiquitous. Typically, motor deficits are regarded as a consequence of movement related pain, however, there is evidence that pain affects motor control and has the ability to negatively influence the neuroplasticity associated with motor output [12-14]. While the presence of acute pain during motor learning may interfere with skill acquisition [9, 15, 16], our recent studies [20, 430] demonstrated that motor learning acquisition improved in the presence of acute pain. A limitation of previous work [19, 430, 431] is that learning saturation occurred with these typing tasks as baseline accuracy was high. If the learning task difficulty is not high enough, differences between groups may not be observed, and a type 2 error may be likely [19, 430]. To address this we developed and validated a more difficult motor tracing task. This tracing task has been used by Holland et al. [339] who demonstrated continued motor learning acquisition throughout the training period with a significant consolidation of motor performance at retention and by Andrew et al. [431] who showed that the tracing task was a more effective learning instrument than a typing task. The application of a complex motor tracing task combined with behavioural and electrophysiological outcomes will allow us to examine the impact of acute pain on motor learning as well as the cortical, subcortical, and cerebellar regions involved.

Motor learning acquisition requires sensorimotor integration (SMI) which is the processing of somatosensory information received from the motor task and integrating this information with the motor command in order to fine tune and improve motor task performance. Early somatosensory evoked potentials (SEPs) are electrical field potentials produced by neurons and are induced by electrical stimulation of their receptors [49]. SEPs reflect pre-cognitive sensory processing [26], and can be used to study the neuroplastic outcomes of the interactive effects of acute pain and motor learning acquisition. SEPs offer the highest temporal

resolution available in non-invasive research [432] and include peripheral (N9), spinal (N11, N13), subcortical (N18) and cortical (N20, P25, N24, N30) peaks for the upper limb. Recent work has found significant changes in spinal and cortical SEP peaks following tracing [431] and typing tasks [430]. Studies using experimental muscle pain [74, 235] and acute cutaneous pain [430] have found decreases in early SEP peak amplitudes in healthy individuals. Additionally, we recently determined that following a motor learning acquisition typing task there was a significant increase in a cortical (N20) SEP peak for a control group that was not observed for a capsaicin-induced pain group and we hypothesized that acute pain may have negated a change that would have otherwise occurred [430]. It has been proposed that motor learning acquisition can reverse the effects of pain, and conversely that acute pain undermines the capacity for learning [421]. There is a gap in the understanding of whether early SEP peaks change in the presence of acute experimental cutaneous pain in healthy humans and whether acute pain impacts SEP changes observed in response to a complex motor learning acquisition task, which will be addressed by the current study.

Another limitation of several previous studies is that they have not measured retention even though it is known that an offline or consolidation period is a critical process for learning [9, 20]. A few studies have examined the impact of capsaicin application on retention utilizing motor adaptation [18] or reaching [21] tasks and found that acute pain during motor learning acquisition had a negative impact on retention despite not having a negative impact on baseline performance measures [21] or acquisition [18]. More recently, Bilodeau et al. [17] investigated the effect of heat pain on motor sequence learning using the fingers and found that acquisition and retention were unaffected by acute pain during the acquisition stage. In addition, our recent work [430] found improved retention for a local pain group as compared to a remote pain group.

This provides support for improved motor learning retention with mild acute pain and we hypothesized that local acute pain increased attention to the body part utilized in motor learning acquisition [430]. Factors improving or decreasing motor learning acquisition are not necessarily predictive of motor retention [294, 295] and from a practical perspective, it is retention that indicates whether learning has been impacted positively or negatively. It is therefore important to investigate how retention is affected using a complex motor tracing paradigm.

The interactions between motor control and pain are complicated and only a few studies have examined the effect of acute experimental pain on motor learning acquisition and retention in healthy humans. Inducing acute experimental pain in healthy participants is therefore instrumental in isolating the motor consequences of acute pain and the conditions in which motor learning in conjunction with pain becomes either adaptive or maladaptive. We investigated the primary hypothesis that a motor learning acquisition task performed while in acute pain (capsaicin group) in comparison with a control group would demonstrate differential alterations in SEP peak amplitudes. Our secondary hypothesis was that participants performing a novel motor learning acquisition task while in acute pain would have improved accuracy pre-motor learning acquisition, post-motor learning acquisition and at retention when compared to a control group.

9.2.3 Methods

Methods Overview:

Two groups of twelve participants, [14 males, 10 females; aged 19 – 27 (M 20.3 SD 2.5)], were recruited from the student population at the University of Ontario Institute of Technology. Each participant filled out a health history form in order to identify any medical conditions which could impact normal somatosensation. This included: recent cervicothoracic injury, neurologic conditions, current use of medication, or currently suffering from chronic pain.

Written informed consent was attained for all participants and the study was approved by the University of Ontario Institute of Technology Research Ethics Board. This study was performed according to the principles set out by the Declaration of Helsinki for the use of humans in experimental research.

Acute experimental pain was induced by applying capsaicin cream and SMI was assessed through the use of SEPs in healthy humans. The effect of acute pain on the amplitudes of SEPs from baseline, at 20 minutes post-application, and following the motor learning task (35 minutes from baseline) was investigated (See Figure 1 for a schematic illustration of the protocol). Prior to performing the motor learning acquisition task, participants in the capsaicin group received a topical application of capsaicin (0.075% Zostrix, New York, USA) while the control group received a topical application of control skin lotion (Life Brand, Shopper's Drug Mart, Ontario, Canada). The topical cream was applied to the lateral aspect of the right elbow.

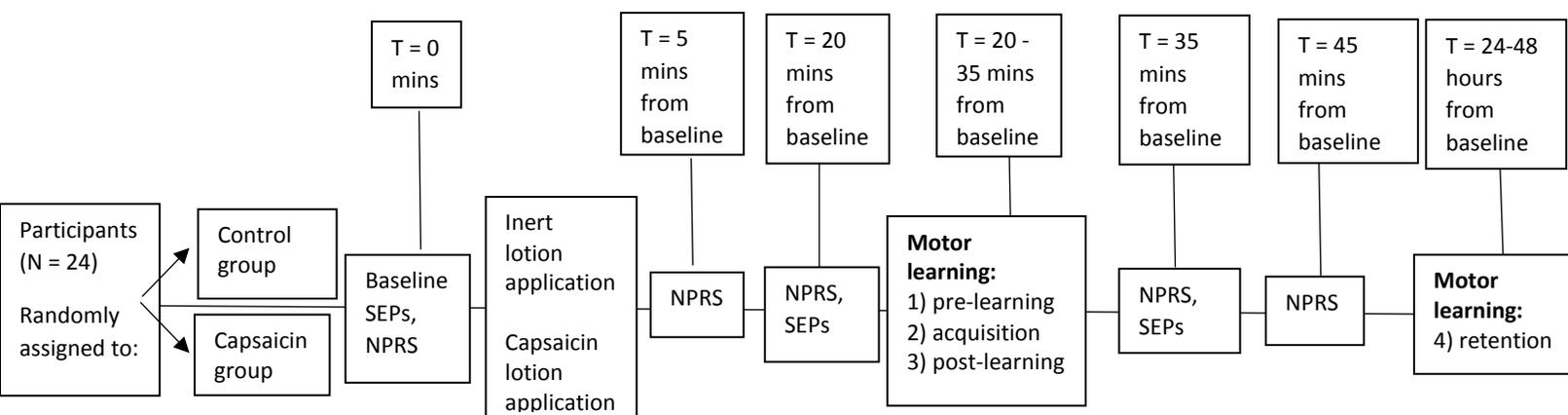


Figure 1: Schematic of the protocol.

Outcome Measures

The outcome measures for this experiment included motor learning accuracy, the amplitude (μV) of the early SEP peaks, and pain (Numeric Pain Rating Score).

Motor learning task:

The motor learning tracing task was run through a custom Leap Motion software tool (Leap Motion, Inc., San Francisco, CA). This task required participants to trace sequences of sinusoidal waves with varying amplitude and frequency with their thumb on a wireless touchpad (Logitech, Inc., Fremont, CA) and included a pre-motor learning acquisition test, a motor learning acquisition phase, a post-motor learning acquisition test and a retention test 24-48 hours later. The pre-motor learning acquisition, post-motor learning acquisition, and retention tests were four minutes long while the motor learning acquisition phase (that occurred between the pre-motor learning acquisition and post-motor learning acquisition tests) was 15 minutes long. The traces consisted of a series of dots and each trial consisted of a total of 500 dots. Each tracing task consisted of four sinusoidal patterns of varying frequency and amplitude, as

established previously [339]. Pre-motor learning acquisition, post-motor learning acquisition, and at retention, each of the versions, 1-4, were performed once; while for the motor learning acquisition phase each version was performed three times totalling 12 traces. The participants utilized the abductor pollicis brevis (APB) muscle as they were required to sweep their thumb from left to right in order to complete this motor learning task.

Pain:

Pain was measured using a Numeric Pain Rating Scale (NPRS) in which participants graded the intensity of their pain from 0–10 [416]. All participants rated their pain at baseline, post-application (5 minutes), post-application (20 minutes), following motor learning acquisition (35 minutes), and following the last round of SEP measurements (45 minutes).

Stimulation of median nerve to elicit SEPs

Ag/AgCl ECG conductive adhesive electrodes (MEDITRACE™ 130 by Ludlow Technical Products Canada Ltd., Massachusetts, USA) (impedance $<5\text{ k}\Omega$) were placed over the median nerve at the wrist of the dominant hand, with cathode proximal and delivered electrical stimuli 0.1ms in duration delivered at frequencies of 2.47Hz . Following the 5 minute 2.47 Hz session, stimuli were delivered at a frequency of 4.98Hz for 15 minutes. SEPs were recorded at two different rates in order to record the N24 and N30 SEP peaks. The rate of 2.47 Hz does not attenuate SEP peaks while the 4.98 Hz rate attenuates the N30 SEP peak, allowing us to identify the N24 SEP peak [46, 364]. The stimulus intensity was increased until motor threshold was attained and this was defined as the lowest stimulation intensity that evoked a visible muscle contraction of the APB muscle.

SEP recording parameters

SEP recording electrodes (1.8m long Traditional Grass™ Lead, 10mm disc, 2mm hole gold cup EEG electrodes, Grass Technologies, An Astro-Med, Inc. Subsidiary, Massachusetts, USA) (impedance <5 kΩ) were placed in accordance with the International Federation of Clinical Neurophysiologists (IFCN), using Grass Technologies EEG adhesive conducting paste (Type TEN20™). Recording electrodes were placed on the ipsilateral Erb's point, over C5 spinous process, the anterior neck (trachea), 2cm posterior to contralateral central C3/4 (a parietal site referred to as Cc'), and a frontal site (6cm anterior and 2cm contralateral to Cz) [74, 433]. The C5 spinous process was referenced to the anterior neck (trachea) and all other electrodes were referenced to the ipsilateral earlobe. A 1.8288m Traditional Lead, 10mm disc, 2mm hole gold cup EEG electrode was also used as a ground, and was placed in the mouth of participants. SEPs were assessed at baseline, 20 minutes post-application, and following the motor tracing acquisition task (45 minutes from baseline).

For each stimulation rate, 1000 sweeps were averaged using a Signal® configuration (Cambridge Electronic Design, England, UK). The SEP signal was amplified (Gain 10,000) and filtered (0.2-1000 Hz). We analyzed the peak-to-peak amplitude (μV) and latencies of the following SEP peaks: the peripheral N9, the spinal N11 and N13, the N18, the parietal N20 and P25, and the frontal N24 and N30 SEP peaks. SEP peak amplitudes were measured according to the IFCN guidelines [26] and were measured from the averaged traces beginning at the peak of interest to the preceding or succeeding peak [417]. For each of the SEP peaks, the latencies were measured from the time of stimulation to their maximal trough or peak.

Statistical Analysis

SEP peak amplitudes were normalized to baseline values to account for variability between participants and to allow for between participant comparisons. The Shapiro-Wilk test for normality was run on each of the SEP peaks. The main effect of interest was the interactive effect of pain and motor learning acquisition on SEP peak amplitudes which was tested using a repeated measures ANOVA with factors TIME (baseline versus post-motor learning acquisition) and GROUP (control versus capsaicin). In order to ensure that the observed interactions were due to the interaction of capsaicin and motor learning acquisition and not simply due to capsaicin application rather than the interactive effect, a separate repeated measures ANOVA with factors TIME (baseline versus post-application) and GROUP (control versus capsaicin) was performed on each SEP peak.

The Shapiro-Wilk test for normality was run on the accuracy data. To investigate and compare performance accuracy, a repeated measures ANOVA with factors TIME (pre-motor learning acquisition versus post-motor learning acquisition versus retention) and GROUP (control versus capsaicin) was performed on the accuracy data.

A Friedman test with pairwise comparisons was run on the capsaicin group NPRS ratings. IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp) was utilized for statistical analysis. Statistical significance was set at $p < 0.05$. Eta-squared was calculated in SPSS, as a measure of effect size with values of 0.01 representing a small effect size, 0.06 a medium effect size and 0.14 or greater, a large effect size [434].

9.2.4 Results

24 participants were tested with 12 participants in the capsaicin group [8 females, 4 males; aged 18-27 (M 20.8 SD 3.3)] and 12 participants in the control group [6 females, 6 males; aged 18-24 (M 22.8 SD 2.0)].

Neurophysiological data: SEPs

The N9, N30, and P25 SEP peaks were normally distributed. For the N11, N24 SEP peaks only the capsaicin group (post-application) was non-normally distributed. For the N13, N20 SEP peaks only the control group (post-motor learning acquisition) was non-normally distributed. For the N18 SEP peak only the capsaicin group (post-motor learning acquisition) was non-normally distributed. All other categories were normal. When only one set of measurements in a repeated measures design are non-normally distributed, it is recommended to still run an ANOVA which is robust against departures from normality [435], as conclusions drawn from the ANOVA will be accurate. That is, deviations in kurtosis will only affect power if the sample size is too low and type I and type II errors will not be likely if the data are skewed [435]. Therefore we conducted a repeated measures ANOVA on all SEP peaks.

Cerebellum: P25, N18, N24

P25:

Following motor learning acquisition, there was no main effect of TIME on the P25 SEP peak amplitude ($p=0.96$). Following the cream application, there was no main effect of TIME on P25 SEP peak amplitude ($p=0.22$), however, the interaction effect of TIME by GROUP was significant [$F(2,23) = 5.12, p<0.05, \eta^2=0.19$], with post-hoc ANOVA tests demonstrating that the capsaicin and control groups differed post-application [$F(1,11) = 5.93, p<0.05, \eta^2=0.35$] with the capsaicin group P25 SEP peak decreasing significantly by 15.3% following the application of

capsaicin cream [$F(1,11)=5.05$, $p<0.05$, $\eta^2=0.32$], while there was a non-significant 10.0% increase in the P25 SEP peak for the control group ($p=0.28$).

N18:

Following motor learning acquisition, there was a significant main effect of TIME [$F(2,23) = 5.66$, $p<0.05$, $\eta^2=0.21$], and a significant TIME by GROUP interaction effect [$F(2,23) = 7.09$, $p<0.05$, $\eta^2=0.25$]. Post hoc ANOVA tests demonstrated that the capsaicin and control groups differed following motor learning acquisition [$F(1,11)=5.86$, $p<0.05$, $\eta^2=0.35$] with the capsaicin group SEP peak significantly decreasing by 18.5% following motor learning acquisition [$F(1,11)=17.76$, $p<0.01$, $\eta^2=0.62$] while the control group showed a non-significant 1.7 % increase in the N18 SEP peak ($p=0.86$). Following the application of the creams, there was no main effect of TIME on the N18 SEP peak amplitude ($p = 0.59$).

N24:

Following motor learning acquisition, there was a significant TIME effect [$F(2,23) = 5.88$, $p<0.05$, $\eta^2=0.21$], and a significant interaction effect of TIME by GROUP [$F(2,23) = 98.92$, $p<0.005$, $\eta^2=0.29$]. Post hoc ANOVA tests demonstrated that for the N24 SEP peak the capsaicin and control groups differed following motor learning acquisition [$F(1,11)=8.14$, $p<0.05$, $\eta^2=0.42$], with the control group N24 SEP peak decreasing significantly by 28.9% following motor learning acquisition [$F(1,11)=52.47$, $p<0.001$, $\eta^2=0.83$] while the capsaicin group showed a non-significant 3.0 % increase in the N24 SEP peak ($p = 0.80$). Following the cream application, there was no main effect of TIME on N24 SEP peak amplitude ($p = 0.19$).

Primary Somatosensory area (SI): N20

Following motor learning acquisition there was a significant TIME effect [$F(2,23) = 4.42$, $p<0.05$, $\eta^2=0.17$], and a significant TIME by GROUP interaction effect [$F(2,23) = 4.42$,

$p < 0.05$, $\eta^2 = 0.35$]. Post hoc ANOVA tests demonstrated that the capsaicin and control groups differed following motor learning acquisition [$F(1,11) = 14.02$, $p < 0.005$, $\eta^2 = 0.56$] with the control group N20 SEP peak significantly increasing by 48.9% following motor learning acquisition [$F(1,11) = 11.32$, $p < 0.05$, $\eta^2 = 0.51$] while there was a non-significant 11.5% decrease in the N20 SEP peak for the capsaicin group ($p = 0.29$). Following the cream application for both groups, there was no main effect of TIME on N20 SEP peak amplitude ($p = 0.97$).

Sensorimotor integration (SMI) and the Motor cortex (MI): N30

Following motor learning acquisition there was a significant main effect of TIME [$F(2,23) = 23.84$, $p < 0.001$, $\eta^2 = 0.52$], while the interaction effect of TIME by GROUP was not significant ($p = 0.37$). Following motor learning acquisition the N30 SEP peak increased by 23.8% for the control group and by 16.2% for the capsaicin group. Following the application of the creams, there was no main effect of TIME on the N30 SEP peak amplitude ($p = 0.62$). For the N9, N11, and N13 SEP peaks no significant changes were seen for either group. There were no significant changes in latency data for any SEP peak in either the control group or the capsaicin group (See Table 2).

Figure 2 illustrates the raw data from a representational capsaicin participant indicating SEP peaks and Figure 3 illustrates the raw data from a representational control participant indicating SEP peaks. The normalized averages for the SEP peaks are illustrated in Figures 4 A and 4 B. Table 1 indicates the mean amplitudes of significant SEP peaks and their associated p -values. Table 2 indicates the mean latencies of the SEP peaks.

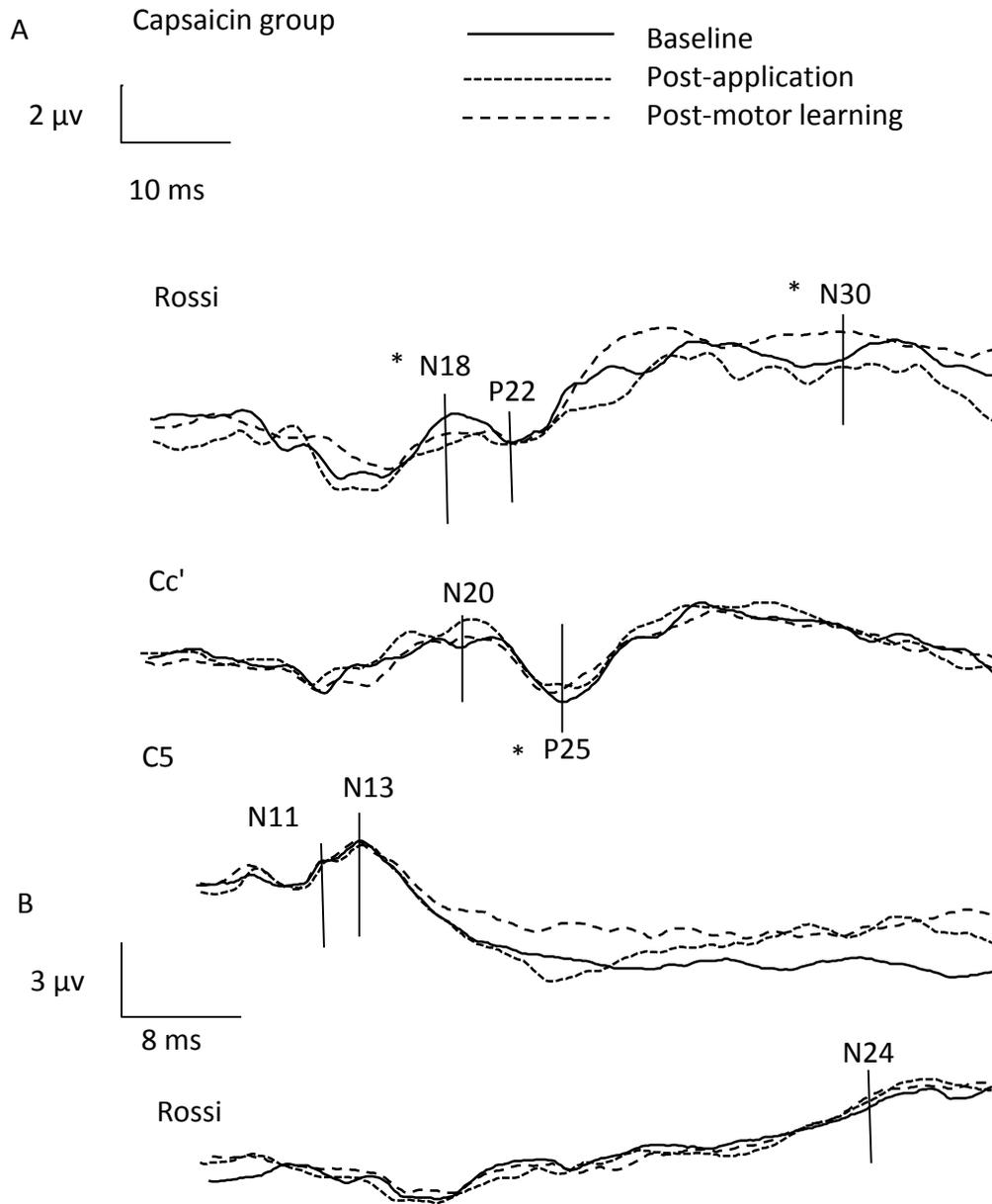


Figure 2: raw data from a representational capsaicin participant. Note the significant differences for the P25 SEP peak ($p < 0.05$) following capsaicin application and the N30 peaks ($p < 0.001$) and N18 ($p < 0.01$) post-motor learning acquisition as indicated by asterisks.

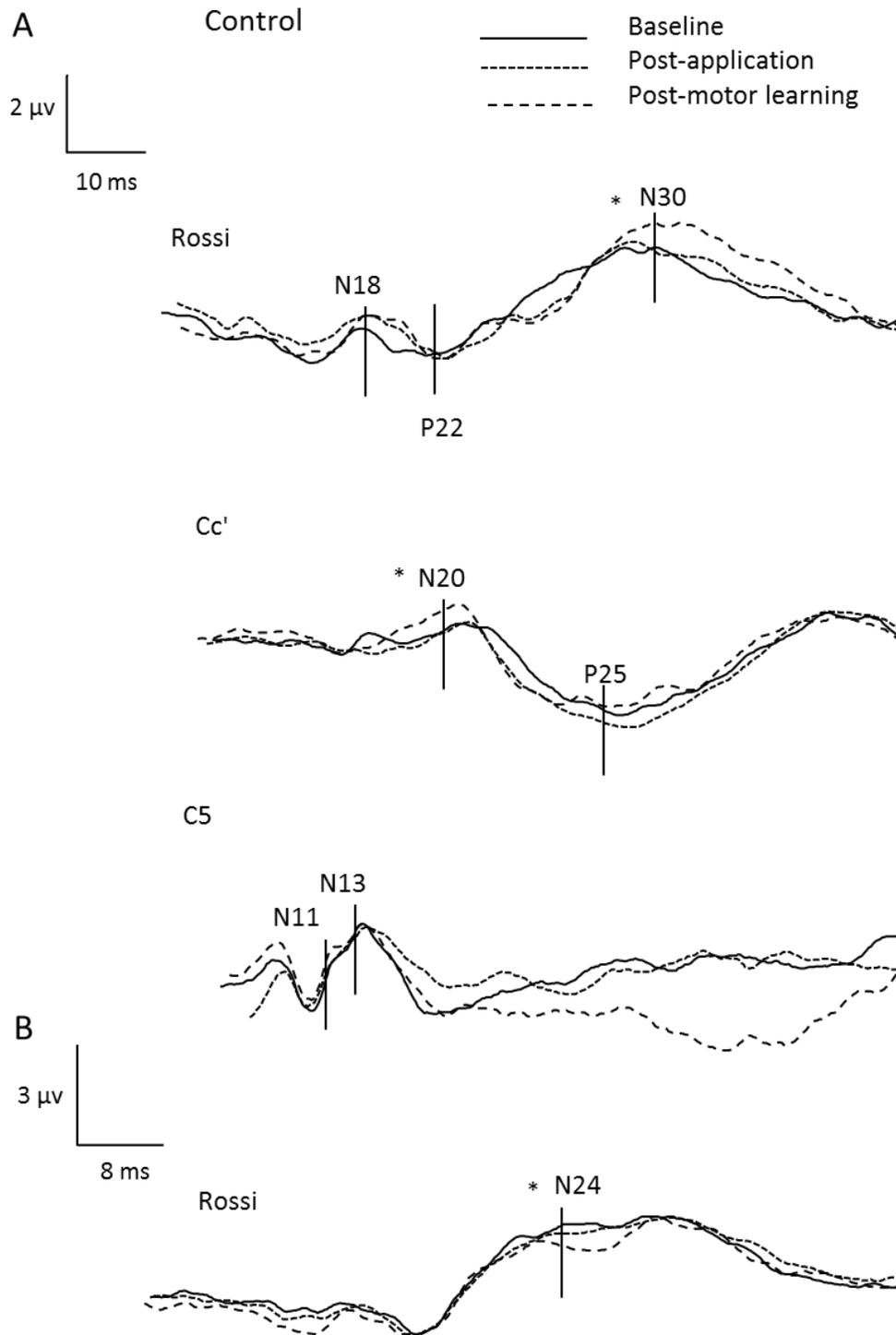


Figure 3: raw data from a representational control participant. Note the significant differences for the N20 ($p < 0.05$), N24 ($p < 0.001$), and N30 ($p < 0.001$) SEP peaks post-motor learning acquisition as indicated by asterisks.

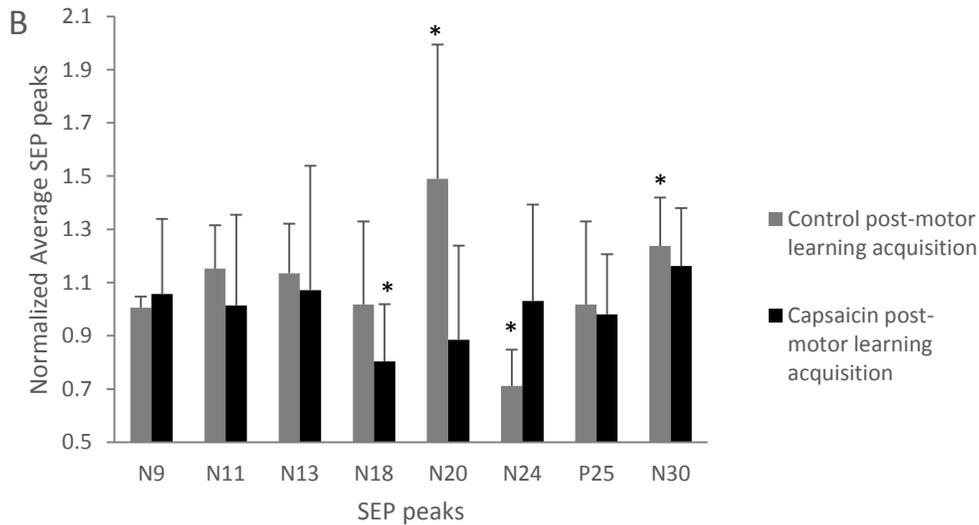
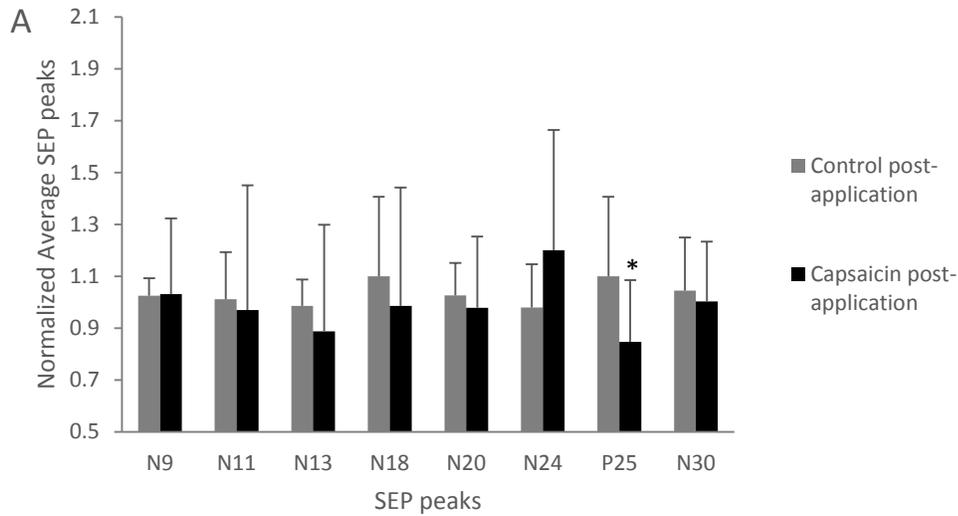


Figure 4: Bar-graph of averaged normalized SEP ratios showing capsaicin versus control groups post-application (A), and post-motor learning acquisition (B). A: No significant differences for the control group post-application while there was a significant decrease for the P25 SEP peak ($p < 0.05$) for the capsaicin group post-application B: Following motor learning acquisition, significantly different changes from baseline are indicated by asterisks for the N20 ($p < 0.05$), N24 ($p < 0.001$), and N30 ($p < 0.001$) SEP peaks for the control group and significantly different changes from baseline are indicated by asterisks for the N18 ($p < 0.01$) and N30 ($p < 0.001$) SEP peaks for the capsaicin group. Error bars represent the standard deviation.

SEP Peak	Group	Post-Application Mean	Post-application p-value	Post motor learning Mean	Post-motor learning p-value
P25	control	1.10 ± 0.31	0.28	1.02 ± 0.31	0.96
	capsaicin	0.85 ± 0.24	P<0.05	0.98 ± 0.23	0.96
N18	control	1.10 ± 0.31	0.59	1.02 ± 0.31	0.86
	capsaicin	0.99 ± 0.45	0.59	0.80 ± 0.25	P<0.01
N20	control	1.03 ± 0.13	0.97	1.49 ± 0.50	P<0.05
	capsaicin	0.98 ± 0.27	0.97	0.89 ± 0.35	0.29
N24	control	0.98 ± 0.17	0.19	0.71 ± 0.14	P<0.001
	capsaicin	1.20 ± 0.46	0.19	1.03 ± 0.36	0.80
N30	control	1.04 ± 0.20	0.62	1.24 ± 0.18	P<0.001
	capsaicin	1.00 ± 0.23	0.62	1.16 ± 0.22	P<0.001

Table 1: Significant SEP peak amplitude means +/- SD and p-values.

SEP peak	Control			Capsaicin		
	Pre-motor learning acquisition	Post application	Post-motor learning acquisition	Pre-motor learning acquisition	Post application	Post-motor learning acquisition
N9	10.1 ± 0.5	10.0 ± 0.6	10.2 ± 0.4	9.8 ± 0.6	9.7 ± 0.5	9.8 ± 0.7
N11	11.9 ± 0.7	12.0 ± 0.6	12.0 ± 0.5	11.6 ± 0.7	11.4 ± 0.7	11.5 ± 0.2
N13	13.2 ± 0.8	13.1 ± 0.7	13.3 ± 0.9	13.0 ± 0.6	13.1 ± 0.7	13.1 ± 0.4
N18	18.1 ± 0.4	18.3 ± 0.6	18.4 ± 0.4	17.8 ± 0.9	17.7 ± 1.1	17.4 ± 1.0
N20	20.1 ± 0.7	19.9 ± 0.6	20.4 ± 0.8	19.3 ± 0.9	19.2 ± 1.1	19.5 ± 1.0
N24	23.7 ± 0.6	23.5 ± 0.8	23.6 ± 0.7	24.1 ± 0.8	23.9 ± 0.9	23.8 ± 0.8
P25	24.9 ± 1.0	24.4 ± 1.3	25.0 ± 1.1	25.2 ± 0.8	25.5 ± 1.1	25.4 ± 0.7
N30	31.2 ± 0.8	31.4 ± 0.9	31.1 ± 1.1	30.4 ± 1.2	30.8 ± 1.1	30.5 ± 1.1

Table 2: Mean SEP latencies +/- SD for each peak.

Behavioural data:

Accuracy

The Shapiro-Wilk test for normality demonstrated that both groups at all time points were normally distributed. The behavioural data demonstrates that motor learning occurred as both the control [$F(1,11)=79.193$, $p<0.001$, $\eta^2=0.88$] and capsaicin [$F(1,11)=12.42$, $p<0.001$, $\eta^2=0.51$] groups improved in accuracy. The interaction effect of TIME by GROUP was significant [$F(2,23)=6.28$, $p<0.05$, $\eta^2=0.51$], with post-hoc ANOVA testing demonstrating that both pre-motor learning acquisition (which occurred after the capsaicin cream had already been applied) [$F(2,23)=8.32$, $p<0.05$, $\eta^2=0.36$] and post-motor learning acquisition [$F(2,23)=9.49$, $p<0.05$, $\eta^2=0.58$] the capsaicin group was more accurate than the control group. For the retention session the capsaicin group outperformed the control group and this approached significance ($p=0.06$) (See Figure 5). Post-hoc ANOVA tests on the percent change in motor error demonstrate that there wasn't a significant difference between the groups following motor learning acquisition ($p=0.31$), however the groups differed significantly from each other at retention ($p=0.036$) with the control group showing a 70.5% decrease in motor error and the capsaicin group a 46.0% decrease in motor error at retention relative to pre-motor learning acquisition values.

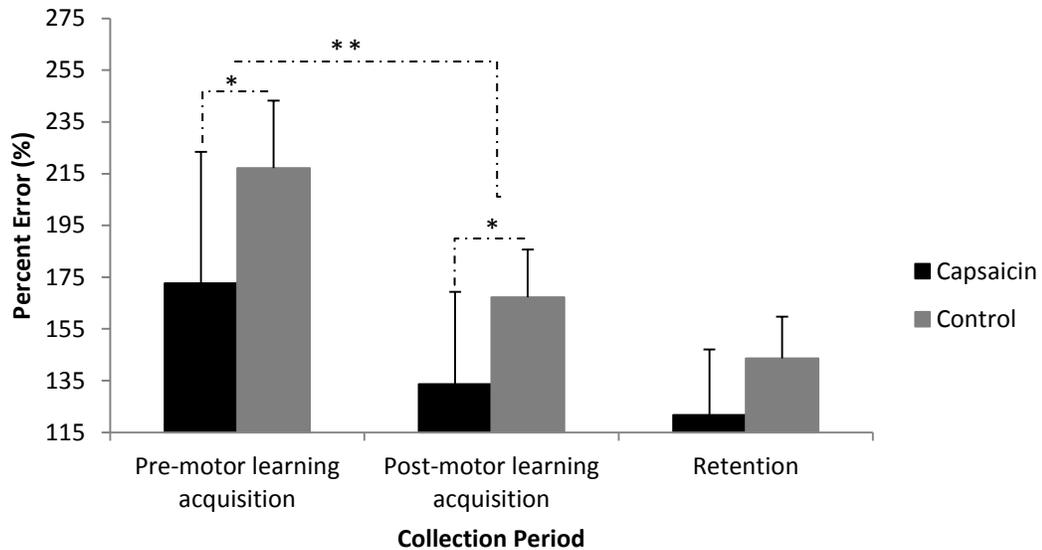


Figure 5: Bar-graph depicting the percent error by group. Both groups improved in accuracy following motor learning acquisition ($p < 0.001$) as indicated by a double asterisk. The capsaicin group outperformed the control group pre-motor learning acquisition ($p < 0.05$) and post-motor learning acquisition ($p < 0.05$) as indicated by asterisks. Error bars represent the standard deviation.

Pain ratings:

The Friedman's test on the NPRS ratings demonstrated a significant effect for the capsaicin group [$\chi^2 (df = 4, p < 0.001) = 39.4, \eta^2 = 0.69$], with pairwise comparisons indicating that from baseline there was a significant increase in NPRS ratings 20 minutes post application ($p < 0.001$), post motor learning acquisition ($p < 0.001$), and post motor learning acquisition (45 minutes from baseline) ($p < 0.05$). The increase 5 minutes post-application of the cream was not significant ($p = 0.27$). The average NPRS ratings are illustrated in Figure 6. None of the participants in the control group reported any pain.

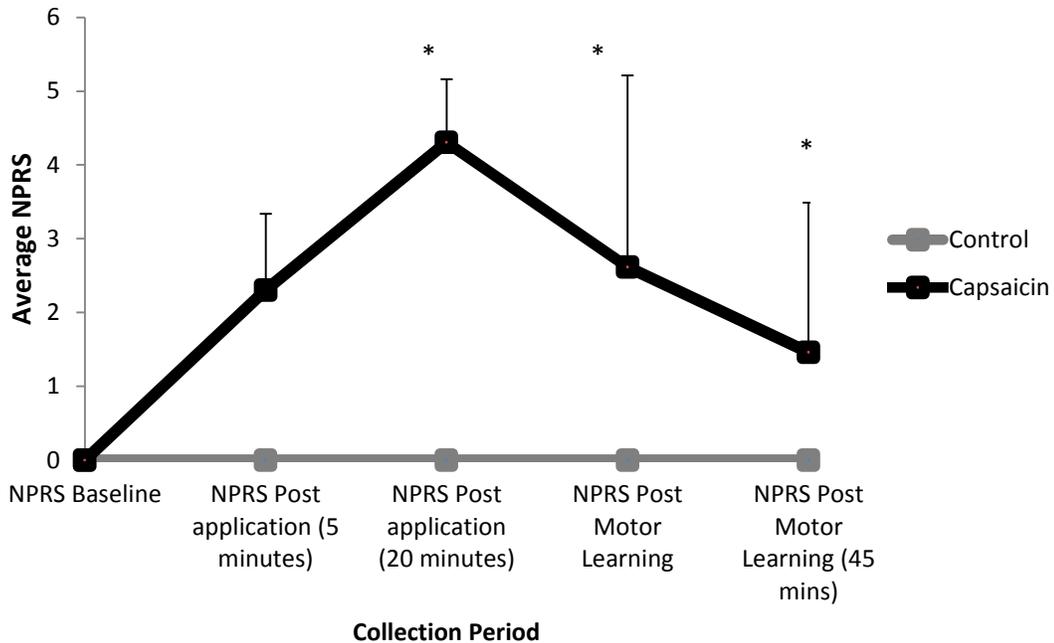


Figure 6: Line-graph depicting averaged NPRS ratings of participants in the control and the capsaicin groups. Significant differences post application for the capsaicin group ($p < 0.001$) relative to baseline are indicated by asterisks. Error bars represent the standard deviation. Error bars represent the standard deviation.

9.2.5 Discussion

Our findings corroborate our hypothesis of differential changes in early cortical SEP peaks evoked following motor learning acquisition as we observed a decrease in the N18 SEP peak for the capsaicin group, whereas the control group had an increase in the N20 SEP peak and a decrease in the N24 SEP peak following motor learning acquisition. In addition, there was an increase in the N30 SEP peaks for both groups following motor learning acquisition and we found a significant decrease for the P25 SEP peak following the capsaicin intervention. There were significant differences in SEP peaks that represent activity in several pathways related to motor control including the SI (N20), cerebellum (N18, N24, P25), and MI (N30) and this highlights the role of these structures in motor learning acquisition and pain processing. Significant improvements in accuracy were observed for both groups and we can therefore

conclude that motor learning acquisition has occurred. We observed significantly greater accuracy for the capsaicin group (who performed their initial pre-motor learning acquisition session in the presence of pain) when compared with the control group. In absolute terms, the capsaicin group continued to outperform the control group following motor learning acquisition, with a strong trend at retention, however in relative terms, the control group actually experienced a greater percent learning following motor learning acquisition. This highlights the interactive effect of pain on the extent of improvement. This is in line with our secondary hypothesis that participants performing a novel motor learning acquisition task during acute pain would demonstrate improved accuracy following motor learning acquisition when compared to a control group.

Neurophysiological Data:

Primary somatosensory area (SI): N20

The N20 reflects the earliest cortical processing within the SI [49] and responds to contralateral tactile stimuli [67]. Our finding of a significant increase in the N20 SEP peaks for the control group following motor learning acquisition highlights the role of the SI in motor learning acquisition. This is corroborated by a recent study [431] that demonstrated a significant increase in the N20 SEP peak following 10 minutes of tracing and 10 minutes of typing and it corroborates our previous work in which we found a significant increase in the N20 SEP peak for a control group following a typing task [430]. The task used for the Andrew et al. [431] study and the current study is more complex than the typing tasks used in previous work [20] that did not find an increase in the N20 SEP peak. A functional magnetic resonance imaging (fMRI) study found that a complex motor task results in an increase in the number of cortical areas that are activated when compared to a simpler task [338] and the number of overlapping cortical

areas that are altered with learning is greater with fine rather than gross motor learning [311].

Cerebellum: P25, N18, N24

The P25 SEP peak amplitude was significantly decreased following capsaicin application. This peak reflects the process of the activation of the cell body along the pyramidal cells of area 3b [77] and therefore cerebellar-induced SEP changes can be found within the 3b area of the SI [72]. The decrease in the P25 SEP peak following capsaicin application is indicative of the role that the SI and the cerebellum plays in somatosensory processing. This finding is in line with our previous work [19, 430] and with our finding of a significant decrease in the N18 SEP peak following motor learning acquisition for the capsaicin group. The N18 SEP peak originates in the brain stem, between the lower medulla and midbrain-pontine region (e.g. the dorsal column nuclei and/or the accessory inferior olives), reflects activity in the olivo-cerebellar pathways [50, 62] and has the potential to show alterations in cerebellar activation [63]. Imaging research demonstrates that there is a significant increase in cerebellar activity with the passive manipulation of a limb [323] and with tasks requiring sensory discrimination [418]. In addition, previous research suggests that the cerebellum processes nociceptive input as most fMRI studies demonstrate activation in the cerebellum in response to pain [214, 242]. Our finding of a significant decrease in the P25 SEP peak following capsaicin application and a decrease in the N18 SEP peak for the capsaicin group following motor learning acquisition supports the role that the cerebellum plays in pain processing, sensorimotor processing, and motor learning acquisition. This is interesting in light of the significant differences in the N20 and N24 SEP peaks following motor learning acquisition for the control group that was not observed for the capsaicin group. The N24 SEP peak shows alterations in cerebellar activity as it reflects activation of the pathway between the cerebellum and the SI [74]. Source localization

identifies the posterior wall of the central sulcus in area 3b of the SI as the source for the N24 SEP peak [71]. This area receives input from the cerebellar cortex and cerebellar nuclei [72]. We hypothesize that our finding of a significant decrease following motor learning acquisition for the N24 SEP peak demonstrates the role that the cerebellum plays in this cortical peak. Research demonstrates that the cerebellum is involved in motor learning acquisition [275, 436, 437] as animal studies demonstrated that motor training is correlated with increases in the number of synapses within the cerebellum [307-309] and plays a role in learning and motor adaptation in humans [299]. The cerebellum modifies extra-cerebellar output through inhibition from GABAergic neurons [298]. Imaging studies confirm that the cerebellum is activated with motor sequence tasks [298] and finger-tapping tasks [316-318]. The resulting increase in activation patterns can come with as little as 5-10 minutes [333] and are more pronounced if the task is novel [1]. Our finding is consistent with the work of Baarbé et al. [438] who demonstrated disinhibition of cerebellar projections to MI following a motor acquisition task and with a study in that found a significant decrease in the N24 SEP peak following motor learning acquisition [431].

We hypothesize that acute pain may have negated the alterations in cortical SEP peaks (N20 and N24) that occurs in the pain-free condition following motor learning acquisition. We hypothesize that the neuroplasticity associated with pain and motor learning acquisition share neural mechanisms and interact with each other [421]. This corroborates previous research that demonstrated that performing an attention-demanding task attenuates the impact of negative stimuli [383, 423-425] increases pressure pain thresholds in healthy participants [420] and suppresses the activity in limbic areas by the frontal cortex [422]. Pain fibers project to the SI

and may produce inhibition of the MI via thalamocortical or cortico-cortical inhibitory inputs [402].

Sensorimotor integration (SMI) and the motor cortex (MI): N30

The evidence demonstrates that the frontal N30 SEP peak reflects the activation within a complex network linking the thalamus, basal ganglia, premotor areas, and the MI [439, 440] and reflects SMI [74]. Primate [82, 183], and human [81] intracortical recordings led to the hypothesis that the N30 SEP peak is generated at the MI. In contrast, there are topographic [426, 427] and intracerebral [79, 428] studies which support that this peak is generated in the SI. Cebolla et al. [83] found that the N30 peak is produced through network activity in the MI as well as the premotor and prefrontal cortex through the use of swLORETA (standardized weighted Low Resolution Brain Electromagnetic Tomography). The amplitude of the N30 SEP peak was significantly increased following motor learning acquisition for both groups. Significant increases in the N30 peak following motor learning acquisition for both groups is in line with previous research that has shown significant changes in the N30 SEP peak following motor activity [198, 363] and motor learning acquisition [19, 430, 431].

Behavioural Data

Significant increases in accuracy were observed for both groups, and we conclude that motor learning acquisition has occurred. There was an effect of pain on the extent of improvement as the capsaicin group outperformed the control group significantly pre-motor learning acquisition, following motor learning acquisition, and approached significance at retention. Previous studies has shown motor learning deficits in conjunction with acute experimental pain in humans [9, 15, 16] and animals [396, 397]. We observed an increase in learning accuracy pre-motor learning acquisition (performed in the presence of capsaicin) and

following motor learning acquisition for the capsaicin group which is in line with our previous research [20, 430]. This work differed from our previous work [20, 430] as the current study utilized a different task (tracing versus typing) that was more complex and had lower baseline accuracy. It is significant that in the research demonstrating impaired acquisition the motor task evoked pain [9, 396] and therefore impacted their ability to perform the motor learning acquisition task. The painful stimulation used in the present study and our previous work [20, 430] and used by another study which demonstrated no impact of pain on motor learning acquisition and retention [17] induced cutaneous pain unrelated to movement. This may help to explain why there was not an adverse effect of pain on motor learning acquisition outcomes as acute pain typically elicits motor responses that protect from further damage which may impair motor learning acquisition [14].

This research suggests that the effects of pain on motor learning acquisition plasticity may differ depending on the type of pain (i.e. cutaneous versus muscle pain). Research indicates that the cortical maps of muscles affected by pain are modified in the sensory and motor systems and that pain and neuroplastic alterations can be reversed through motor learning acquisition [180]. The re-establishment of sensorimotor representations and reduced pain following motor learning acquisition are also in line with research involving sensory training in individuals suffering from phantom limb pain [181]. There is an interdependence of sensory and motor systems and the effects of motor learning on pain may be due to cortico-thalamic loops, producing inhibition on sensory systems. Although it has been hypothesized that pain may interfere with learning-induced neuroplasticity [9], other studies indicate that pain may improve motor performance and learning acquisition [20, 430] or have no effect if the quality of movement is maintained [18, 413].

Research demonstrates that neuroplasticity accompanying motor learning acquisition is altered by attention [372-375] as motor learning depends on attentional resources [376, 377]. We hypothesize that improved motor learning acquisition outcomes for the capsaicin group is due to attention to the arm undergoing motor learning [372-375]. Growing evidence demonstrates that affective processing is modulated by attention and cognitive regulation [379] and that stress leads to a narrowing of attention [380, 381] decreasing the processing of task-irrelevant stimuli [382]. Previous work has found that the application of tactile-proprioceptive noise improved sensorimotor performance [391] and one sensory modality (tactile noise) enhances the response of another sensory modality (visual evoked potentials) [392]. In addition, Passmore [395] had participants recreate Morse code patterns and determined that when paresthesia was present under transfer conditions, performance improved. These results indicate that a secondary stimulus may draw increased attentional resources toward discerning the meaningful stimulus [391, 392, 395]. Cognitive load studies confirm that under high load conditions, there is decreased activation in brain regions associated with emotion (amygdala) and increased activation in executive control areas (prefrontal cortex) [383-385].

9.2.6 Conclusion

This study provides confirmation of SMI areas in motor learning acquisition as demonstrated by significant differences in the N30 SEP peaks amplitude following motor learning acquisition for both groups, and for the N20 and N24 SEP peaks (control group) and the N18 SEP peak (capsaicin group). A significant decrease in the P25 SEP peak was found following the application of capsaicin cream demonstrating the effect of acute pain on SEP peaks. As there were significant differences in SEP peaks that represent activity in the cerebellum (N18, N24, P25), an important future experiment would be to investigate changes in excitability between the cerebellum and the MI following motor learning acquisition in the

presence of pain using TMS techniques. This will enable us to measure cerebellar inhibition [438] and to see if pain changes excitability in the cerebellum to MI pathway. In addition, the findings of improved motor learning acquisition during acute pain may be caused through increased attention or arousal during the painful stimulation. Therefore an important future direction is the comparison of the effects of local versus remote acute pain combined with a complex motor learning acquisition task. In addition, as pain can be viewed as a sensory perturbation that improves motor learning acquisition it would be interesting to explore whether motor learning acquisition in conjunction with tactile noise would also lead to significant differences in SEP peaks when compared with a control group. These results help to explain why activation of the motor system through therapeutic exercise (focusing on movement) can assist in decreasing pain. As motor learning acquisition is accompanied by pain in a multitude of settings, the effect of pain on motor learning and neuroplasticity is vital to consider to ensure that therapeutic interventions lead to adaptive and not maladaptive changes.

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Preface to Manuscript 3:

The second thesis study demonstrated improved motor learning acquisition with capsaicin providing support for the enhancement of motor learning while in acute pain. Furthermore, the changes in SEP peaks suggests that SEP peak alterations reflect neurophysiological alterations accompanying both motor learning acquisition and mild acute pain. The findings of improved motor learning acquisition during acute pain may have been the result of increased attention or increased arousal, and therefore we concluded that it was important to compare the effects of local versus remote versus contralateral acute pain in conjunction with a complex motor learning task. The third study investigated whether a novel motor learning task performed during local versus remote versus contralateral acute pain would show differential changes in early SEP peaks. I investigated whether participants performing a motor learning task during local and remote acute pain would have improved accuracy during motor learning acquisition and at retention when compared to the contralateral group due to increased attention to the limb performing the task.

9.3 Manuscript 3: The effect of local, remote, and contralateral tonic pain on motor learning and sensorimotor integration using a motor tracing task

THE EFFECT OF LOCAL, REMOTE, and CONTRALATERAL
TONIC PAIN ON MOTOR LEARNING AND SENSORIMOTOR
INTEGRATION USING A MOTOR TRACING TASK

Author(s): Erin Dancey, Bernadette Murphy, Paul Yelder

Affiliation(s): University of Ontario Institute of Technology
Faculty of Health Sciences
Oshawa, ON Canada
L1H 7K4

Corresponding Author Address: Paul Yelder, University of Ontario Institute of Technology, 2000 Simcoe Street North, Oshawa, Ontario, Canada.

Email: Paul.Yelder@uoit.ca

Telephone: (905) 721-8668 x 2768

Fax: (905) 721-3179

9.3.1 Abstract

Background: Previous work demonstrated improved learning and alterations in early somatosensory evoked potentials (SEPs) when motor learning acquisition occurred in the presence of tonic cutaneous pain, but less is known about how the location of pain affects the neuroplasticity of somatosensory processing and motor learning acquisition and retention. The aim of this experiment was to investigate the interactive effects of local (overlying the muscle performing the task) versus remote (body segment involved) versus contralateral (body segment not involved) acute experimental cutaneous pain on motor learning acquisition, retention, and sensorimotor processing.

Methods: Three groups of twelve participants (N= 36) were randomly assigned to either a local capsaicin group (capsaicin cream over thumb muscle), remote capsaicin group (capsaicin cream over dominant elbow) or contralateral capsaicin group (capsaicin cream over contralateral elbow). SEP amplitudes were collected at baseline, post-application of capsaicin cream, and following a motor learning acquisition task. Participants performed a motor tracing acquisition task followed by a pain-free retention task 24-48 hours later while accuracy data was recorded.

Results: The P25 ($p < 0.001$) SEP peaks significantly decreased following the application of capsaicin cream for all groups. Following motor learning acquisition the interaction effect of TIME by GROUP was significant for the N18 ($p < 0.05$) SEP peak with post hoc tests demonstrating that the N18 SEP peak differed significantly for the remote group ($p < 0.05$) but not for the contralateral ($p = 0.13$) or the local ($p = 0.89$) groups. The N30 ($p < 0.005$) SEP peaks were significantly increased following motor learning acquisition for all groups. The local, remote and contralateral capsaicin groups improved in accuracy following motor learning with no significant differences between the groups ($p < 0.001$).

Conclusion: The changes in SEP peak amplitudes demonstrates that early SEP alterations are markers of SMI accompanying acute pain and motor learning acquisition. Improved motor learning acquisition with capsaicin provides evidence for improved motor learning acquisition while in acute pain. There were no significant differences between the three groups in motor learning accuracy and we hypothesize that improved motor learning while in acute pain was due to an increase in arousal as opposed to increased attention to the limb performing the task.

KEYWORDS

Somatosensory evoked potentials (SEP); motor learning; remote pain; local pain

9.3.2 Introduction

Motor learning difficulties are a significant problem for many people undergoing rehabilitation but are usually regarded as a consequence of pain. Research has shown that pain has the ability to negatively influence the neuroplasticity associated with motor control [12-14] and can interfere with motor learning acquisition [9, 15, 16]. However, recent work demonstrated that local and remote acute pain did not have a negatively impact [17, 18] and can also improve motor learning acquisition [20, 430]. The acute tonic pain used in these studies [17, 20, 430] induced cutaneous pain that didn't impact movement which may help to explain why pain did not negatively influence motor learning acquisition. The discrepancies in the literature underscore the need for further research investigating different types of pain (incorporating pain unrelated to motor performance and remote versus local pain) in order to determine the impact of acute pain on motor learning acquisition, retention, and neuroplasticity. Therefore, a tonic pain model (capsaicin cream) that does not cause increased pain in response to specific movements was selected for this study. The topical application of capsaicin cream is a widely used experimental pain model [441-443] that elicits activation in C-nociceptors and induces central sensitization and an area of hyperalgesia [444, 445].

Research has established that the neuroplasticity accompanying motor learning acquisition is mediated by changes in attention [372-375]. In addition, attention demanding activities reduces perceived pain in individuals with chronic [446, 447] and acute [448, 449] pain while directing attention towards the acute pain increases the perceived pain intensity [450]. We hypothesized that improved motor learning acquisition while in remote acute pain (capsaicin applied to the elbow of the same arm performing the task) as compared to a control condition [19, 430] and with local pain (overlying the muscle performing the task) as compared to remote pain (elbow of

the arm performing the task) [430] was due to increased attention to the limb performing the task.

A limitation of previous work [20, 340, 430] is that learning saturation likely occurred with these typing tasks as baseline accuracy was high and we used a remote location on the same arm that performed the motor learning acquisition task. In order to address these limitations, we developed and validated a more difficult motor tracing task which was used in our most recent work [451]. Our work utilizing this tracing task demonstrated that a remote capsaicin group (applied to the elbow of the arm performing the task) outperformed a control group pre-motor learning acquisition, post-motor learning acquisition and approached significance at retention. As our findings of improved motor learning acquisition during acute pain [19, 430, 451] may have been caused through attentional mechanisms as capsaicin cream was applied to the same body segment performing the task, the comparison of local versus remote versus contralateral capsaicin application is important to consider and this is the aim of the current study.

Experimentally induced acute pain impacts neuronal properties and organization in the somatosensory area (SI) and motor cortex (MI) [43, 178]. The evidence suggests that there are differing effects of acute local versus remote acute pain on MI excitability. During local muscle pain, the amplitude of motor evoked potentials (MEPs) increase [260-262], decrease [6, 7, 258, 259, 263], or don't change [264]. While there are numerous studies which look at the effect of local versus remote pain on MI excitability, there are few studies that have examined how tonic cutaneous pain applied to remote versus local locations affect sensorimotor integration as measured by SEPs. Early SEPs are a measurement of sensory processing, and therefore provide a tool for assessing activation within the sensorimotor integration areas of the brain [26]. Studies using local acute experimental muscle pain [74, 235] and our previous studies that induced local

and remote cutaneous pain [430, 451] found decreases in early SEP peaks following an acute pain stimulus. There is a gap in the literature in terms of the response of SEP peaks to local versus remote versus contralateral acute tonic pain in healthy individuals which will be explored by the current study.

Recent work has found significant changes in SEP peaks following tracing [340, 451] and typing tasks [20, 430]. Our most recent work found differential changes in cortical SEP peaks for a control group that were not observed for the capsaicin group and we hypothesized that acute pain may have negated alterations that would have otherwise occurred following motor learning acquisition [19, 430, 451]. There is a gap in our understanding of the interactive effect of remote versus contralateral versus local acute tonic pain and motor learning on the response of SEP peaks in healthy humans, which will be investigated by this experiment.

We investigated the primary hypothesis that motor learning during local versus remote versus contralateral acute pain would show differential changes in early SEP peaks. Our secondary hypothesis was that participants performing a novel motor learning task during local and remote acute pain would have improved accuracy during motor learning acquisition and at retention when compared to the contralateral group due to increased attention to the limb performing the task. The results of this study may contribute to our understanding of how the location of acute pain may affect neuroplasticity during motor learning, and may provide insight as to how well the motor skill has been retained when acquired during acute pain.

9.3.3 Methods

Methods Overview:

Three groups of twelve participants, [(12 males, 24 females; aged 19 – 27 (M 21.2 SD 2.1)], were volunteers recruited from the student population at the University of Ontario Institute of Technology. We recruited a healthy population under 50 years (18-50 years) as peripheral

conduction velocities decrease after the age of 50 [452]. Each participant filled out a confidential health history form in order to identify any conditions which could impact somatosensation. This included neurologic conditions, recent cervicothoracic injury, medication use, and chronic pain.

The University of Ontario Institute of Technology Research Ethics Board approved this experiment and informed consent was obtained for all participants. This experiment was performed according to the principles set out by the Declaration of Helsinki for the use of humans in experimental studies.

SMI was assessed by recording early SEPs in humans and acute experimental pain was induced by applying capsaicin cream. The effect of acute pain and motor learning on signal transmission was assessed by investigating alterations in the amplitude of SEP peaks 20 minutes post-application, and then following the motor learning task (45 minutes from baseline).

Participants received a topical application of capsaicin (0.075% Zostrix, New York, USA) which was applied to a 50 cm² area and massaged into the skin. The capsaicin cream was applied either to the skin overlying the abductor pollicis brevis (APB) muscle (local capsaicin group), to the lateral aspect of the elbow of the dominant arm (remote capsaicin group), or the elbow of the non-dominant arm (contralateral capsaicin group).

Outcome Measures

The outcome measures for this study included the amplitude (μV) of the early SEP peaks, motor learning accuracy, and pain (Numeric Pain Rating Score).

Motor training task:

The tracing task was run through a custom Leap Motion software tool (Leap Motion, Inc., San Francisco, CA). This task required participants to trace sequences of sinusoidal-pattern waves with varying amplitude and frequency using their thumb on a wireless touchpad (Logitech, Inc.,

Fremont, CA) pre-motor learning acquisition, an acquisition phase, post-motor learning acquisition and a retention test 24-48 hours later. The pre-motor learning acquisition, post-motor learning acquisition, and retention tests were four minutes long while the acquisition phase was 15 minutes long. Pre-motor learning acquisition, post-motor learning acquisition, and at retention, versions, 1-4, were performed once. For the acquisition phase each version was performed three times totalling 12 traces. The traces consisted of a series of dots and each trial consisted of 500 dots. Each tracing task consisted of four pre-selected sinusoidal patterns of varying frequency and amplitude, as determined by prior research [339].

Pain:

Participants graded the intensity of their pain from 0–10 using a Numeric Pain Rating Scale (NPRS) [416]. Participants in all three groups rated their pain at baseline, post-application (5 minutes), post-application (20 minutes), following motor learning acquisition (35 minutes from baseline), and following the last round of SEP measurements (45 minutes from baseline).

Stimulation of median nerve to elicit SEPs

Ag/AgCl ECG conductive adhesive electrodes (MEDITRACE™ 130 by Ludlow Technical Products Canada Ltd., Massachusetts, USA) (impedance <5 kΩ) placed over the median nerve at the wrist of the dominant hand, with anode proximal. Electrical square pulses 1ms in duration were delivered at frequencies of 2.47Hz followed by followed by a session where the stimuli were delivered at a frequency of 4.98Hz. SEPs were recorded at two different rates in order to record both the N24 and N30 SEP peaks. The slower rate of 2.47 Hz does not attenuate SEP peaks while the faster rate (4.98 Hz) attenuates the N30 SEP peak, allowing for the identification of the N24 SEP peak [46, 364]. The stimulus intensity was increased until motor threshold was attained and this was defined as the lowest stimulation intensity that evoked

a visible muscle contraction of the APB muscle.

SEP recording parameters

SEP recording electrodes (1.8m long Traditional Grass™ Lead, 10mm disc, 2mm hole gold cup EEG electrodes, Grass Technologies, An Astro-Med, Inc. Subsidiary, Massachusetts, USA) (impedance <5 kΩ) were placed in accordance with the International Federation of Clinical Neurophysiologists (IFCN), with Grass Technologies EEG adhesive conducting paste (Type TEN20™). Recording electrodes were placed on the ipsilateral Erb's point, over the C5 spinous process, the anterior neck (trachea), 2cm posterior to contralateral central C3/4 (a parietal site referred to as Cc'), and a frontal site (6cm anterior and 2cm contralateral to Cz) [74, 433]. The C5 spinous process was referenced to the anterior neck (trachea) while all other electrodes were referenced to the ipsilateral earlobe. A 1.8288m Traditional Lead, 10mm disc, 2mm hole gold cup EEG electrode was also used as a ground, and was placed in the participant's mouth. SEPs were recorded at baseline, 20 minutes post-application, and following motor learning acquisition (45 minutes from baseline).

A total of 1000 sweeps per stimulation rate were averaged using a purpose written Signal® configuration (Cambridge Electronic Design, England, UK). The SEP signal was amplified (Gain 10,000) and filtered (0.2-1000 Hz). We analyzed the peak-to-peak amplitude (μV) and latencies (ms) of the following SEP peaks: the peripheral N9, the spinal N11 and N13, the far-field N18, the parietal N20 and P25, and the frontal N24 and N30 SEP peaks. SEP peak amplitudes were measured according to the IFCN guidelines [26] and were measured from the peak of interest to the preceding or succeeding peak of opposite deflection [417]. For each of the SEP peaks, the latencies were measured from the onset of stimulation to their peak or trough.

Statistical Analysis

SEP peak amplitudes were normalized to baseline to account for variability between participants and to allow for between participant comparisons. The Shapiro-Wilk test for normality was run on the SEP peak amplitude data. To explore the effects of pain location, a repeated measures ANOVA with factors TIME (baseline versus post-application) and GROUP (local capsaicin versus remote capsaicin versus contralateral capsaicin) was performed on each SEP peak. To explore the interactive effect of pain location and motor learning acquisition a repeated measures ANOVA with factors TIME (baseline versus post-motor learning acquisition) and GROUP (local capsaicin versus remote capsaicin versus contralateral capsaicin) was performed.

The Shapiro-Wilk test for normality was run on the accuracy data. To investigate accuracy, a repeated measures ANOVA with factors TIME (pre-motor learning acquisition versus post-motor learning acquisition versus retention) and GROUP (local capsaicin versus remote capsaicin versus contralateral capsaicin) was performed on the accuracy data.

For the NPRS measurements, a repeated measures ANOVA with factors TIME [baseline, post-application (5 minutes), post-application (20 minutes), post-motor learning acquisition (35 minutes), post-motor learning acquisition (45 minutes)] and GROUP (local capsaicin versus remote capsaicin versus contralateral capsaicin) was performed. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp).

Statistical significance was set at $p < 0.05$.

9.3.4 Results:

A total of 36 participants were tested. The local group (aged M 21.2 SD 2.2) consisted of 8 females, 4 males, the remote group (aged 20.3 SD 2.5) consisted of 8 females, 4 males, and the contralateral group (aged M 21.4 SD 2.4) consisted of 8 females, 4 males.

SEP peaks

The amplitude of the P25 SEP peak decreased following capsaicin application for all three groups. Following motor learning acquisition the N18 SEP peak decreased for the remote capsaicin group while the amplitude of the N30 SEP peaks increased significantly for all groups. The N13, N20, P25 and N30 SEP peaks were normally distributed. For the N11, N24 SEP peaks only the remote group (post-application) was non-normally distributed. For the N18 SEP peak only the contralateral group (post-application) and for the N9 SEP peak only the contralateral (post-motor learning acquisition) SEP peak was non-normally distributed. All other categories were normally distributed. When only one set of measurements in a repeated measures design are non-normally distributed, it is recommended to still run an ANOVA which is robust against departures from normality [435], as conclusions drawn from the ANOVA will be accurate. Therefore, type I and type II errors will not be increased if the data are skewed and deviations in kurtosis will only affect power if the sample size is too low [435]. Therefore we ran ANOVAs on all SEP peaks.

P25:

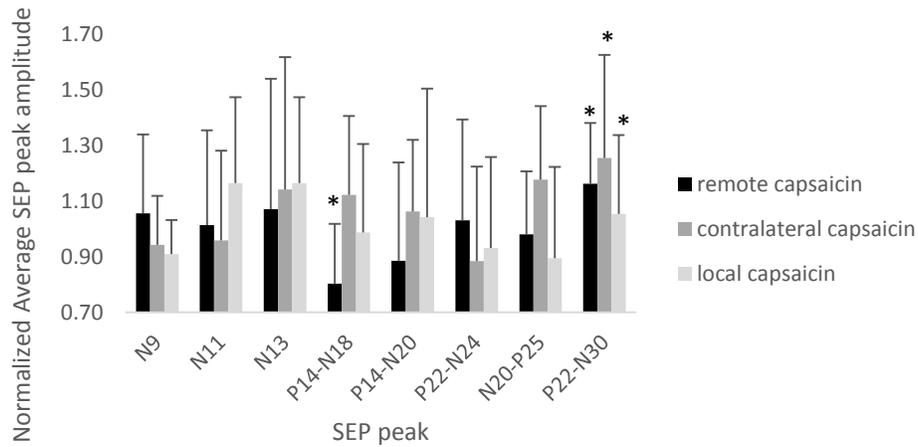
Following capsaicin application, there was a main effect of TIME on P25 SEP peak amplitude [$F(3,35) = 16.63, p < 0.01$], while the interaction effect of TIME by GROUP was not significant ($p = 0.76$). There was a 21.3% decrease in the P25 SEP peak following the local application of capsaicin cream, a 15.3 % decrease in the P25 SEP peak following the remote application of capsaicin cream and a 14.1 % decrease in the P25 SEP peak following the contralateral application of capsaicin. Following motor learning acquisition, there was no main effect of TIME on P25 SEP peak amplitude ($p = 0.30$).

N18: Following the application of the capsaicin creams there was not a significant effect of TIME ($p = 0.48$). Following motor learning acquisition, the effect of TIME was not significant ($p = 0.53$), however the interaction effect of TIME by GROUP was significant [$F(3,35) = 4.16$, $p < 0.05$]. Post hoc tests demonstrated that the N18 SEP peak decreased significantly by 18.7% following motor learning acquisition for the remote capsaicin group [$F(1,11) = 7.98$, $p < 0.05$] with a non-significant increase of 12.2% for the contralateral capsaicin group ($p = 0.16$) and a non-significant decrease of 1.2% for the local capsaicin group ($p = 0.89$).

N30: Following the application of the capsaicin creams, there was not a significant effect of TIME ($p = 0.59$). Following motor learning acquisition, there was a significant effect of TIME [$F(3,35) = 11.14$, $p < 0.005$], while the interaction effect of TIME by GROUP was not significant ($p = 0.23$). Following motor learning acquisition, a 5.4 % increase in the N30 SEP peak was observed for the local capsaicin group, a 16.2% increase in the N30 SEP peak was observed for the remote capsaicin group and a 25.4 % increase was observed for the N30 SEP peak for the contralateral capsaicin group.

The normalized averages for the peaks are illustrated in Figures 1. Figure 2 illustrates the raw data from a representational remote participant indicating cortical peaks.

B: Post-motor learning acquisition



A: Post-application SEP peaks

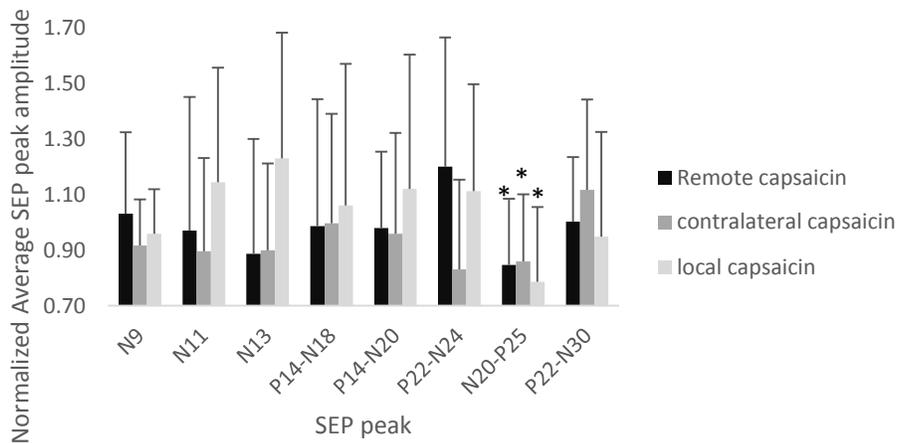


Figure 1: Bar-graph of averaged normalized SEP ratios showing capsaicin versus remote versus contralateral groups post-application (A), and post-motor learning acquisition (B). A: The P25 ($p < 0.001$) SEP peaks significantly decreased following the application of capsaicin cream for all groups as indicated by asterisks. B: Following motor learning acquisition, significantly different changes from baseline are indicated by asterisks for the remote group N18 SEP peak ($p < 0.05$) and for all three groups the N30 ($p < 0.005$) SEP peaks were significantly increased. Error bars represent the standard deviation.

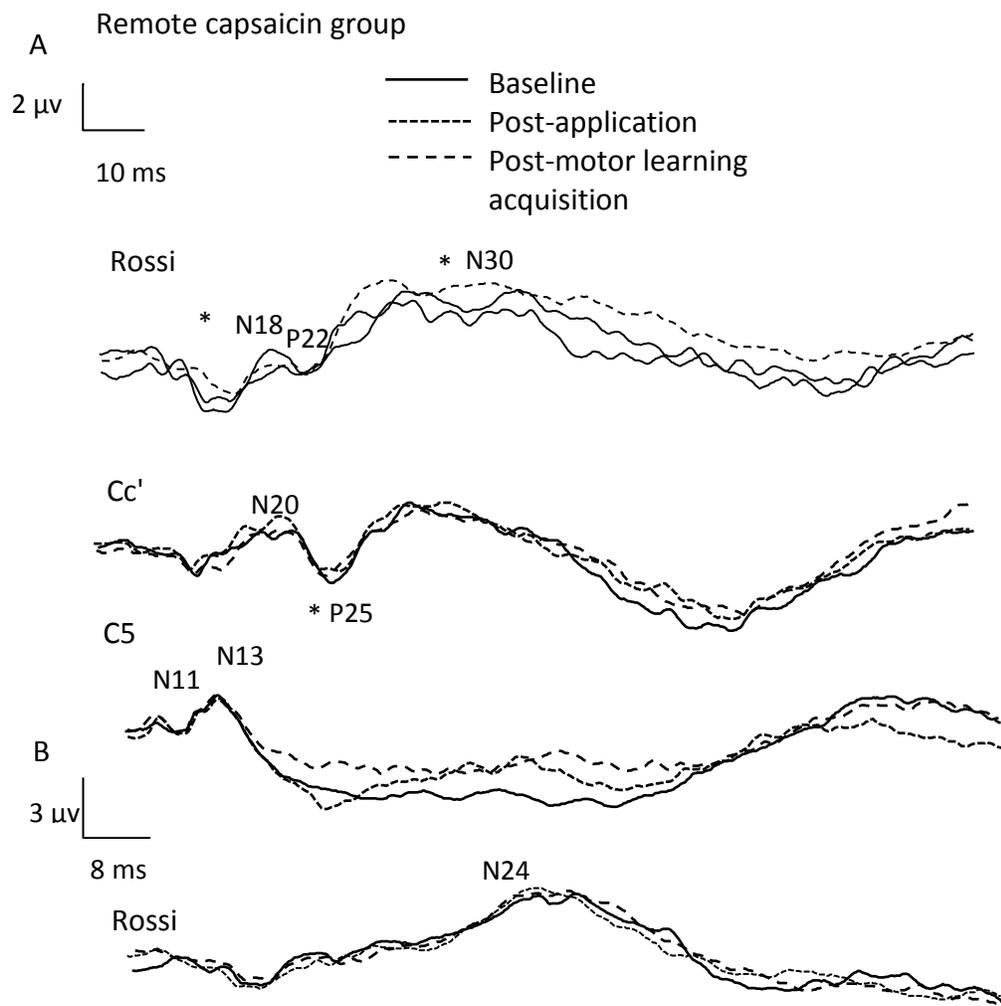


Figure 2: raw data from a representational remote participant indicating SEP peaks. Note the significant differences for the P25 SEP peak following capsaicin application and for the N18 and N30 SEP peaks following motor learning acquisition as indicated by asterisks.

Motor performance:

Accuracy

The Shapiro-Wilk normality test indicated that the accuracy data demonstrated that all of the groups and conditions were normally distributed except for the local capsaicin (pre-motor learning acquisition) group and thus an ANOVA was performed. The behavioural data demonstrates that the remote, local, and contralateral capsaicin groups improved in accuracy following motor learning acquisition [$F(3,35)=28.53, p<0.001$] and at retention [$F(3,35)=45.97, p<0.001$] with no significant differences between the groups (See Figure 3). Post-hoc tests on the percent change in motor error demonstrate that there wasn't a significant difference between the groups following motor learning acquisition ($p=0.89$) or at retention ($p=0.90$). The remote group had a 39.7% decrease in motor error following motor learning acquisition, and an additional 11.9% decrease in motor error at retention. The contralateral group had a 32.4% decrease in motor error following motor learning acquisition, and an additional 13.7% decrease in motor error at retention. The local group had a 28.3% decrease in motor error following motor learning acquisition, and an additional 24.6% decrease in motor error at retention.

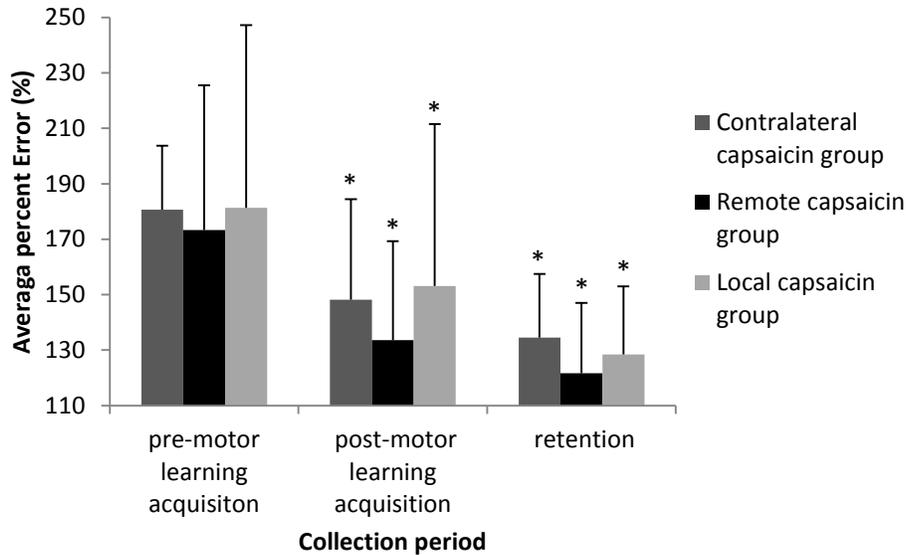


Figure 3: Bar graph depicting the percent error by group. The remote, local, and contralateral and capsaicin groups improved in accuracy following motor learning acquisition ($p < 0.001$) and at retention ($p < 0.001$) as indicated by asterisks. Error bars represent the standard deviation.

Pain ratings:

Significant differences in subjective pain levels relative to baseline were observed for all three groups 5 minutes post-application [$F(3, 35) = 32.11, p < 0.001$], 20 minutes post-application [$F(3,35) = 149.89, p < 0.001$], post-motor learning acquisition (35 minute mark) [$F(3,35) = 114.01, p < 0.001$] and post-motor learning acquisition (45 minute mark) [$F(3,35) = 52.74, p < 0.001$]. There was a significant interaction effect of TIME by GROUP at 20 minutes post application [$F(3,35) = 4.93, p < 0.05$] with post hoc tests indicating that the local and contralateral groups differed ($p = 0.021$). At the 20 minute time-point the contralateral group had an average pain of 5.17, the remote group had an average pain of 4.25, while the local group had an average pain of 2.67. The average NPRS ratings are illustrated in Figure 4.

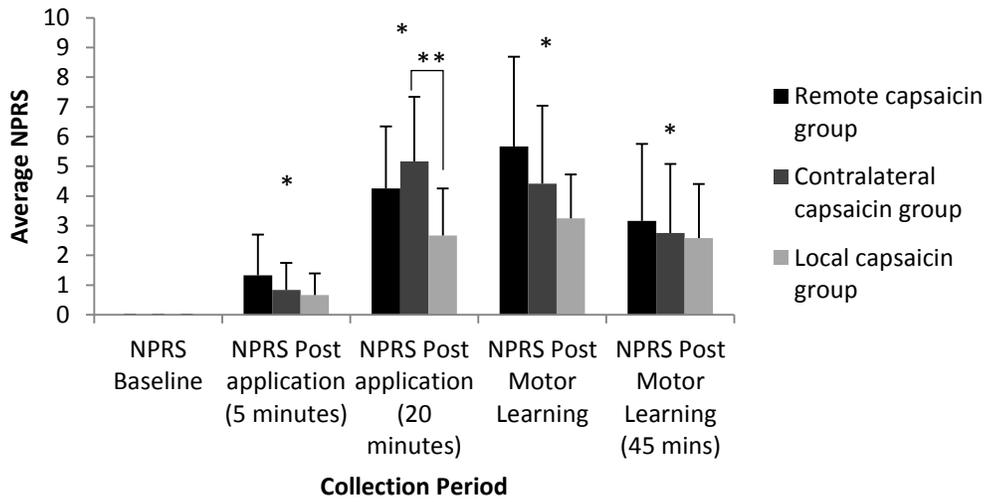


Figure 4:

Bar-graph depicting averaged NPRS ratings of participants by group. Significant differences in subjective pain levels relative to baseline were observed for all three groups 5 minutes post-application ($p < 0.001$), 20 minutes post-application ($p < 0.001$), post-motor learning acquisition (35 minute mark) ($p < 0.001$) and post-motor learning acquisition (45 minute mark) ($p < 0.001$) as indicated by an asterisk. At 20 minutes post application there was a significant difference between the local and contralateral groups differed ($p = 0.021$) as indicated by a double asterisk. Error bars represent the standard deviation.

9.3.5 Discussion

Our findings are in line with our hypothesis of differential changes in early cortical SEP peaks evoked following motor learning acquisition for the different groups as there was a decrease in the N18 SEP peak (remote capsaicin group) following motor learning acquisition. In addition, there was an increase in the N30 SEP peak for all three groups following motor learning acquisition. Significant improvements in accuracy were observed for all three groups suggesting that motor learning had occurred, however there wasn't an interactive effect of the location of pain on the extent of the improvement, which isn't in line with our secondary hypothesis that

participants performing a novel motor learning task during local and remote acute pain would have improved accuracy during motor learning acquisition and at retention when compared to the contralateral group.

SEP peaks: P25

The P25 peak was significantly decreased following capsaicin application. This peak reflects the activity in area 3b [77] and it is hypothesized that cerebellar-induced SEP changes originate within the 3b area of the SI [72]. The decrease in the amplitude of the P25 peak following capsaicin application is indicative of the role that the SI and the cerebellum play in somatosensory processing and is in line with our previous work [19, 430] and with our finding of a significant decrease in the N18 SEP peak (another peak that reflects cerebellar activity) for the remote capsaicin group following motor learning. The cerebellum plays a role in the processing of sensory input [323, 418]. Previous work found that the cerebellum is activated in response to nociceptive input as most fMRI studies show activation in the cerebellum in response to nociceptive stimuli [214, 242].

SEP peaks: N18

The N18 SEP peak originates in the brain stem and reflects activity in the olivo-cerebellar pathways [50, 62] and therefore alterations in this peak reflects changes in cerebellar activity [63]. Our finding of a decrease in the amplitude of the N18 peak for the remote capsaicin group supports the functional role of the cerebellum in somatosensory processing and motor learning acquisition. The cerebellum is connected to other parts of the CNS through afferent and efferent connections and receives cortical input through the brainstem [453]. Animal studies demonstrate that motor training is associated with increased synapses in the cerebellum [307-309] and the MI [310]. Work with human participants has shown that the increase in excitability can come with as

little as 5-15 minutes of motor training [9, 333]. fMRI evidence shows that the cerebellum also plays a role in sensory processing as discriminating sensory information significantly increases cerebellar activation [418] and demonstrates that acute pain leads to activation within the cerebellum [454]. Cortical projections to the pons pertain to acute pain, including somatosensory, motor, and cognitive contributions [455]. An animal study demonstrated that stimulation of cutaneous nociceptors activated climbing fibers that terminate in the cerebellum [456]. Cerebellar activation in response to acute pain is involuntary, and doesn't require pain perception [457]. In addition, the cerebellum is activated with the anticipation of pain [458]. This study found an interactive effect of acute pain and motor learning acquisition which corroborates an interaction between the cerebellum and cortical regions of the brain when combining acute pain and motor learning acquisition.

A possible explanation for why we did not see a significant change in the N18 SEP peak for the local capsaicin group was that they had a lower average NPRS level at the 20 minute mark.

Acute tonic pain may influence sensorimotor integration through complex central pain processes [413].

SEP peaks: N24

Our previous finding of a significant decrease following motor learning acquisition for the N24 SEP peak reflects the role that cerebellar input plays in this SEP peak [430]. Research demonstrates that the cerebellum is activated with motor learning acquisition [275, 436, 437] as animal studies have demonstrated that motor training is correlated with increases in the synapses within the cerebellum [307-309] and plays an active role in motor adaption and motor learning in humans [299]. We hypothesize that acute pain may have negated the alterations in cortical SEP peaks (N20 and N24) that occurs in the pain-free condition following motor learning acquisition

that we observed in our previous work [430]. It is interesting that for the current experiment we did not see any significant differences in these SEP peaks (N20 and N24) following motor learning acquisition and that all three groups were in acute pain while undergoing motor learning. This corroborates our previous findings of an interactive effect of pain and motor learning on neuroplasticity as measured by SEPs [19, 430, 451]. We hypothesize that the neuroplasticity associated with pain and motor learning acquisition share neural processes and interact with each other [421]. This validates previous research that demonstrated that performing an attention-demanding task attenuates the impact of negative stimuli [383, 423-425] increases pressure pain thresholds in healthy participants [420] and decreases activity in limbic regions [422].

SEP peaks: N30

The literature demonstrates that the N30 SEP peak is a result of a supraspinal network that links the thalamus, basal ganglia, premotor areas, and MI [439, 440] and reflects sensorimotor integration [74]. The amplitude of the N30 peak was significantly increased following motor learning acquisition in all three groups. Our finding supports previous research that found significant increases to the N30 SEP peak following motor learning acquisition [20, 340, 430, 431, 451]. In addition, previous research demonstrated that the N30 SEP peak amplitude increased with finger-to thumb opposition training [459] and during a gripping task [460].

Behavioural Data

Significant increases in accuracy were observed for all of the groups, suggesting that motor learning had occurred. There wasn't an effect of the location of pain on the degree of improvement following motor learning acquisition or at retention. Previous research has shown deficiencies in motor learning with acute experimental pain in animal [396, 397] and human models [15, 16]. We observed an increase in accuracy following motor learning acquisition and

at retention for the capsaicin groups which is in line with our previous research [20, 430, 451]. This work differed from our previous work as the current study utilized a complex tracing task and examined how the location of acute pain affects motor learning acquisition and retention. The literature demonstrates that neuroplasticity of the MI is mediated by alterations in attention [372-375] as motor learning is dependent on attentional resources [376, 377]. Previous work has established that there are disruptions in neuroplasticity when participants focused their attention to a body part that wasn't required for the motor learning task [375] while increased attention increases neuroplasticity [374]. However, as there were no significant differences between the three groups we hypothesize that improved motor learning acquisition during pain as observed in previous studies [20, 430, 451] and with our current study is due to an increase in arousal as opposed to solely an increase in attention to the region of the body undergoing learning. The noradrenergic arousal system has vast cortical projections [461], and imaging research demonstrate brain stem, cingulate, thalamic, prefrontal, and parietal activation with arousal [462]. Furthermore, manipulating arousal through pharmacological means can improve performance [463]. Of interest is the increased activation of the posterior area of the cortex in response to arousal, which is also activated in response to an attention task [462]. Thus arousal and attention share similar neural mechanisms and are not mutually exclusive explanations for improved motor learning while in mild acute pain. A limitation of this study is that participants in the local capsaicin group had a lower average NPRS level at the 20 minute mark and this may have had an impact on their motor learning acquisition. In future studies, capsaicin cream should be re-applied if the NPRS ratings starts to drop below 3 at any point during an experiment. Although previous work has shown that pain may negatively impact training-induced neuroplasticity [9], other studies indicate that pain may improve motor learning [20, 430, 451] or

have no effect if the quality of movement is maintained [413].

9.3.6 Conclusion

This experiment demonstrates that sensorimotor integration areas are vital in motor skill acquisition as there was a significant decrease in the N18 SEP peak for the remote capsaicin groups and for the N30 SEP peak amplitude following motor learning acquisition for all three groups. Motor learning occurred with mild acute pain and there were no significant differences in motor learning acquisition or retention between the three groups. As there were no differences between the three groups we hypothesize that improved motor learning while in acute pain is not due to increased attention to the limb performing the task but instead may be caused through increased arousal during the painful stimulation. A future direction for a study would be to measure cortisol levels in response to acute cutaneous pain and motor learning acquisition as endogenous stress hormones are a component of a memory modulating system [464].

Acknowledgements:

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Preface to Manuscript 4:

The second study *The interactive effect of acute pain and motor learning acquisition on sensorimotor integration and motor learning outcomes* demonstrated that following motor learning acquisition, the amplitude of the N20 SEP peak significantly increased ($p < 0.05$) and the N24 SEP peak significantly decreased ($p < 0.001$) for the control group while the N18 SEP peak significantly decreased ($p < 0.01$) for the capsaicin group. The N30 SEP peak was significantly increased ($p < 0.001$) following motor learning acquisition for both groups. The P25 SEP peak decreased significantly ($p < 0.05$) following the application of capsaicin cream. The third study showed that the effects of pain on motor learning acquisition and retention were the same regardless of whether the capsaicin was applied locally, remotely on the same limb performing the task, or on the contralateral limb. The fourth study sought to explore whether the impact of capsaicin and motor learning acquisition on the sensory system extended to the motor system. It was hypothesized that the changes in SEPs following motor learning acquisition that was observed in our first three studies would be reflected in increased cortical excitability following motor learning acquisition. For the fourth study the effect of acute pain on neuroplasticity of the MI was determined using input-output curves elicited using TMS. Therefore, if our TMS experiment corroborates our parallel SEP experiments, it would add further weight to our findings in terms of the interactive effects of pain and motor learning on neuroplasticity. The fourth study investigated whether a novel motor learning acquisition task performed in a pain-free condition (controls) as compared to acute pain (capsaicin group) would show differential changes in the slope of the TMS input-output curves. This study also investigated whether participants performing a novel motor learning acquisition task during acute pain would show

improved motor learning accuracy as compared to a control group, in keeping with our previous studies.

9.4: Manuscript 4: The effect of tonic pain and motor learning on corticospinal excitability

THE EFFECT OF TONIC PAIN AND MOTOR LEARNING ON
CORTICOSPINAL EXCITABILITY

Author(s): Erin Dancey, Bernadette Murphy, Paul Yelder

Affiliation(s): University of Ontario Institute of Technology
Faculty of Health Sciences
Oshawa, ON Canada
L1H 7K4

Corresponding Author Address: Paul Yelder, University of Ontario Institute of Technology, 2000 Simcoe Street North, Oshawa, Ontario, Canada.

Email: Paul.Yelder@uoit.ca

Telephone: (905) 721-8668 x 2768

Fax: (905) 721-3179

9.4.1 Abstract

Objectives: Previous work demonstrated improved motor learning acquisition and differential alterations in early somatosensory evoked potentials (SEPs) when motor learning acquisition occurred in the presence of acute tonic pain, however, it is unknown how the interactive effect of acute pain and motor learning acquisition impacts cortical excitability as measured by TMS and this is the aim of the current study.

Methods: Two groups of twelve participants (N= 24) were randomly assigned to either a capsaicin (capsaicin cream) or control (inert lotion) group. TMS input-output curves were performed at baseline, post-application and following motor learning acquisition. Following the application of the creams, participants in both groups performed a motor tracing acquisition task followed by a pain-free retention task within 24-48 hours.

Results: Following motor learning acquisition there was no main effect of TIME on the input-output curves ($p = 0.38$) however there was a significant interaction effect of TIME by GROUP ($p < 0.05$), with post-hoc tests showing that following motor learning acquisition there was a significant increase in slope for the control group ($p < 0.05$), and no significant change for the capsaicin group ($p = 0.57$). Both groups improved in accuracy following motor learning acquisition ($p < 0.001$). The capsaicin group outperformed the control group at baseline ($p < 0.005$), following motor learning acquisition ($p < 0.005$) and at retention ($p < 0.005$).

Conclusions: The acute pain in this study was shown to negate the increase in slopes that was observed for the control group despite having a positive impact on motor learning acquisition. The improved motor learning acquisition with capsaicin provides support for the enhancement of motor learning while in mild acute tonic pain.

Significance: Acute tonic pain may increase focal attention to the body part utilized in motor learning.

KEYWORDS

Transcranial magnetic stimulation (TMS); motor learning; acute pain; sensorimotor integration (SMI)

9.4.2 Introduction:

There is evidence that pain negatively impacts the neuroplasticity associated with motor output [12-14] and negates the increases in somatosensory evoked potential (SEP) peaks that would otherwise occur following motor learning acquisition [20, 430]. The interactions between pain and motor learning are complicated and few experiments have examined the interactive effect of acute tonic pain and motor learning acquisition on motor cortical excitability in healthy humans. In addition, while there is some evidence that acute pain during motor learning interferes with acquisition [9, 15, 16], other work has shown no impact of pain on acquisition [17, 18] and improved acquisition in the presence of acute pain [20, 430]. Inducing acute tonic pain in healthy participants is instrumental in isolating the motor consequences of acute pain and the conditions under which motor learning acquisition while in pain becomes either adaptive or maladaptive. An acute tonic pain model (capsaicin cream) that does not lead to increased pain with movement was chosen for this study.

Transcranial magnetic stimulation (TMS) has been utilized to study alterations in cortical excitability that occurs with acute and chronic pain. TMS studies of patients in chronic pain generally demonstrate decreased MI excitability [239, 240] although increased MI excitability has been found in patients suffering from phantom limb pain [240]. In terms of acute experimental pain, research demonstrates that experimental muscle pain modulates neuromuscular control through decreased coordination of muscle groups [251, 254-256]. However, there are differing effects of acute experimental pain on MI excitability. Acute experimental pain reduces excitability in some [7, 263, 465] but not all [264] research studies. Acute pain leads to inconsistent alterations in excitability for differing muscles and these changes in excitability may lead to protective motor strategies. Inconsistencies in the literature

highlights the necessity for research that investigates different pain models (i.e. pain not affected by the motor task) in order to investigate the effect of pain on cortical excitability.

Motor learning acquisition induces neuroplasticity in the motor cortex (MI): an expansion of motor representations [139, 173, 466, 467], changes in the kinematics of movements [3, 468], and facilitation of the motor evoked potentials (MEPs) [3, 468, 469]. Recent work has demonstrated that an input/output curve is a robust measure of cortical excitability [136]. The slope of the linear aspect of the sigmoid shaped curve represents cortical excitability [137] and therefore, this is a useful way of exploring the interactive effect of motor learning acquisition and pain on cortical excitability.

Our primary hypothesis was that a motor learning acquisition task performed in a pain-free condition (controls) as compared to acute pain (capsaicin group) would show differential changes in the slope of the TMS input-output curves. Our secondary hypothesis was that participants performing a novel motor learning acquisition task during acute pain would demonstrate improved motor learning accuracy as compared to a control group, in keeping with our previous studies.

The results of this study may contribute to our understanding of how acute pain impacts motor cortical plasticity in response to motor learning acquisition, as well as providing insight on the relationship between cortical plasticity changes and the impact on motor skill retention when acquired during acute pain. A better understanding of the impact of pain on motor learning acquisition and retention is vital in guiding effective rehabilitation interventions.

9.4.3 Methods:

Methods Overview:

Two groups of twelve participants, [6 males, 18 females; aged (M 20.2 1.31 SD)], were recruited from the student population at the University of Ontario Institute of Technology. Each participant filled out a confidential health history form in order to identify any conditions which may impact normal somatosensation. This included recent cervicothoracic injury, chronic pain, neurologic conditions and medication use. Informed consent was obtained for all participants and the study was approved by the University of Ontario Institute of Technology Research Ethics Board. This study was performed according to the principles set out by the Declaration of Helsinki for the use of humans in experimental studies.

Acute tonic pain was generated through the application of capsaicin cream and cortical excitability was investigated using TMS input-output curves. The effect of acute tonic pain on cortical excitability was assessed by performing TMS input-output curves at baseline, at 20 minutes post-application, and then following the motor learning acquisition task (45 minutes from baseline). Participants in the capsaicin group received a topical application of capsaicin (0.075% Zostrix, New York, USA) while the control group received a topical control skin lotion (Life Brand, Shopper's Drug Mart, Ontario, Canada). The topical creams were applied to a 50 cm² area on the lateral aspect of the dominant elbow.

Electromyography recording

Surface electromyography (EMG) recordings were obtained from the abductor pollicis brevis (APB) muscle with surface Ag–AgCl electrodes. The electrodes were placed over the APB, while the reference electrode was placed over the lateral epicondyle of the same limb. We

requested that participants maintain a relaxed position throughout the study. The EMG signal was amplified band-pass filtered (1,000x) and digitized.

2.3 Transcranial magnetic stimulation

Focal TMS was applied over the hand region of the dominant MI using a figure-eight coil (outer diameter 10 cm), linked to two Magstim 200 stimulators connected with a BiStim unit (Magstim Co., Whitland, Dyfed, UK). The coil was positioned rotated approximately 45 away from the mid-sagittal line with the handle pointed backwards. This coil orientation allowed the induced current to be perpendicular to the central sulcus and therefore the TMS coil stimulates corticospinal neurons trans-synaptically [104, 470]. The optimal coil position for inducing MEPs in the APB muscle was established as the site where stimulation at a suprathreshold level generated the largest MEPs (after averaging ten stimuli). The site was marked on a cap with a marker in order to confirm correct placement of the coil throughout the study. Resting motor threshold (rMT) was found by finding the lowest stimulator intensity that elicited a MEP of at least 0.05 mV in at least five out of ten trials, while the participant was at rest.

Input-output curve

The intensities used to develop the TMS input-output curves were determined for each participant using their rMT attained at the beginning of the experiment (See Table 1). As pain intensity changes throughout the experiment, it was important to collect recruitment curve data as efficiently as possible. Therefore, in keeping with previous research [137, 339] magnetic stimuli were applied in 10 % increments between 90 and 140 % of rMT, as this range encompasses the linear portion of the curve for the majority of participants. Twelve stimuli were given at each stimulus intensity. Therefore, a single input-output curve block consisted of 72 stimuli.

	Control	Capsaicin
Participant		
1	36	62
2	49	54
3	58	60
4	62	49
5	44	52
6	64	53
7	45	40
8	53	42
9	43	56
10	59	64
11	57	47
12		53
Averages	51.82	52.67
SD	9.02	7.40

Table 1: Resting motor threshold (rMT) values that were determined by finding the lowest stimulator intensity that elicited a MEP of at least 0.05 mV in at least five out of ten trials.

Outcome Measures

The outcome measures for this study included the slope of the input-output curves, motor learning accuracy and pain (Numeric Pain Rating Score).

Motor learning task:

The tracing task was run through a custom Leap Motion software tool (Leap Motion, Inc., San Francisco, CA) and required participants to trace sequences of sinusoidal waves with varying amplitude and frequency using their thumb on an external touchpad (Logitech, Inc., Fremont,

CA) pre-motor learning acquisition, an acquisition phase, post-motor learning acquisition and a retention test 24-48 hours later. The pre-motor learning acquisition, post-motor learning acquisition, and retention tests were 4 minutes in duration while the acquisition phase was 15 minutes in duration. The traces consisted of a series of dots and each trial was 500 dots. Each tracing task consisted of four pre-selected sinusoidal patterns of varying frequency and amplitude and frequency, as determined previously [339]. Pre-motor learning acquisition, following motor learning acquisition, and at retention, each of the versions, 1-4, were performed once; for the acquisition phase each version was performed three times totalling 12 traces. The participants used the APB muscle as they were required to sweep their thumb from right to left.

Pain:

Pain was measured using a Numeric Pain Rating Scale (NPRS) in which participants classified the intensity of their pain from 0–10 [416]. Participants in both groups rated their pain at baseline, post-application (5 minutes), post-application (20 minutes), following motor learning acquisition (35 minutes), and following the last round of SEP measurements (45 minutes).

2.8 Statistical Analysis

MEP amplitude measurements were taken peak-to-peak and averaged for each intensity. This file was exported to Microsoft Excel, where the 12 stimuli for every intensity were averaged and visually graphed. The slope of the linear aspect of the input-output curve was calculated and exported to IBM SPSS Statistics for statistical analysis. The plateau phase was excluded for those participants that had levelling off at the lower (90% rMT) pulse intensities, and to disregard this plateau, only the slope of the curve from the 100% intensity to 140% intensity was included in the analysis. MEP amplitudes were normalized to baseline values to account for variability between participants at baseline and to allow for between participant comparisons. To

explore the interactive effect of acute tonic pain and motor learning acquisition on the input-output slopes, a two-way repeated measures ANOVA with factors TIME (baseline, post-application, post-motor learning acquisition) and GROUP (control versus capsaicin) was performed.

To investigate and compare accuracy, a repeated measures ANOVA with factors TIME (pre-motor learning acquisition versus post-motor learning acquisition versus retention) and GROUP (control versus capsaicin) was performed on the accuracy data.

For the NPRS measurements, a repeated measures ANOVA with factors TIME [(baseline, post-application (5 minutes), post-application (20 minutes), post-motor learning acquisition (35 minutes), post-motor learning acquisition (45 minutes)] and GROUP (control versus capsaicin) was performed. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp). Statistical significance was set at $p < 0.05$.

9.4.4 Results

A total of 24 participants participated with 12 participants in the capsaicin group [9 females 3 males; aged 19-22 (M 19.9 SD 0.9)] and 12 participants in the control group [9 females, 3 males; aged 19-23 (M 20.7 SD 1.4)].

Behavioural data:

The behavioural data demonstrates that motor learning occurred as both the control [F(1,11)=59.93, $p < 0.001$] and capsaicin [F(1,11)=23.16, $p < 0.001$] groups improved in accuracy. The interaction effect of TIME by GROUP was significant [F(2,23)=3.23, $p < 0.05$], with post-hoc testing demonstrating that pre-motor learning acquisition [F(2,23)=18.88, $p < 0.005$] post-motor learning acquisition [F(2,23)=15.32, $p < 0.005$] and at retention [F(2,23)=17.04, $p < 0.005$] the capsaicin group was more accurate than the control group (See Figure 1). Compared to the pre-motor learning acquisition values, the control group had a 48.7% decrease in motor error

following motor learning acquisition and a subsequent 21.9% decrease at retention while the capsaicin group had a 35.2 % decrease in motor error following motor learning acquisition and a subsequent 10.7% decrease in motor error at retention.

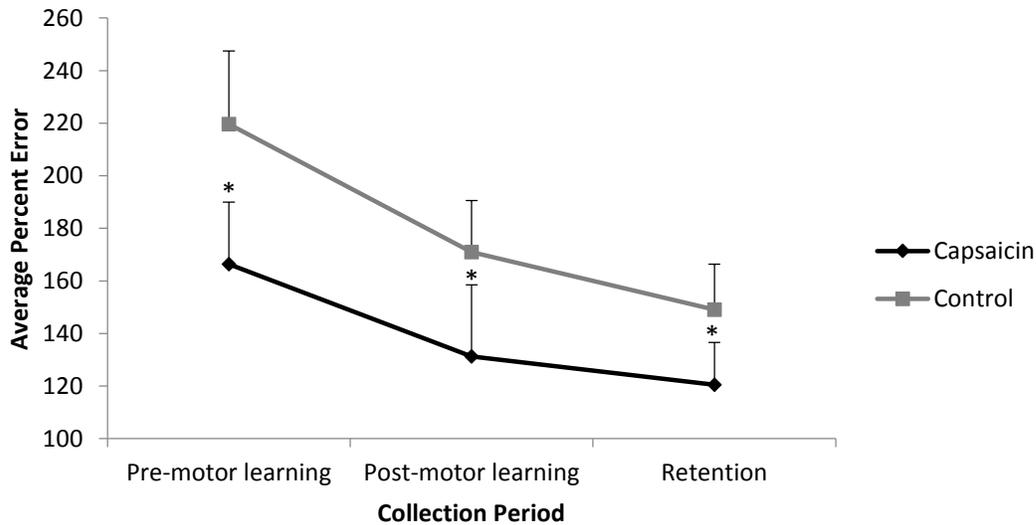


Figure 1: Line-graph depicting the average percent error for the capsaicin and control groups. Pre-motor learning acquisition ($p < 0.005$) post-motor learning acquisition ($p < 0.005$) and at retention ($p < 0.005$) the capsaicin group was significantly more accurate than the control group as indicated by asterisks. Error bars represent the standard deviation.

Pain ratings:

There were significant differences in subjective pain levels relative to baseline for the capsaicin group 5 minutes post-application [$F(1, 11) = 54.55, p < 0.001$], 20 minutes post-application [$F(1, 11) = 286.00, p < 0.001$], post-motor learning acquisition (35 minute mark) [$F(1, 11) = 11.64, p < 0.01$] and post-motor learning acquisition (45 minute mark) [$F(1, 11) = 7.05, p < 0.05$]. The average NPRS ratings are illustrated in Figure 2. None of the participants in the control group reported any pain.

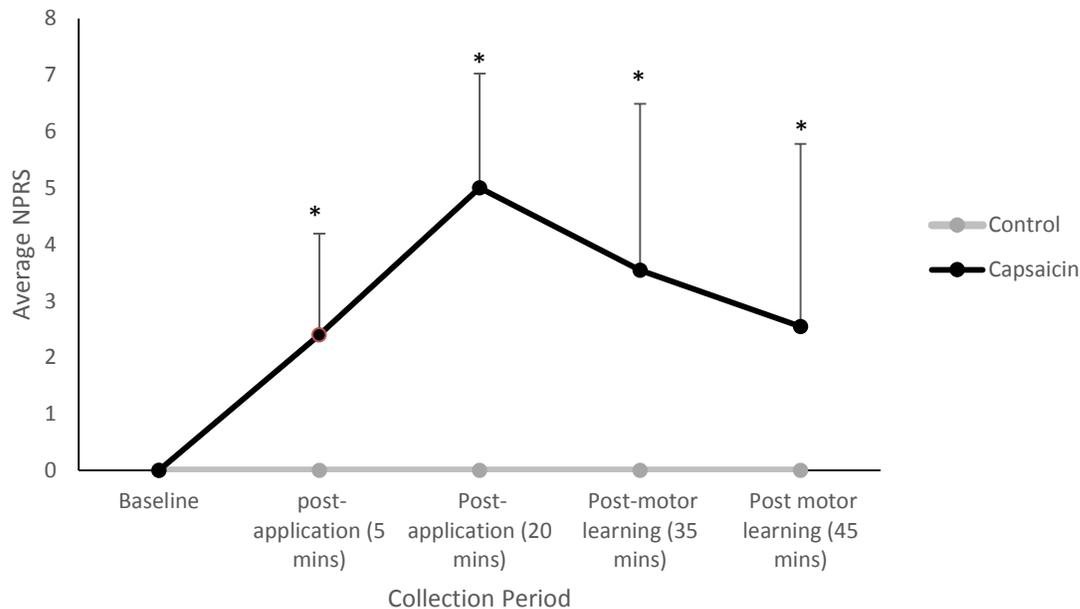


Figure 2: Line-graph depicting the average NPRS ratings for the capsaicin and control groups. Significant differences 5 minutes post-application ($p < 0.001$), 20 minutes post-application ($p < 0.001$), post-motor learning acquisition (35 minute mark) ($p < 0.01$) and post-motor learning acquisition (45 minute mark) ($p < 0.05$) for the capsaicin group are indicated by asterisks. Error bars represent the standard deviation.

Input-output curves:

Following the cream application, there was no main effect of TIME on the input-output slopes ($p = 0.89$). Following motor learning acquisition there was no main effect of TIME on the input-output curves ($p = 0.38$) however there was a significant interaction effect of TIME by GROUP [$F(2,23) = 3.42, p < 0.05$], with post-hoc tests showing that following motor learning acquisition there was a significant increase in slope for the control group [$F(1,11) = 4.42, p < 0.05$], and no significant change for the capsaicin group ($p = 0.57$). The average input-output curves are illustrated in Figure 3. Figure 4 depicts the input-output curves of a control (A) and capsaicin (B) participants.

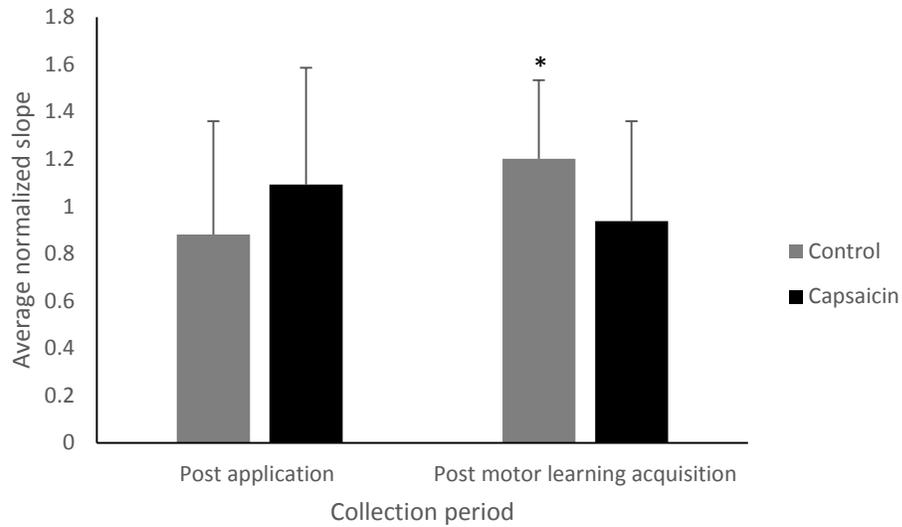
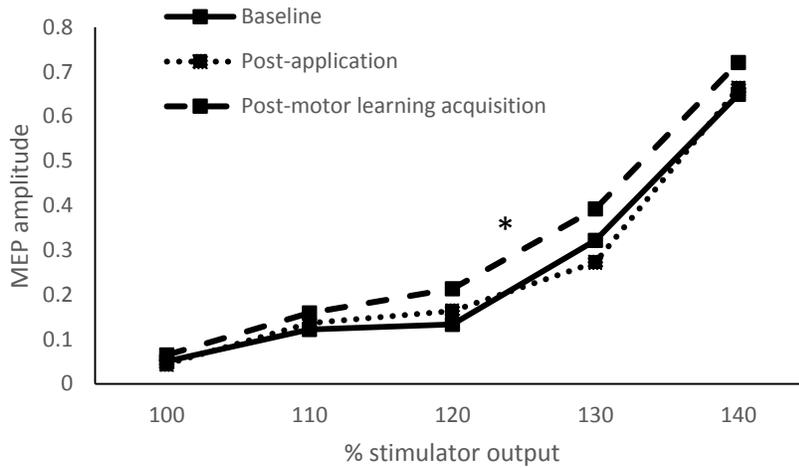


Figure 3: Bar-graph depicting the normalized average slope post-application and post-motor learning acquisition for the control and capsaicin groups. There was a significant increase in slope for the control group ($p < 0.05$) following motor learning acquisition as indicated by an asterisk. Error bars represent the standard deviation.

A : Control participant



B: Intervention participant

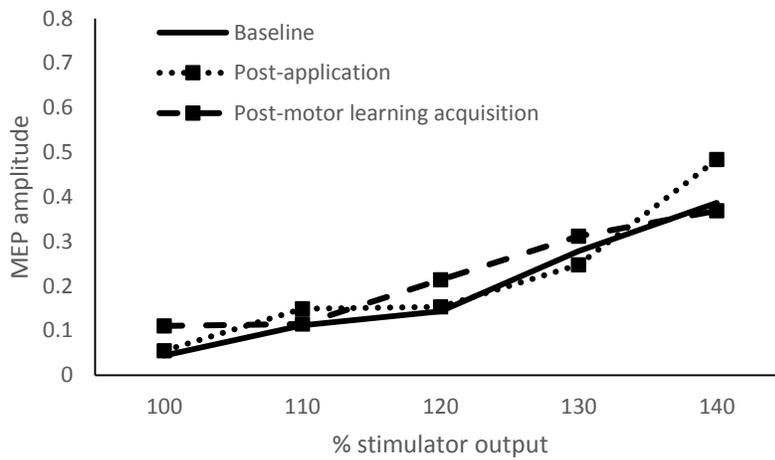


Figure 4: input-output curves of a control (A) and capsaicin (B) participants. Significant increases in slope for the control (A) participant is indicated by an asterisk.

9.4.5 Discussion

Our findings support our hypothesis that a novel motor learning acquisition task performed in a pain-free condition (controls) as compared to acute pain (capsaicin group) would show differential changes in the slope of the TMS input-output curves.

Input-output curves

There is an increase in MEPs following motor performance of the Purdue pegboard [471] or finger tapping [139] tasks. MEP facilitation associated with motor learning is correlated with improvements in performance [139, 190, 469] and reflects the initial stages of motor learning acquisition in the MI [472]. Currently, the mechanism responsible for changes in cortical excitability in the MI are not fully understood. It is known that the increases in MEPs associated with motor performance is influenced by GABAergic intracortical inhibition [190] and it is hypothesized that disinhibition in the MI plays an important role in motor learning [473-475]. Synaptic neuroplasticity in the MI is also mediated by the activation of the N-methyl-D-aspartate (NMDA) receptor [476]. Evidence for this comes from motor training studies which have demonstrated increases in MEPs that are inhibited by dextromethorphan (an NMDA receptor blocker) and reduced by lorazepam (a GABA_A modulator) [468]. This is relevant to this research as a decrease in GABA inhibition and an increase in glutamate provides a mechanism for the increase in MI excitability following motor learning acquisition.

Our results demonstrated an increase in the slope of the input-output curve for the control group following motor learning acquisition which is in line with the theory that plastic changes in the MI accompany motor learning. However, there was no change in the slope of the input-output for the capsaicin group suggesting that although plastic changes in the MI often accompany motor learning acquisition they are not essential for motor learning to occur. This finding is in

line with Cirillo et al. [477] who did not find an association between MEPs and improved motor performance. This corroborates previous work that found differential effects of pain and motor learning on neuroplasticity as measured by SEPs when compared to a control group, without a negative impact on motor performance [19, 430, 451]. Research demonstrates that neuroplasticity accompanying motor learning is mediated by changes in attention [372-375] as motor learning is dependent on attention resources [376, 377]. Focused attention to the task has been correlated with activation of prefrontal cortex and pre-SMA but not with either the SI or MI [378]. We hypothesize that improved motor learning outcomes for the acute tonic pain group is due to attention to the limb undergoing learning [372-375]. Increasing evidence establishes that affective processing is modulated by attention and cognitive regulation [379] and that stress leads to a narrowing of attention [380, 381] decreasing the processing of task-irrelevant stimuli [382]. It may be that factors such as increased arousal or attentional focus [478] are more important in mediating improved motor learning acquisition. The improved performance while in acute pain could be attributed to increased attention to the limb performing the motor learning task or increased arousal. Endogenous stress hormones provide a potential explanation as they are a component of a memory modulating system that results in memory strength proportional to memory importance [464] and manipulating arousal through pharmacological means can affect task performance [463]. We hypothesize that acute pain may increase arousal which improves motor learning outcomes. The noradrenergic arousal system has projections to the cortex [461], and imaging research demonstrates brain stem, cingulate, thalamic, prefrontal, and parietal activation with arousal [462]. Additionally, increased arousal from nociceptive input leads to increased activity in the posterior parietal area, which is also activated in response to an attention task [462].

Since the neuroplastic changes associated with early stage motor learning are not present once a skill has been learned it is also possible that the capsaicin group learned the motor skill earlier on and thus we do not see a significant increase in slope post-motor learning acquisition for this group.

Behavioural Data

Motor learning is the acquisition of new muscle patterns in order to improve motor performance [1]. Significant increases in accuracy were found for both groups, demonstrating that motor learning had occurred. There was an effect of pain on motor performance throughout the study as the capsaicin group outperformed the control group significantly pre-motor learning acquisition, post-motor learning, and at retention. Research demonstrates that deficits in motor learning occur with acute pain in animal [396, 397] and human models [9, 15, 16]. We observed an increase in accuracy pre-motor learning acquisition (performed while in pain), post-motor learning acquisition, and at retention for the capsaicin group which corroborates our previous research [20, 430]. In absolute terms, the capsaicin group outperformed the control group pre motor learning acquisition, post-motor learning acquisition and at retention, however in relative terms, the control group actually experienced a greater percent learning post-motor learning acquisition. This highlights the effect of acute pain on motor performance. This work differed from our previous work [20, 430] as the current study utilized a different task (tracing versus typing) that was more difficult and had lower baseline accuracy. The painful stimulation used in the present study and our previous work [20, 430] and used by another study which demonstrated no impact of pain on motor learning acquisition and retention [17] induced tonic cutaneous pain unrelated to the motor task. This may help to explain why there wasn't an adverse effect of pain on motor learning outcomes as pain has is thought to elicit protective motor responses [14].

This research suggests that there may be contradictory effects of acute pain on motor learning

plasticity. Research indicates that cortical representations in the SI and MI are altered in response to pain, and that pain perception and the associated alterations in neuroplasticity can be reversed by motor learning acquisition [180]. Clearly there is an interdependence of sensory and motor systems and the effects of motor learning on pain may be due to cortico-thalamic loops, inhibiting nociceptive input. Although it has been hypothesized that pain interferes with learning-induced motor plasticity [9], other studies indicate that pain may improve motor learning [20] or have no effect if the quality of movement is maintained [18, 413].

9.4.6 Conclusion

We found an increase in slope for a control group following motor learning acquisition that was not observed for the capsaicin group and we hypothesized that increased arousal or attentional focus [478] are more important in mediating improved motor learning acquisition. A future direction for a study would be to measure cortisol levels in response to acute cutaneous pain and motor learning as endogenous stress hormones are a component of a memory modulating system that results in memory strength proportional to memory importance [464]. In addition, performing TMS input-output curves midway through the motor learning acquisition phase is important in order to see if there is an increase in slope for a capsaicin group at this time point.

10 – GENERAL DISCUSSION AND CONCLUSIONS

The first study provides supportive evidence for early SEP peaks as markers for SMI and acute pain. *Experiment 1*: N11, N13 and N30 SEP peak amplitudes increased following motor learning for both groups while the N20 SEP peak increased for the control group. *Experiment 2*: The P25 SEP peak decreased for the local group following application of capsaicin cream while the N30 SEP peaks increased following motor learning for both groups. Motor performance was better in the presence of pain at baseline (*Experiment 1*) and motor learning retention improved in the presence of local pain (*Experiment 2*). This study suggests that acute pain may increase focal attention to the body part utilized in motor learning; contributing to our understanding of how the location of pain impacts somatosensory processing and the associated motor learning. The development of a motor learning task with lower baseline accuracy to prevent learning saturation led to the development of the tracing task that was used in the subsequent studies. The first study increases our comprehension of how acute pain affects motor learning and pain in different locations affecting motor learning which has important implications for rehabilitation.

The second study provides evidence for SMI areas in motor learning acquisition as we found significant differences in the N30 SEP peak amplitude following motor learning for both groups, and for the N20 and N24 SEP peaks (control group) and the N18 SEP peak (capsaicin group). A significant decrease in the P25 SEP peak was found following the application of capsaicin cream demonstrating the effect of acute pain on SEP peaks. Research demonstrates that neuroplasticity accompanying motor learning is a consequence of increased attention [372-375] as motor learning depends on attentional resources [376, 377]. It was hypothesized that improved motor learning outcomes for the capsaicin group was due to increased attention to the region of the body used for the motor learning task [372-375]. The evidence has established that affective

processing is modulated by attention and cognitive regulation [379] and that stress leads to a narrowing of attention [380, 381]. As the findings of improved motor learning acquisition during acute pain may be caused through increased attention or through an increase in arousal during the painful stimulation, an important direction for the third study was the comparison of the effects of local versus remote acute pain relative to the muscle(s) performing a complex motor learning task. The results of this study help to explain why activation of the motor system (focusing on movement) can assist in decreasing pain. As motor learning is accompanied by pain in a variety of settings, the effect of pain on learning and neuroplasticity is important to consider to ensure that therapeutic interventions lead to adaptive and not maladaptive changes.

The third study provides evidence for SMI areas in motor learning acquisition as there was a significant decrease in the N18 SEP peak for the remote capsaicin groups and for the N30 SEP peak amplitude following motor learning for all three groups. Motor learning occurred in the presence of mild acute pain and there were no significant differences in motor learning acquisition or retention between the three groups. As there were no differences between the three groups it was hypothesized that improved motor learning while in acute pain is caused through an overall increase in arousal during the painful stimulation.

For the fourth study the effect of acute pain on neuroplasticity of the MI was determined using input-output curves elicited using TMS and we demonstrated that acute experimental pain negated the increase in slope that was observed for the control group despite having a positive impact on motor learning acquisition.

The increased cortical excitability following motor learning acquisition is in line with our findings of differential changes in SEPs following motor learning acquisition while in acute pain that we observed in our first three studies. Our TMS findings support the findings of our parallel

SEP experiments and adds further weight to the interactive effects of pain and motor learning acquisition on neuroplasticity. This study also demonstrated improved motor learning acquisition in the presence of capsaicin providing support for the enhancement of motor learning while in acute tonic pain.

Limitations

Inducing acute tonic pain in healthy participants is instrumental in isolating the motor consequences of acute pain. An acute tonic pain model (capsaicin cream) that does not lead to increased pain with movement was chosen for this thesis in order to determine the interactive effect of acute experimental pain and motor learning on neuroplasticity without the confounding effects of musculoskeletal pain. However, musculoskeletal pain may be more relevant to rehabilitation as it provides a deep pain that may have a more direct impact on SMI and therefore this a limitation of the current work.

Given that capsaicin causes vasodilation that is mediated locally it is possible that some of the effects of SEPs that were observed in response to acute experimental pain may have been due to other local effects (i.e. skin temperature) as opposed to a direct effect of pain on SEP peak amplitude.

For Experiment 3 we studied the effect of local versus remote versus contralateral capsaicin application on SEPs and motor learning outcomes. A limitation of this study are the differences in innervation between the remote (elbow) versus local (thumb) application site. The glabrous skin over the thumb lacks C-fibers and A-delta mechano-heat nociceptors [479]. These differences may account for the lower pain ratings for the local group and may also explain the lack of change in P25 SEP amplitude for the local pain group.

Future Directions

A future direction for the next study would be to measure cortisol levels in response to acute pain and motor learning acquisition as endogenous stress hormones are a component of a memory modulating system that results in stronger memories for certain events [464]. While there is some evidence that the presence of acute pain during motor learning acquisition interferes with skill acquisition [9, 15, 16], other work has shown no impact of pain on motor skill acquisition [17, 18] and improved motor learning acquisition in the presence of acute pain [20, 430]. Our third study found improved motor learning acquisition in the presence of pain with no significant difference between remote, local and contralateral pain groups. It was hypothesized that improved motor learning acquisition during pain as observed in previous studies [20, 430] was due to an increase in arousal. The noradrenergic arousal system has widespread cortical projections [461], and neuroimaging studies have confirmed that brain stem, thalamic, cingulate, prefrontal, and parietal activations associated with arousal [462]. Inducing acute tonic pain in healthy participants combined with the measurement of cortisol is therefore instrumental in determining whether altered cortisol levels are altered following the application of capsaicin cream and provides a possible explanation for improved motor learning acquisition while in acute tonic pain.

TMS has been used extensively to study changes in neural plasticity including plastic changes in the MI following motor learning acquisition. These changes include an expansion of motor representations [139, 173, 466, 467] changes in the kinematics of movements evoked by TMS [3, 468], and increased MEPs following motor learning [3, 468, 469]. Currently, the mechanisms responsible for changes in cortical excitability in the MI are not well understood. However, it is known that task-induced MEP facilitation is influenced by GABA_A intracortical inhibition [190]

and practice induced changes in MEPs are reduced by lorazepam (a GABA_A agonist) [468]. Disinhibition may play an important role in motor learning acquisition by unmasking excitatory connections to corticospinal neurons in the MI [473-475]. Short-interval paired-pulse TMS in humans has been used to explore the excitability of various inhibitory [112, 114, 134] and facilitatory [110, 111, 480] neuronal circuits. Studies have shown that the first phase of SICI is due to a synaptic inhibition mediated by the GABA_A receptor [113, 117-119]. In contrast to SICI, SICF reflects direct excitation of axon initial segments of excitatory intracortical interneurons by the S2, which is depolarized and made hyperexcitable by the preceding TS [110]. Studying SICI and SICF in association with a motor learning acquisition task will allow us to determine if improved motor learning acquisition is mediated by GABA_A disinhibition in the MI and if there is an interactive effect of pain and motor learning acquisition on inhibition and facilitation at the level of the cortex.

Conclusion and Significance

These results may aid our understanding of the impact of pain on motor learning and contribute to our understanding of how acute cutaneous pain may contribute to adaptive or maladaptive plasticity during motor learning.

Appendix:

A.1 Pain: from the periphery to the cortex

Based upon the rexed lamination (division of the dorsal horn into laminae based on the morphological properties of the cells) the dorsal horn can be divided into six different laminae (see Figure 21) [25].

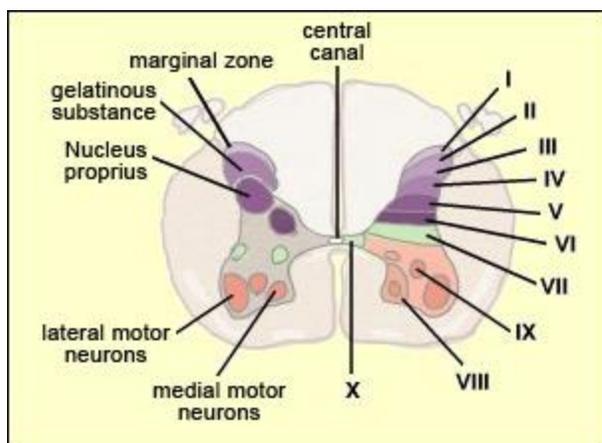


Figure 22: the rexed laminae system of the dorsal horn grey matter (Reproduced from <http://www.thr.brain.mcgill.ca>)

Most nociceptive input is transferred from the nociceptors to lamina I, II, and V of the dorsal horn. The nociceptive input projects to the thalamus in the contralateral spinothalamic tract (STT) and to the brainstem via spinoparabrachial, spinoreticular, spinomesencephalic and cervicothalamic tracts [481]. The tracts have differing purposes linked to where they originate in the dorsal horn and to their destination at the level of the cortex [450].

A.1.1 Nociceptors

Acute pain is initiated by intense thermal, mechanical, or chemical stimuli which activates a withdrawal reflex, increased arousal as well as emotional and autonomic responses [269]. Tissue irritation results in the release of chemicals from sensory neurons in the skin, muscles, and joints

which stimulate nociceptors and elicits the perception of pain [207]. Similar to other sensory neurons, nociceptors are pseudounipolar: the dorsal root ganglion or trigeminal ganglion sends an axon that innervates the skin and another axon synapses on second-order neurons in the dorsal horn or the trigeminal subnucleus caudalis (See Figure 21). An electrical potential is generated by the membrane of nociceptors that contain proteins that convert nociceptive input [25]. There is a direct pathway between electrical potentials in the periphery and the spinal cord [38] and projects to the thalamus and then on to the cortex [482].

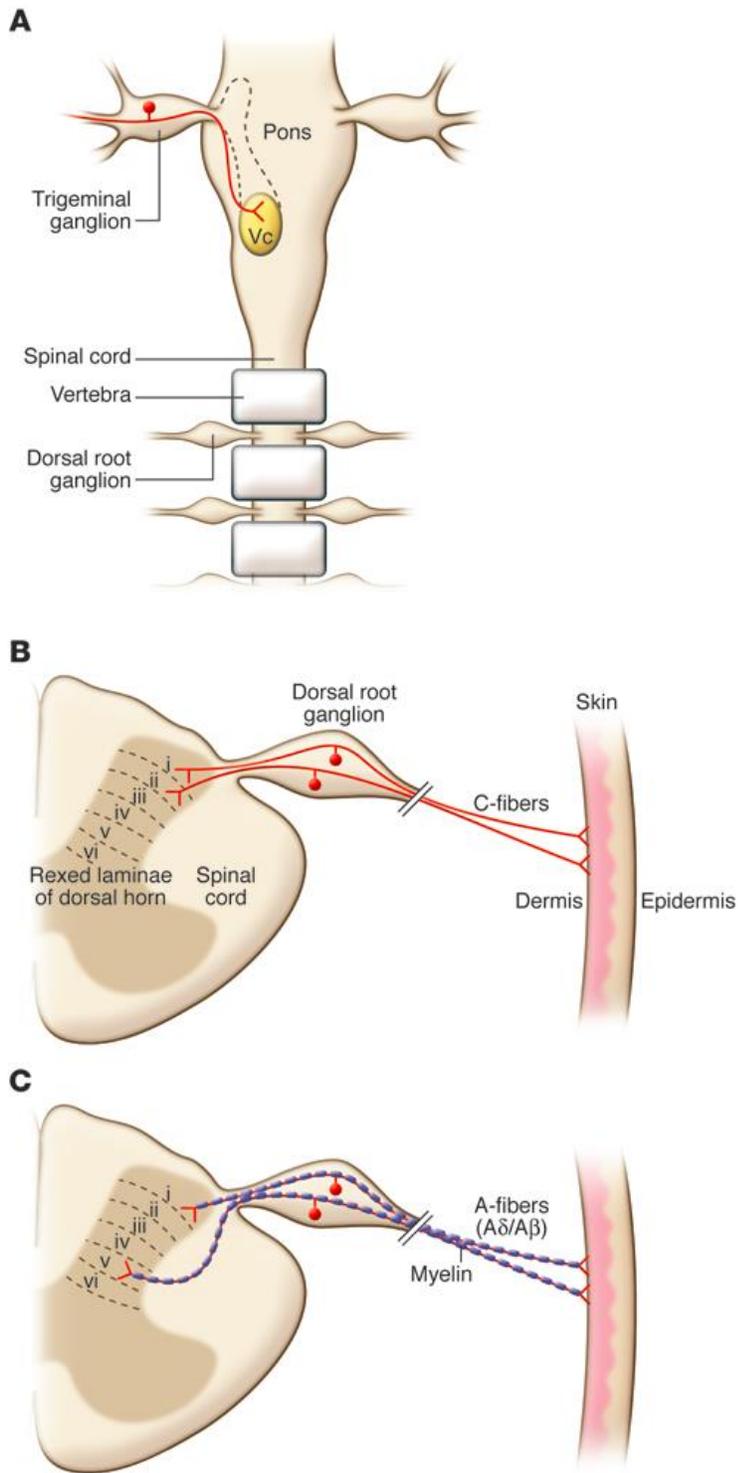


Figure 23: Anatomy of nociceptors.
[38]

Activation of nociceptors occurs when stimuli depolarize peripheral neurons sufficiently to transmit action potentials [483]. All nociceptors have a specific threshold that has to be reached before transduction occurs and this depends on the chemical, mechanical, or thermal nature of the receptor. The stimulus intensity is encoded in action potentials and the transmission speed correlates to the myelination and diameter of the axons [484]. Activation of nociceptors does not necessarily lead to the pain perception as perception requires the activation of supraspinal centers. Activation of supraspinal centers is contingent on the frequency of action potentials and central influences [38].

A.1.2 Classes of nociceptors

Noxious stimuli such as intense pressure, temperature extremes, and chemicals activate nociceptors [38]. Somatosensory neurons are classified into three groups: C, Ab, and Ad fibers [485].

A.1.3 C-fibers

Intense mechanical stimulation, temperature and chemical irritants activate unmyelinated, small diameter C fibers with conduct with velocities of 0.4–1.8 m/s [202]. C fibers are broadly distributed preventing precise localization and are surrounded by Schwann cells [485]. C fibers respond to thermal, chemical, mechanical, and stimuli and can be subdivided based on the expression of neuropeptides (substance P) [485]. C fibers are unmyelinated and they therefore conduct with slower velocity than A fibers resulting in a pain that is described as burning, throbbing, or aching [212]. As C fibers transmit signals from chemical irritants it is hypothesized that they play a major role in inflammation. Nociceptors that are insensitive to heat or mechanical input are sensitized by inflammatory mediators [38].

A.1.4 A-d fibers:

Intense mechanical stimulation and thermal stimulation activates thin myelinated A-delta fibers with conduction velocities of 2–33 m/s [202]. A-delta fibers terminate as free-nerve endings and do not usually transmit signals from chemical stimulation [485]. Nociceptive input is transduced into an electrical signal. As the axons of A-d fibers are myelinated, they can transmit signals much faster than the unmyelinated C fibers and impart the CNS with rapid information regarding the location of the noxious stimulus resulting in pain that is described as brief, localized and sharp [212].

A.1.5A-b fibers:

A-b fibers are large diameter, myelinated fibers with conduction velocities of 30-70 m/sec. A-b fibers innervate structures in the periphery, including Merkel cells, keratinocytes, corpuscles, and hair follicles. They are highly sensitive to light touch, vibration, stretch, and hair movement [485]. These fibers provide information to the CNS regarding light touch sensation. However, with sensitization these A-b fibers can function as nociceptors and transmit a stimulus interpreted by the CNS as pain. Allodynia is a light touch stimulus that is processed as painful and is mediated by A-b fibers [212].

A.1.6 Transducer molecules:

Pain starts in the periphery with the activation of nociceptors that depolarize in response to noxious stimuli resulting in action potentials that are sent to the CNS. The term transduction refers to the transitioning of tissue damage into an electrical signal and is mediated by transducer proteins that respond to irritant chemical and noxious heat in sensory neurons (noxious transducers) [269]. Ionotropic and metabotropic receptors are involved, including TRP (transient receptor potential) channels, P2X receptors, and ASIC (acid-sensing ion) channels resulting in cation influx leading to depolarization [486]. ATP activates nociceptors via activation of the P2X

channels [450]. Reductions in tissue pH (that occurs as a result of ischemia or inflammation), can stimulate nociceptors and sensitize them to heat and other somatosensory input [450].

TRP channels play a role in pain and thermosensation and they are activated by noxious heat and capsaicin [486]. The receptor was first called a vanilloid receptor subtype-1 (VR1) but is now called TRPV1 as it is the first member of the TRPV subfamily of ion channels [487]. TRPV1 is a cation channel and its activation increases their Ca^{+2} concentration [487].

A.1.7 Mediators:

Peripheral inflammation is a consequence of tissue injury and characterizes an initial step in the healing process. Following tissue damage, vasodilation, edema, and pain occur and immunologic factors are brought in to clear damaged tissue. Cytokines are proteins generated in the inflamed tissue and in lymphoid organs that can have stimulatory or inhibitory effects on immune function and the activation of nociceptors [450]. Other mediators are released from mast cells (histamine and serotonin), basophils (histamine and serotonin), the arachadonic acid pathway (prostaglandins, leukotrienes, and thromboxane), plasma (bradykinin, kallidin, interleukins, thrombin, trypsin, and hydroxyacids), calcitonin gene-related peptide and substance P and have an effect on nociceptors [38].

Prostaglandins are mediators and regulate smooth muscle [488]. Prostaglandin E2 (PGE2) binds to an EP1/EP2 receptors and this increases cation channel opening [489]. Serotonin (5-HT) is a neurotransmitter with a vital role in the descending control of dorsal horn excitability.

Serotonergic descending pathways mediate dorsal horn excitability and can have either antinociceptive and pronociceptive effects [450].

Kinins lead to increased vascular permeability and the dilation of blood vessels and are referred to as inflammatory mediators [490]. With inflammation, bradykinin is generated from high-

molecular weight kininogen through cleavage by kalikrein [491]. Bradykinin modulates vasodilation, and increases vascular permeability [450] and is generated after injury to the tissues. Bradykinin binds to B1/B2 receptors, initiating an intracellular cascade that results in increased sensitivity of the Na⁺ channels [492].

A.1.8 Sensitization in the periphery:

Nociceptive pathways can be sensitized, resulting in increased responses to stimuli [269]. With acute pain, if there is no longer nociceptive input then there is no longer transduction and transmission to the CNS. Peripheral sensitization occurs when nociceptive input is transmitted to the spinal cord out of proportion to or in the absence of a noxious stimulus [38] and is often the result of exposure to mediators. Peripheral inflammation increases the number of nociceptors that can be activated. One third of the nociceptors cannot be activated unless sensitization activates the receptors [493]. Mediators affect the nociceptors in several ways. Mediators activate nociceptors resulting in transduction and can also function indirectly. Initially, a nociceptive stimulus initiates transduction and leads to peripheral inflammation. Inflammation restarts the pathway and can cause more inflammation and activates the nociceptors [38].

Convergent interactions of TRPV1 with multiple stimulators results in greater activation levels [450]. Increased transmission of pain results as a greater number of nociceptors are activated by a given stimulus. With continuous inflammation, peripheral sensitization and nociceptor pathophysiology results [212]. There are two types: modification and modulation. Modulation is a reversible alteration in the excitability of neurons that is the result of alterations in receptors [269]. This occurs through the phosphorylation of receptors such as TRPV1 [269]. Modulation results in enhanced responsiveness of nociceptors leading to facilitated responses to nociceptive and innocuous inputs [494]. After an injury, there is increased pain as a result of decreased C

fiber activation threshold. A zone of flare develops around the injury and there is pain in response to innocuous input (secondary allodynia) and increased pain in response to nociceptive input [38].

Activation of nociceptors can lead to biochemical changes that alters gene expression leading to modification [38]. Another general mechanism by which TRPV1 can be modified with of inflammation is through changes in the phospholipid content of membranes [450].

A.1.9 The spinal cord:

The sensory receptor, axon, and cell body, together with the synaptic contacts in the dorsal horn are known as the primary afferent [495]. This first order neuron is located in the dorsal horn and connects a receptor with the spinal cord [26].

Neurons that relay distinctive sensations terminate in distinct laminae [205]. The substantia gelatinosa (lamina II) consists of interneurons which may respond to nociceptive input or innocuous stimuli [25, 496]. Lamina III and IV (nucleus proprius) receive input from A-b fibers and respond to innocuous input [25]. Lamina V contains axons that ascend to the brain stem and the thalamus and have input from A-b, A-d, and C fibers [25]. Neurons in lamina VI receive inputs from muscles and joints [25]. Laminae VIII and VII are responsive to nociceptive input, and have complex responses because the inputs are polysynaptic [25].

Action potentials are transmitted to the CNS, whereby they initiate neurotransmitter release. In the dorsal horn, nociceptors have afferent terminals containing large dense core vesicles that store peptides and small vesicles storing glutamate [25]. These neurons release glutamate as well as peptides (i.e., substance P, somatostatin) [38]. Fast synaptic transmission in nociceptive pathways results from glutamate acting on AMPA and kainate ligand-gated ion channels [269].

Glutamate acts on neurons that are nearby while neuropeptides can diffuse considerable distances and can enhance the actions of glutamate [25].

A.1.10 The spinal cord: Central sensitization

The evidence demonstrates that nociceptive input may sensitize CNS structures involved in pain perception [152]. The perception of pain is a dynamic process: sensory stimuli acts on neurons that have been altered by past inputs, and the output is affected by these prior inputs. Persistent pain may be driven by central sensitization and neuroplasticity [497]. Acute pain activates the spinal cord which then facilitates neurons leading to increased sensitivity to pain [482, 498, 499]. Woolf [482] demonstrated that the increases in spinal cord excitability caused through injury can be maintained even after local anaesthesia of the injured location, providing proof that acute injury can lead to spinal changes. These changes include an expansion of dorsal horn receptive fields (in lamina I and V) producing changes in excitability that can be maintained without nociceptive input [152, 500]. Acute experimental pain has also been shown to cause changes in spinal motor neuron activity [176]. In addition, Burstein et al. [501] found changes in the spinal nucleus pars caudalis that receives the fibres of the sensory root of the trigeminal nerve that descends as the spinal tract of trigeminal nerve.

The major excitatory neurotransmitter released by nociceptive neurons is glutamate which acts on AMPA evoking fast synaptic potentials in dorsal horn neurons [25]. Fast excitatory postsynaptic potentials (EPSPs) indicate the onset, intensity, duration, and location of the pain [269]. With central sensitization nociceptive neurons exhibit erratic firing patterns underlying the neuropathic processing of sensory stimulation within the spinal cord. Nociceptive neurons then release elevated quantities of substance P and glutamate, leading to a hyperexcitable state within the dorsal horn [211]. Sustained nociceptive input results in slow EPSPs in the dorsal horn

neurons through the release of peptide transmitters (and glutamate) [25]. Substance P and neurokinin A (NKA) activate neurokinin 1 (NK1) and neurokinin 2 (NK2) receptors. Following nociceptive input there is an increase in the internalization of NK-1 receptors in the dorsal horn and this is increased with inflammation or injuries to the nerve leads to temporal summation [433]. The removal of the Mg block of the channels allows for Ca^{2+} influx [494]. NMDA receptors help produce the hyperexcitability of dorsal horn neurons [502]. As a result increase their response to nociceptive input as a result of physiological changes within the cord [211]. This leads to a windup of action potentials [269]. The use of a NMDA antagonist (e.g. Ketamine) impedes wind-up in some individuals but not the preliminary pain [492].

Sensation is initiated from impulses in peripheral sensory nerves. If the peripheral nerves are injured, neuropathic pain occurs due to abnormal ectopic discharge. [503]. Spontaneous ectopic discharge occurs and an injured sensory neurons begin to fire spontaneously following an injury. Alterations in gene expression and protein trafficking create repetitive firing at ectopic locations [450]. Dorsal horn neurons demonstrate an induction of genes encoding transduction factor c-fos in response to peripheral nociceptive input [504]. In addition, upregulation in the expression of peptides, neurotransmitters, and receptors occurs [25]. The physiology of nociceptors are altered leading to spontaneous pain [25].

The literature on pain and plasticity indicates that nociceptive input can produce sensitization in the spinal cord [152, 397, 505]. The dorsal horn neurons are specifically involved and dynamic changes occur in these neurons contributing to chronic pain [482, 498, 500, 501]. Questions remain as to what happens at the supraspinal level and to what extent descending systems contribute.

A.1.11LTP:

An increase in synaptic strength is referred to as long term potentiation (LTP) and this occurs after the repetitive use of a synapse [506]. There may also be a reduction in synaptic strength which is referred to as long term depression (LTD) [507]. LTP has been demonstrated in nociceptive pathways at synapses between primary afferent A-d or C fibers and second order neurons and is a cellular mechanism for pain amplification [508].

A.1.12 Changes in excitability at the supraspinal level:

Changes in excitability in response to pain is observed in various components of the somatosensory system: at the spinal cord level, in supraspinal structures, and at the cortex [152, 215]. Wall et al. [215] proposed that injury alters neuronal structures at both subcortical and cortical locations and that peripheral injuries cause rapid alterations in peripheral, spinal, and brainstem structures which are more extensive than cortical alterations. The result is that injuries affect different components of the CNS and can change their response to subsequent inputs.

Changes in excitability in response to pain have been demonstrated at the supraspinal level [216-218, 222] (See Figure 21). Neurons in the thalamus of patients with neuropathic pain present with spontaneous high firing rates [216] and changes in the amygdala [217], and ACC [218] have also been described in response to pain. Further studies with humans have revealed increases in excitability in other supraspinal structures (parabrachial nucleus, PAG, superior colliculus, prefrontal cortex) [222].

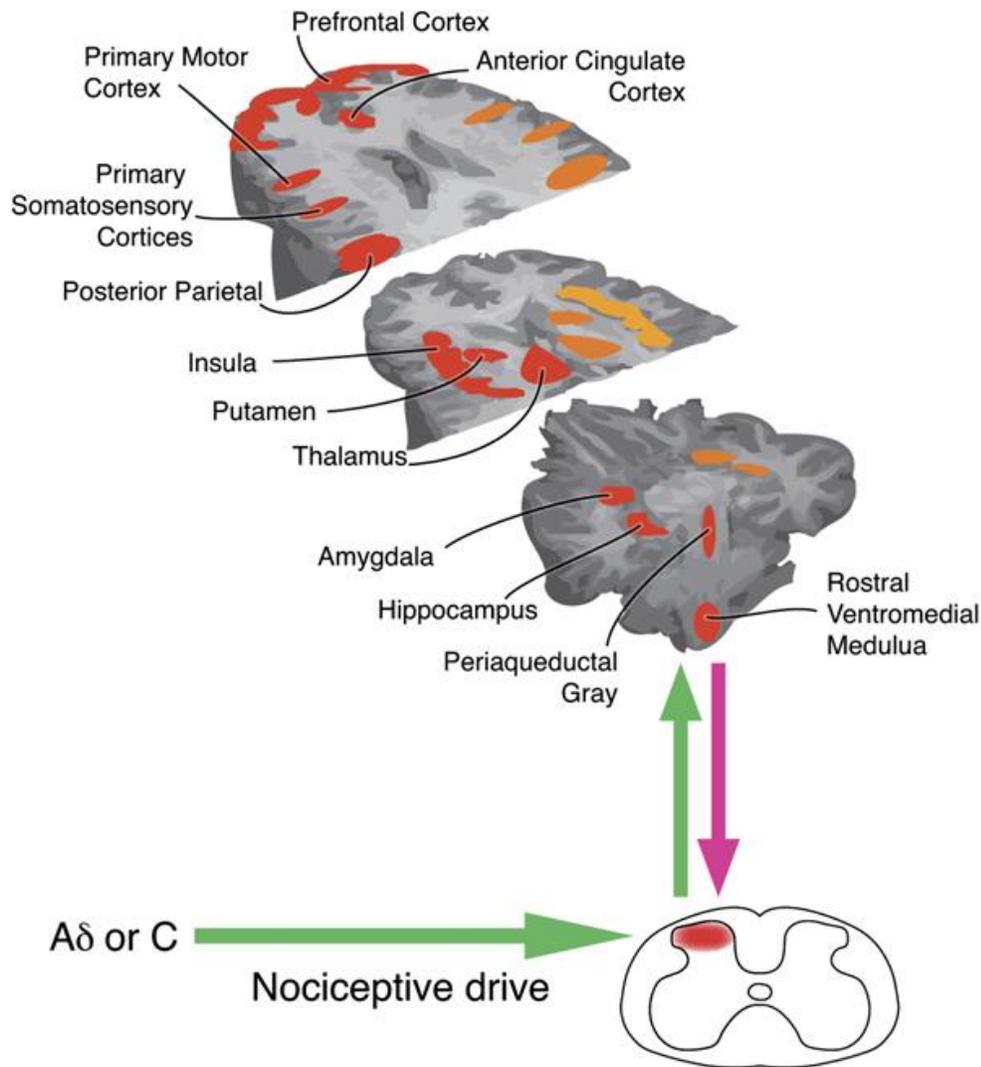


Figure 24: Activated regions during nociceptive input

A.1.13 Brainstem:

The brainstem includes the medulla oblongata, pons and midbrain and is continuous with the spinal cord [509]. The motor and sensory tracts pass through the brainstem [510] including the corticospinal tract, the posterior column, the spinothalamic, spinomesencephalic, spinoreticular and spino-ponto-limbic tracts. Sensory inputs from the brainstem are part of the ascending systems carrying nociceptive input to supraspinal areas [36]. These tracts connect to the brainstem and integrate nociceptive input with homeostatic, autonomic, and arousal activities.

This allows for communication of nociceptive input to the cortex. Tracts to the brainstem can impact forebrain and spinal activity and thus play a direct role in affecting pain perception [36]. Neurons of the brainstem receive convergent inputs from nociceptive and innocuous activation and have large receptive fields.

A.1.14 Pain inhibition

The literature demonstrates that the brainstem plays a central part in altering pain perception. The brainstem can inhibit or facilitate nociception and is influenced by the diencephalon, hypothalamus, amygdala, insula, ACC, and prefrontal areas [36]. Pain inhibition or facilitation is achieved through a descending pain modulatory system (an anatomical network that regulates nociceptive processing) (See Figure 22) [36]. Pain inhibition can prioritize nociceptive input relative to other demands and contributes to the fight-or-flight response [36] and typically include autonomic, endocrine, and motor responses [450].

Three major areas of the brainstem are constitute the brainstem pain modulatory centers: the LC, the PAG, and the RVM [213]. Descending projections of the brainstem block neurons in laminae I and V through inhibition [511]. In the dorsal horn, the PAG-RVM network is important for opioid analgesia which involves an interaction with endogenous opioid circuits [512]. The ceruleospinal inhibits spinothalamic activation in the dorsal horn. The binding of the transmitter norepinephrine suppresses the release of nociceptive neurotransmitters [512].

The RVM is comprised of the nucleus raphe magnus and the reticular formation. Activation of the RVM inhibits neurons in the dorsal horn, including nociceptive neurons [511]. Research has demonstrated that neurons within the PAG or RVM leads to pain inhibition [513, 514].

The PAG integrates information from different areas of the CNS [515]. The spinal cord conveys direct projections from the dorsal horn to the ventrolateral and lateral PAG that relay innocuous

and noxious input [516]. Extensive afferent inputs to the PAG are from the forebrain, including prefrontal and the insula as well as the amygdala and hypothalamus [517]. Afferents to the PAG from the brainstem arise from the medulla, in particular the RVM. The PAG makes excitatory connections to the RVM. This then connects to the dorsal horn and makes inhibitory connections with neurons in the dorsal horn. [450].

Stimulation of the PAG in animals produces analgesia via activation of the RVM [518]. This is pain specific analgesia as there is still a response to pressure, touch, and temperature. Stimulation of the PAG blocks withdrawal reflexes that are normally evoked by nociceptive input and recruits descending pathways that inhibit neurons in the dorsal horn [25]. Stimulation of the PAG also produces autonomic changes including hypertension and altered heart rate [450].

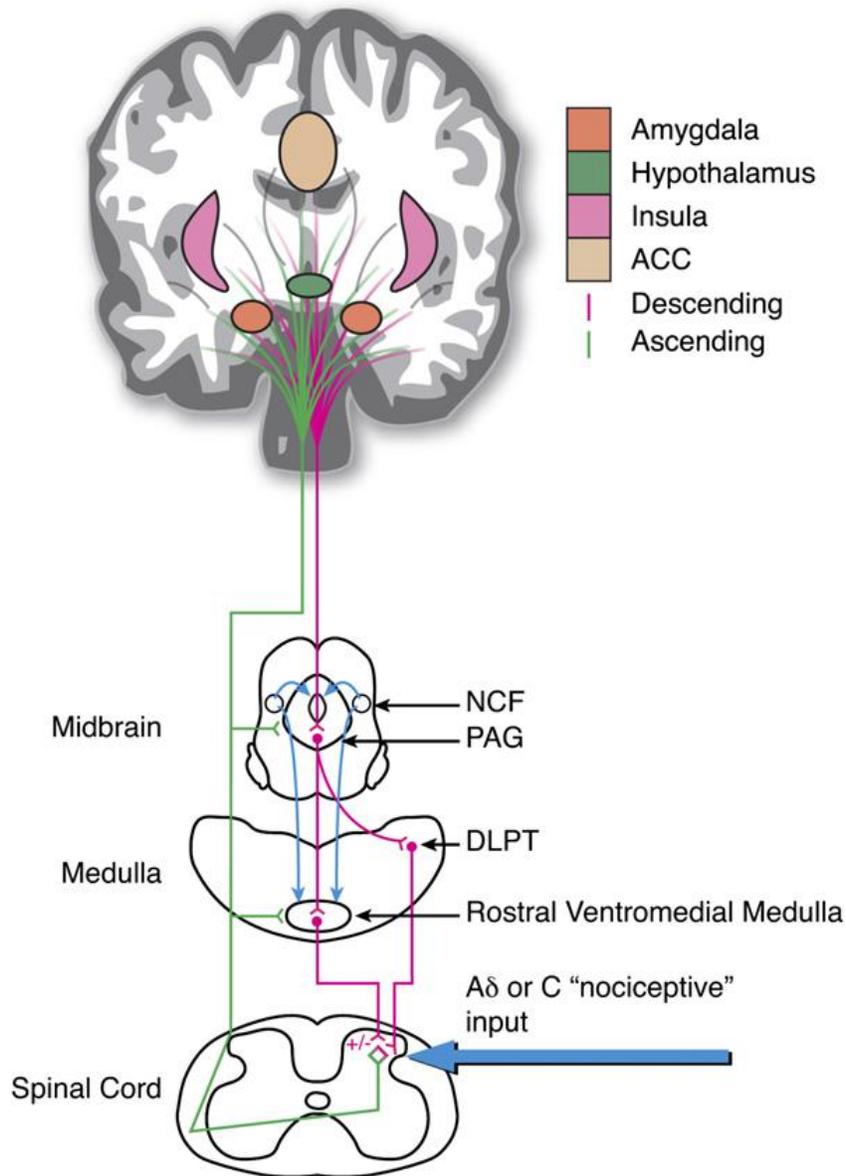


Figure 25: pro and anti-nociceptive influences respectively. [36]

Pain facilitation

The frontal cortex, insula, ACC, hypothalamus, amygdala, PAG, RVM and nucleus cuneiformis, are regions involved in pain facilitation [36]. The PAG, parabrachial nucleus, RVM, NCF and dorsal reticular nucleus are involved in central sensitization [36]. Specifically, the RVM is involved in hyperalgesia and allodynia associated with inflammation [512].

The application of capsaicin to the PAG has a pronociceptive effect and activates descending facilitation [512]. Pronociception occurs with systemic infection or inflammation and sustained activation of these descending pathways facilitates pain transmission and is thought to contribute to chronic pain [450].

Animal work demonstrates increased activity within pain modulatory centers with inflammation which corroborates their function as part of a negative feed-back loop to control painful input [213]. Changes within the descending pain modulatory network through either increased descending facilitation or through system decreased descending inhibition have been associated with chronic pain conditions [36].

A.1.14 The historical record of pain research: The two major main pain models

A.1.15 The specificity theory

The theory of pain at the beginning of the 20th century was the same as proposed by Descartes [519]. This was referred to as the specificity theory that posited that injury activates specific pain receptors specific to pain and fibers which transmit nociceptive input from the spinal cord to a pain center [520]. This was considered a passive system in which the perception of pain was proportional to the peripheral input [152] and proposed that psychological components did not impact pain perception. This theory did not include neuroplasticity, in which neurons and synapses are modified and then impact subsequent perception [152].

A.1.16 The gate control theory

The specificity theory was the dominant pain model until the last half of the 20th century. This changed in 1965, when Melzack and Wall developed what is referred to as the gate control theory of pain [521]. This is the second major pain model which proposed that pain is enabled or prevented through the opening or closing of gate controls. The synapse in the spinal cord is an

important center of study with regard to pain physiology. Nociceptive input travels along sensory neurons until reaching the dorsal horn where they synapse. Following activation, these spinal circuits initiate motor patterns which produce avoidance behaviours that promote survival [211]. This theory proposed that pain is not simply the result of the activation of nociceptive neurons but can be modified by activity in other somatosensory neurons that do not transmit nociceptive information. The spinal cord was viewed as a gate that was either open (propagation of the noxious stimuli) or closed (blocking the peripheral noxious stimuli from being processed). Neurons in lamina V receive input from non-nociceptive A-b fibers and nociceptive C and A-d and fibers. This theory proposes that the status of the gate was determined by the interaction of many signals, and that non-nociceptive input close and nociceptive input opens the gate to noxious input [25]. If the cumulative inhibitory signal was stronger than the net excitatory signal, then the gate was closed. If the cumulative excitatory signal was stronger than the net inhibitory signal, then the gate was opened. The excitatory and inhibitory signals came from the periphery and centrally from higher brain structures [212]. An important contribution of the gate control theory was its emphasis on the CNS. This theory proposed that the dorsal horns were dynamic sites through which inhibition, excitation, and modulation occurs and focused on the brain as an vital component in pain processes which filters and modulates inputs. This theory also included psychological factors as an important part of pain processing [152]. Although the mechanism is more complex than the status of a single gate either being open or closed, the principles of the gate control theory still hold true [212]. The gate control theory of pain aided in the understanding of pain physiology and provides a neurophysiological foundation for the finding that there is a reduction in pain following vibration that activates large-diameter afferents [25].

A.1.17 The counterirritant theory:

The counterirritant theory integrated findings from studies generated by the gate control theory [519]. Inhibition of noxious input by non-noxious afferents occurs in the spinal cord. Pressure stimulates mechanoreceptors and branches of these afferents stimulate interneurons releasing the neurotransmitter enkephalin [522]. Enkephalin binds with receptors on the non-noxious afferents and interneurons of the nociceptive system, and there is therefore decreased release of substance P thus inhibiting the transmission of nociceptive signals [512].

A.1.18 Current view of pain:

The current view is that pain is a submodality of sensation and is a collection of sensory, cognitive, and emotional experiences associated with actual or potential injury [36, 38]. Pain is a complex phenomenon that affects sensory, immune, affective, and motor processing [40]. Pain is perceived when the supraspinal structures are activated; and pain relief takes place when these supraspinal structures are interrupted [197]. Pain isn't linearly associated with nociceptive input but is individual and subjective and is influenced by genetics, memories, emotions, and cognitive factors. The modality and locality of the stimulus can impact the qualities and temporal features of pain [36, 38]. The highly subjective and individual nature of pain makes it challenging to treat and define [25].

The sensations we call pain; pricking, stinging, burning, and aching are distinctive [25] and are evoked at pressures, temperatures, and by toxic substances extreme enough to potentially injure tissues [38]. The somatosensory system serves an important protective function by allowing us to differentiate between noxious stimuli, such as a hot object or an intense pinch, and innocuous stimuli [485]. The threshold for nociception must be high so that normal activities can be carried out without resulting in pain, but with enough sensitivity to alert if an injury is imminent [523].

Pain is an adaptive mechanism as it alerts us and generates a reflex withdrawal away from the damaging stimulus and results in behavioural adaptations to avoid further pain [38]. If pain is a consequence from a particular behaviour, then animals are liable to avoid that behaviour in the future. The intensity of the pain response emphasizes dangerous situations which is important for survival and maintaining homeostasis [38]. Nociceptive pathways are subject to plasticity, and results in an increase in the response of the system to continuous input [211]. Plasticity in the nervous system may have resulted in the capacity to detect and remember danger [269, 486]. It is conceivable that neuronal pathways within the nervous system have evolved to support nociception and learning and that all types of learning may be derived from neuroplasticity within nociceptive pathways [211].

Peripheral tissue damage which affects components of the PNS and CNS and increases pain sensitivity is referred to as central sensitization. Sensitization of the system results in persistent pain and occurs after repeated painful stimuli, so that subsequent inputs are enhanced as the threshold for activation falls [25]. Central sensitization becomes pathological when it is upheld in the absence of pathology and persists after an injury has healed. This is chronic pain that occurs from damage to the PNS or CNS and is also referred to as neuropathic pain. Pain can occur without nociceptive input, with non-noxious input (allodynia), or as an amplified response to nociceptive input (hyperalgesia) [485, 486]. In contrast, acute pain occurs with peripheral input and is referred to as nociceptive pain. It is with central sensitization that syndromes like chronic tension-type headache, sciatica, LBP, or phantom limb pain occur [211].

The gate control theory hypothesized that the dorsal horn can modulate nociceptive input [524]. The brain has modulation circuits functioning to regulate pain perception. Modulation initially occurs in the dorsal horn whereby connections between nociceptive and non-nociceptive

pathways mediate whether the nociceptive input reaches the cortex [25]. For each individual nociceptor that has been stimulated, there are thousands that have not been stimulated [212]. Descending inhibition and facilitation and excitatory and inhibitory interneurons in the dorsal horn controls nociceptive input contributing to the perception of pain [38].

Descending pathways from supraspinal structures send excitatory and inhibitory signals down to the dorsal horn [525]. Descending modulation utilizes the following systems: serotonergic, noradrenergic and opioidergic. The system exerts anti-nociceptive and pronociceptive effects. Excitatory signals include peptides (substance P, somatostatin, bombesin, galinin, and vasoactive intestinal peptide), amino acids (glutamate, aspartate), nitric oxide, and prostaglandins [212]. Inhibitory signals include endorphins, amino acids (GABA and glycine), serotonin, and adenosine [212].

A.1.19 Ascending Pathways

A.1.20 The spinothalamic tract

The spinothalamic tract is an ascending nociceptive pathway and is comprised of nociceptors and wide-dynamic-range neurons from laminae I and V-VII [44]. It ascends contralaterally in the white matter ending in the thalamus as clusters of terminals and eventually reaching the SI, SII, prefrontal cortex, posterior and mid-insula, posterior parietal cortex, and mid-cingulate cortex [202, 203]. One section of this tract ends in the posterior and ventroposterior thalamus. This lateral system projects to the SI which mediates the sensory component of a pain sensation (e.g. location, texture, and intensity [24]. Nociceptive neurons in SI have input from the lateral system are mostly found in Brodmann area 1, but there is some evidence that Brodmann area 3a may also have some nociceptive input [24]. Historically, thermal and pain sensations had been considered as sub served by common pathways within both the peripheral and the CNS through

the spinothalamic pathway. However, a segregation of thermal and noxious inputs has been demonstrated [44].

A.1.21 Spinoreticular tract

The spinoreticular tract is comprised of neurons in laminae VIII and VII and ascends in the anterolateral white matter and is positioned closely to the lateral spinothalamic tract terminating in the reticular formation and the thalamus [202, 203]. In contrast to the spinothalamic tract, most of these axons do not decussate [25].

A.1.22 Spinomesencephalic tract

The spinomesencephalic tract is comprised of neurons in lamina I and V and it ascends in the anterolateral white matter to the mesencephalic reticular formation and PAG, and through the spinoparabrachial tract, it ascends to the PB. The PB neurons transmit to the amygdala, a major element of the limbic system that is involved in emotional processing. Therefore the spinomesencephalic tract contributes to the affective aspects associated with pain. A significant portion of this tract projects in the lateral funiculus rather than in the anterolateral white matter [25].

A.1.23 Cervicothalamic tract

The cervicothalamic tract arises from the lateral cervical nucleus, located by the upper two cervical segments. Nociceptors in laminae III and IV relay input to the lateral cervical nucleus. A significant portion of this tract decussates and ascend in the brain stem to the midbrain and to the thalamus.

A.1.24 The pain matrix:

Pain is complex, subjective, multifactorial, and involves a distributed supraspinal network. This was initially described as a pain neuromatrix, but now it is referred to as the pain matrix. The pain matrix consists of areas that regulate and process nociceptive input and can create pain perception without nociceptive input [512].

The pain matrix is supported by invasive and non-invasive electrophysiological studies in humans, including MEG and EEG studies and stereotactic procedures [450]. This network receives parallel inputs from multiple nociceptive pathways [214]. This includes a number of regions that are activated in response to nociceptive input and this is correlated to the intensity of the nociceptive input [197, 526]. PET and fMRI neuroimaging techniques demonstrated that there are alterations in a number of areas of the cortex in response to nociceptive input: the SI, SII, posterior parietal cortex, thalamus, posterior and mid-insula and the mid-cingulate cortex [24, 27, 217]. In addition, other areas such as the prefrontal area, BG, cerebellum, amygdala, and hippocampus have yielded activation by experimental pain [36].

The evidence indicates that information about the intensity of the nociceptive input is transmitted independently from the thalamus to multiple cortical areas [527, 528]. Neurological research has demonstrated that there are multiple pathways that ascend to the cortex. Although some aspects of pain processing may be disrupted with injuries of the SI, SII, ACC, or the insula, there is still an awareness of pain intensity [529, 530]. Thus communication of nociceptive input through any one of these areas of the cortex is not compulsory for pain perception. Neurons in the spinal cord, thalamic nuclei, SI, SII, and ACC exhibit increased responses to increasing nociceptive input [493, 531]. Therefore, cortical regions including the SII SI, ACC, insula, and premotor regions constitutes a parallel, distributed mechanism for pain intensity processing [526].

Historically, components of the lateral thalamus and the SI were considered responsible for the sensory components of nociception (location, quality, and intensity), whereas medial aspects of the thalamus, limbic system, and the prefrontal cortex are responsible for processing the affective components of pain. Though the pain matrix has lateral (sensory) and medial (affective) components [36] it is simplistic to assign specific pain components to specific brain regions [217, 526]. Although imaging studies show differential activation of the medial and lateral systems of the pain matrix [512] other regions of the brain are also responsible for the processing of sensory information. Additionally, neurons within the medial thalamus encode pain of sufficient intensity for discrimination [219]. Furthermore, the SI is the area responsible for sensory-discrimination, but it is not necessary for determining the intensity of pain. Patients with lesions of the SI can evaluate pain intensity [532] and the removal of the postcentral gyrus through surgery does not help patients suffering from chronic pain [533].

A.1.25 Thalamus:

The third ventricle is surrounded by the thalamus; the gate and critical relay site to the cortical and subcortical structures for ascending sensory pathways including nociceptive input [534]. Divisions of the thalamus are based on their link to particular dorsal horn laminae [36]. The lateral and medial nuclear groups of the thalamus are crucial in the processing of nociceptive input [25]. Neuroimaging studies and patient surgeries confirm the importance of the thalamus in pain processing [36]. Following deafferentation there is increased representation of intact regions towards deafferentated regions of the thalamus [450] and research has demonstrated that peripheral injuries can produce acute and chronic neuroplastic changes to the thalamus [36, 213]. The lateral group includes the ventroposterior medial nucleus, the ventroposterior lateral nucleus, and the posterior nucleus which project to the SI in order to discern the sensory component of a

pain sensation, i.e. intensity and location [26]. The lateral nuclear group receives input from the spinothalamic tract, including nociceptors and wide dynamic range neurons in laminae I and V [36]. The lateral nuclear group and the spinal neurons that project to them have small receptive fields, consistent with spatial localization. Thus the ability to localize stimuli is impaired following lesions limited to the lateral nuclear group, and can cause the inability to localize painful stimuli despite feeling the affective aspects of pain [512]. However, as pain is processed in a parallel distributed fashion, lesions of the lateral portion of the thalamus in humans does not produce analgesia [535].

The medial group of the thalamus is composed of the central lateral nucleus and the intralaminar complex [536]. Neurons in the laminae VII and VIII project to this area of the thalamus. This is considered the spinoreticulothalamic tract as it comprises inputs from the reticular formation. These neurons respond to nociceptive input but also have projections to other cortical areas [25]. Stimulation of the medial nuclear group of the thalamus elicits fear-like responses associated with escape behaviour. In humans, lesions of the medial thalamus can provide relief from intractable pain [520]. However, thalamic lesions can also lead to allodynia and hyperalgesia in the periphery [213].

The thalamus plays a major role in chronic pain; there are changes in the thalamic processing of somatosensory inputs leading to increased perception of pain. Research has demonstrated that there is decreased blood flow in the thalamus contralateral to the site of nociceptive inputs, and following lesions to the PNS or CNS leading to pain, thalamic hypoperfusion occurs [36]. Additionally, several studies have found decreased rCBF in the thalamus of fibromyalgia patients as compared to healthy participants [537, 538]. Other studies have demonstrated that there is an increase in activation of the thalamus associated with attention [539] or analgesia

[540]. Increased rCBF in the bilateral ventrolateral nuclei and contralateral ventroanterior nuclei in healthy participants has been observed [541] and it was hypothesized that these thalamic activations represent increased motor output in healthy participants. Additionally, lesions of the posterior inferior region can also result in analgesia [219].

A.1.26 The limbic system

The limbic system includes the hippocampus, thalamic nuclei, ACC, amygdala, limbic cortex, septum, and fornix and plays a role in emotion, behaviour, memory, and olfaction [542].

Across different clinical pain conditions there is pronounced ACC and insula activation [36]. For people in acute and chronic pain, emotions and mood impact pain perception and coping abilities. In addition, depressive disorders can occur with chronic pain and it is hypothesized that central neuronal plasticity may underlie both conditions [36].

A.1.27 Anterior Cingulate Cortex (ACC):

The ACC is a component of the limbic system and is the frontal part of the cingulate cortex and is situated above and around the corpus callosum [197]. Lesion and imaging studies demonstrate that the ACC has autonomic and cognitive functions including empathy, emotions, and reward anticipation [197]. Activity in the ACC reflects the sensory, cognitive and affective components of pain, comprising attention, anticipation, and evaluation of pain including the initiation and of coping efforts [197, 217]. Animal studies have shown that ACC lesions do not have an effect on nociceptive discriminative functions but impair the ability of the animal to identify the noxious qualities of the input [543]. Similarly, in humans, lesions within the ACC do not have an effect on nociceptive discriminative functions, but has an effect on the perceived pain [544]. ACC neurons that respond to noxious stimuli have a limited role in spatial discrimination and therefore have large bilateral receptive fields [545].

The ACC is activated in imaging studies in studies of acute experimental pain [546-548]. There is an increased rCBF response in the ACC in chronic pain patients and in response to noxious stimuli in healthy participants [549, 550]. PET studies in humans has found that nociceptive heat activates ACC which is consistent with the encoding of the perceived pain [551, 552].

The ACC is critical in the retention of fear memories related to pain as it is activated by the expectancy of pain [220, 553]. The ACC is subdivided into areas responsible for cognition (Brodmann area 24 and 32) and affect (Brodmann area 24, 25, 33) [554]. Lane et al. [555] determined that the rostral portion of the ACC plays a vital role in emotional awareness. These areas of the ACC that are involved in emotion have connections with the amygdala, PAG, and brainstem [554] In humans, Rainville et al. [547] found that hypnosis can alter the unpleasantness of pain and that there is a decrease in pain-evoked activity in the ACC suggesting that there is a representation of the nociceptive input within the ACC that can be modified through cognition.

In addition to responding to pain, the ACC also participates in nociceptive control, specifically the rostral ACC [556]. The role of the ACC in nociceptive inhibition is dependent on the connection of the rostral ACC with subcortical structures and descending inhibition of nociception through the actions of a variety of neurotransmitters, including dopamine, glutamate, and opioids [557]. The ACC receives projections from the medial thalamic nucleus and connects with the descending modulation system, including the PAG [556] and therefore plays a role in processing pain-related emotion. The ACC is also hypothesized to contributed to the motor responses as it has projections to motor areas and thus activation of the ACC during pain may impact motor control [558].

A.1.28 Insula:

The insula is part of the cerebral cortex which is located within the lateral sulcus between the frontal lobe and the temporal lobe near the SI [559] and is divided into two parts: the posterior insula and the larger anterior insula. The insula has interconnections with regions involved in autonomic regulation [560] and connects with the frontal, temporal, parietal, lobes, amygdala, cingulate gyrus, and the thalamus. Animal research studies confirm that the anterior insula connects to the brainstem; the PAG, RVM, NCF, and the parabrachial nucleus [36]. The insula is a limbic integration area and through these connections are linked to sensory, motor, motor association, somatosensory, vestibular, and language areas [561]. The insula therefore plays a role in emotion and homeostasis. These roles include perception, motor control, cognitive functioning, executive functions, and respiratory control [562]. Additionally, the insula participates in the multidimensional experience of pain [217] and receives direct afferent projections from the thalamus [553]. Homeostatic afferent input ascends to the mid-posterior insula, and then represented in the anterior insula, an area that is responsible for interoception (evaluation of the physiological state) [217].

In healthy individuals, the insula is activated during sadness, happiness, fear, and anger [563], as well as in normal anticipatory anxiety [564, 565]. PET studies of emotion [555] and anxiety [566] demonstrate activation of the insula. It is hypothesized that the insula plays is instrumental in processing the emotional component of pain and is implicated in the evaluation of distressing sensory and cognitive and interoceptive input [564]. The insula is more active when there are threats to what the body needs for survival and thus plays a role in pain perception and contributes to the autonomic aspects of pain [553].

PET imaging research indicates that the cingulate gyrus and the insula are activated following nociceptive input [25] and that activation in the insula is correlated with the subjective pain

experience [557]. The insula is activated with anticipation of pain [458], and with pain empathy [567]. The posterior insula is somatotopically organized; is activated in response to acute and chronic pain and stimulating it can cause the perception of pain [217]. Research has shown that chronic pain is more rostrally located in the anterior insula in comparison with healthy participants [36]. Lesions of the insula result in pain neglect behaviour [529], which is referred to as asymbolia for pain. Individuals with this syndrome are able to distinguish nociceptive input as painful but do not display the appropriate emotional responses [25].

Research has shown that the insula has a role in pain modulation [557]. Animal research demonstrates that the insula is connected to the brainstem and the parabrachial nucleus [36] which may explain how emotions may impact pain perception. Evidence indicates that opioid, GABA and dopamine neurotransmitters are utilized in pain modulation by the insula [557].

A.1.29 Amygdala:

The amygdala are a part of the limbic system and consist of nuclei within the medial temporal lobes that play a role in the memory of emotional responses [568]. The lateral (LA), central (CeA), and basolateral (BLA) nuclei are particularly important for the processing of sensory information. Nociceptive input is received through different lines of input. Nociceptive input reaches the CeA from the brainstem and spinal cord [569]. Nociceptive information from the thalamus and cortical areas target the LA which is an area that is important in anxiety and fear. Neuroplasticity in the amygdala as a result of nociceptive input leads to anxiety [217].

The amygdala is also important for pain inhibition as it has connections to the brainstem's pain control areas.

A.1.30 Cerebellum:

The cerebellum lies posterior to the pons and medulla oblongata [570] and is divided into three main lobes: anterior, posterior, and the flocculonodular. The three lobes are subdivided into 10

lobules, I to X [570]. The cerebellum modulates range and force of movement, and plays a role in motor learning and the coordination of movements [512]. Cerebellar lesions do not induce paralysis but impair voluntary movements, reflex movements, spatial accuracy and the coordination of movement [25]. The cerebellum is involved in cognition. Although previous research has demonstrated that the cerebellum is activated in response to nociceptive input as most fMRI studies show activation of the cerebellum in response to pain [214, 242] the function of the cerebellum in the processing of nociceptive input is unknown [455].

Pathways to the cerebellum are via the mossy and climbing fibers. Mossy fibers transmit input from the pontine nuclei [455]. The Purkinje cell is the processing unit of the cerebellum that provides the sole neural output from the cerebellar cortex and is responsible for integrating information from the pons and inferior olive and exerts an inhibitory effect on intracerebellar and vestibular nuclei [570].

Research confirms that the cerebellum receives inputs from cutaneous primary afferents [571], and receives nociceptive input [205]. In an animal study, activation of nociceptors activated climbing fibers [456]. C fiber nociceptors convey input as part of a spinoolivocerebellar pathway and can reach Purkinje cells [572].

It is unknown how nociceptive input is encoded however it is suggested that the cerebellum plays a modulatory role. An animal study demonstrated that electrical stimulation of the cerebellum modulated the encoding of nociceptive input to the thalamus [573]. Another animal study found that electrical stimulation of the cerebellum can raise nociceptive thresholds [574]. Injection of morphine into the cerebellum of animals results in acute analgesia [575] and stimulation of the cerebellum using electrical stimulation increases neural responses to a nociceptive stimulus around nociceptive neurons in the dorsal horn [576]. Another study has

demonstrated functional changes in the SI following cerebellar lesions [73]. The cerebellum is now thought to function in the processing of sensory input as discriminating sensory information significantly increased cerebellar activation [418]. Furthermore, the passive manipulation of a limb produces cerebellar activation of the limb [323] and it is hypothesized that increased cerebellum activation associated with motor control may be due to the processing of sensory information.

A.1.31 The Cerebellum and Basal ganglia (BG)

The cerebellum and the BG are groups of subcortical nuclei with significant roles in motor control [349]. Nociceptive input evokes the experience of pain and behavior is almost always provoked. It has been hypothesized that there are innate motor patterns that are triggered by nociceptive input. Chudler et al.[577] found evidence for nociceptive processing by pallidal neurons and other research studies have found that the globus pallidus is activated in patients suffering from chronic pain [578-580]. In addition, some patients with chronic pain have abnormalities of posture and motor control that are similar to those occurring in patients with lesions of the BG [581].

The cerebellum and BG integrate inputs from the prefrontal, temporal and parietal lobes and provide output to the MI through the thalamus [582, 583]. Research has indicated that cerebellar output targets the MI and prefrontal areas [584-587]. In addition, Alexander et al. [588] hypothesized that BG output targets prefrontal areas implicated in cognition. Different cortical regions ascend to specific cerebellar and BG regions that project to the same cortical regions creating closed-loop circuits [349, 589, 590]. In addition, there is a connection linking the output of the cerebellum to an input stage of BG processing [591] and another connection linking the

sub thalamic nucleus of the BG to the cerebellar cortex [592]. Bostan et al. [592] determined that the connection that links the cerebellum with the BG integrate cerebellar and BG functions.

A.1.32 Hypothalamus:

The hypothalamus consists of a number of small nuclei; forms the ventral part of the diencephalon, situated beneath the thalamus and above the brain stem [593] and is the main center for neuroendocrine and autonomic regulations. The hypothalamus is a link to the endocrine system via the pituitary gland [509]. Body temperature, thirst, hunger, sleep, and circadian cycles are regulated by the hypothalamus through synthesizing and secreting hormones which stimulate or inhibit the pituitary gland [213]. The hypothalamus has nociceptive afferents and efferent projections to the brainstem and dorsal horn [213]. The hypothalamus is involved in the complex regulations that accompanying chronic pain and stress and animal research studies have reported alterations in hypothalamic function in individuals suffering from chronic pain [213].

A.1.33 Primary and Secondary Sensory Cortex (SI and SII):

The somatosensory cortex is divided into SI and SII areas [553]. The SI and SII areas encode spatial information about nociception as well as the severity of the nociception [553]. In humans, neuroimaging studies have shown that nociceptive input activates the SI and SII while animal work confirmed that these areas receive direct nociceptive input [547].

The SI and SII are areas responsible for encoding the sensory and discriminations aspects of pain [531, 594, 595]. Research indicates that SI and SII are implicated in encoding the intensity and location of the stimulus. However, the response of the SII nociceptive neurons are dependent on the arousal levels [596, 597]. Decreased activation in the SII was found when participants were distracted from the nociceptive input [597] while increased attention increases the SII responses

[598]. It is established that the perception of pain can be altered by a patient's attention to their pain [596, 599, 600].

Activity within thalamus, SII, SI and insula in response to nociceptive input is associated with the discrimination characteristics of pain, while the ACC may be linked with the affective and attentional processing of pain [541].

A.1.34 SI:

The SI is situated in the postcentral gyrus and is subdivided into distinct and well-defined loci (Brodmann's area 3a, 3b, 1 and 2) [553]. These areas each contain a separate body representation [601] characterized by a distinct connectivity [602]. The SI plays a critical role in processing somatosensory information and is important in somatosensory acuity, detection and discrimination [28, 217]. The ability to locate somatic input in time and space is impaired following lesions to the SI or the ventral posterolateral thalamus (although these lesions do not generate analgesia) [535]. The SI is important in the processing of both tactile and nociceptive stimuli [603, 604]. Ploner et al. [605] found that in humans there was concurrent activation of SII and SI and SII to nociceptive input.

Research indicates that chronic pain is related to alterations in cortical organization. For example, the presence and magnitude of phantom limb pain is correlated with reorganization within the SI. Flor et al. [150], found that activation in the SI was correlated with pain among individuals with phantom limb pain. Additionally, in individuals suffering from phantom pain, stimulation of the forearm produces sensations in the phantom hand [152]. As the hand and forearm are somatotopically close within the SI, it is hypothesized that the phantom pain is a result of the neuroplasticity in terms of the organization of the forearm and hand in the CNS. Similar cortical reorganization is thought to occur in other chronic pain conditions [553].

A.1.35 SII:

The SII is near the SI in the parietal lobe. SII neurons are some of the first neurons at the level of the cortex to receive nociceptive input [553]. In contrast to touch, pain evokes activation of SII and the insula and this may function in the sensory-discrimination components of pain [214]. It is hypothesized that the SII is important for the recognition of pain as evoked potential recordings in humans demonstrated that the SII was activated concurrently with the SI [606]. Retrograde and anterograde tracer studies in animals demonstrate that the noxious stimulus reaches the SII more through a more direct pathway than non-noxious stimuli [450].

A.1.36 The prefrontal cortex:

The prefrontal cortex (PFC) lies on the surface of the brain, on the anteriorly of the premotor and motor areas (Brodmann's area 9-12 and 44-47). This region has been implicated in executive functions, such cognition cognitive behaviours, reward expectancy, goal directed behaviours, personality, decision making, and social behaviour [553]. The PFC does not have a primary sensory role however it is implicated in higher order processing of sensory input and has projections to motor and limbic systems [26, 27].

Research suggests that the prefrontal cortex encodes the cognitive aspects of pain [553]. The interaction of the prefrontal cortex with the midbrain, thalamus, and the limbic system is dependent upon the motivational and emotional context [220]. PFC activation is observed with experimental pain [217] including following capsaicin application [220]. It is hypothesized that during acute experimental pain the prefrontal cortex interacts with mechanisms of descending inhibition, governed by the brainstem [220]. The literature demonstrates that activation within the PFC that occurs with nociceptive input is negatively correlated with how severe the pain is perceived to be. This is in line with the view that the PFC has an inhibitory function [553]. In

contrast, PFC deactivation is seen in patients suffering from chronic pain and it is hypothesized that there is a decreased capacity to inhibit pain [217]. Chronic back pain and fibromyalgia is correlated with decreased grey matter in the PFC, linked with decreased pain inhibition [553]. In addition, Luoto et al.[607] found decreased speed of information processing among patients with chronic lower back pain.

Animal studies demonstrate that there is an increase in descending inhibition during acute inflammation in comparison with chronic inflammation even under similar pain intensities [220]. This is in line with the theory that the PFC exerts a top-down influence on the PAG in the brainstem, reducing pain perception [553]. Animal research demonstrated that stimulation of the pathway from the PFC to the midbrain mediates anti-nociceptive effects [220].

A.1.37 mPFC

The paracingulate mPFC has been implicated in emotional processing [608, 609], response conflict, and detection of adverse outcomes [610]. The mPFC plays a role in a sustained emotional response, and modulates the time course of emotional response [611]. The mPFC is activated during anticipation of pain [419], and with increasing nociceptive input [220]. Factors that lead to persistent mPFC activation without a nociceptive input include: the nature of the pain, enhanced spinal prefrontal projections through the spinoparabrachial, spinostratial, and spinoreticular pathways [612], and the interaction between mPFC and DLPFC [613].

A.1.38 DLPFC

DLPFC is implicated in cognition, including willed actions and with reappraisal [614]. Dias et al. [615] found that the DLPFC plays a role in the inhibition of nociceptive input. The DLPFC is also important for working memory [616]; and is inversely correlated with mPFC activation

[617]. The interaction between the DLPFC and mPFC in which the activity of DLPFC is inversely related with mPFC activity has been demonstrated with pain [220].

A.1.39 OFC

The orbitofrontal cortex (OFC) is divided into lateral and medial divisions by the medial orbital gyrus [618], and is linked with paralimbic regions. The orbitofrontal cortex receives input from the hypothalamus, hippocampus, amygdala, olfactory cortex, thalamus, and the SII. Animal and human research demonstrates that the OFC plays a role in inhibition and protects goal-directed behaviour from interference [619, 620] controls the autonomic responses that are associated with emotional experience [621] and is proposed to play a role in anxiety [564].

A.1.40 Anticipation, empathy, attention: prefrontal cortex and the limbic system

It is hypothesized that pain utilizes attentional resources as it is relevant for survival. Event-related potentials [622], imaging research [546], and cognition studies [623, 624] have found increased error rates with pain. Furthermore, it is hypothesized that pain uses up the attentional resources and this may alter the coordination of movement. Several studies have shown that stress may affect motor control [625, 626].

fMRI research demonstrated that attention and distraction can modulate pain related activation in diverse pain processing areas, with associated alterations in perception [36]. Research provides evidence that pain can be modulated by cognition (attention, expectation, or memory) and emotional state (fear and anxiety). Changes in the PFC that occur in response to pain results in the conscious awareness and evaluation of pain and the emotional–affective consequences of pain [217]. Furthermore, there is evidence that medial prefrontal areas are activated by the expectancy of pain [220].

Under specific conditions such as hypnosis [547], or pain anticipation [458], the ACC and the insula underlie the affective and attention components of nociceptive processing. This is in contrast to the sensory-discriminative component of pain which demonstrate activation of the SI and SII. Following painful stimuli to different parts of the body there is a somatotopic organization of hemodynamic activity in the SI and SII [627] supporting their role in sensory-discriminative functions. Imaging research looking at the effects of attention show that there is a decrease in the activation of numerous cortical areas, including limbic, sensory, and prefrontal regions [628, 629]. These studies show reduced activation in sensory regions of the cortex while the frontal regions show increased activity [220] suggesting that attention can mediate pain through frontal structures resulting in reduced perceived pain. There is an interaction between the ACC and frontal regions with the PAG and the thalamus which can reduce the activity in cortical sensory regions resulting in a decreased perception of pain [450].

Anticipation and attention are important in pain processing [151] and nociceptive pathways are stimulated by the anticipation of pain in the absence of a pain stimulus [202, 419]. These results demonstrate nociceptive networks are influenced by cognitive factors and provides evidence for top-down influences that can modulate the nervous system. Pain empathy includes the affective but not the sensory aspects. Studies in pain empathy have shown that many cortical areas are activated for experiencing pain and knowing that another participant was experiencing pain [450].

Improved mood usually reduces pain, while a deterioration in mood increases pain [450].

Cortical input can also gate out nociceptive input. The cortical input can be manipulated pharmacologically, with the administration of drugs that are normally used in the treatment of depression [151].

A.2 Consent form



Professor Bernadette Murphy
University of Ontario Institute of Technology
Faculty of Health Sciences
2000 Simcoe St. North
Oshawa, Ontario
CANADA L0B 1J0
Email: Bernadette.Murphy@uoit.ca
Phone: (905) 721-8668 Fax: (905) 721-3179

Central sensitization evokes changes in the properties of nerve conduction

Purpose of the Study

The physiologic mechanisms of pain are poorly understood. Central sensitization is an important, if not fundamental, mechanism in expression of pain yet there is currently no objective measure of central sensitization. Central sensitization is defined as an 'increased excitability' of nerves in the central nervous system. The purpose of this study is to investigate the effect of central sensitization on the characteristics of nerve conduction in humans. Specifically, we are interested in finding out what, if any, changes occur to the properties of nerve impulses after sensitization as it may provide insight into novel methods of quantifying sensitization. We are also interested in understanding if sensitization affects motor performance, that is, the way your muscles perform when learning a novel task. You are invited to participate in this study being conducted by Dr Bernadette Murphy (Faculty of Health Sciences, University of Ontario Institute of Technology). It has received Ethical Approval from the University of Ontario Institute of Technology (REB# 11-067).

Procedure

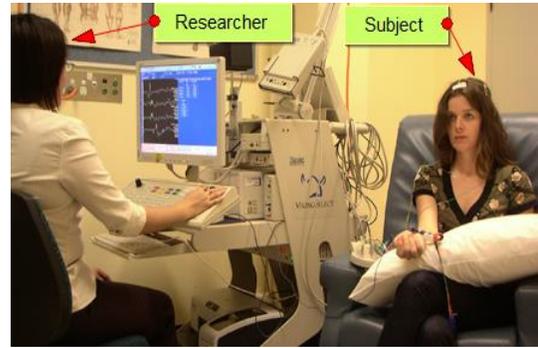
Prior to the commencement of the study, you will be required complete a general health questionnaire which gives us a profile of your current health status and how this may affect your results. You may fill this form out at home prior to arriving for the study. You will also be required to undergo a brief physical examination by one of the presiding clinicians to ensure that you are eligible to participate in this study. This exam will involve standard orthopaedic and neurologic testing to ensure that you do not have any conditions which may affect the way you process sensations on the skin. The study will require approximately two hours of your time.

We will require access to your arm, shoulder, upper back and neck regions; please wear appropriate clothing that allows for exposure of these areas. In the event you do not have such clothing, you will be provided appropriate gowns for this study. In addition, you will have complete and sole privacy in the Human Neurophysiology lab for the duration of this study.

You will be seated in a comfortable reclining chair for the recording of the nerve impulses. There are three different types of nerve impulses which we wish to test. You may choose to participate in **one, two or three of the measurement types**.

They are: **1) Somatosensory evoked potentials, (SSEP)**.

Surface electrodes will be placed on your skin at selected points along your arm, spine and scalp; these electrodes are sticky electrodes that affix directly to your skin. We will then apply a small electrical pulse to the electrode in the arm, and measure this pulse at the other electrodes along the arm, spine and scalp. The pulse will be very mild and may feel like a brief pin prick or irritation. These will be your 'baseline' readings. A typical SSEP experimental setup is illustrated above.



2) Transcranial Magnetic Stimulation (TMS) During the evaluation session we will collect some information about the way your brain is processing information from your upper limb, and how it is controlling hand and forearm muscles. To do this it will be necessary to place some electrodes on your skin over these hand, and forearm, muscles to record the signals from your brain to these muscles. You may experience some mild discomfort as your skin is prepared for the electrodes by rubbing them with special abrasive tape and then wiping the area with alcohol. It is important to note that these are recording electrodes only and do not pierce the skin and do not run current through your body. The stimulation will only be over your scalp. Occasionally, some people experience mild, transient nausea or scalp discomfort, due to the activation of the scalp muscles by the stimulator. If you feel uncomfortable at any time during the experiment, please notify the experimenter. Each evaluation session will take approximately 2-3 hours and you will be given feedback about your results at each session.

3) H-reflexes: An H-reflex is similar to the tendon reflex except that it is elicited by electrically stimulating your nerve rather than tapping your tendons. The same electrical stimulator used for SSEP recordings will be used to stimulate the median nerve on the front of your elbow area in order to elicit a reflex in the flexor carpi radialis muscles which flexes your wrist. We will place recording electrodes over your flexor carpi radialis muscle which will record the muscle contraction evoked when we stimulate the nerve to this muscle at the front of your elbow. You may experience some mild discomfort as your skin is prepared for the stimulating and recording electrodes by rubbing them with special abrasive tape and then wiping the area with alcohol.

After recording the baseline readings for each type of experiments, you will randomly be assigned to have one of two types of topical cream to a specific area of your elbow. This cream will either be a moisturizing cream or Zostrix, an over-the-counter cream commonly used for reducing muscle and joint pain. The active component of this cream is a substance called capsaicin, which is derived naturally from chilli peppers and acts to mildly irritate the pain receptors in the skin. The irritation of pain receptors results in central sensitization and this process will not harm you in any way. SEP recordings will be taken again at 15 and 30 minutes after the application of the Zostrix cream.



The investigator applying the capsaicin cream will wear gloves at all times. After the application of the cream, please do not touch or scratch the treated area for 3 hours to avoid getting the capsaicin on your hands and potentially transferring it to other parts of your body. Capsaicin is mildly irritating to the skin, especially sensitive

areas such as mucous membranes, mouth, eyes and groin. Please ensure you wash your hands vigorously with warm soapy water after the study is complete.

Typing task intervention

Some experiments will include a typing task which will take place after the cream has been applied. The intervention will consist of a repetitive typing task where you will be required to press keys on an external numeric keyboard with your thumb for a period of 20 minutes. There will be sequences of four letters arranged in random order that come up on a computer monitor and you will be asked to reproduce them with the numeric key pad. We will be monitoring the typing rate and number of errors to determine the effects of capsaicin on your ability to type these sequences.

Tracing task intervention

Some experiments will include a tracing task which will take place after the cream has been applied. You will be required to trace sequences of sinusoidal-pattern waves with varying frequency and amplitude using only you thumb on an external wireless touchpad for a period of 20 minutes. We will be monitoring accuracy in order to determine the effects of capsaicin on your ability to trace these sequences.

Cortisol

Cortisol is a steroid hormone released during stressful episodes such as acute pain. Cortisol elevation is a normal part of the physiological response to stress. Elevations in cortisol production is linked with changes in the way the brain functions which can affect task performance. The researchers will use swabs under your tongue to collect your saliva three times throughout the experiment. These samples of your saliva (spit) will then be put in the freezer and will be later tested at a laboratory for the stress hormone cortisol.

Potential Risks and Discomforts

It is important to disclose any/all potential risks associated with this research study prior to participation. You may experience some local effects in the areas treated with the lotion. Specific symptoms may include a mild to moderate tingling and/or warmth sensation. The tingling will subside within 2 hours of application but may be mildly rekindled if warmed (eg. warm baths) within the first 24 hours after treatment at the site of treatment. You may also experience redness in the areas where the topical lotion was applied which corresponds to increased local blood flow. These symptoms can be effectively minimized or eliminated by icing the treated area(s) with a 10 min of icing (ON) followed by 10 min OFF pattern, as required symptomatically.

You may also feel some mild discomfort as your skin is being prepared for SSEP, TMS or H-reflex recordings. This will involve mild debridement (scraping) of the skin to remove debris and dead cells. The stimulating electrode on the arm will be used to stimulate some of the hand and arm muscles by passing a mild current through them. You will likely feel a mild tingling sensation on the skin over the nerve. While it is not painful or harmful, you may feel some of the hand and/or forearm muscles twitch mildly. This will not be painful nor is there any risk of harm or damage to the nerve and/or muscle, due to the very mild intensity of the stimulus.

Potential Benefits to Participants and/or to Society

While there are no direct benefit to subjects, this study will provide us with valuable information on the effects of sensitization in the nervous system. You will be provided with a summary of findings at the end of the study, if you so desire. Please advise us of your preferable format for communication (check one and provide details in the space provided):

- email _____
- fax _____
- written _____

Compensation for Participation

You will be offered your choice of \$10 gasoline voucher or a Tim card to thank you for your participation in this experiment.

Confidentiality

Every effort will be made to ensure confidentiality of personal information that is obtained in connection with this study. Confidentiality will be secured by the use of participant ID Codes on all correspondence. Data will be kept indefinitely on a password-protected computer in the researcher's laboratory and all written material secured in a locked cabinet on site for a period of seven years, after which it will be shredded.

Participation and Withdrawal

You may choose whether to be involved with this study or not. If you volunteer, you may withdraw at any time without consequence. You may exercise the option of removing your data from the study up to and including the point where it is anonymously coded and can no longer be identified. You may also refuse to answer any questions you don't want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise that warrant doing so.

Rights of Research Participants

You may withdraw your consent at any time and discontinue participation without penalty. This study has been reviewed and received ethics clearance through the University of Ontario Institute of Technology Research Ethics Board REB 11-067.

Any questions regarding your rights as a participant, complaints or adverse events may be addressed to Research Ethics Board through the Compliance Officer compliance@uoit.ca (905 721 8668 ext 3693).

Thank you very much for your time and help in making this study possible. If you have any queries, concerns about side effects or you wish to know more please contact Dr Bernadette Murphy, an Associate Professor at the University of Ontario Institute of Technology, Faculty of Health Sciences, 2000 Simcoe St North, Oshawa, Ontario, L1H 7K4 Phone (905) 721-8668 ext 2778 or email : Bernadette.Murphy@uoit.ca or Dr John Srbely (at 416-760-7418).

Please read the following before signing the consent form and remember to keep a copy for your own records.

- I understand that taking part in this study is voluntary (my choice) and that I am free to withdraw from the study at any time without giving a reason. If I am a student, I understand that this will in no way affect my academic progress, irrespective of whether or not payment is involved.
- I have read and I understand the consent form for volunteers taking part in the study designed to investigate central sensitization. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.
- I will be attending **at least one session** where measurements will be taken of the electrical activity in my nervous system before and after the application of cream, which may be either capsaicin or control cream.
- I understand that by signing this consent form I am not waiving any legal rights.
- I have completed an eligibility checklist to ensure I am eligible to participate in this research.
- I understand that I can withdraw any data I supply up to and including the completion of my last measurement session.
- I understand that my participation in this study is confidential to the researchers and that no material which could identify me will be used in any reports on this study.
- I have had time to consider whether to take part.
- I know who to contact if I have any side effects to the study.

- I know who to contact if I have any questions about the study.

I give consent for the data from this study to be used in future research as long as there is no way that I can be identified in this research. (tick one)

YES NO

I would like to receive a short report about the outcomes of this study (tick one)

YES NO

(Name of Participant)

(Date)

(Signature of Participant)/

(Signature of Research

A.3 Pain questionnaire



COLLEGE OF BIOLOGICAL SCIENCE
Department of Human Health and Nutritional Sciences

RESEARCH STUDY CONFIDENTIAL HEALTH HISTORY

Subject CODE: _____

How old are you?

You are: Male Female

Are you: Left Handed Right Handed

Do you play a musical instrument Yes No

If yes, how many times a week?

Do you play competitive sports? Yes No

If yes, please indicate what sport and how often?

Do you suffer from any joint or muscle pain? Yes no

How long have you had the above pain?

Is your pain getting: better worse

Was this pain a result of an accident, fall or injury? Yes no

Does the pain wake you at night? Yes no

Do you experience pain/discomfort in morning? Yes no

What does the pain feel like? Burning numb/tingling deep/achy sharp/stabbing

What seems to help your pain? Physiotherapy chiropractic massage acupuncture
medication rest exercise Other: _____

Do you have any allergies to topical ointments? Yes no

Are you allergic to deep heat crèmes? Yes no

Are you allergic to capsaicin (active ingredient in some deep heat crèmes and chili peppers)?

Yes no

Do you have a history of:

-Use of anticoagulant medication or therapy yes no

-Stroke or transient ischemic attacks yes no

-Serious cervical spine trauma/fracture/dislocation yes no

A.4 TMS safety checklist

TMS safety checklist:

The following questions are to ensure it is safe for you to have TMS applied. If you answer yes to any of the questions below, we may need to exclude you from TMS experiments.

QUESTION	ANSWER	
1. Do you suffer from epilepsy, or have you ever had an epileptic seizure?	Yes	No
2. Does anyone in your family suffer from epilepsy?	Yes	No
3. Do you have any metal implant(s) in any part of your body or head? (Excluding tooth fillings)	Yes	No
4. Do you have an implanted medication pump?	Yes	No
5. Do you wear a pacemaker?	Yes	No
6. Do you suffer any form of heart disease?	Yes	No
7. Do you suffer from reoccurring headaches**?	Yes	No
8. Have you ever had a skull fracture or serious head injury?	Yes	No
9. Have you ever had any head surgery?	Yes	No
10. Are you pregnant?	Yes	No
11. Do you take any medication or use recreational drugs (including marijuana)*?	Yes	No
12. Do you suffer from any known neurological or medical conditions?	Yes	No

Comments _____

Name _____

Signature _____

Date _____

*Note if taking medication or using recreational drugs please read through the medication list on the next page to see if you use contraindicated drugs or medications. You do not need to tell the researcher which medications or drugs you use, unless you wish to. However, all researchers have signed confidentiality agreements and this information will not be recorded in writing, if you do wish to discuss this issue.

**Dr. Murphy will meet with participants who answer yes to this question to seek further information.

Medications contraindicated with magnetic stimulation:

1) Tricyclic antidepressants

Name	Brand
amitriptyline (& butriptyline)	Elavil, Endep, Tryptanol, Trepiline
desipramine	Norpramin, Pertofrane
dothiepin hydrochloride	Prothiaden, Thaden
imipramine (& dibenzepin)	Tofranil
iprindole	-
nortriptyline	Pamelor
opipramol	Opipramol-neuraxpharm, Insidon
protriptyline	Vivactil
trimipramine	Surmontil
amoxapine	Asendin, Asendis, Defanyl, Demolox, Moxadil
doxepin	Adapin, Sinequan
clomipramine	Anafranil

2) Neuroleptic or Antipsychotic drugs

A) Typical antipsychotics

Phenothiazines:	Thioxanthenes:
o Chlorpromazine (Thorazine)	o Chlorprothixene
o Fluphenazine (Prolixin)	o Flupenthixol (Depixol and Fluanxol)
o Perphenazine (Trilafon)	o Thiothixene (Navane)
o Prochlorperazine (Compazine)	o Zuclopenthixol (Clopixol and Acuphase)
o Thioridazine (Mellaril)	• Butyrophenones:
o Trifluoperazine (Stelazine)	o Haloperidol (Haldol)
o Mesoridazine	o Droperidol
o Promazine	o Pimozide (Orap)
o Triflupromazine (Vesprin)	o Melperone
Levomepromazine (Nozinan)	

B) Atypical antipsychotics

Clozapine (Clozaril)	Quetiapine (Seroquel)
• Olanzapine (Zyprexa)	• Ziprasidone (Geodon)
Paliperidone (Invega)	• Amisulpride (Solian)
• Risperidone (Risperdal)	

C) Dopamine partial agonists: Aripiprazole (Abilify)

D) Others

Symbyax - A combination of olanzapine and fluoxetine used in the treatment of bipolar depression.

Tetrabenazine (Nitoman in Canada and Xenazine in New Zealand and some parts of Europe)

Cannabidiol One of the main psychoactive components of cannabis.

Regular Cannabis use more often than once per week and/or cannabis use in the past 4 days.

Regular use of other recreational drugs, or single episode within the past three weeks.

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