An Investigation of Experimentally Induced Central Sensitization and Motor Unit Activity Using High-Density Surface Electromyography Decomposition

by

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ABSTRACT

AN INVESTIGATION OF EXPERIMENTALLY INDUCED CENTRAL SENSITIZATION AND MOTOR UNIT ACTIVITY USING HIGH-DENSITY SURFACE ELECTROMYOGRAPHY DECOMPOSITION

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Central sensitization (CS) is an amplification mechanism for pain in the central nervous system. I hypothesized that motor unit firing rates in the tibialis anterior would decrease during experimental CS without changes in recruitment thresholds, de-recruitment thresholds, or electromyography amplitude. Two participants were assigned to the “CS” group and received topical application of capsaicin. One participant was randomly assigned to the “placebo” group and a non-noxious cream was applied. Participants dorsiflexed their ankle isometrically and electromyography from the tibialis anterior was collected and decomposed to identify motor units before and after cream application. The mean recruitment thresholds of the CS group were reduced and de-recruitment thresholds and firing rates were unchanged. EMG amplitude in both groups increased. This does not support my hypothesis. I suggest the use of a more advanced decomposition algorithm, larger sample, and more robust controls for psychological factors that influence pain.

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Central Sensitization	CS
A-delta fibers	Aδ
Amino-3-hydroxy-5-methyl-4-isoxazole propionate	AMPA
N-methyl-D-Aspartate	NMDA
Long-Term Potentiation	LTP
Neurokinin 1 receptor	NK1
Calcium	Ca2+
Sodium	Na+
Transient receptor potential cation channel subfamily V member 1	TRPV1
Electroencephalogram	EEG
Electromyography	EMG
Surface Electromyography	sEMG
Indwelling Electromyography	iEMG
High-Density Electromyography	HDEMG
Persistent Inward Current	PIC
Neurokinin 1 receptor	NK1
Protein kinase A	PKA
Protein kinase C	PKC
Phospholipase C	PLC
Extra cellular signal regulating kinase	ERK
Numerical Pain Rating Scale	NPRS
Visual Analog Scale	VAS
Decompose-Synthesize-Decompose-Compare	DSDC
Precision Decomposition 1	PD1
Precision Decomposition 3	PD3

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Chapter 1: Background and Literature Review

1.0 Literature Review

1.1 Prevalence and Incidence of Chronic Pain

Worldwide, chronic pain has become an epidemic (Burma et al., 2017). Burma et al., (2017) estimate that 20% of adults experience chronic pain which accounts for approximately 1.5 billion people affected globally. Chronic pain conditions such as fibromyalgia, myofascial pain syndrome, and osteoarthritis effect 18.9% of Canadians (Schopflocher et al., 2011). Direct medical costs of treating chronic pain are over $6 billion dollars annually (Dion et al., 2010). Indirect costs reach nearly $37 billion dollars each year (Lynch, 2011).

Currently, treatment for chronic pain conditions consists of opioid prescription (Els et al., 2017). The prevalence of opioid prescription has been increasing (Voon et al., 2017). Increased opioid prescription has resulted in an opioid epidemic characterized by misuse leading to addiction or death. Voon, Karamouzian, and Kerr (2017) investigated the ongoing opioid epidemic in Canada. Their review investigated the number of individuals with non-cancer related chronic pain who took prescription opioids for non-medicinal purposes. An estimated number of up to 60% of these individuals suffering from chronic pain may be abusing opioids (Voon et al., 2017). When compared to the general population, approximately 11% to 19% of the population takes prescription opioids (Els et al., 2017). A better understanding of pain is required to guide treatment alternatives to reduce the number of opioids that are prescribed to people with chronic pain.

1.2 Chronic Musculoskeletal Pain

Musculoskeletal (MSK) pain; pain which effects the muscles, tendons, ligaments, bones, and joints, is considered one of the leading causes of disability according to the World Health

Organization (Bhayankaram et al., 2019; Zumwalt & Reddy, 2019). Between the ages of 10 and 80-years old, MSK pain is ranked in the top 10 causes of disability worldwide. (Bhayankaram et al., 2019). MSK conditions occur from acute or chronic damage to joints, muscles, ligaments, or tendons and result in inflammation and pain (Zumwalt & Reddy, 2019). Chronic MSK pain develops from a variety of different factors. Lifestyle factors, occupation, anatomy, stress, family history and many other factors all play a role in determining whether MSK pain will develop.

1.3 Central Sensitization

Many people with chronic pain may experience central sensitization (CS). Central sensitization is an amplification mechanism for nociception within the central nervous system (Scerbo et al., 2018). CS can create a disproportionate perception of pain in response to a noxious stimulus. The International Association for the Study of Pain (IASP) identifies CS as amplified responsiveness of nociceptive neurons within the central nervous system (CNS) in response to normal afferent input (Jensen et al., 2011). Normal afferent input may lead to the perception of pain in those with CS in the absence of a painful stimulus. CS develops from persistent afferent input from peripheral nociceptors and this persistent input creates increased excitability of the dorsal horn of the spinal cord (Latremoliere & Woolf, 2009; Woolf, 2011). This persistent activity causes activity-dependent and cellular-mediated responses which explain the observed increased excitability of dorsal horn nociceptors (Latremoliere & Woolf, 2009). Arendt-Nielsen et al. (2018) explain that CS can present either within a specific spinal segment (e.g., C3 level of the cervical spine and all regions supplied by the C3 spinal nerve, L4 level of the lumbar spine, etc.) or with widespread effects throughout the body. These widespread effects can be seen in multiple spinal segments or within multiple body regions (Arendt-Nielsen et al., 2018).

Within the realm of chronic pain conditions are disorders such as myofascial pain syndrome, generalized widespread pain sydrome, fibromyalgia, whiplash, and musculoskeletal

disorders such as osteoarthritis (den Boer et al., 2019). In each of these conditions, persistent nociceptive input may lead to central sensitization (Woolf, 2011). Studying the development of CS is necessary to learn more about the pathophysiology of chronic pain to guide future treatment and pain management. The current challenge in studying CS is determining the underlying mechanisms of CS and its effects for future translation into pain management.

CS can elicit phenomena known as allodynia and hyperalgesia. Allodynia is a misinterpretation of mechanical stimuli being recognized as a nociceptive input (den Boer et al., 2019). Hyperalgesia is an inappropriately high magnitude of response to a painful stimulus (den Boer et al., 2019). A person with allodynia may feel that a paint brush stroke on the skin is painful, a technique known as brush allodynia. A person with hyperalgesia may feel that touching something hot such as a cup of coffee, which would normally be somewhat painful, is extremely intense. The following sections of this review will explain in greater detail the value of studying CS, the anatomy and cellular mechanisms of its development, and its clinical presentation and assessment. A comparison between the segmental and widespread effects seen across the spectrum of CS will be addressed in a later section of this review.

1.4 Anatomy of Central Sensitization

Persistent nociceptive input to the dorsal horn of the spinal cord originating in the periphery leads to the development of CS (Woolf, 2011). The cell bodies of peripheral nociceptors are localized in the dorsal root ganglion (DRG) (Latremoliere & Woolf, 2009). Peripheral nociceptors synapse in the dorsal horn of the spinal cord with receptors projecting from the periphery (skin, muscle, joints, organs) (Latremoliere & Woolf, 2009). The two main types of nociceptors are C-fibers and Aδ fibers (Kandel et al., 2000). The C-fibers are activated by tissue damage and noxious temperature (extreme heat or cold). These fibers are unmyelinated and small diameter leading to slow conduction velocity relative to other proprioceptive neurons

such as 1a afferents (Basbaum et al., 2009). Aδ fibers respond to “sharp” stimuli and have a higher conduction velocity than C-fibers as they are small diameter but are myelinated. C-fibers synapse in layers I and II of the dorsal horn laminae whereas Aδ fibers synapse in layers I and V. Synapses in the dorsal horn are organized in layers divided based on function. Layers I and II respond to noxious stimuli primarily as well as layer V which is a hybrid of both nociception and non-noxious stimuli (Basbaum et al., 2009). Layers III and IV are primarily for non-noxious stimuli.

From the aforementioned laminar layers, axons project to higher order brain centres by way of the spinal cord. The spinothalamic tract and the spinomesencephalic tract project from layers I and V of the dorsal horn lamina to the thalamus and the midbrain respectively (Kandel et al., 2000). The spinothalamic tract then projects from the thalamus to the somatosensory cortex to fulfill its role in discriminating between various types of pain sensation (heat, cold, damage, toxicity, etc.) (J.D. Schmahmann & D.N. Pandya, 2006; Mense et al., 2010). The spinomesencephalic tract, however, transmits nociceptive inputs from the periphery to the reticular formation, the periaqueductal grey matter, and the parabrachial nucleus. From here, nociceptive information projects to the amygdala and contributes to the conscious perception of pain (J.D. Schmahmann & D.N. Pandya, 2006).

1.5 Cellular Mechanisms of Central Sensitization Development

Nociceptive afferents release both glutamate and substance P when forming a synapse (Latremoliere et al., 2009). Glutamate crosses the synapse and binds with α–amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors while substance P binds with neurokinin 1 (NK1) receptors on the membrane of post synaptic neurons within the dorsal horn (Latremoliere et al., 2009). Both receptors are ligand-gated sodium channels. Binding of their respective ligand (glutamate or substance P) leads to an increase in electrical potential in these cells bringing

them closer to a membrane potential of 0 (depolarization). This is achieved by allowing positively charged sodium (Na+) ions to flow through the opened channels (Latremoliere et al., 2009). Glutamate leads to a relatively faster rate of depolarization with a relatively shorter duration making it beneficial for transmission of nociceptive inputs into the spinothalamic and spinomesencephalic tracts (Kandel et al., 2000). These tracts transmit signals from the spinal cord to the thalamus and the midbrain respectively. Substance P, however, is responsible for widespread depolarization with a longer duration (Kandel et al., 2000). The cumulative local depolarizations produced by binding of glutamate and substance P leads to propagation of action potentials to the thalamus by way of the spinothalamic tract (Mense et al., 2010).

During longer duration barrages of nociceptive input, N-methyl-D-aspartate (NMDA) receptors on the post-synaptic cell membranes become active. These receptors are gated by a magnesium ion (Mg+) (Mayer et al., 1984). These receptors are both ligand-gated as well as voltage-gated. Release of glutamate and substance P leads to local depolarization. If this local depolarization surpasses the threshold of the receptor while glutamate is bound, the Mg+ block will be cleared allowing Na+ and Ca2+ influx into the post-synaptic cell (Latremoliere et al., 2009; Mayer et al., 1984). Increased intracellular Ca2+ is a necessary component to begin a cellular cascade resulting in long-term potentiation (LTP) of the post-synaptic cell. LTP increases the excitability of the post-synaptic neuron resulting in larger responses to the same input (Latremoliere et al., 2009).

Within the post-synaptic neuron, intracellular Ca2+ influx through the NMDA receptors activates protein kinase A (PKA) and protein kinase C (PKC) (Carvalho et al., 2000). These enzymes increase excitability of the post-synaptic neuron by phosphorylating the NMDA and AMPA receptors (Leonard et al., 1997; Tingley et al., 1997). Phosphorylation increases the permeability of these receptors allowing greater influx of Na+ and Ca2+ and, therefore, greater

relative excitability of the neuron. This increased excitability explains the primary hyperalgesia demonstrated in regions effected by CS, where a painful stimulus now feels more painful even though the stimulus is unchanged, or where a stimulus that would be subthreshold for a nociceptive response is now suprathreshold due to the increased excitability of central nociceptors.

Another phenomenon shown in CS, however, is secondary hyperalgesia. This arises from hetero-synaptic hypersensitivity which is when neighbouring synapses become hyperexcitable along with the primary route of transmission (Latremoliere et al., 2009). There are many cellular cascades that contribute to secondary hyperalgesia by increasing intracellular Ca2+. PKC, phospholipase C (PLC), extracellular regulating kinases (ERK) and mitogen activated protein (MAP) kinases all contribute to opening Ca2+ channels on the endoplasmic reticulum releasing more Ca2+ into the cell and contribute to depolarization (Latremoliere et al., 2009). Factors upstream of ERK are phosphorylated when intracellular Ca2+ increases (Gao et al., 2009) which triggers production of translational and post-translational factors that sustain long-term plastic changes via increased expression of c-Fos, NK1, tropomyosin receptor kinase B (trkB), and cyclooxygenase-2 (Cox-2) (Ji et al., 2009).

Along with the previously mentioned changes contributing to sustained CS, cAMP response binding protein (CREB) acts on PKA causing phosphorylated AMPA receptors to embed in the cell membrane allowing more routes for Na+ influx and a further increase in membrane excitability (Latremoliere et al., 2009). Channels found in post-synaptic neurons, mGluR, contribute to secondary hyperalgesia as they are coupled with Ca2+ channels on the membrane of the endoplasmic reticulum (Derjean et al., 2003). These channels serve as the location where extracellular regulating kinases (ERK) are phosphorylated (Latremoliere et al., 2009). Increased Ca2+ release from the endoplasmic reticulum has the potential to disperse through the cell to

other synapses and generate sensitization (Latremoliere et al., 2009). Nitric oxide (NO) may also be a key contributor to the generation of CS as it acts as a retrograde signal between the post-synaptic neuron back to the presynaptic neuron (Mense et al., 2010). The ability of NO to diffuse across a synapse may be the reason that it can sensitize neighbouring synapses. NO acts to cause calcium influx into the presynaptic cell which, in sufficient amounts, results in depolarization and increases the volume of glutamate that is released into the synapse (Mense et al., 2010). A greater volume of glutamate in the synapse results in more NMDA and AMPA receptors opening, greater Na+ and Ca2+ influx, and an amplified response (Mense et al., 2010). All these converging cellular cascades contribute to allow more Na+ and Ca2+ to enter the cell, increasing the excitability of these neurons because of persistent input.

Within the dorsal horn of the spinal cord, dorsal root reflexes (DRR) contribute to CS by causing hetero-synaptic facilitation (Rees et al., 1996). Interneurons within the spinal cord can cause presynaptic inhibition of incoming nociceptive signals under normal, healthy conditions, as first described in Melzeck and Wall's (1965) Gate Control theory. In sensitized conditions, however, this interneuronal pathway generates DRRs in close temporal proximity to an incoming afferent (Rees et al., 1996). DRRs are antidromic action potentials travelling away from the spinal cord along the axons of nociceptive afferents resulting in the release of substance P and calcitonin gene-related peptide (CGRP) in the periphery (Rees et al., 1996). Release of substance P and CGRP into the periphery results in peripheral sensitization of nociceptive afferents which may demonstrate that DRRs may create hetero-synaptic facilitation in a CS state resulting in secondary hyperalgesia (Rees et al., 1996).

Overall, many factors contribute to both the homo-synaptic and hetero-synaptic facilitation observed in CS. On a cellular level, many cascades and cycles of phosphorylation result in greater excitability in both the post-synaptic and pre-synaptic neurons along the

nociceptive pathway. All these effects culminate to increase excitability within the dorsal horn of the spinal cord, resulting in an amplified central response to a peripheral stimulus.

1.6 Assessment and Quantification

The challenge in assessment and quantification of CS is measuring both primary and secondary hyperalgesia. Assessing increased excitability in the dorsal horn of animals is possible directly in preparations but indirect measures are necessary for assessment in humans. Increased areas of secondary hyperalgesia can be assessed in humans by measuring the increased size of receptive fields (Woolf, 2011). While decerebrate preparations or direct measurement of dorsal horn neurons is impossible in humans, the nociceptive reflex (NR) (Lim et al., 2011; Sandrini et al., 2005) and the contact heat evoked potential (CHEP) (Madsen et al., 2014; Madsen et al., 2012) are both feasible techniques for indirect measurement.

For measurements of the nociceptive reflex, a suprathreshold electrical stimulus is applied, typically in the lower limb, over the surface of the skin to activate nociceptors in an accessible nerve (Sandrini et al., 2005). This surface electrical stimulation activates a spinal reflex to generate a coordinated muscle activation to withdraw the limb and protect it from the noxious condition (Sandrini et al., 2005). The threshold required to generate this withdrawal reflex is defined as the NR threshold and a relationship between experimental CS and reduced NR thresholds has been demonstrated previously (Biurrun Manresa et al., 2014; Gronroos et al., 1993). Similarly, in conditions associated with CS by Woolf (2011) such as FM, MPS, whiplash, osteoarthritis and others, reduced NR thresholds have been shown (Banic et al., 2004; Desmeules et al., 2003; Biurrun Manresa et al., 2013; Neziri et al., 2010). This work demonstrates the sensitivity of the NR to identify CS in various patient populations.

Using a similar philosophy as the NR, CHEPs are another tool for assessment of CS. Application of a heated thermode to the skin elicits a response that can be measured by

electroencephalogram (EEG) (Jutzeler et al., 2016). Following the use of capsaicin to induce CS experimentally, Madsen et al., 2014 demonstrated a reduction in the latency of EEG responses to the thermal stimulus indicating sensitization of both the C fibers and Aδ fibers (Madsen et al., 2012). Madsen et al., (2012) describe both short and long latency responses occur more quickly following heat application justifying the conclusion that both C fibers and Aδ fibers are hyperexcitable. CHEPs are another useful indirect measure of the excitability of dorsal horn neurons but the complexity of EEG has led to limited use of CHEPs for CS assessment.

More practical but more subjective measures of CS are the battery of available quantitative sensory testing (QST) modalities. CS creates an area of secondary hyperalgesia surrounding an affected area and this allows the use of brushing of the skin or pinprick testing to be used to map and measure the size of the increased receptive field (Woolf, 2011). The brush allodynia technique has been used to indicate CS by applying repeated brush strokes to an affected limb to map the areas where the non-noxious stimulus elicits a pain sensation (Buonocore et al., 2016). These quantitative sensory tests combined with measures of temperature perception, both hot and cold, have been combined to create a greater array of QSTs (Rolke et al., 2006). Brush allodynia and pinprick testing are feasible tools to confirm that experimental induction of CS was successful (Dirks et al., 2003; Srbely et al., 2010). Multiple studies have also been successful in using these tools to identify CS in a clinical population (Pfau et al., 2009; Rolke et al., 2006) and for establishing a relationship between the dose of capsaicin and the size of the area of secondary hyperalgesia (Lamotte, 1992; Scanlon et al., 2006; Simone et al., 1989). These QST techniques are promising and clinically feasible. However, they rely on subjective reporting from the participant rather than directly measuring excitability in the dorsal horn.

1.7 Capsaicin as an Experimental Tool

Central sensitization can be induced experimentally using a capsaicin model. Capsaicin is a chemical extracted from hot peppers that is applied topically in an alcohol-based cream (O'Neill et al., 2012). This topical cream is absorbed through the skin to activate TRPV1 receptors which usually respond to noxious heat (Kandel et al., 2000). Activation of TRPV1 receptors on nociceptors causes C-fibres and Aδ fibres to signal that the skin has been exposed to a noxious level of heat (typically 44 degrees Celsius or higher) (Kandel et al., 2000). The persistent input from these fibres causes experimentally induced CS. At the end of an experiment the cream can be washed off to eliminate the nociceptive input and allow the participant to return to baseline sensitivity. The capsaicin model allows researchers to temporarily induce and then remove central sensitization from a participant.

1.8 Anatomy and Cellular Mechanisms

The structure of the capsaicin molecule consists of a benzene ring and a polar amide group making up a hydrophobic tail (Hayman et al., 2008). The hydrophobic tail of the capsaicin molecule makes it ideal for distribution within alcohol for topical application (O'Neill et al., 2012). Capsaicin, applied topically, can absorb through the skin and bind to TRPV1 receptors expressed on both Aδ fibers and C-fibers (Kandel et al., 2000). When attached to the TRPV1 receptor, capsaicin acts as a ligand to open the channel and allow Na+ and Ca2+ influx (Szallasi et al., 1999). TRPV1 receptors typically respond to capsaicin and noxious heat (>43°C) but also respond to other stimuli such as pH<5.2 and other inflammatory mediators (Szallasi et al., 1999). Any of these stimuli can allow Na+ and Ca2+ influx leading to depolarization of both Aδ fibers and C fibers (Kandel et al., 2000). Most nociceptive neurons express multiple types of TRP channels on their membranes allowing them to respond to a variety of noxious stimuli (Kandel et al., 2000).

In TRPV1-knockout mice, there was no response to injection of capsaicin and no development of allodynia, either mechanical or thermal (Caterina et al., 1997). This work demonstrated the utility of the TRPV1 receptor in responding to capsaicin. Similarly, work done in monkeys directly measuring activity in the dorsal horn have shown increased activity in the dorsal horn following injection of capsaicin (Dougherty et al., 1992). Further work determined that the NK1 receptors in these monkeys became active in response to capsaicin injected into the hind paw, leading to increased dorsal horn excitability (Dougherty et al., 1994). In other direct measures of the dorsal horn, specifically in rats, the same trend of increased dorsal horn activity was demonstrated with injected capsaicin (Carstens, 1997).

This body of evidence suggests that capsaicin is a valid tool for inducing CS in animals as validated by direct measures of the dorsal horn. Direct measures of the dorsal horn are not possible in humans.

1.9 Methods of Application

Capsaicin is often used as a topical cream (Dirks et al., 2003), applied within the viscera (O'Neill et al., 2012), or by injection, either within the skin or directly in the muscle (Lamotte, 1992; Neziri et al., 2009). Topical creams allow CS to be induced experimentally, using low doses (<1%) to create a transient effect (O'Neill et al., 2012). The advantage of topical application is the transient effects, allowing CS to be induced experimentally and then removed after testing. A variety of studies have tested topical application for its ability to induce CS experimentally and demonstrated its effectiveness (Andersen et al., 1995; Gronroos et al., 1993; Dirks et al., 2003; Arendt-Nielsen et al., 1996).

Similarly, injected capsaicin demonstrates a comparable effect with reductions in NR thresholds and generation of areas of secondary hyperalgesia (Biurrun Manresa et al., 2014; Scanlon et al., 2006) as confirmed by infrared thermography (Serra et al., 1998) and both brush

and pinprick allodynia (Scanlon et al., 2006). Unlike topical application, intradermal and intramuscular injected capsaicin, while still transient, has a longer duration and cannot be removed with water like topical capsaicin. O'Neill et al., (2012) confirmed that applying capsaicin directly to the gastrointestinal system elicited the same effects as other routes of application, however, work using this method of application is uncommon.

Despite the multiple routes of application, and the demonstrated efficacy of using capsaicin to induce CS, a standard dose or route of application has not been established. Currently, a variety of different doses are used experimentally in both topical and injected capsaicin protocols (Culp et al., 1989; Simone et al., 1989). The varied methods of capsaicin application make comparisons between efficacy of different routes of application challenging. Across all methods of application, similar effects have been shown in both humans and animals which suggests that capsaicin is effective in inducing CS experimentally regardless of the method employed to apply the molecule (Latremoliere et al., 2009; Dirks et al., 2003; Simone et al., 1989).

1.10 Dose-Dependency

The amount of TRPV1 receptors activated by capsaicin application is dependent on the dose applied in animal models (Voets et al., 2004). Likewise, a higher amount of TRPV1 channels activated corresponds to an increase in excitability of dorsal horn neurons (Wood et al., 1988). Translating this to humans becomes more challenging as whole-cell preparations are not available. Secondary hyperalgesia can be used as an indirect analog of dorsal horn excitability with a larger area of secondary hyperalgesia corresponding to a greater degree of excitability (Simone et al., 1989). Like the animal studies demonstrate, studies evaluating the size of the area of secondary hyperalgesia in humans using capsaicin have shown dose-dependency (Lamotte, 1992; Scanlon et al., 2006; Basbaum et al., 2009). To assess dose dependency, pinprick

allodynia and brush allodynia have been used (Lamotte, 1992; Scanlon et al., 2006) as well as infrared thermography (Culp et al., 1989) and measured a significant relationship between dose and the size of the area of secondary hyperalgesia. Work by Sumikura et al., (2003) also demonstrated dose-dependency with higher skin temperature resulting from higher doses of capsaicin.

This body of literature demonstrates that there is a dose-dependent relationship between capsaicin and the magnitude of CS induced experimentally. This evidence suggests that the dose of capsaicin applied should be taken into consideration in the study design process.

1.11 What is Wind Up?

Repetitive stimulation of a neuron can result in a phenomenon known as wind up (Herrero, 2000). Wind-up occurs from persistent input within a set frequency range (Herrero, 2000). Wind-up, like CS, leads to an amplified response to stimuli at a consistent intensity but it is restricted to a set range of frequencies (Herrero, 2000). Initial studies of wind-up were performed in cats (Mendell et al., 1965). This work used preparations of unmyelinated nociceptors and subjected them to repetitive activation at a range of frequencies between 0.3 Hz and 0.5 Hz. Stimulation at these frequencies created amplified responses to each subsequent stimulus until reaching a ceiling effect at the 16^{th} stimuli where responses did not continue to increase in intensity (Schouenborg et al., 1983). Direct measurement from neurons of the dorsal horn is not possible in humans as it requires removal and preparation of neurons, so indirect measures are necessary to assess windup. Indirect measurement of wind-up in humans is termed temporal summation (Mendell et al., 1965). Work by Mendell at al., (1965) suggests that repeated stimulation at a controlled frequency may summate to create an amplified response, and this may explain the varying degrees of sensitivity in CS (Mendell et al., 1965). Repeated stimulation of C-fibers showed increasing amplitudes of responses in the dorsolateral

column of the spinal cord (Mendell et al., 1965). The summation of stimuli created enough excitatory post-synaptic potentials in the post-synaptic neurons of the dorsal horn to surpass the excitation threshold and generate an action potential (Thompson et al., 1991; Sivilotti et al., 1993).

Although wind-up is quite similar to CS, as both are characterized by an amplified response to a nociceptive signal, wind-up is a different phenomenon with a different method of induction. Arendt-Nielsen et al., (1996) demonstrated that, while these conditions are different, experimentally inducing CS and then initiating windup resulted in temporal summation occurring at a lower stimulus intensity. This highlights the potential for wind-up to be used as validation for the successful induction of CS by testing windup both before and after the application of capsaicin to verify that a higher degree of windup has occurred after experimental sensitization.

1.12 Assessment and Quantification

A variety of methods exist for the assessment of wind-up. Although wind-up was initially shown in isolated dorsal horn neurons (Herrero, 2000), temporal summation is measured as a human correlate of wind-up as direct measures are not possible (Price, 1972). Temporal summation can be elicited in humans through electrical stimulation as well as by weighted pinpricks (Price et al., 1992). Magerl et al., (1998) demonstrated that different weights generated temporal summation after different numbers of repetitions, while Herrero (2000) determined that temporal summation typically occurs within the first 4 consecutive pinpricks.

1.13 Motor Unit Physiology

Edward Lidell and Charles Sherrington first described the motor unit in 1925 as the functional component of a muscle under voluntary control, comprised of a single neuron and the muscle fibres that it innervates (Duchateau & Enoka, 2011;Farina et al., 2020). The

recruitment order for each motor unit can be described by Henneman's size principle (Henneman, 1985). Motor units are recruited with the lowest threshold units activating earlier than the high threshold units. This allows for a graded response to control the level of force production and contribute to smooth control of movement in conjunction with rate coding in which a MU progressively increases its firing rate to create a progressive increase in force (Patla et al., 1993). In an isometric ramp contraction, this relationship is demonstrated by a motor unit being recruited and undergoing rate coding to increase force, while further motor units are recruited and begin to contribute to force production (Patla et al., 1993). Different muscles have different recruitment and rate coding profiles which determine the degree to which a muscle will increase rate coding or increase recruitment. For example, during an increasing intensity of contraction, the tibialis anterior recruits motor units continuously until 70% MVC where further force is produced by an increase in rate coding (del Vecchio et al., 2018). Conversely, in an isometric ramp contraction up to 40% MVC in the vastus lateralis, males continued to recruit further MUs until 25.7% MVC while females stopped recruiting further MUs at 27.5% MVC with further increases in force up to 100% MVC likely achieved by increases in rate coding (Trevino et al., 2019). This work by Del Vecchio et al., (2018) and Trevino et al., (2019) showed that different muscles, in this case the tibialis anterior and vastus lateralis, displayed different strategies for increasing force through rate coding and motor unit recruitment. The ratio between increased rate coding and increased recruitment can also change depending on the task. For example, a task requiring a rapid rate of force development in the tibialis anterior leads to a high MU firing rate as well as near maximal recruitment occurring simultaneously (Del Vecchio et al., 2019). Regardless of strategy or the ratio between increased recruitment and increased rate coding, both contribute to the control of force production (Patla et al., 1993)

Within the tibialis anterior, there are approximately 140 motor units contributing to force production at 50% MVC as identified by indwelling EMG (Chino et al., 1999). The firing characteristics of these motor units is dependent on activation level and recruitment threshold during voluntary isometric contractions (De Luca & Hostage, 2010). Hostage and De Luca (2010) describe the "onion skin principle" in which motor units demonstrate an inverse relationship between recruitment thresholds and firing rates. According to this work, a motor unit with a higher threshold will tend to have a lower firing rate (Hostage & De Luca, 2010).

However, it is not clear whether measurements that demonstrate the "onion skin principle" are valid, whether this principle applies to all muscles, or what the physiological benefit would be to promote this type of motor control. Data collected from eight males between the ages of 19 and 30 using a bipolar indwelling electrode inserted into the extensor digitorum does not appear to support the "onion skin principle" (Willem Monster & Chan, 1977). A positive correlation between motor unit recruitment threshold and firing rate was observed with the higher threshold motor units increasing firing rate more rapidly than lower threshold units (Willem Monster & Chan, 1977). In work by Gydikov & Kosarov (1973), motor units were collected from the biceps brachii of 15 participants (8 males, 7 females). The authors observed a higher firing rate in low threshold motor units at low force compared to the higher threshold motor units, but as force continuously increased up to maximum voluntary effort the firing rate of low threshold motor units did not increase further while the firing rates of high threshold motor units continued to increase. Therefore, at higher force levels the high threshold motor units had a higher firing rate than the low threshold motor units despite having a lower firing rate at low force levels (Gydikov & Kosarov, 1974). Therefore, it is possible that the activation pattern creating the "onion skin principle" is the result of low threshold motor units rate coding at their maximum firing rates, while the higher threshold MUs have not yet reached their

maximum firing rate, thus creating the illusion that the high threshold MUs must have a lower firing rate. It does not seem likely that the “onion skin principle” is displayed at near maximal force levels where higher threshold MUs increase firing rate above the maximum firing rate of a low threshold unit (Gydikov & Kosarov, 1974)

De Luca & Contessa (2015) assert that the motor unit control scheme known as the “onion skin principle” provides a biomechanical and evolutionary advantage that is not provided by a control scheme where high threshold motor units have a higher firing rate, often referred to as the “reverse onion skin principle”. The assertion is that limiting the firing rate of high threshold motor units prevents the muscle from reaching maximal activation and reserves high threshold MUAP summation at a high firing rate to occur in emergency scenarios to provide a short-term rapid increase in muscular strength (de Luca & Contessa, 2015). This potentially explains anecdotes of unexpected strength in life-threatening situations. However, assuming an evolutionary explanation for the existence of the “onion skin principle” could be considered a logical fallacy. Assumptions that evolution is actively driving biological processes towards optimal function are commonly seen in biological discussions and scientific literature. Evolution exists as a random series of mutations with no inherent goal or driving force and using evolution as a justification for why the “onion skin principle” should exist is not valid evidence but does provide “a convenient shortcut when describing how things work” (Dubochet, 2011).

Furthermore, there is evidence to refute the idea that high threshold motor units do not reach maximum firing rate as this would imply that muscles do not achieve maximal voluntary activation excluding extreme situations. To determine full muscle activation the interpolated twitch technique (ITT) can be employed experimentally. The ITT is used to detect muscle inactivation by superimposing an electrically stimulated twitch during a maximal voluntary contraction (Behm et al., 1996). If the electrical stimulation leads to an increase in maximal force

it can be concluded that the muscle was not at full activation or else recruiting more motor units or increasing firing rate to produce more force would not be possible. Work by Behm et al., (2002) investigated whether the knee extensors, plantarflexors, dorsiflexors, and elbow flexors of twelve physically active males from a university population could reach full activation as confirmed by the ITT. They determined that on average the knee extensors had the highest inactivation at 15.5% while both the elbow flexors and plantarflexors had 5% of the muscle inactive, and the most active muscles were the dorsiflexors which had only 1.3% of the muscle inactive (Behm et al., 2002). The authors note that several studies have shown full activation of the quadriceps is possible (Behm et al., 2002). The authors propose this finding could be related to the relatively higher percentage of type II fibers in the vastus lateralis (surface: 67.3%, deep: 53.1%) compared to postural muscles such as the tibialis anterior (surface: 26.6%, Deep: 27.3%), soleus (surface: 13.6%, deep: 11%), and even less posturally relevant muscles like the biceps brachii (surface 57.7%, deep: 49.5%) (Johnson et al., 1973). Therefore, they suggest that a higher percentage of high-threshold MUs in the quadriceps may lead to increased difficulty in achieving full activation. Regardless, this work demonstrates that across muscles there is a varying degree of difficulty in achieving full activation and that the tibialis anterior appears to be able to reach full activation more easily. Combined with other work (Dowling et al., 1994; De Serres et al., 1998; Chapman et al., 1985), it appears that full muscle activation can reasonably be achieved and provides further doubt to the claim that high threshold motor units do not regularly reach full activation due to the “onion skin principle”.

While both the “onion skin principle” and the “reverse onion skin principle” have evidence supporting them, it appears that the “onion skin principle” could be a consequence of technological limitations rather than serving a physiological purpose. Due to the difficulty in identifying individual motor units at near maximal contraction intensities the “onion skin

principle" may be a result of high-threshold motor units not yet achieving a high firing rate as it is not warranted by the contraction intensity (Gydikov & Kosarov, 1974). It is difficult to claim with certainty that either the "onion skin" or the "reverse onion skin" theories are correct, or even that one control strategy is exhibited across all muscles or across the entire population.

Firing rates can also be modified by non-task dependent factors. The firing rate of an individual motor unit can be modulated by descending control. Work in hemiplegic and intact participants by Frontera et al. (1997), investigated firing rates of individual motor units at 25% MVC in both the paretic and intact limbs in the tibialis anterior as a model of motor units unaffected by descending control. In the intact participants, MUs recruited below 25% MVC fired at a rate of 5 to 10 Hz (Frontera et al., 1997). As intensity approached 25% MVC the mean firing rates of the active MUs increased to an average of 11.1 Hz (Frontera et al., 1997). In the hemiplegic participants, those that could reach 50% MVC demonstrated a mean firing rate of 13.9 Hz while those requiring extraordinary effort to reach 50% MVC, the high threshold MUs fired at only 5-7 Hz (Frontera et al., 1997). While this mirrors the findings of Gydikov & Kosarov (1974), it also suggests that motor unit firing rates are heavily influenced by descending control from upper motor neurons, as those with limited voluntary control of lower limb muscles experienced greater difficulty reaching 50% activation, and exhibited a lower firing rate (Frontera et al., 1997).

Like this work in hemiparetic participants, Burke et al., (1992) investigated the firing rates of the motor neurons innervating the tibialis anterior in both intact conditions and with a nerve block to test the effects of acute deafferentation on motor unit activity. The purpose of this work was to determine how afferent feedback is incorporated into motor unit behaviour. Participants performed isometric contractions with the foot secured in a brace to prevent movement. Following insertion of a needle electrode into the common peroneal nerve and

recording of baseline firing rates, a nerve block was applied to prevent incorporation of peripheral feedback from the dorsiflexors. Subjects then repeated the voluntary contractions with the nerve block and firing rates were recorded with the same needle electrode. It was estimated that 12 of the measured motor neurons were relatively low threshold, likely activating before 10% MVC, while 6 were considered high threshold (Burke et al., 1992). The low threshold motor neurons demonstrated a significantly higher firing rate than the high threshold motor neurons (Burke et al., 1992). Low threshold motor neurons displayed a mean firing rate of 21.7 Hz while high threshold MUs had a mean firing rate of 14 Hz. Once the motor neurons were deafferented, firing rates were significantly lower than in intact conditions with a mean firing rate of 18.6 Hz (Burke et al., 1992). This work, along with work by Frontera et al., (1997), suggest that normal motor unit firing rates in the tibialis anterior are significantly modulated by both the influence of descending drive from upper motor neurons and the incorporation of peripheral feedback. Therefore, it is possible that both peripheral and central mechanisms such as pain can affect both descending drive to the tibialis anterior and modulate activity through the afferent feedback of nociceptors. These models both show that firing rates in the lower limb can differ from “normal/healthy” behaviour without damage to the muscle itself.

Along with peripheral feedback and descending control, motor unit firing rates can be influenced by age. Connelly et al., (1999) investigated the effects of aging on motor unit firing rates by comparing six young and six older adult males with mean ages of 20.8 and 82 respectively. Participant’s feet were fixed in an isometric dynamometer while torque generated by both voluntary dorsiflexion and electrical stimulation over the common peroneal nerve was measured with a strain gauge. The electrical stimulation achieved roughly 10% of the participant’s maximal voluntary contraction torque. The older adult group demonstrated a significant reduction in firing rates at matched relative torques (Connelly et al., 1999). This

indirectly demonstrates the age-associated motor unit remodeling theory in which there is a loss of fast-twitch motor neurons resulting in an increased number of muscle fibers innervated by slow-twitch motor neurons (Connelly et al., 1999). Less fast-twitch fibers combined with other age-related factors such as reduced excitability of the corticospinal tract and reductions in conduction velocity could explain why age appears to impact motor unit behaviour (Rossini et al., 1992; Connelly et al., 1999). Of note is that the authors highlight that the older adults in this study were recreationally active as often as the younger males, suggesting that these participants may outperform the average older adult population and that the age-related decline in motor unit firing rate and increased innervation ratio may be more extreme than observed in this study (Connelly et al., 1999). This work provides further evidence that motor unit firing rates can be affected by several factors beyond achieving a desired voluntary force.

Overall, previous work in the field demonstrates that motor unit firing rates are modulated by a variety of factors. While the effect of motor unit firing rates has not yet been investigated in the presence of central sensitization, it is possible that central nervous system hyperexcitability and amplification of peripheral nociceptive signals may combine to influence firing rates. The following section of this review will focus on changes of motor unit properties because of pain.

1.14 Properties of a Motor Unit and Pain

A variety of factors including descending control, peripheral sensation, and age can modify motor unit activity. Nociceptors transmit information from the periphery to the central nervous system, which, if above a certain intensity threshold, reaches conscious awareness. Pain, which is a conscious emotional response to this nociceptive input, like these aforementioned factors, has been investigated to determine if it too, like other forms of peripheral feedback influences motor unit activity.

Within the literature studying pain and motor output there is no established trend. In studies defining relative effort as “low intensity”, some studies show an increase in muscle activation while others observed muscular inhibition (Roland, 1986; Lund et al., 1991). These review papers lead to the formation of two theories to explain the observed movement changes that accompany pain. These two theories are referred to as the “Vicious Cycle” theory and the “pain-adaptation” theory (Hodges & Tucker, 2011).

In a review paper on conditions of spinal pain, Roland (1986) theorized that pain leads to muscle spasms (muscle hyperactivity) which leads to further pain due to ischemia and progressively increasing metabolite concentrations. The proposed mechanism of this hyperactivity is through type III and type IV nociceptors synapsing onto gamma motoneurons leading to increased muscle spindle sensitivity. This theory is supported by work showing increased stretch reflex amplitudes in multiple muscles during pain (Hodges & Tucker, 2011). This work proposing that pain creates more pain provides the basis of the “Vicious Cycle” theory.

In opposition of the “Vicious Cycle” theory, theories proposed by Lund et al., (1991) contradict this claim. In musculoskeletal pain conditions such as tension headache, fibromyalgia, and chronic back pain, Lund et al., (1991) concluded that pain resulted in reduced agonist activity accompanied by increased antagonist activity. The authors advise that this behaviour is not proof of pain leading to “dysfunction”, but serves as a protective adaptation (Lund et al., 1991). This observation gives rise to the opposing “Pain-Adaptation” theory.

It is clear from these reviews that the evidence is inconclusive when it comes to whether pain leads to hyperactivity and further pain, or whether pain leads to reduced activity to prevent further damage. A later review of the literature by Hodges & Tucker (2011) investigated this

controversy and suggested that the central nervous system can adapt to pain in a task-dependent manner. By activating specific muscle regions while inactivating others, Hodges & Tucker (2011) explain that task-specific changes may serve as a protective mechanism to prevent further muscle damage. Overall, work in humans has shown that measured EMG activity can increase, decrease, or remain the same depending on the muscle tested or the model of pain used (Hodges & Tucker, 2011). Therefore, it can be concluded that muscular responses to pain are task-dependent and not generalizable across the body or the population.

To directly test the potential effects of pain on motor unit activity, Farina et al., (2004) performed a study using isometric contractions of the tibialis anterior both with and without experimental pain. Participants were positioned in a chair with the ankle secured in a brace at 90^{o}. Force data was collected from a torque transducer within the brace. Participants performed 3 maximum voluntary contractions and the highest torque was considered their maximum. Surface EMG was collected using a linear array of 16 electrodes placed on the distal portion of the tibialis anterior to avoid the innervation zone of the muscle. Intramuscular EMG was collected with 4 wire electrodes inserted into the muscle proximal to the linear array at different depths with the goal of collecting as many individual motor units as possible. Each participant performed 6 contractions with a duration of 20s at an isometric force of 10% MVC. Experimental pain was then induced by injecting hypertonic saline into the muscle belly of the right leg in 3 different volumes (0.2, 0.5 and 0.9 mL). After the 0.2mL injection, the participants repeated 2 more 20s long isometric contractions at 10% MVC. Then, the 0.5mL injection was used and the participants performed 2 more contractions. Finally, the 0.9mL injection was used and the last 2 contractions were performed for a total of 6. As a control condition, the same protocol was performed on the left leg except isotonic saline was used in place of hypertonic saline, as isotonic saline would not stimulate a pain response. After performing spike triggered averaging

on the EMG signal collected from their 12 young, healthy participants they were successful in tracking a total of 55 motor units in the right leg and 49 from the left. The firing rate of the active MUs ranged from 7.4 – 14.8 Hz at 10% MVC and did not change significantly from the baseline after the control condition of isotonic saline injection. In the right leg however, when hypertonic saline was injected to induce pain there was a significant reduction in firing rate of approximately 1 Hz on average. Furthermore, there was an inverse relationship between participant-reported pain perception and reduction in firing rate with those participants perceiving more pain demonstrating a greater reduction in firing rate. This data suggests that experimental muscle pain may lead to a reduction in motor unit firing rate while a non-painful injection does not produce the same effect. Farina et al., (2004) investigated further by analyzing the conduction velocity of each motor unit and they did not observe a significant change in any experimental condition. This data suggests that while experimental pain may affect firing rate, it likely does not affect conduction velocity. The authors explain that based on this data reductions in firing rate during experimental muscle pain are likely a result of a central inhibitory control mechanism rather than an alteration in membrane properties of the motor unit itself (Farina et al., 2004). It was concluded by this research group that the observed reduction in firing rate while maintaining a constant force could be explained by changes in contractile properties of the muscle fiber, recruitment of additional motor units, or possibly alterations in the activity of dorsiflexion antagonists namely a reduction in co-contraction (Farina et al., 2004). However, previous work has established a link between surface EMG amplitude and motor unit recruitment, demonstrating no observed increases in amplitude, suggesting recruitment of further motor units is unlikely (Fuglevand et al., 1993; Woods and Bigland-Ritchie 1983). However, Farina et al., (2004) further explain that as firing rate contributes to surface EMG amplitude, it is possible that firing rate reductions balanced out

further recruitment in motor units. Moreover, surface EMG may not detect the deepest motor units, and approximately 27% of deep motor units are of the larger fast-twitch variety in the tibialis anterior. It is possible that motor units were recruited without being detected by the surface signal. Another possible explanation is a change in behaviour of the entire muscle group of the ankle, however, previous work showing a change in synergist and antagonist activation during experimental pain was performed in dynamic contractions so it is not clear if the same effect would be observed in an isometric task (Graven-Nielsen et al., 1997; Zedka et al., 1999). This explanation also does not account for the observed reduction in firing rates of synergistic muscles during an isometric task which should lead to a reduction in force or a reduction in surface EMG amplitude from a reduction in crosstalk, neither of which were observed in this study (Farina et al., 2004; Ciubotariu et al., 2004). While this work demonstrates that pain, like other forms of afferent feedback, can affect central control of motor unit activity, it also highlights the fact that the exact mechanism for this change is still unclear.

Prior research has attempted to uncover the neurological mechanism by which pain affects muscle activity. Schechter et al., (2013) induced neuropathic pain in a mouse model by surgically damaging the sciatic nerve in the hindlimb. Several tests were performed in this preparation of mice to determine both how the dorsal horn neurons contribute to reflexive muscle activation in the neuropathic pain state, as well as how electrical stimulation of the spinal cord may attenuate this effect.

Mechanical hypersensitivity was tested both before and after surgery using weighted pinpricks. Sharp pins of progressively increasing weight were applied to the paw until the mouse exhibited a nociceptive withdrawal reflex, in which they produced an involuntary, coordinated muscle action to remove their paw from the painful stimulus. This threshold was recorded as the baseline measure of sensitivity and again following the surgery. Only those mice who showed at

minimum a 50% reduction in withdrawal threshold (n=97) were included in the study as this level of reduction was assumed to represent successful induction of hypersensitivity (Schechter et al., 2013). During the initial surgery a stimulating electrode was inserted into the dorsal columns of the spinal cord to provide electrical stimulation and potentially lead to an analgesic effect. Schechter et al., (2013) did observe a reduction in pain hypersensitivity following dorsal column stimulation leading to the mice displaying a greater tolerance for pinprick stimulation before withdrawing due to pain.

Schechter et al., (2013) who primarily were interested in the potential analgesic effect of spinal cord stimulation, observed a reduction in sensitivity and suggested that it may be due to failure of action potential propagation as measured directly within the dorsal horn of the prepared mice (Schechter et al., 2013). The dorsal columns, which carry sensory information from the periphery to the brain, modulating action potentials in the dorsal horn is supported by the "Gate-Control Mechanism" of pain first theorized by Melzack & Wall (1965), in which pathways carrying tactile sensory information can inhibit neighbouring nociceptive pathways.

Therefore, the neurons of the dorsal horn may play a role in not only pain perception, but also involuntary changes in muscle activity due to pain. This idea is supported by the fact that increasing or decreasing activity in the dorsal horn corresponded to changes in pain sensitivity, and thus also affected the occurrence of a nociceptive reflex. In this study, a mouse model was used because direct measurement of the dorsal horn within humans is not possible. While it does show an interesting benefit of non-noxious stimulation on spinal cord activity, there are some limitations to its generalizability. Besides using an animal model which inherently has both benefits and limitations, this work also primarily focused on sensory inputs over motor outputs. It does suggest that the dorsal horn is included in the nociceptive reflex loop but the characteristics of the muscle activity itself was not tested. As such, it is not clear if

the dorsal horn's contribution to muscle activity is limited simply to the withdrawal reflex or whether it contributes to the observed firing rate changes observed by Farina et al., (2004). Furthermore, the nociceptive withdrawal reflex is an involuntary response, which raises further questions about whether activity at the dorsal horn can also influence voluntary actions. Overall, this work provides the valuable conclusion that the dorsal horn is involved in some involuntary muscle action. It also allows speculation that the dorsal horn may be worth investigating further to determine if it contributes to motor control beyond simple reflexes. Finally, increases and decreases in the dorsal horn influencing involuntary muscle activation supports the idea that phenomena such as central sensitization may affect not only sensory inputs, but also motor outputs, but it is far from uncovering the mechanism behind the observed reductions in motor unit firing rates during experimental pain.

As this work in the field is yet to determine the mechanism behind the behaviour of individual motor units during pain, Martinez-Valdes et al., (2020) further investigated the role of experimental pain in motor unit activity by testing both low and high threshold motor units during isometric contractions in the tibialis anterior. At the time of this research and the formulation of this literature review, there is conflicting evidence making it especially unclear how a motor unit is likely to behave when someone experiences pain, and by what mechanism the variety of behaviours occur.

This research group opted to use a model of pain induction by hypertonic saline as it appears to only cause moderate levels of pain for a period of 10 minutes, making it a reasonable choice for the length of a single data collection (Martinez-Valdes et al., 2020). While a capsaicin model is often used as a model of central sensitization; acute, peripherally sensitized pain is most commonly modeled through saline injection (Roberts et al., 2010). As saline is injected into the muscle directly, it leads to peripheral sensitization causing pain within the dermatome in

which it is injected, while capsaicin causes central sensitization, and thus produces pain in both the dermatome it is applied, as well as neighbouring regions (Roberts et al., 2010). For example, a hypertonic saline injection into the tibialis anterior would sensitize the deep peroneal nerve root stemming from the L5 region of the spinal cord. In contrast, capsaicin applied to the skin innervated by the L5 nerve root, if induction of CS is successful, would result in sensitivity in areas supplied by the L5 nerve root, as well as areas supplied by L4, S1 and potentially other dermatomes or the contralateral L5 nerve root regions (Shenker et al., 2008). As the purpose of this work was not to study central sensitization specifically, either hypertonic saline injection or capsaicin application would be sufficient to produce the desired pain sensation in the tibialis anterior.

As a control condition, isotonic saline (0.5 mL, 0.9%) was used in place of the hypertonic saline (0.5 mL, 5.8%). Each of the 15 young, healthy participants performed all 4 conditions in the order: baseline, isotonic saline, hypertonic saline, and post-pain. The right leg of all participants was fixed in a dynamometer with a 90^{o} joint angle at the ankle. Participants performed 3 maximum voluntary contractions and the highest was taken as the true maximum and used to standardize the following contractions to their maximum torque. Participants then performed trapezoidal ramp contractions with visual feedback to ensure that they maintained a consistent increase in force of 10%/s up to an isometric hold of either 20% or 70% MVC followed by a decrease in force at a rate of 10%/s. A 64-electrode high-density EMG array (OT Biolettronica, Torino, Italy) was placed over the central region of the tibialis anterior directly between the proximal and distal tendons. The array was oriented in a way that the columns of electrodes were parallel with the participant's tibia.

The HDEMG signal was decomposed using a custom blind source separation algorithm to identify individual motor units that contributed to the overall surface EMG signal. To ensure

motor units were tracked across conditions, trials for each participant were merged across all 4 conditions and then decomposed to identify only those motor units found in all conditions. Firing rates of each MU were taken during the plateau region of each contraction. Recruitment and de-recruitment threshold of each MU were considered as the force level at which the MU first began firing, and then ceased firing during the decrease in force at the end of the trapezoidal contraction. Firing rates at recruitment and de-recruitment were calculated using the time elapsed during the first 6 or the last 6 identified spikes respectively.

Several comparisons were performed on this data, the first being comparing motor units identified below 20% MVC to those identified between 50%-70% MVC. Further data analysis performed a linear regression analysis to examine the rate of change in firing rate of each MU in both the low force (<20%) and the high force (50%-70%) conditions. This analysis was performed to see if the rate of firing rate increases varied from the rate of force increases which would suggest a change in the gain of the motor neuron pool, potentially from descending command from supraspinal brain regions. Then behaviour of the low and high threshold units was compared to determine their relative contribution to the overall EMG signal and force. Lastly, the entire motor unit pool's behaviour during high force contractions was investigated by pooling the data from all motor units identified during the high force trials.

Activity of the motor neuron pool was also simulated in both 20% MVC and 70% MVC conditions as an estimate of activity both before and after pain. The simulated model had been used in numerous previous studies as a modification of an original simulation created by Cisi & Kohn (2008) (Negro & Farina, 2011; Dideriksen et al., 2012; Negro et al., 2015, 2016). In the model, a simulated pool of 450 motor units recruited across the entire range from 0% MVC to 70% MVC were used to mirror the force profile produced by the real participants. The simulated motor neuron pool received a mean drive of 13.6 nA to mimic the descending neural drive

required to produce the collected force profile without any inhibition. This was performed to simulate the baseline trial in which they predicted there would not be descending inhibition. To simulate the pain condition, descending inhibition to the motor neuron pool was introduced by using a variable inhibitory current with the lowest threshold motor unit receiving the most inhibition (8 nA) and the highest threshold motor unit receiving the least inhibition (0.1 nA). The variable degree of inhibition was selected based on an experiment by Lee & Heckman (2000) and a simulation by (Powers & Heckman, 2015) which both showed that supraspinal and spinal inhibition the motor neuron pool is non-uniform.

In the hypertonic saline injection model of pain, Martinez-Valdes et al., (2020) report that participants reached peak pain sensation approximately 1-minute following injection of saline which was enough time to persist throughout the entirety of the pain condition data collections. Of note is that 3 of the participants reported feeling referred pain as far away from the injection site as the lateral malleoli of the ipsilateral limb. This dermatome is near the border between the L5 and S1 dermatome. While not explained further, it is possible that these 3 participants may have experienced some central sensitization, presenting with referred pain in a neighbouring dermatome, although this was not confirmed in this study.

From the 20% MVC contractions, a total of 568 motor units from the group of participants were identified across all 4 conditions. The 70% MVC trials identified fewer motor units across all conditions, with a total of 494 from all the participants. From each participant an average of 14 MUs were tracked across conditions at the 20% MVC force level, with an average of 11 MUs identified in each participant at the 70% force level. Martinez-Valdes et al., (2020) report that some MUs were identified in some, but not all conditions, although they report that this was relatively rare. This information could lead to the drawing of three conclusions. Firstly, and perhaps the most likely, is that while the decomposition algorithm used was well-designed,

it does not always identify every single active MU in every trial, leading to some MUs not being active in some trials. Secondly, it is possible that this phenomenon was not an equipment error but a result of natural variability, with certain MUs displaying a recruitment threshold near the 20% or 70% MVC level, and thus being activated in some trials but not others. I believe that this is possible due to natural variability in force. During a 20% MVC isometric hold for example, a participant may have briefly surpassed 20% MVC, and potentially recruited another MU. In the following trial, if they did not vary to such a significant degree, this MU would not have been recruited. The authors report a variability in torque at 20% MVC of +/- 0.8% MVC, and at 70% a variability of +/- 0.04% (Martinez-Valdes et al., 2020). While this variability was not significant ($P>0.05$), it is possible it was a large enough variability to recruit further MUs in some cases. The third possible explanation is that perhaps different conditions lead to different recruitment strategies, although this is speculation and I predict that this phenomenon is a result of imperfections in the decomposition algorithm.

In a single participant, which Martinez-Valdes et al., (2020) explain is representative of the overall trend, the motor units identified in the low force (20% MVC) condition displayed a significant reduction in firing rate after induction of pain compared to their baseline values (group statistics $P=0.0003$). Interestingly, in the high force (70% MVC) condition, a divergent trend emerged (Martinez-Valdes et al., 2020). The low threshold motor units, defined as those recruits below 20% MVC, displayed a reduction in firing rate while the high threshold MUs (those recruited above 50% MVC) displayed a significant increase in firing rate in the pain condition as compared to baseline values (group statistics $P=0.02$). This data is summarized below in a figure taken from Martinez-Valdes et al., (2020) which displays the mean discharge

rate of MUs in the low force and high force trials across all 4 conditions.

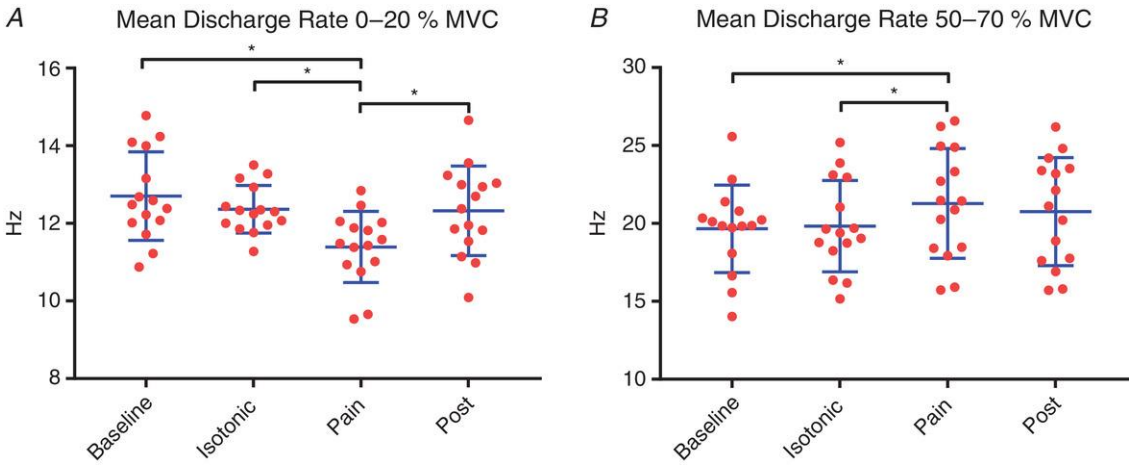

Figure 1: A. This graph displays the mean discharge rate of all motor units with a recruitment threshold between 0%-20% MVC. The data shows a significant reduction in firing rate (P<0.05) in the pain condition when compared to the baseline or isotonic conditions. It also shows a return to baseline values post-pain with a significant increase in firing rate between the pain and post pain conditions (P<0.05). B. This graph displays the mean discharge rate of motor units recruited between 50%-70% MVC across all 4 experimental conditions. It shows a significant increase in firing rate (P<0.05) in the pain condition when compared to the baseline or isotonic conditions. There is not a significant change between the pain and post pain conditions, displaying a prolonged elevation in firing rate after experimental pain. Taken from the paper "Divergent response of low- versus high-threshold motor units to experimental muscle pain", authored by Martinez-Valdes et al., (2020) .

Investigating the firing rates at recruitment and de-recruitment expose further differences following experimental pain. Across all conditions and force levels, firing rate at recruitment remained stable (20% MVC condition P=0.18; 70% MVC P=0.22). However, a significant decrease in firing rate was observed at de-recruitment for exclusively those motor units recruited between 50% - 70% MVC when comparing the pain condition to the baseline or isotonic conditions (P=0.02). This finding further displays the variability in the activity of low-threshold and high-threshold MUs as there was not a significant change at de-recruitment for the motor units identified below 20% MVC (Martinez-Valdes et al., 2020).

Further investigation by Martinez-Valdes et al., (2020) lead to comparisons of recruitment and de-recruitment thresholds of MUs between all 4 conditions and both force levels. At the low force level of 20% MVC, there did not appear to be a significant change in either recruitment (P=0.59) or de-recruitment thresholds (0.08) after experimental pain

compared to either the isotonic or baseline conditions (Martinez-Valdes et al., 2020). However, the divergent response of low- and high- threshold motor units was displayed again during analysis of the 70% MVC condition. The motor units recruited above 50% MVC displayed a significant decrease in both recruitment threshold ($P=0.02$) and de-recruitment threshold ($P=0.008$) following injection of hypertonic saline as compared to either the baseline or isotonic conditions (Martinez-Valdes et al., 2020). De-recruitment threshold maintained this significant reduction into the post-pain condition while recruitment threshold returned to baseline values. This finding further displays the disparities in motor unit activity, with MUs recruited at different thresholds displaying different changes following experimental pain along with there being apparent differences between recruitment and de-recruitment thresholds.

As an estimate of net synaptic input, Martinez-Valdes et al., (2020) performed a linear regression analysis at both the 20% MVC and the 70% MVC force levels comparing the change in torque to the change in firing rate for each motor unit between recruitment firing rate and plateau firing rate. At the low force level, there was a significant difference between the pain condition and all 3 other conditions, with the slope of the baseline, isotonic, and post-pain conditions having a significantly greater slope than in the pain condition ($P<0.001$). This finding demonstrates that in the pain condition the MU firing rate did not increase as greatly between recruitment and plateau compared to the other conditions. At the high force level, the opposite trend was observed with the pain condition showing a greater slope, and therefore a more rapid increase in firing rate in the pain condition when compared to either the baseline ($P<0.0001$) or the isotonic ($P=0.02$) conditions. The rate at which the firing rate increased in the high force contraction following pain was not significantly different from the post-pain condition, suggesting that following pain the return to baseline activity is slower in the high force contractions when compared to the low force conditions (Martinez-Valdes et al., 2020). While

these changes may be attributed to changes in synaptic drive, with greater descending inhibition in the low force condition and greater descending excitation in the high force condition, it may also be explained by the method of analysis. As the rate of change was calculated between recruitment threshold and plateau, the changes in linear regression may be attributed to other changes in control. Specifically, in the pain condition at 20% MVC there was a significant reduction in firing rate, which would lead to the rate of change being calculated over the same time window with a lower firing rate and therefore a lower rate of change. Likewise, in the pain condition at 70% MVC, the recruitment threshold decreased while the firing rate increased. When calculating the rate of change, the rate would be calculated starting at a lower force level in the pain condition compared to the baseline and would end with a greater firing rate than measured at baseline. Both these factors may have influenced this finding instead of, or in conjunction with, altered synaptic drive.

To further study the divergent effects observed across high and low force conditions, the simulated motor unit activity was analyzed to investigate potential mechanisms behind the observed changes. In the simulations, the motor units in the baseline conditions were not altered while the pain condition was represented by estimated descending inhibition. When the lowest threshold units received the most inhibitory drive and the high-threshold units received the least, a trend was observed in which the low threshold units experienced a reduced firing rate while the high-threshold units experienced an increase. Martinez-Valdes et al., (2020) suggest based on this simulation that these changes are a compensatory mechanism. With graded levels of inhibition, the low threshold motor units can reduce firing rate, while the high threshold units are activated earlier and increase their firing rate, allowing maintenance of force despite alterations in firing rate. While this observation mirrors the behaviour observed from the human participants, this theory was formed based on simulated data. It does, however,

provide some insight into the mechanism behind the observed changes. While neural drive was not explicitly measured in this study, the simulated control scheme did create changes comparable to those observed in the human participants. It is not definitive evidence, but it is possible that alterations in motor unit activity during pain are a result of descending inhibition.

Overall, this work by Martinez-Valdes et al., (2020) was the first to study the effects of pain on motor unit activity during both low and high force contractions. Previous work primarily focused on low force contractions due to the difficulty in accurately tracking motor units across conditions at high forces. The most important finding in this study is the divergent response of low and high threshold motor units (Martinez-Valdes et al., 2020). This research group makes a significant contribution to the literature with their novel approach and findings. It also contributes to the general trend seen across a range of studies that motor unit activity is task-dependent as in this work it was shown that the pool of motor units within the same muscle can behave differently when in pain (Martinez-Valdes et al., 2020).

While much of this work is building towards determining the exact control mechanism behind motor unit activity during pain, it warrants an investigation into the psychological component of the pain experience. Comparing self-reported perceived pain intensity from the participants in the Martinez-Valdes et al., (2020) study to the firing rate changes observed in the pain condition revealed a significant correlation between perceived pain and the magnitude of altered motor unit activity. In the 20% MVC condition there was a significant correlation, with higher pain intensity corresponding to a greater reduction in firing rate ($r=0.74$, $P=0.0018$). Likewise, in the 70% MVC condition there was also a significant correlation, with a higher perceived pain appearing to correspond to a greater increase in firing rate ($r=0.59$, $P=0.02$) (Martinez-Valdes et al., 2020). This finding suggests that there is a relationship between perceived pain intensity and activity of the motor neuron pool. All participants received the

same dose of saline but experienced different levels of pain, and there appears to be a relationship between the level of pain and the degree of change in motor unit activity. Therefore, it is likely that there is a psychological contribution to the observed changes. The dosage of hypertonic saline elicited different responses in different people suggesting that there is a dose-response relationship between pain and motor unit activity changes with an elevated perception of pain creating a greater change in activity (Martinez-Valdes et al., 2020).

To further investigate the psychological aspect of pain and motor unit activity, Tucker et al., (2012) investigated the relationship between pain expectation and motor unit firing rates. Working with the knowledge obtained from animal studies that nociceptive input from the periphery onto motor neurons can influence activity, Tucker et al., (2012) set out to investigate the effects of descending control. This research group was interested in the possibility that descending control can influence motor unit firing rates during pain, and the possibility that these changes may persist after cessation of pain. In this case, it would be possible that descending control alone can modulate motor unit activity without direct nociceptive input from the periphery. From this work, it would appear that motor unit activity can be influenced by descending control during pain, but also from anticipation of pain (K. Tucker et al., 2012). The model used to discover this phenomenon consisted of indwelling electrodes inserted into the muscle belly of the quadriceps in a total of 9 participants. Isometric knee extension torque was generated at two force levels. The first being enough force to recruit up to 4 motor units, and the second a force level recruiting up to 8 motor units. Subjects performed these isometric contractions across 3 conditions, those being anticipation of pain, experimental pain, or a control condition. The expectation of pain condition was created using electrical stimulation to the skin over the infrapatellar fat pad. The stimulus intensity delivered by a Digitimer (DS7A, Digitimer, UK) was increased until participants reported a 5/10 pain or greater with a 0 being

anchored as painless and a 10 being anchored as the worst pain imaginable. While the participants created isometric knee extension torque, they were told that the anchored 5/10 electrical stimulation would occur at random intervals during the contraction. Participants maintained the requested isometric contraction while the painful stimulus was delivered at random intervals, until two windows of 5 seconds each in which no stimulus was delivered. These 5 second windows represented the anticipation of pain condition, as participants were not aware that no painful stimulation was going to be delivered.

For the pain condition, hypertonic saline was injected into the infrapatellar fat pad as to create experimental pain in the same location as the electrical stimulation. Isometric contractions began once pain reach a 3/10 on the same anchored pain scale. The control trials with no pain and no expectation of pain were collected once participants reported 0/10 pain from the hypertonic saline injection.

As a further control, participants returned for a second testing session to avoid any confounding variables such as wind up (temporal summation). A concern of the Tucker et al., (2012) research group was that the protocol may have created the wind-up phenomenon in which repeated nociceptive stimulation of certain frequencies creates a sustained and amplified perception of pain reminiscent of central sensitization. The control collection consisted of the same experimental protocol including painful electrical stimulation up to a 5/10 pain, with the alteration that participants were informed that they would not have electrical stimulation delivered during the isometric knee extension task. This collection was used to ensure that participants knew they would not be receiving any pain during the contraction (Tucker et al., 2012). However, this control condition preceded by pain, followed by no anticipation of pain, does create a methodological concern. Specifically, Tucker et al., (2012) performed the second control condition to ensure that wind-up had not occurred due to the electrical stimulation.

They did, however, deliver electrical stimulation of a high enough intensity to create moderate pain immediately before collecting the control trial. Therefore, it is possible that if wind-up was created by the repeated stimulations during the first data collection, it may have also been created by the electrical stimulus delivered during the second control-only data collection.

This work did contribute valuable information about the descending control of pain, specifically in the absence of noxious stimulus. Tucker et al., (2012), observed that when participants were anticipating a painful stimulus, there was a significant decrease in MU firing rate ($P<0.01$) in the quadriceps muscles in the absence of a noxious stimulus. The authors report that of the 23 total motor units observed, 7 experienced an increase in firing rate during the anticipation of pain despite the mean firing rate of the entire group decreasing significantly. These motor units maintained their increased firing rate during the control contractions in the absence of pain (Tucker et al., 2012). The authors do report however that there appeared to be two different motor unit pools active during these contractions. They observed some motor units becoming active only in the control condition, while others were active only during the pain and anticipation of pain conditions. While this warrants further investigation, it is possible that a different group of MUs is activated during pain or anticipation of pain than the group that is activated in normal conditions. This observation creates further questions about motor unit activity during pain and is a possible avenue for future work to investigate as it is not clear why some motor units would be de-recruited during pain while others were recruited. Tucker et al., (2012) do suggest that this is a possible sign of altered recruitment thresholds, with higher-threshold motor units activating earlier during pain and creating the observed effect of a group of MUs appearing in only the pain trials. This serves to support the finding mentioned briefly by Martinez-Valdes et al., (2020) in which some motor units were identified in the pain trial but not in the controls. This study did not specifically investigate recruitment thresholds; therefore, this

suggestion cannot be confirmed, nor can their suggestion that this may be a sign of non-orderly recruitment of motor units (Tucker et al., 2012).

The significant reduction in mean firing rate during the anticipation of pain was exacerbated when hypertonic saline was injected into the infrapatellar fat pad, as the firing rate decreased further (Tucker et al., 2012). The most important finding from this study is that anticipation of pain appears to create similar effects on the motor unit pool as the experience of pain. Therefore, in the absence of nociceptive input from the periphery, descending control can still create significant changes in motor unit firing rates without any nociception. As pain is both a physical and emotional experience, it would appear that descending control modulates motor unit activity due to exclusively psychological factors in the absence of physical perception. Further, as those 7 motor units which increased firing rate maintained this elevated activity into the control condition, and participants did not report perceiving pain, it creates questions about the control strategy of the nervous system during pain. As discussed in this work, one potential explanation for the sustained changes is a protective effect like that purported by the "Pain-Adaptation Model" of Lund et al., (1991). It is possible that this sustained increase in firing rate is a method to maintain a higher force output in expectation of further pain or injury. However, as the authors reported concern of wind-up occurring during this study, this cannot be ruled out. It is possible that the sustained increase in firing rate due to pain is a result of wind-up, leading to prolonged effects in the absence of pain. This would be supported by the "Vicious Cycle Model" proposed by Roland (1986) in which pain leads to hyperactivity and further pain.

With this data, it is clear that descending control of pain has a significant impact on motor unit discharge in the absence of nociceptive input, but nociceptor activity may contribute to greater changes than descending command alone. Under the lens of central sensitization, this creates a plausible phenomenon in which those affected by chronic pain experience long-term

alterations in motor unit activity. Speculatively, those with chronic pain may anticipate pain more often, leading to significant changes in motor control strategies, while also experiencing hyperalgesia or allodynia, creating further change through direct nociceptive input. As this has not been measured directly it cannot be confirmed. Likewise, it is not clear whether this strategy would serve as a protective effect as purported by the "Pain Adaptation Theory", whether these changes are maladaptive as Roland (1986) proposed in the "Vicious Cycle Theory", or whether either proposition is exclusively correct. As has been the trend throughout the literature, it is not unlikely that different situations could lead to different control strategies, either protective or maladaptive.

While the work in the tibialis anterior and quadriceps showed a reduction in firing rate at low force levels, other studies have shown a less clear trend (Martinez-Valdes et al., 2020; Tucker et al., 2012). Moving beyond the lower limb, work in the muscles of the face showed a variety of changes in firing rate in the masseter muscle following injection of hypertonic saline (Minami et al., 2013). Hypertonic saline was injected into the masseter while participant's bite force was measured by a dynamometer inside the mouth. EMG data was collected using indwelling electrodes inserted into the masseter. Some of the identified MUs increased discharge rate while some decreased, and some remained the same. They theorized that this change represents a reorganization of motor unit activity in response to pain. Work by Malik et al., (2018) further demonstrates a reorganization of MU activity in response to noxious stimulus or pathological conditions. Different regions of the masseter displayed increases or decreases in MU firing rate during experimental pain, which supports the work by Minami et al., (2013) and proposes that changes due to pain may be location specific (Malik et al., 2018). The research showing reorganization is primarily based around muscles of the jaw but simple observations of motor units recruiting and de-recruiting across conditions in the lower limb suggest that

reorganization may happen throughout the body (Tucker et al., 2012; Martinez-Valdes et al., 2020). Ferreira et al., (2019) observed a similar trend where there was some evidence that different motor units were recruited due to noxious stimulation while others were de-recruited (Ferreira, 2019). This finding supports the earlier proposition by Tucker et al., (2012) that some MUs may cease firing altogether while higher-threshold MUs are recruited to maintain the desired force. Further evidence is shown within muscle groups. Gallina et al., (2018) demonstrated that within the same muscle group (quadriceps), muscle activity of the individual muscles within the group can be modulated by pain (Gallina et al., 2018). When comparing firing rates of vastus medialis to vastus lateralis in a group of patients suffering from patellofemoral pain, the pain group had an increase in vastus lateralis MU firing rate compared to controls (Gallina et al., 2018). Vastus medialis firing rate remained the same between control and patellofemoral pain groups. This work shows that even within synergistic muscles, different strategies can be adopted during a pain state.

A mechanism is proposed by Tucker et al., 2009 to explain how force is maintained during pain while low threshold MUs appear to experience a reduction in discharge rate. The proposed mechanism is that the decreased discharge rate of MUs is accompanied by increased recruitment of new MUs to maintain the desired amount of force (K. Tucker et al., 2009). This is supported by their later work which demonstrated this finding during quadriceps pain, as well as an observation in the tibialis anterior during an isometric contraction while experiencing pain that also showed increased recruitment of some motor units while others de-recruited (Tucker et al., 2012; Martinez-Valdes et al., 2020). This is inconsistent with previous theories that the motoneuron pool experiences generalized inhibition because of inhibition by nociceptors (K. Tucker et al., 2009). It is possible that equal inhibition to all MUs from nociceptors synapsing directly to motor neurons is incorrect, and it has been shown that descending control can affect

inhibition of motor neurons without nociceptive input (Tucker et al., 2012). In simulations of motor unit activity during pain it appears that the generalized inhibition is not equal for all motor units but is proportional to recruitment threshold (Martinez-Valdes et al., 2020). Low threshold motor units experiencing the most significant inhibition while high threshold units experience the least explains much of the previously unexplained findings in the literature. If future work in human participants can replicate the results of this simulation by Martinez-Valdes et al., (2020), it would explain why low threshold motor units experience a reduction in discharge rate while high threshold motor units experience an increase during pain. It would also explain why it appears that some low threshold motor units cease firing entirely during pain, while higher threshold motor units are recruited. At the time of this review this explanation cannot be confirmed, but it does present a plausible explanation for previously unexplained findings.

Much of the previous literature suggests motor unit pool inhibition descending from the brain is a key contributor to alterations in MU firing rates and recruitment thresholds during pain. To study activity in the motor cortex during pain, Romaniello et al., (2000) induced skin pain using injection of hypertonic saline into the left masseter with topical application of capsaicin over the same area. This study was designed to determine how muscle and skin pain in areas innervated by the trigeminal nerve affect the motor cortex. Participants clenched their jaw on a force transducer while motor-evoked potentials were stimulated in the masseter at 15, 30, and 45% MVC force levels. The clenching forces were created using transcranial magnetic stimulation and measured at the masseter using indwelling EMG. Experimental muscle pain in these participants did not result in a significant change in motor-evoked potential amplitude or latency (Romaniello et al., 2000). However, experimental muscle pain did increase EMG amplitude on the contralateral side to injections and capsaicin when compared with the

baseline values. Romaniello et al. (2000) suggest that this increased EMG amplitude may be a compensatory mechanism for changes in force producing characteristics of the effected (painful) side. The authors of this work explain that compensation for pain in the brainstem and at the level of the cortex to account for experimental pain may be the cause of increased masseter EMG amplitude on the opposite side of the body. Like the "pain adaptation model" proposed by Lund et al., (1991), this compensatory mechanism may be beneficial to contribute more bite force on the side opposite pain to avoid using the painful muscle. The findings of this work do not support the theory that central sensitization leads to hyperresponsiveness of muscles effected by topical capsaicin (Romaniello et al., 2000). However, as central sensitization was not confirmed it is unclear whether topical capsaicin applied to the face created hyperresponsiveness to pain in the dorsal horn. Likewise, as this study specifically targeted the trigeminal nerve, the nociceptive input bypassed the spinal cord, meaning the dorsal horn did not receive persistent nociceptive input. Therefore, central sensitization likely was not induced.

Later work by Svensson et al., (2002) once again used a capsaicin model to induce muscle pain in the jaw region and investigate potential effects on stretch reflexes. This work suggested a velocity-dependence of changing reflex amplitudes due to pain. Stretch was induced in the masseter and temporalis muscles at various rates before, during, and after induction of pain by injected capsaicin. Svensson et al., (2002) hypothesized that, if there is hyperexcitability of the muscles in response to pain, there should be an effect of velocity and displacement of stretch. Their data showed that the stretch reflex amplitude was greater during experimental pain, however, this effect was only shown with a high velocity stretch. There was not an effect of displacement when velocity was maintained while reflex amplitude increased as stretch velocity increased (Svensson et al., 2002). Therefore, it is possible that effects of experimental pain induction on motor behaviour may be dependent on the intensity of stimulus.

This work provides evidence that while pain can generate a simple reflex response, namely the nociceptive withdrawal reflex, it also appears to be capable of increasing the sensitivity of the muscle spindles during stretch reflexes.

To relate this work to motor function, Wang et al., (2010) set out to investigate the effects of glutamate and capsaicin on resting EMG, maximum bite force, and maximum voluntary jaw opening force. Resting EMG activity was evaluated 5 minutes after injection of glutamate followed by capsaicin into the left or right masseter and showed a significant increase in amplitude (Wang et al., 2010). To test the relative contribution of glutamate, a second condition was used in which isotonic saline was injected in its place, followed by capsaicin. When comparing changes caused by injection of glutamate and capsaicin to injected isotonic saline then capsaicin, the percent change from baseline resulting from the pairing of glutamate and capsaicin was greater than capsaicin alone (Wang et al., 2010). There was a significant reduction in both bite force and jaw opening force following injection of capsaicin and glutamate with no effect of the order of injection. Regardless of which noxious agent was injected first, force was significantly reduced (Wang et al., 2010). This work suggests that induction of peripheral pain can alter force producing capabilities of muscles which is not surprising based on previous work showing reductions in MU firing rate during pain.

To identify the mechanism of firing rate reductions in single motor units in response to pain, Svensson et al., (2003) injected capsaicin into the masseter of participants and evaluated twitch amplitude, half-relaxation time, and contraction time. Capsaicin injections led to a significant increase in twitch amplitude while half-relaxation time and contraction time remained unchanged in a pain condition. This work suggested that twitch potentiation in response to pain could be elicited to compensate for reduced firing rates in response to pain to maintain constant force (Svensson et al., 2003). This presents another potential mechanism for

force maintenance despite reductions in firing rate. It is possible that compensation for pain prevents reduction in force through other mechanisms such as increased twitch amplitude, recruitment of additional motor units, or increased firing rate of high threshold MUs (Svensson et al., 2003; Martinez-Valdes et al., 2020).

On the scale of gross movement, it appears that movement accuracy is impaired when pain is induced through injection of saline solution directly into a muscle (Bozec et al., 1987). Inducing pain within a muscle through injection of saline led to an observed increase in the number of errant movements and caused more mistakes during an upper limb reaching task aimed at a target. These mistakes include increases or decreases in movement velocity, reduced strength, and decreased smoothness of movement (Bozec et al., 1987). It is possible that reduced firing rate of low threshold motor units coupled with increased firing rate and further recruitment of high threshold motor units is displayed as the movement inaccuracies observed during this study.

Most of the work investigating the effect of pain on motor unit activity has been performed in isometric contractions. During dynamic contractions in the upper trapezius, Martinez-Valdes & Farina (2005) tested action potential conduction velocity in those effected by neck pain compared to controls. Those effected by neck pain had a significantly higher muscle fiber conduction velocity than controls as well as a more rapid reduction in conduction velocity over time as repetitive shoulder flexion was performed (Martinez-Valdes & Farina, 2005). Fatigue of the upper trapezius has been previously implicated in neck pain development whilst this work suggests a more rapid reduction in conduction velocity because of pain (Martinez-Valdes & Farina, 2005). It is possible therefore, that neck pain is related to fatiguability of the upper trapezius, but it is unclear whether pain leads to fatiguability or whether the pain is a result of fatigue.

Prior to this work, Farina et al., (2003) investigated both conduction velocity and firing rate simultaneously in the tibialis anterior. Experimental pain was induced through injection of hypertonic saline at doses of 0.2, 0.5, or 0.9 ml and had participants perform six isometric contractions of 20 seconds each. Isotonic saline was used as a control condition. Injection of isotonic saline did not induce a significant change. However, injection of hypertonic saline resulted in a significant reduction in motor unit firing rates from the baseline range of 7.4 -14.8 Hz (Merletti et al., 2003). This reduction supports work by Svensson et al., (2003). When comparing firing rate changes to subjective pain scores, there was a significant inverse relationship in which greater reductions in firing rate correlated to increased pain scores (Farina et al., 2003). No dose of injected hypertonic saline caused a significant reduction in conduction velocity which does not support later work by Martinez-Valdes & Farina (2005) and raises questions about the differences between chronic neck pain and experimentally induced pain (Farina et al., 2003). This disparity could demonstrate that chronic pain and experimental pain are not interchangeable.

Recent work by Evans et al., (2021) sought to investigate the effects of central sensitization specifically on motor unit behaviour in the upper limb. Using both surface EMG and indwelling EMG, normal activity of the trapezius and infraspinatus were evaluated. Following contractions of the trapezius created through a shrugging motion, capsaicin cream was applied over an area spanning the T3-T8 dermatomes on the left side of the body, roughly covering the skin over the scapula in a normal seated position with arms relaxed. A total of 9 participants completed the trial for the CS group. Placebo group participants received application of a non-noxious cream over the same area and a total of 11 participants were included in this group. Brush allodynia and visual analog scale measures were recorded to confirm that those in the capsaicin group experienced brush allodynia and an increase in perceived pain as this was

considered successful induction of CS. The same scores were used to confirm that the placebo group was not sensitized. The shoulder shrug motion was repeated 20-minutes following application of either cream. EMG data were decomposed using the NeuroMap software (Delsys, Massachusetts, USA) to identify individual motor units contributing to the overall surface signal. Motor units from the baseline and post-intervention conditions were matched using a custom cross-correlation algorithm created by the researchers in MatLab (MathWorks, version 2018a). All matches with a correlation of less than 0.8 were rejected as they were deemed unlikely to be the same motor unit (Evans et al., 2021). The recruitment order of each MU was determined in NeuroMap for both the baseline and post-intervention trials by ordering the motor units in the order that they were first identified. The primary objective of this work was to determine if recruitment order was influenced by central sensitization. The recruitment order of each MU from each group was used to create a scatter plot comparing recruitment order between the baseline and post-intervention conditions (Evans et al., 2021). A slope of 1 meant that recruitment order was identical before and after intervention. In the trapezius muscle, the capsaicin group showed a slope of 0.72 after the capsaicin intervention when compared to baseline recruitment order while the placebo group showed a slope of 0.93 suggesting a significant ($p=0.03$) change in recruitment order after application of capsaicin. These trends were not shown within the infraspinatus. One possible explanation is that in many cases the surface EMG decomposition identified very few motor units, or in some cases none, from some participants (Evans et al., 2021). Another explanation for why no change was observed in the infraspinatus is due to the relatively low activation level during a shrug, leading to motor units being below the required threshold for identification from the HDEMG system. The authors also report that the cross-correlation cut-off of 0.8 may have been too high and eliminated some of the matching MUs, as when they reanalyzed the data with a cut-off of 0.7, they were able to

identify a larger pool of matching MUs although introducing a greater likelihood of erroneous matching. As such, they did not make any conclusions from the infraspinatus data as it requires further work to ensure validity of their findings (Evans et al., 2021). The reduced slope of the trapezius data provides evidence that CS can change the recruitment threshold of individual motor units in the upper limb and also suggests that CS can affect motor units across multiple spinal levels as T3-T8 were sensitized and changes were observed in the trapezius which is supplied by C3/C4 (Evans et al., 2021). This work demonstrates that while previous work has shown that CS amplifies afferent input, it also appears to influence motor output (Evans et al., 2021). The recruitment thresholds of each MU in this study were not determined as the focus was on recruitment order. This leaves an outstanding gap in the literature investigating the physiological mechanism that created a difference in recruitment threshold during CS, leading to the observed changes from this work by Evans et al., (2021).

Overall, the current body of literature suggests that both clinical pain conditions and experimentally induced pain can lead to changes in various aspects of motor behaviour including control, force, firing rates, and recruitment. While this evidence shows that pain leads to changes, more work is required to identify the causal mechanisms of these changes, whether it be local control, potentiation, descending control, or any other combination of factors and compensatory mechanisms.

1.15 Experimental Techniques for Studying MU Behavior

Common methods for studying electrical activity of a muscle are surface electromyography (sEMG) and indwelling electromyography (iEMG). sEMG uses electrodes placed on the surface of the skin to record activity of the muscle underneath. It represents a sum of all motor unit action potentials occurring underneath the recording electrodes. iEMG allows precise recording by invasively inserting a needle electrode directly into a muscle. This

allows recording of the activity of individual motor units. This technique is valuable for testing the recruitment threshold of individual motor units. Each motor unit has an electrical profile that can be used to differentiate it from neighbouring units.

A great advantage of indwelling EMG is that a wire can be inserted by a needle, and then the needle removed. This allows the recording electrode to remain in place during a contraction while sEMG may change position over the muscle as the muscle moves below the skin. One disadvantage of indwelling EMG is that it is an invasive approach, and it may potentially act as a therapeutic intervention. An inert needle inserted into a muscle is an acupuncture treatment. A recording electrode inserted into a muscle by way of a needle may exhibit the same effects. As acupuncture has been shown to reduce motor unit firing rates in athletes, as well as effectiveness at temporarily reducing pain perception if the participant believes it will cause an effect, its applicability during the study of pain may be limited (Rehme Siqueira et al., 2018). There is some debate in the field about the effectiveness of acupuncture, and further about dry needling in which the needle is inserted directly into a trigger point (Kietrys et al., 2013; Stieven et al., 2020). Regardless, a meta-analysis of small, poorly controlled studies by Kietrys et al., (2013) suggested that it may be more effective than a sham treatment, while a meta-analysis using larger studies suggests that it is no more effective than a sham. Regardless, it appears as though if someone believes that a needle inserted into a muscle will cause an effect, it will alter their pain perception. Further, as stated by Tucker et al., (2012), expectation of pain appears to reduce MU firing rates in the quadriceps due to descending inhibition. It is possible then that if a participant anticipates insertion of a needle will be painful, they may elicit a compensatory response. Likewise, it is possible that if they believe a needle is a therapeutic intervention, they may perceive less pain. This is supported by the meta-analysis by Stieven et al., (2020) which concluded that even telling someone they would receive a needle

reduced their self-reported disability severity. Beyond the expectations of an individual participant, while an invasive approach like iEMG may be valid for most situations, insertion of a needle into a muscle of a centrally sensitized participant may be uncomfortable or painful and so this method may not be ideal for the study of central sensitization. Finally, while iEMG allows the observation of individual motor units, to measure the entirety of a muscle requires many needles inserted in different locations and at different depths to maximize the yield of motor units. While generally a valuable tool, it may not be the best option specifically for pain research.

Both sEMG and iEMG are valuable tools, but like any experimental tool there are limitations to consider. To minimize the limitations and maximize the advantages, there has been a trend in the electrophysiology research community of developing both hardware and software that incorporate both methods. A new method of measuring electrical activity which incorporates the strengths of both sEMG and iEMG has been proposed, known as high-density EMG (HDEMG) decomposition. This new method uses an array of 4 or more surface electrodes placed on the skin surface over the muscle of interest. This network of electrodes collects the sum of electrical activity beneath the recording site like regular sEMG (Merletti et al., 2003). While this allows measurement of sEMG over a larger area, advances in software have allowed extraction of motor unit activity similar to the data that could be acquired through iEMG (Merletti et al., 2003). The HDEMG electrode array collects the summed signal and then decomposes that sEMG signal into its MUAP component parts and conduction velocities (Merletti et al., 2003). The great advantage of this technology is that it creates a non-invasive method to acquire data like that acquired by iEMG, without breaking the skin surface. For specific situations such as the study of pain, where inserting a needle may confound the experiment, sufficiently advanced HDEMG hardware and software may be a suitable alternative.

Furthermore, being able to identify individual motor units within a summed signal allows researchers to observe multiple aspects of motor unit behaviour. With advanced enough algorithms, the firing rate of a motor unit can be observed, as well as observation of its recruitment threshold by determining the force level at which each MU becomes active. HDEMG decomposition, therefore, allows for the testing of recruitment thresholds and firing rates of individual MUs in both healthy and centrally sensitized conditions without the potential for an unintended therapeutic or painful effect from the insertion of iEMG electrodes. In ideal conditions, the different types of hardware and software that are available allow a more representative measurement of motor unit pool excitability than either iEMG or sEMG alone. However, as mentioned there are differences in both hardware and software that are worth discussing, as this technology is constantly being developed, improved, and debated.

Firstly, early examples of HDEMG hardware can be seen in studies using a linear electrode array (Merletti et al., 2003). The authors explain that the use of a pair of linear arrays allow the detection of action potentials in two domains (parallel with muscle fibers and perpendicular with muscle fibers) as well as in time which represents a third domain. A group of 6 electrodes placed in a line parallel with the direction of muscle fibers allows the action potential to be tracked as it travels beneath the electrodes. Likewise, the perpendicular array allows tracking of action potentials in a second direction, giving multiple views of the same action potential from different distances as well as showing if the arrays are oriented directly parallel to the muscle fibers. This spatial representation of action potentials allowed manual identification of motor units by their unique twitch profiles but was not able to extract complete firing patterns (Merletti et al., 2003). Since this early work, HDEMG technology has advanced significantly leading to the creation of decomposition algorithms and the hardware becoming commercially available. Currently there are two different commercially available sets of arrays.

The first system is produced and sold by Delsys (Delsys Incorporated, MA, USA). Delsys produces a 5-electrode sensor used and developed by the research group of De Luca et al. Comparable to the early work of Merletti et al., (2003), this system uses spatial orientation of action potentials to identify unique motor units. The Delsys system orients their electrodes on a reusable sensor in the shape of a square. Four electrodes are equally spaced to create the square with a fifth electrode in the center. When placed over a muscle, it uses its 2-dimensional orientation of electrodes to measure action potentials in two planes, as well as in the time domain. This data must then be analyzed to identify individual motor units.

Examples of the entire system of sensor and algorithm being used are described by Kline & De Luca (2014) but original explanation of the algorithm itself were first described by De Luca et al., (2006). The algorithm used, named "Precision Decomposition III" (PD3), is an advanced version of an algorithm originally used to identify individual MUAPs within an indwelling EMG signal. While this algorithm was reliable and valid in iEMG analysis, the PD3 variety was specifically designed for surface signals (De Luca et al., 2006). While the PD1 version was capable of decomposing simulated HDEMG signals, it was unable to decompose a real biological signal leading to the recommendation that any decomposition algorithm should be validated with biological, rather than simulated signals (De Luca et al., 2006).

The algorithm itself consists of four processing stages. First, a digital band-pass filter is used on the incoming data before it is passed through the second stage, a maximum a posteriori probability (MAP) equation. De Luca et al., (2006) explain that this equation is used to avoid false positives by not identifying any action potentials that seem improbable. The authors do admit, however, that due to variability in the shape of a MUAP, the MAP equation often misidentifies a single MUAP train as multiple different motor units. It also is common for the MAP equation to fail to classify some MUAPS if they are overlapping, as the overlapped signals

do not fit within the templates produced by the algorithm. The following processing steps are designed to correct these errors. After the MAP step, the third step begins in which the algorithm compares the identified MUAP trains to each other. It then attempts to estimate based on the MUAP templates whether it is probable that these motor units were erroneously split, or whether they are different. If it is probable that it was an error, the MUAP trains are recombined to resolve the first type of error commonly created by the MAP. Finally, the algorithm attempts to correct errors caused by overlapping MUAPs. The already identified twitch profiles are correlated with the overlapping MUAPs to estimate the probability that the twitch profile with no matches is actually a combination of other MUAPs rather than a different MU entirely. If the system determines that this is a probable error of the MAP, it is added into the MUAP train from which it likely belongs. After the algorithm is completed, the user can use an interactive editor to judge the data visually. If they notice a gap in the data that they believe to be an error, they can visually inspect the templates and insert an action potential into the train if they think there was a mistake. Due to the ability to modify what the algorithm creates, in theory it is possible to reach nearly 100% decomposition accuracy (De Luca et al., 2006). However, this claim is based on the assumption that using probability and manual editing of data to fill in gaps is correcting errors, and not producing more.

To reduce error in the Delsys system, it was used to collect biological activity from the upper and lower limb. The 5-electrode sensor was placed over the first dorsal interosseus (FDI) and the vastus lateralis (VL) of 6 participants (Kline & De Luca, 2014). Participants performed isometric contractions at 5, 10, 15, 20, 25, and 30% MVC in the FDI, and at the force levels 20, 25, 30, 35, 40, and 50% MVC in the VL. Force increased at a rate of 10%/s, was held for 35s at the desired force, and then reduced back to 0% at the same rate. This force profile created a trapezoidal contraction with a sustained hold at the desired force (Kline & De Luca, (2014). A

technique known as the “Decompose-Synthesize-Decompose-Compare” (DSDC) method was introduced. In this method the biological signal is decomposed as described previously, then the twitch profiles and MUAP trains identified by the algorithm are used to create a synthetic HDEMG signal. Estimated levels of noise are added to the synthetic signal, and then it is decomposed a second time. The advantage of this technique is that it is a built-in test for accuracy. The overarching assumption is that if the process of decomposing the biological signal was accurate, then that data is used to create a synthetic signal to be decomposed again, then both of the decomposed signals should be nearly identical. Kline & De Luca, (2014) make the claim that this technique leads to 97% accuracy in correct identification of MUs and MUAP trains.

While the Delsys system claims only 3% error, there are opponents in the field who do not believe that the algorithm is as valid as previously claimed. As such, these aforementioned opponents challenged Delsys to provide further validation. A simulated signal composed of multiple computer-generated motor unit action potentials (MUAPs) was produced by an opposing research group led by Dr. Dario Farina. The algorithm developed by Delsys appeared to be successful in decomposing the synthetic signal with a high-level of accuracy, ranging from 92%-98% (De Luca et al., 2015). Still, Farina et al., (2015) purport that a more valid model than the DSDC technique for decomposition is the two-source test originally proposed by Kline & De Luca (2014) and described by Farina et al., (2015) as the “only current reliable approach to assess the accuracy of a surface EMG decomposition algorithm”. In the two-source test the HDEMG decomposition results are compared between two different concurrently collected EMG channels (Farina et al., 2015; Negro et al., 2016). For example, a 10% MVC isometric ankle dorsiflexion task with surface EMG on the tibialis anterior and an indwelling electrode inserted into the muscle would have some of the same sources in both channels, along with some

different sources due to the shape of MUAPs differing when measured from over the skin compared to internally. If both the surface and indwelling signals yield the same results, it can be assumed that the decomposition was accurate as it is not likely that the same shape would be erroneously attributed to a MU in both channels (Negro et al., 2016). The decision of Delsys to use their own DSDC system as validation over something like the two-source test raises concerns about their system's validity.

It appears that the greatest objection from the group of Farina, Merletti, and Enoka, (2015) is that the DSDC technique relies on creating an artificial signal based on the first decomposition, to then create the second for comparison. They argue that the DSDC approach is not a valid method of decomposition due to the inherent assumption that 100% similarity between the first and second decompositions means that the algorithm produced a valid measure of motor unit activity (Farina et al., 2015). To illustrate this point, Farina et al., 2015 use the example of an HDEMG signal without any residual noise. In this hypothetical situation, the DSDC algorithm would reconstruct the surface signal absent of noise, which is inherent in any system, and then proceed to decompose it and claim 100% accuracy. Further criticism comes from a hypothetical scenario in which there is only noise without muscle activity. The DSDC system would reconstruct the signal but in the absence of any MUAP trains the synthesized signal would just be noise. The noise would then be decomposed and compared to the original and declared 100% accurate despite not having identified any motor units (Farina et al., 2015). Overall, the issue with the DSDC technique used by Delsys is that any error in the first decomposition, including human error introduced by manually filling gaps in MUAP trains, are then carried over into the second decomposition. While both decompositions may be nearly 100% similar, that does not necessarily mean that there weren't errors in the original decomposition, producing two virtually identical decompositions that are both invalid.

The opponents to the work by Delsys are led by Dr. Dario Farina and his research group. This group uses their own hardware for HDEMG collection and their own custom-made algorithms (OT Biolettronica, Turin, Italy). The OT Biolettronica group expands on the paired linear array idea first used by Merletti et al., (2003) by creating an array of 64 surface electrodes of various shapes and sizes to sufficiently cover most muscles. This equipment comes equipped with its own decomposition algorithm that is not based on the PD3 or DSDC used by Delsys. (Negro et al., 2016). The algorithm itself is known as convolutive blind source separation. The goal of the algorithm is to identify the maximum number of unique sources within a convoluted EMG signal (Negro et al., 2016). Within each EMG channel, the algorithm attempts to use the duration of the trial, the duration of suspected action potentials, corresponding suspected spike trains, and noise in the channel to estimate the number of sources contributing to the signal and therefore the active motor units in the given channel. Each newly identified source is assumed to be unique when first identified, and then correlated with other channels to determine if the waveform is repeated at a slight delay in other channels. For example, if an HDEMG array is applied on the tibialis anterior and a suspected motor unit is identified in the most proximal electrodes it would be compared to the channels located distally. If the suspected source found in the proximal electrodes is highly correlated with those found in the more distal electrodes at a slight time lag, it is likely that this is a MUAP. This process is repeated for each potential source to determine how many MU spike trains contribute to the overall signal.

While the PD3 algorithm used by Delsys reported struggling with overlapping MUAPs, OT Biolettronica claims that the convolutive blind source separation technique can handle these without an error (De Luca et al., 2006; Negro et al., 2016). Negro et al., (2016) explain that it is very unlikely that action potentials summating within a channel will then highly correlate with the same two action potentials summating at a plausible time lag in a neighbouring channel.

Therefore, they do not suspect that in the condition that synchronization of MUAP occurs, it would present a significant challenge for the algorithm (Negro et al., 2016).

As previously mentioned, the two-source method is currently considered the gold standard of HDEMG decomposition validation (Negro et al., 2016). Therefore, the OT Biolettronica system was validated using this technique and yielded an estimated accuracy of 90%. Negro et al., (2016) do report that a limitation of this approach is the repeated comparison to the original source. It is possible that repeatedly comparing to an incorrect source could lead to accidental identification of a false motor unit, however, it is unlikely that the same waveform would be seen across multiple channels with a plausible time lag, so this potential error is likely uncommon. Of note is the report that the two-source test used to validate this algorithm cannot be used to compare two different sEMG signals. As two sets of sEMG electrodes would both collect action potentials from the skin surface, the waveforms would not differ significantly between channels. Therefore, the two-source test can be used to compare HDEMG decomposition to indwelling EMG, but it cannot be used to compare two HDEMG channels.

While there are plenty of concerns raised about the algorithm used by Delsys, De Luca et al., (2014) also provide some criticisms about the two-source test and blind source separation used by OT Bioelettronica. Primarily, the criticism raised is drawn from a paper by Farina et al., (2014) in which they discuss improvements in their decomposition algorithm. In this work, they report information regarding the quality of the decomposition produced by their algorithm (Farina et al., 2014). De Luca et al., (2014) focus on the statement that their algorithm identified an average of 0.7 MUs per trial. Achieving an average of less than 1 means that in some trials the algorithm was unable to identify any MUs which De Luca et al., (2014) state is a fundamental drawback of this system and that they question its usefulness.

Regardless of the possible drawbacks that the De Luca group and the Farina group present in the other's algorithm, both groups appear to be successful in using their systems to obtain a high number of motor units. Both groups assert that a major advantage of using HDEMG decomposition is the relatively high yield of motor units that can be identified compared to indwelling EMG (De Luca et al., 2009). De Luca et al., (2009) demonstrated that using their small array of 5 electrodes they were able to identify a range of 20 to 30 motor units with a maximum yield of about 40. This work using just 5 electrodes shows that the current technology for HDEMG decomposition can reliably produce a high yield of individual motor units with a small sensor size and with high accuracy. The authors suggest that HDEMG decomposition could be a novel tool for studying motor control as well as serve as a tool for clinical investigations of motor unit behaviour (De Luca et al., 2009).

Like any research tool, there are potential limitations of HDEMG to consider beyond the differences in algorithms. A review by Merletti, Holobar, and Farina (2008) provides a list of potential limitations of using HDEMG that should be considered when thinking of using it for a research study. Firstly, HDEMG heavily relies on skin-electrode contact. Due to this fact, the application of HDEMG arrays require a higher degree of experience for setup compared to a more basic bipolar or monopolar setup. Secondly, recording many channels at a high sampling rate requires significant storage space and processing time as well as a great amount of computational power. Thirdly, like other forms of surface EMG, detection depth is approximately 1-2cm leading to collection of the most superficial motor units which has the potential to bias the signal dependant on the organization of fiber type of the muscle of interest. Finally, higher levels of effort, depending on the muscle of interest, leads to a reduction in accuracy and therefore higher levels of effort are more difficult to study (Merletti et al., 2008).

Further evidence of contraction intensity limits is provided by McNeil et al., (2005). Within the tibialis anterior, there was a progressive decline in the yield of motor units as contraction intensity increased. The greatest yield of motor units identified appeared to be at a contraction intensity of approximately 25% MVC. The authors suggest that it is integral to consider contraction intensity and control for effort during research using high density EMG to ensure a representative sample of the true number of motor units is obtained (McNeil et al., 2005).

Overall, the technology of HDEMG is still developing and improving. In its current state there are several potential limitations, but it does provide an alternative to indwelling EMG. While it will likely continue to be improved upon, commercially available HDEMG software can be a valuable research tool if the limitations are acknowledged and controlled.

1.16 Conclusion

Chronic pain presents a significant burden on both the healthcare system and the economy. Despite the prevalence and impact of chronic pain, it is still poorly understood. Current literature describes in detail the various factors that can influence motor unit properties. Factors such as ageing and fatigue can modulate firing rates of motor units. It has also been shown that descending control has the potential to modulate the activity of a motor unit in the lower limb. Pain, like these aforementioned factors, has the potential to cause similar changes in motor unit properties. Experimental pain has been shown to reduce motor unit firing rates without a change in recruitment threshold. While much of the current work uses a variety of modalities to induce experimental pain such as injections of saline or capsaicin, or topical application of capsaicin, little of the previous work has directly investigated central sensitization. Therefore, future work is required to investigate the effects of central sensitization on motor unit excitability.

For this work, high-density electromyography presents a possible tool for non-invasive investigation of motor unit behaviour. Like any experimental tool, there are limitations. Some limitations include the inability to identify motor units at high levels of effort and the fact that a large amount of processing is required. However, the ability to measure an entire muscle and obtain a high yield of motor units makes high density electromyography decomposition a potential tool for the study of motor unit activity in a centrally sensitized population.

More work is required in the field of pain research to determine the effects of experimental pain on motor unit activity and how this translates to a central sensitization population. Confirmation of central sensitization through wind up ratio and brush allodynia, accompanied by high-density EMG composition, has the potential to progress the field of central sensitization research. More work is needed to clearly establish the effects of central sensitization on motor unit pool excitability.

Chapter 2: Introduction

2.0 Introduction

Chronic pain has become a global epidemic (Burma et al., 2017). Approximately 1.5 billion people worldwide are affected, which accounts for an estimated 20% of adults (Burma et al., 2017). Within Canada, direct medical costs of treating chronic pain surpass $6 billion dollars annually while the indirect costs reach nearly $37 billion dollars (Lynch, 2011). Specific chronic pain conditions such as fibromyalgia, myofascial pain syndrome, and osteoarthritis affect 18.9% of Canadians (Schopflocher et al., 2011). The long-term persistence of pain observed in these specific conditions is likely due to the development of central sensitization (CS) (Desmeules et al., 2003; Gerwin, 2001; Gwilym et al., 2009). Central sensitization is a state of hyperexcitability in the dorsal horn of the spinal cord resulting in progressively increasing amplification of nociceptive input and it is a theorized contributor to both the transition from acute to chronic pain as well as the persistence of pain even in those who's body has fully healed (Scerbo et al., 2018; Loeser et al., 2011). A similar phenomenon to central sensitization is peripheral sensitization. Both conditions display hyperalgesia, an amplified perception of pain to the same amount of noxious stimulus. Peripheral sensitization can be differentiated clinically from central sensitization by the presence of secondary hyperalgesia (Lluch et al., 2018). Primary hyperalgesia occurs when a repeated noxious stimulus creates an amplified perception of pain in the area being stimulated and occurs in both central and peripheral sensitization (Jensen & Finnerup, 2014). Secondary hyperalgesia, which is unique to central sensitization, occurs when areas surrounding the site of a painful stimulus are also hyperexcitable to stimuli, both noxious and non-noxious (Jensen & Finnerup, 2014). Therefore, a simple differentiator between the two types is that while peripheral sensitization effects just the area where the painful stimulus is

applied, central sensitization effects a larger area. In a centrally sensitized state non-noxious afferent input can be perceived as a noxious stimulus, a phenomenon known as allodynia (Loeser et al., 2011). Allodynia is the key contributor to long-term pain after nociceptive signalling from the periphery has ceased, as non-noxious stimulus providing proprioceptive information is interpreted as pain (Loeser et al., 2011; Jensen & Finnerup, 2014). The physiological development of CS is an analog of long-term potentiation (LTP) in the hippocampus (Ji et al., 2003). Both CS and LTP result from a barrage of input and persists after the afferent barrage has ceased (Ji et al., 2003). The persistent barrage of input leads to the strengthening of synapses and neurogenesis to create a greater amplitude post-synaptic response to the same amount of peripheral input (Ji et al., 2003). While in the case of LTP, this strengthened synaptic connection is beneficial for forming memories, in CS specifically, the strengthening of connections is maladaptive, leading to the amplified response to input typical of CS (Desmeules et al., 2003). Through this process, pain can lead to CS in the dorsal horn creating the long-lasting pain phenotype observed in conditions such as fibromyalgia, myofascial pain syndrome, and osteoarthritis (Desmeules et al., 2003; Gerwin, 2001; Gwilym et al., 2009; Ji et al., 2003).

The effects of a CS state on noxious and non-noxious afferent input are well-established, along with the effects of pain on efferent motor output (Desmeules et al., 2003; Martinez-Valdes et al., 2020). However, pain and peripheral sensitization are not synonymous with CS. It can be concluded that peripheral sensitization influences motor unit activity (Martinez-Valdes et al., 2020). It is unclear, however, whether central sensitization produces the same effects on motor output as experimental pain. On the scale of gross movement, some investigations into the effects of central sensitization have been conducted, and this work suggests that CS may affect motor control (Van Hilten, 2010). The suggested effects include movement inaccuracy,

such as slowed velocity and less accurate target reaching, as well as reduced voluntary range of motion (Van Hilten, 2010). The exact mechanism of these movement changes is not well understood.

There is a large body of evidence investigating the changes in motor unit activity due to experimental pain, but it is not clear whether the participants in this work were centrally sensitized (Martinez-Valdes et al., 2020; Scerbo et al., 2018). Newly published Work by Evans et al., (2021) sought to investigate the effects of CS specifically in the upper limb using HDEMG decomposition and concluded that CS may alter motor unit thresholds compared to a no-pain condition as shown by some motor units activating in a different order (Evans et al., 2021). Altered recruitment thresholds shown by Evans et al., (2021) provides evidence that CS influences motor unit activity but the exact modification leading to this change is not clear. This evidence published by Evans et al., (2021) also showed that CS appears to affect motor units across multiple spinal levels in the upper limb and this finding warrants more investigation whether central sensitization may create similar changes in the lower limb. My work combined with work from Evans et al., (2021) may explain the movement inaccuracies observed in a CS state. As central sensitization is common in chronic pain, it is valuable to compare pain and CS to identify any potential differences, as experimental pain represents acute pain while CS represents chronic pain.

Past research has used saline injections or capsaicin, either injected or in a topical application over the skin, to induce pain experimentally. Much of the previous work in the field is in experimental muscle pain in the jaw (Svensson, 2002). In this group of musculature, pain has been shown to affect motor unit pool excitability as demonstrated by increases in reflex amplitude, increased baseline electromyography (EMG), and reductions in firing rate when compared to control data collected in the absence of pain (Romaniello, Cruccu, Mcmillan,

Arendt-Nielsen, & Svensson, 2000; Svensson, 2002; Wang, Svensson, Sessle, Cairns, & Arendt-Nielsen, 2010). Supporting work in the lower limb suggests that MU excitability in the quadriceps is altered in a pain state as shown by reductions in firing rate and a reorganization of MU in matched force conditions (Tucker et al., 2009). Tucker et al., (2009) explain that reorganization in the quadriceps was shown when some motor units that were active without pain appeared to de-recruit at the same force level during pain, while other MUs not identified in the pre-pain trials became active in conjunction with increased activity in knee extension synergists. This work suggests that during experimental pain some motor units de-recruit while others recruit (Tucker et al., 2009). It is possible that during pain additional MUs are recruited to maintain force production as the firing rate of low threshold MUs decreases (Tucker et al., 2009). These studies are limited to low force (<50% maximal voluntary contraction) contractions but suggest that experimental pain can reduce MU firing rates in the face, and the lower limb, during pain.

Recent work by Martinez-Valdes et al., (2020) investigated motor unit firing rates at recruitment and de-recruitment, as well as recruitment thresholds following experimental induction of pain in the tibialis anterior using injected hypertonic saline. This work found that firing rate changes for each MU appear to be dependent on their recruitment threshold with low-threshold MUs showing a reduced firing rate while high-threshold units showed an increase during experimental pain (Martinez-Valdes et al., 2020). Changes in recruitment thresholds were also shown to be related to force level as high-threshold units were recruited at a lower force (%MVC) during pain while low-threshold units were unaffected (Martinez-Valdes et al., 2020). This work shows that changes in motor unit behavior due to pain are dependent on the recruitment threshold of motor unit groups. There is a large body of evidence in both the muscles of the face and the lower limb suggesting that experimental pain can alter motor unit

firing rates and recruitment thresholds. Recent work by Evans et al., 2021 has demonstrated that CS modifies the recruitment thresholds of individual MUs in the upper limb. Currently, there is an outstanding gap in the literature studying the effect of central sensitization on motor unit activity in the lower limb. It is possible that some participants in the previous work using experimental pain became centrally sensitized, but it has yet to be confirmed. Work by Evans et al., (2021) confirmed induction of CS in the upper limb and showed altered recruitment order when sensitized but did not investigate the recruitment thresholds or firing rates of each identified MU. Following a similar experimental design to the Martinez-Valdes et al., (2020) work, I aimed to test the effects of CS on motor unit activity in the lower limb induced through topical capsaicin application similarly to work by Evans et al., (2021) in the upper limb. With the addition of confirming experimental induction of CS in participants, my work presents a possible framework for investigating not just the effects of pain, but the effects of central sensitization itself. This work is important for identifying differences between acute pain and long-term persistent pain conditions associated with CS, as CS has been shown to alter motor unit activity at multiple spinal levels in the upper limb (Evans et al., 2021). Work by Evans et al., (2021) proposes that CS results in altered motor unit recruitment order, which has practical implications for motor control and fatigue in those affected by chronic pain.

A limitation of investigating the behavior of individual MUs in a state of central sensitization is the relatively low yield of motor units that can be acquired using indwelling EMG (Nawab et al., 2010). An emerging method for the study of MU behavior is high-density surface electromyography (HDEMG) (Negro et al., 2016). This work uses an array of electrodes to collect a relatively large area of summed motor unit action potentials and then decomposes this signal to identify the waveforms and firing rates of the individual motor units (Negro et al., 2016). This work has been validated by comparing it to both intramuscular EMG recordings as well as

simulated signals and has been shown to be reliable for the identification of MUs while also leading to an increased yield of motor units when compared to the amount of MUs that can be identified by intramuscular recording (Farina et al., 2014). While there are some concerns regarding the validation methods and limitations of the algorithms such as misidentifying MUs, it appears that the commercially available devices can successfully identify MUs under ideal conditions (De Luca et al., 2015; Farina et al., 2015; Martinez-Valdes et al., 2020). For this reason, HDEMG decomposition is a useful method for identifying large pools of MUs to study their response to experimentally induced CS when used in conjunction with well-developed algorithms.

The previous work in the field has studied experimental pain and peripheral sensitization. However, it is not clear whether the same results are seen in those effected by central sensitization leading to an outstanding gap in the literature about whether peripheral and central sensitization cause the same effects. Therefore, it is unknown how motor units behave in those with long-term and persistent pain from central sensitization shown in conditions such as fibromyalgia, arthritis, and migraine. Therefore, the purpose of this study is to investigate whether experimentally induced CS alters motor unit recruitment and firing rate similarly to peripheral sensitization during a submaximal isometric dorsiflexion task in the tibialis anterior. I set out to test the hypothesis that during experimentally induced central sensitization, motor units in the tibialis anterior will demonstrate a reduction in firing rate at recruitment and at 20% MVC without a change in recruitment thresholds, de-recruitment thresholds, surface EMG amplitude, or any changes in those given a placebo treatment, similar to the MU behaviour alterations seen during peripheral sensitization (Martinez-Valdes et al., 2020). I propose that, as CS is an amplification mechanism for nociception, experimental central sensitization will create the same or greater changes than peripheral sensitization. The results of

this study may compliment previous work with experimental pain and provide insight into the effects of central sensitization on motor unit recruitment in the lower limb, which may have implications for understanding, diagnosing, and treating chronic pain.

Chapter 3: Methods

3.0 Methods

3.1 Piloting

Prior to the collection of the data included in this thesis, I performed extensive piloting to become familiar with the new experimental setup and attempt to achieve the highest possible data quality. Initial piloting was performed on the biceps brachii. Several different experimental setups were tested to achieve EMG decomposition including: the participant's hand under a desk performing isometric elbow flexion, an isometric hold of dumbbells of weights ranging from 2.5lbs to 20lbs, isometric elbow flexion resisted by another lab member, and dynamic contractions. Different elbow angles and postures were also performed, ranging from full elbow extension to full elbow flexion, 90 degrees of elbow flexion, and the upper arm supported on the surface of the desk with the forearm oriented vertically to create a 90-degree angle for isometric elbow flexion. Further piloting had participants engage in several different degrees of supination and pronation of the forearm. Several pilot trials were collected each week beginning from when the HDEMG setup arrived in December 2019 until the target muscle was changed to the tibialis anterior in late January 2020. From the pilot trials using the biceps brachii and multiple attempts (repeated lab visits) on five different participants, I was not able to identify any motor units in most participants. Those that I could identify motor units from had an average of one motor unit per trial, and the firing plots were not consistent with some MUs only being identified for 3 action potentials across a 30 second contraction. Interestingly, from the participants that yielded few motor units I was consistently able to get a single motor unit across multiple attempts and multiple days, while I was never able to identify any motor units

from the other participants despite multiple attempts. Further, the male participants (N=3) yielded few motor units while the female participants (N=2) did not yield any.

A similar issue occurred when data collections were shifted to the tibialis anterior. While MU yield was higher in the TA, it was still difficult to identify motor units in most participants. Eight different participants attended the lab for piloting, but I was not able to identify MUs from their trials, so their data was not included in this work. In total, 3 participants (separate from the other 8) who attended the piloting yielded usable data from their tibialis anterior before data collections were cancelled due to the COVID-19 restrictions on in-person data collections.

In case of continued issues with collecting quality data, I performed a second study involving different dosages of capsaicin or placebo cream on the upper and/or lower limbs of participants to determine how the severity and location of capsaicin application influenced a participant's sensitivity to noxious and non-noxious stimuli in both the upper and lower limb. This data was not used for the completion of this thesis in favor of using the more limited but more impactful EMG decomposition data from the tibialis anterior of 3 participants.

The overall experimental protocol described in the following sections was determined based on the protocol that appeared to yield the most motor units from the tibialis anterior combined with the collection parameters used for similar work with the same hardware by Martinez-Valdes et al., (2020).

3.2 Participant Selection

All participants provided informed written consent prior to participation. Participants were informed that the pain intensity during the study could range from no-pain to moderate. The sample consisted of 3 participants (3 males, aged 24-25). Each participant attended a single visit. Two participants were allocated to the "central sensitization" group in which experimental pain was induced. One participant was allocated to the "placebo" group in which experimental

pain was not induced. A total of 10 motor units were identified from these participants with 6 motor units identified in the experimental pain participants and 4 identified from the participant given a placebo treatment. Participants completed a standardized health questionnaire prior to inclusion in the study, which screened for recent injuries, pain, or altered sensation. Inclusion criteria required that participants were young, healthy adults between the ages of 18 and 35 without any pre-existing chronic pain, neurologic pathology, or altered sensation in the lower limb. Participants with conditions such as fibromyalgia, osteoarthritis, low back pain, whiplash, or similar conditions were excluded as they could potentially be centrally sensitized prior to beginning the study. Participants were included in both a pre-intervention "baseline" condition and a post-intervention "CS" or "Placebo" condition for comparison within subjects.

3.3 Participant Setup

Participants were positioned upright in a chair with their foot secured in a custom-made ankle dynamometer (*Figure 2)*. Participants had their ankle plantarflexed at 110 degrees and secured with a foot strap. Angles at the knee and hip varied between participants to allow the anterior thigh to be positioned parallel with the ground for sensory testing (pinprick testing). Ankle angle was measured using a handheld goniometer by the researcher who has clinical experience assessing joint angles using protocol by Rome & Cowieson (1996). One arm of the goniometer was aligned with an axis created by palpating the head of the fibula and the lateral malleolus and the other arm of the goniometer was aligned along the axis created by a line between the inferior aspect of the heel and the head of the 5^{th} metatarsal. The center of rotation of the goniometer was placed at the intersect between the lateral malleolus/fibular head axis and the 5^{th} metatarsal head/inferior aspect of the heel axis. The plastic goniometer was not affixed to the testing apparatus to allow direct measurement of the joint angle and not the angle of the footboard. As such, the goniometer is not represented in *Figure 2*. Participants

remained in the same position throughout the protocol to control for effects of changing joint ankles on motor unit recruitment. A strap was placed across the metatarsal heads of the participants foot and tightened until the participants foot was not able to lose contact with the footboard while dorsiflexing, without impairing blood flow to the toes. Pressure was applied by the researcher to the great toe of the participant to cause blanching and then released to ensure venous return was not impaired after tightening of the foot strap to ensure that the foot was immobilized without limiting blood flow. The foot strap was a repurposed generic ankle strap attachment typically used on a cable machine in a gym setting. It was oriented to wrap around the footboard with clearance on either side to avoid contact. The strap consisted of two flat bands secured with Velcro. The footboard itself was immobile, ensuring that when a participant dorsiflexed against the strap all the produced force was transmitted directly to the attached force transducer without the ankle angle changing. The apparatus was designed to allow measurement of dorsiflexion torque in an exclusively isometric task, and this was achieved by ensuring the strap was tight enough to secure the participant's foot against the footboard without being attached to it directly. The side beneath the footboard was secured to the TAS501 S-shaped load cell (Capacity 200kg; Accuracy +/- 0.02%; HT Sensor Technology Co., Xi'an, China) by a carabiner. The two flat bands were overlapped over the dorsal surface of the participant's foot, allowing fine adjustment of tightness, and ensuring that dorsiflexion against the strap created tension on the force transducer. The dynamometer was designed to allow the participant to apply force to the foot strap during dorsiflexion, which created tension on the load cell without allowing the foot to lose contact with the footboard

3.4 Task Overview

Upon arrival at the lab, participants were asked to fill out a confidential health history questionnaire. Participants were seated in front of the lab computer, secured in the

dynamometer (*Figure 2*), and the EMG array was setup over the tibialis anterior. An anatomical landmark frame (ALF) was determined for the tibialis anterior by palpating the tibial tuberosity and intermalleolar line of the tibia. A straight line between these landmarks was defined as the ALF and the electrode was placed in the distal 50% of this range based on work by Barbero et al., (2013). Three maximal voluntary contractions (MVCs) were performed and the highest of the three was taken as their maximum in accordance with the MVC protocol used by Martinez-Valdes et al., (2020). This protocol assumes that the peak force achieved was maximal and that participants achieved near 100% voluntary activation. A superior protocol would be to use a technique such as the interpolated twitch technique (ITT) to ensure the participants reached maximal activation (Herbert & Gandevia, 1999). The peak of the MVC was used to calculate 20% of their maximum force. Since the MVC was assumed, standardizing all contractions to the same force level ensured that contraction intensity was consistent across trials and all data, including recruitment thresholds, de-recruitment thresholds, and firing rates were analyzed based on their degree of change from baseline rather than raw values. This reduced the odds that a participant not reaching true maximal effort during the MVC would impact the trend of the group. To ensure consistent rates of force development during the test trials, real-time visual feedback was given to the participant on a computer screen in front of them. Within the OT BioLab+ software (OT Bioeletrronica, Torino, Italy) the requested trapezoidal force path was produced for participants to follow. The participants were given as many practice contractions as required until they confirmed that they were comfortable tracing the requested path. Three minutes of rest were given between the practice contractions and the first baseline trial in case of fatigue. The baseline trial was then performed which consisted of 3 consecutive trapezoidal contractions with a 30 second rest interval between. The contractions consisted of a ramp up to 20% MVC force at a rate of 2% MVC/second. The target force was held for 10 seconds before

ramping down at the same rate. Following these contractions, capsaicin was applied to experimentally-induce central sensitization. CS and Placebo trials were always performed second to compare to baseline. A timer was started once the intervention had been applied. A series of 3 consecutive trapezoidal contractions with the same profile as the baseline trials were completed 10 minutes after capsaicin or placebo cream had been fully applied. Pinprick tests were performed immediately following the clusters of 3 baseline and capsaicin trials at each time point. Brush allodynia was performed 5 minutes after each series of 3 contractions. Capsaicin trials were repeated at 20-, 30-, and 40-minutes post-capsaicin application but were omitted from statistical analysis as MUs identified in the baseline trials were not observed in each of the later trials. Following the third ramp contraction of the 40-minute post-capsaicin time point, the collection was concluded. Each participant attended one lab visit and each collection lasted approximately 1 hour. A timeline of the experimental protocol is displayed in *Figure 6*.

3.5 Force Collection and Processing

Force data were collected using a uniaxial load cell connected by a cable to the foot strap of the participant (*Appendix A: Figure 13)*. When the participants dorsiflexed against the strap, tension was applied to the load cell. To ensure close coupling of force to activation the foot strap was tightened and adjusted until there was no slack in the cable connecting the foot strap to the load cell. As the foot strap consisted of two overlapping pieces attached to each other by Velcro, increasing or decreasing the amount of overlap allowed adjustment of the cable tension. After the foot strap was properly secured the force amplifier was zeroed. Therefore, any added tension to the cable would be recorded by the system. Analog data from the load cell was connected to a “Forza” model force amplifier (OT Biolettronica, Torino, Italy) for direct input into the OT BioLab+ software for processing. Force data were amplified 100x and digitally low-

pass filtered with a frequency cut-off of 15 Hz based on work by Martinez-Valdes et al., (2020). Within OT BioLab+, force data were automatically normalized to the maximal voluntary contraction and reported within the software as %MVC rather than as raw force data in Newtons.

3.6 High-Density Electromyography Setup

A 64-electrode matrix (OT Biolettronica, Torino, Italy) was used to collect EMG data. The array (GRO8MM1305, OT Biolettronica, Torino, Italy) consisted of 5 columns of electrodes oriented parallel to the long axis of the lower leg of the participant with 13 rows of electrodes oriented perpendicular to the long axis of the leg and an 8mm interelectrode distance (*Figure 3)*. The inferior medial corner of the array did not have an electrode, thus creating a 64-electrode array instead of 65. The 64-electrode matrix was placed over the lower portion of the right tibialis anterior muscle belly after shaving hair if necessary, gentle abrasion, and cleaning with alcohol (*Figure 2*). The location of the array was chosen to not include the innervation zone of the tibialis anterior. Had the array been placed over the innervation zone, motor unit action potentials would have been measured by the array travelling in opposite directions leading to a reduction or extinction of unique identifiable MUAPs. The software would consider these MUAPs two different action potentials as they would be opposite and nearly equal in amplitude, so the innervation zone was avoided to maximize the number of action potentials that could be collected from the tibialis anterior (Mesin et al., 2009). This location was based on work by Barbero et al., (2013) establishing innervation zones of various muscles. An anatomical landmark frame (ALF) was determined for the tibialis anterior by palpating the tibial tuberosity and intermalleolar line of the tibia. A straight line between these landmarks was defined as the ALF. In work by Barbero et al., (2013), the innervation zone of tibialis anterior is always located within the proximal 50% of the anatomical landmark frame. The 64-electrode matrix was placed

to encompass the distal 50% of the tibialis anterior. For grounding of the HDEMG array, a pair of grounding straps were provided by OT Biolettronica. These straps were used as a ground by soaking them in water before data collection and then wrapped around the ankle at the level of the malleoli without making contact with each other. One ground strap was connected directly to the HDEMG array while another was connected to the Quattrocento hardware itself. Directions for use provided with the full HDEMG setup advise that these straps function as a reusable ground for the HDEMG array, and can be placed anywhere on the body, with both on the same limb, regardless of whether it is over an active muscle. For the reasons of cable length, avoiding areas of cream application, and precautionarily attempting to avoid muscle activity, just above the ankle was chosen to apply the grounding straps. The full experimental setup is shown in *Figure 2*.

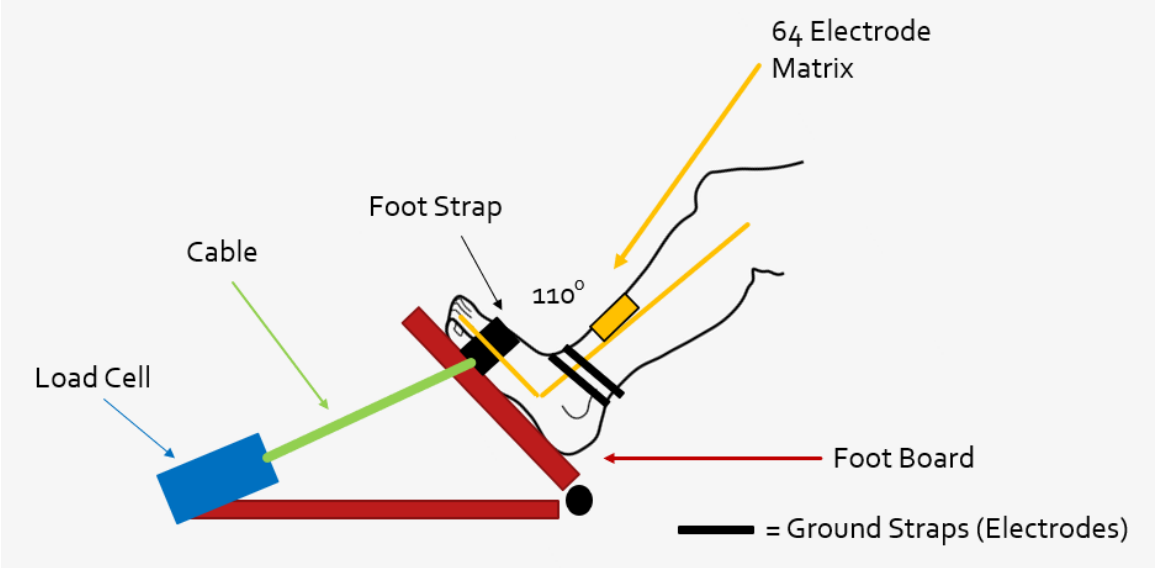

Figure 2: A schematic of the experimental setup showing the location of the load cell, foot orientation, and location of the EMG array in the distal 50% of the tibialis anterior

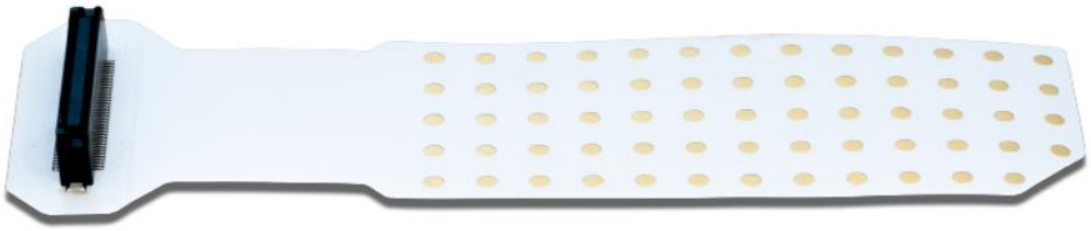

Figure 3: The GRo8mm1305 HDEMG array with 64 individual electrodes and an 8mm interelectrode distance

3.7 Induction of Experimental Central Sensitization

Experimental central sensitization was induced by application of topical capsaicin (0.0.75% concentration, Zostrix). Capsaicin was applied across the L5 dermatome of the right leg and lower back. This dermatome includes the dorsal surface of the foot proximal to the metatarsal heads to avoid interference with the foot strap, the lateral surface of the leg, and the lower back as shown in *Figure 4*. Participants self-reported an initial cold sensation which transitioned to warmth and then a painful burning over the course of ten minutes. They did not report pain from contact with the isometric dynamometer. A control cream was applied to a single “placebo” group participant in the same location. The cream was chosen for its viscosity, colour, and scent to be indistinguishable from capsaicin. Creams were applied to the gloved hand of the researcher in a cabinet and then applied to the participant to prevent their knowledge of which cream they had received.

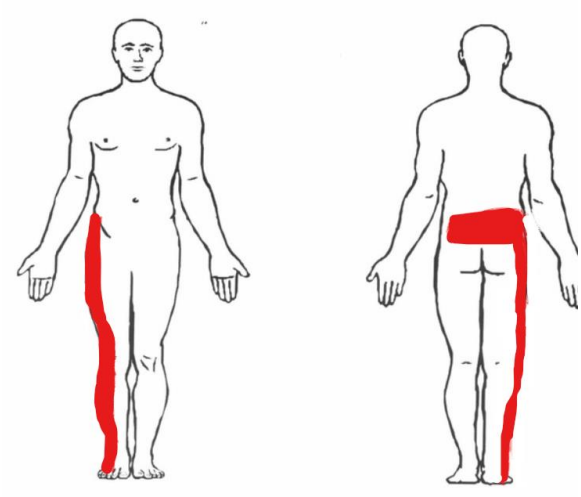

Figure 4: Example of the site of capsaicin or placebo cream application. All areas not covered by the participant's clothing or the experimental equipment in the shaded red area had cream applied. All participants wore shorts rolled as high as possible which covered most of the lateral hip area

3.8 Confirmation of Central Sensitization Induction

To confirm successful experimental induction of CS both weighted pinpricks and brush allodynia were used to test for secondary hyperalgesia. Each participant's right knee was positioned to ensure the thigh was parallel with the floor. The greater trochanter of the participant's right leg was palpated by the researcher. A measuring tape was used to measure the distance between the greater trochanter and the superior edge of the patella while the participant was standing. A mark was made on the lateral thigh at 50% of the distance between the two landmarks as well as on the anterior thigh. At this landmark on the anterior thigh, 4 vertical dots were drawn 5mm apart on the participant's skin. These were used to mark the location of pinprick delivery parallel to but outside of the zone of capsaicin application which was approximately the midline of the thigh to the lateral hamstring tendons. The location of pinprick delivery is displayed visually in *Figure 5*. Multiple stimulus locations (4) were chosen within a test zone to limit skin irritation from repeatedly pinpricking the same location. A different stimulus location was used at each time point. A 128 mN pin was used for all

participants and all trials which, when applied to the thigh, would slide within a protective cover, restricting the researcher from applying more than 128 mN of pressure. The advantage of a weighted pin is that force applied was consistent throughout all trials as the force was applied by the weight of the pin itself and not the researcher. Pinprick testing began with a single weighted pin application. Participants were asked to rate the pain from the stimulus on a scale of 0 to 10. Participants were instructed to consider 0 as “no pain at all” and a 10 as “the worst pain imaginable” to anchor the pain scale. After the initial pinprick stimuli, 16 pinprick stimuli were delivered at a frequency of 1Hz. A 1Hz metronome was started for the researcher to follow and ensure that pinpricks were delivered at the desired frequency, with stimulus delivery coinciding with each beat of the metronome. Participants were instructed to rate every second stimulus on the same 0-10 scale used previously. Rating each stimulus did not allow enough time for participants to verbally report intensity leading to the choice to rate every second stimulus. To further simplify collections for the participants, the metronome alternated between a high pitch and low pitch auditory tone. The participants were instructed to rate the intensity only on the high-pitched tone and the first pinprick was delivered during a low pitch tone. This prevented the participant from needing to count each stimulus, and only focus on rating the pinprick intensity and listening to the metronome.

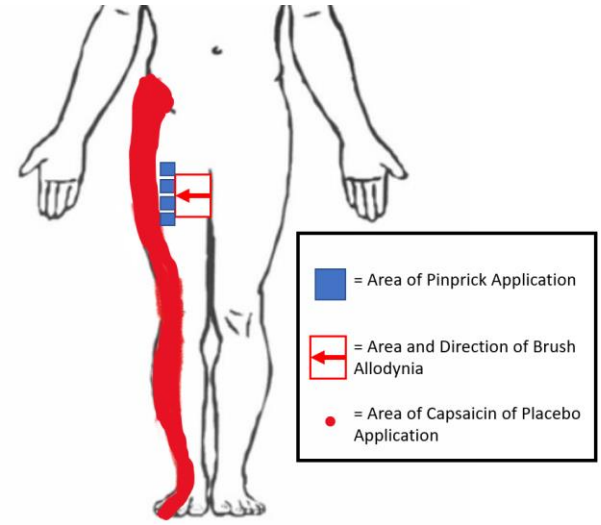

Figure 5: Location of pinprick and brush allodynia application in relation to the site of capsaicin or placebo cream application. Each blue square represents one of the 4 pinprick sites and the red box with red arrow represents the area where brush allodynia was applied and the direction of brushing, moving from the inner medial surface of the thigh towards the center anterior surface

Following the pre-capsaicin trial, a trial of brush allodynia was administered. This consisted of using a paintbrush to brush vertically on the participant's skin beginning on the medial hamstring tendons and brushing in an alternating proximal-distal: distal-proximal pattern along the full circumference of the thigh toward the anterior surface. Brushing continued in the anterior direction until reaching the dots 1cm medial to the site of capsaicin application where pinpricks had been applied. The direction and area of brush allodynia application is displayed in *Figure 5*. The participant closed their eyes and turned their head so that they could not see the location of the paintbrush. The participant was instructed to tell the researcher to stop brushing if they felt an alteration in sensation from the paint brush on their skin. If there was a change in sensation the location was marked with a marker. A ruler was used to measure the distance between the site of pinprick delivery and the location where sensation was altered. Brush strokes stopped short of the area where capsaicin had been applied as a positive sign of central sensitization was allodynia surrounding the painful area not within the area itself.

Following application of capsaicin, brush allodynia was repeated directly between groups of trials at time points 5, 15, 25, and 35 minutes. This time point was chosen to allow enough time for all 3 contractions and weighted pinprick testing to be performed at 10, 20, 30, and 40 minutes without conflicting with brush allodynia testing. After brush allodynia the participant was asked to rate the intensity of the sensation created by the cream on the same 0 to 10 scale as the pinpricks and this numerical pain rating scale was used as a third measure of successful induction of CS. They were also asked to describe how the cream felt in their own words. At 10-, 20-, 30-, and 40-minutes post-cream application the pinprick protocol was repeated beginning with a single stimulus and then a 16-stimuli train. After the 40-minute trial the participant was able to rinse the area of cream application under cold water to remove any lingering sensation caused by the cream.

3.9 EMG Decomposition and Motor Unit Tracking

Data were collected using a Quattrocento analog-digital converter (OT Biolettronica, Torino Italy) and processing was performed in OT BioLab+ (v.1.4.3, OT Biolettronica, Torino, Italy). A band-pass filter with a high-pass cut-off of 10 Hz and a low-pass cut-off of 500 Hz was used to filter raw EMG data with a sampling rate of 2048 Hz as the OT BioLab+ software required all acquisition hardware to use the same sampling rate. The 64 monopolar EMG channels were reanalyzed offline and re-referenced to create 59 bipolar channels. This was performed to obtain the differential between adjacent electrodes oriented in the direction of the muscle fibers of the tibialis anterior. The 59 bipolar EMG channels were decomposed into individual motor unit firing plots using a convolutive blind-source separation technique described by Negro et al., (2016). This process identifies individual firing plots of motor units by decomposing a surface EMG signal into the individual motor unit waveforms and identifying when in the trial each motor unit was active. Decomposition also determines the spatial

representation of each MU within the array to create a unique "fingerprint" to identify MUs. As each MU has a different location within the array and a unique waveform, MUs can be distinguished.

Tracking of motor units throughout trials was performed based on a technique described by Maathuis et al., (2008). Waveforms of each motor unit were exported from OT BioLab+ to Microsoft Excel (Office 365) to be processed in MatLab (Version R2017b, MathWorks, Natick, United States, 2017). A custom code was created by the researcher with assistance from another graduate student (Travis Branigan) to cross correlate each MU "fingerprint". The "fingerprint" is the unique identifier of each motor unit consisting of both the unique waveform of the MU action potential as well as the location within the array. Tracking MUs using their fingerprint from high-density EMG is the most effective method for tracking multiple motor units across multiple trials (Maathuis et al., 2008). The MatLab processing automatically performed correlations between all waveforms from all trials and any waveform with a strong correlation (0.9 or above) was grouped with the MUs that it correlated with. The MatLab processing compared the changes in waveform amplitude across all 59 channels for each motor unit from each trial. Groups in which the motor units were identified in both the baseline and capsaicin/placebo trials were included in the analysis. Each motor unit was required to appear in at least the baseline trial and one post-intervention trial. Any motor unit that was not present in both the baseline and capsaicin/placebo conditions were not included for analysis. Once tracking of MUs across trials was completed, a MatLab script provided a readout of which MUs from which trial were the same. A final check was performed by the researcher to visually check each MU "fingerprint" and confirm that the program successfully grouped the identified MUs. This was performed by confirming that each MU possessed a visually distinct waveform in case any noise in the signal was misidentified as a MUAP. After limiting MU tracking to correlations of

0.9 or higher, no MUs were excluded during the visual check as misidentified MUs were not highly correlated with errors in other trials.

3.10 EMG Amplitude Analysis

EMG amplitude analysis was performed in OT BioLab+. Band-pass filtered EMG amplitude was analyzed during the plateau of each contraction at 20% MVC. Filtered EMG data from all 64 channels were split into 500ms epochs within the OT BioLab+ software. The first and last epochs of the 20% MVC plateau region were not included in case participants surpassed 20% MVC during the ramp phase of the contraction. Following elimination of the first and last 500ms epoch, the OT BioLab+ software merged the 64 channels to create a single overall representation of the sum of action potentials beneath the entire electrode array. This single channel representing the sum of all MUAPs collected by the HDEMG array was then used for EMG amplitude assessment. The root mean square (RMS) of this new channel collected during the 20% MVC plateau region of the contraction excluding the first and last 500ms epoch was then calculated within the software. Each 500ms epoch was used to calculate the RMS within each window and this value was then averaged to determine the mean RMS value of the overall signal during the plateau region of each contraction. The RMS values were used to represent the overall EMG amplitude. The post-capsaicin and placebo trials from each participant were normalized to their baseline trials to determine the %change from baseline after capsaicin or placebo cream application as the MVC trials were used within the software to represent force as %MVC but the EMG and force data from these trials was not presented for analysis with OT BioLab+.

3.11 Motor Unit Recruitment Threshold Analysis

The recruitment threshold (RT) of each MU was determined by exporting binary MU firing plots and force data to Microsoft Excel. Firing plots were exported in a binary format

meaning that in Excel an inactive MU was represented by a 0 and an active MU was represented by a 1. Firing plots and force data were time-locked to each other for each trial so that the first instance of each MU being recruited, represented by the first "1" in the series of 0s, directly corresponded with the force level at that time point unless the next instance of firing was >200ms later than the first in which case the motor unit was not considered recruited at that time point (Martinez-Valdes et al., 2020; Martinez-Valdes et al., 2015). For each MU, the force level at the same time point as the first "1" in its firing plot was considered the recruitment threshold. This process was repeated for each MU. If the motor unit was identified in more than 1 trial for each time point the recruitment thresholds were averaged. Motor units were only included if they began firing during the ramp up or plateau. No motor units fired exclusively during the ramp down. If the same MU was identified by the OT BioLab+ software as two different MUs within the same trial the lowest threshold was taken as per protocol described by Maathuis et al., 2018.

3.12 Motor Unit Firing Rate Analysis

Firing rates of each motor unit were determined through analysis within OT BioLab+ and exported to Excel. Firing rate was assessed at recruitment threshold as well as at plateau. To determine the firing rate at recruitment threshold, the previously identified recruitment threshold was used. The first 6 instances of each MU firing were used to calculate firing rate by dividing 6 by the elapsed time between the first and sixth MUAP as described by Martinez-Valdes et al., (2020). To assess plateau firing rate, data in OT BioLab+ was split into 500ms epochs and all epochs encompassing the plateau range of the contraction (20% MVC) were exported to Excel excluding the first and last epochs where there was an inflection point. Once in Excel, the mean firing rate data for the entirety of the plateau was acquired by dividing the number of MUAPs by the elapsed time. Typically, during EMG decomposition MU firing rates

should display regular discharge rates during a sustained contraction, however, this regularity is not typical of MUs near their recruitment threshold (McGill et al., 2005). Since the MUs in this study were all recruited at nearly 20% MVC, the entire plateau excluding the first and last 500ms was chosen to calculate firing rates to achieve an average firing rate at 20% MVC instead of choosing a smaller time window which may have led to calculating a faster or slower firing rate depending on which time window was chosen.

3.13 Statistical Analysis

The sample size of this work is limited due to cessation of data collection during the COVID-19 pandemic. As such, data is limited to 2 central sensitization group participants and 1 placebo group participant. From these participants, 3 MUs were collected from each CS participant for a total of 6 while 4 were collected from the placebo participant. A larger sample is required before drawing conclusions from the current study. A minimum of 4 participants per group would achieve an 80% statistical power level with an error of 5% and a confidence interval of 95%. With data collections involving experimental pain, the potential for no detection of MUs, and the requirement that CS be successfully induced for study inclusion, I recommend a minimum of 7 participants assigned to each group for a total of 14. This would allow the data of up to 3 participants from each group to be excluded for any reason while still having a large enough sample to achieve 80% statistical power. If the minimum sample size of 4 participants in each group was achieved and samples were equal in number, a two-way repeated measures ANOVA would have been used to determine whether the groups were different between the two treatments, capsaicin or placebo, at either of the two time points, baseline and 10-minutes post treatment. A two-way repeated measures ANOVA would identify if there were differences between time points or between capsaicin and placebo while comparing participants to their own data as the baseline and post-treatment groups included the same participants. The two-

way repeated measures ANOVA would not identify exactly what the differences were, making a post-hoc test necessary if any differences were identified. A paired t-test would be used to compare means of the baseline and 10-minutes post treatment scores for each group to determine if there was a significant change after capsaicin or a placebo cream was applied. An unpaired t-test would be used to compare the two groups at baseline and a second test would be used to compare the 10-minutes post treatment means. For this comparison between groups at both time points, a Bonferroni Correction would be necessary to maintain the 95% confidence interval and reduce the risk of creating a false significant result. In this study, with the limited sample size and different sized groups, t-tests were used to compare group means for the identified MUs in each group.

All statistical analyses were performed in Microsoft Excel (Office 365). Results are expressed as mean (SD). The level of significance was set at $p<0.05$. Statistical analysis consisted of multiple paired t-tests to compare the means of the capsaicin and placebo groups after cream application to their baseline values. As the motor units in both conditions were collected from the same population, a paired t-test was chosen. Firstly, paired 2-tailed t-tests were used to compare recruitment thresholds, decruitment thresholds, recruitment firing rates, and plateau firing rates from both the capsaicin group and the placebo group. The paired 2-tailed t-tests were performed between baseline values and those collected 10 minutes following the application of capsaicin or a placebo cream. Two further t-tests, both being one-tailed t-tests, were performed to compare the RMS values collected 10 minutes after capsaicin to baseline, and the RMS values 10 minutes after placebo to baseline. The paired one-tailed t-test was used to determine whether there was an increase from baseline in RMS values after cream application. A two-tailed t-test was performed to compare RMS values from each group after application of capsaicin or a placebo cream. Comparing differences between groups required

corrections for multiple comparisons and a Bonferroni Correction was used. As two comparisons were performed comparing the CS and placebo group means at both baseline and 10-minutes post cream application, the p-value required to signify a significant difference with a 95% confidence interval was reduced from 0.05 to 0.025.

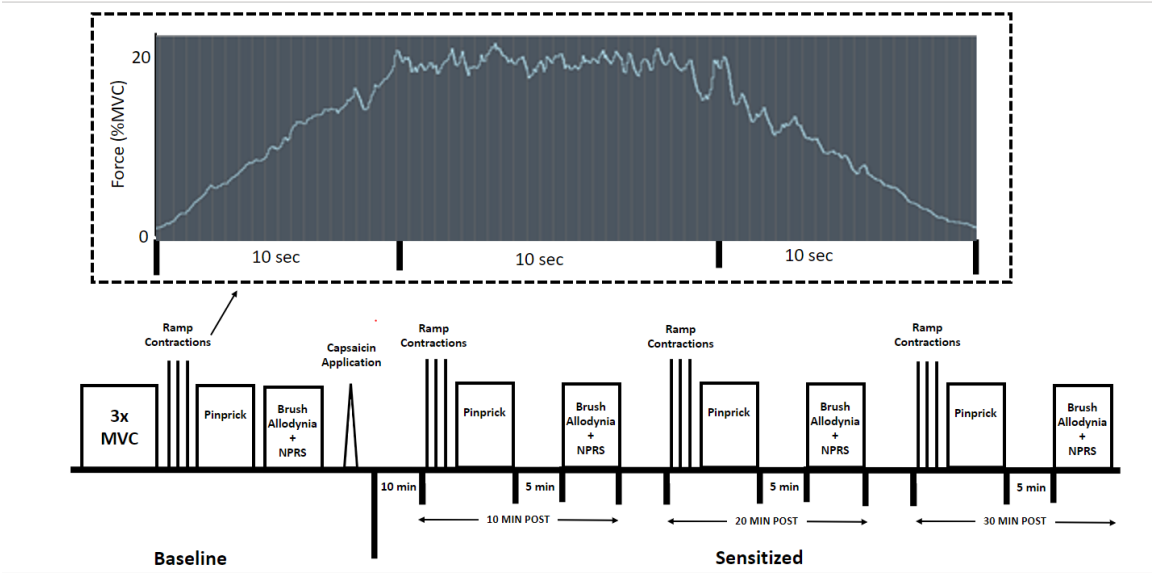

Figure 6: Timeline of experimental protocol. The timeline begins after equipment setup and shows the entirety of the data collection until the final collection of brush allodynia. While all steps in the protocol were performed for each participant, only the baseline and 10-minute post conditions are included for data analysis

Chapter 4: Results

4.0 Results

All results are represented as p-value followed by standard deviation (SD) and effect size (ES). The average values normalized to the baseline data for each outcome measure are presented in *Tables 1-5* with levels of significance and standard deviations. All values at the 10-minute time point are represented as their change from the baseline (pre) values with an increase being >1 and a decrease being <1. Three male participants between the ages of 24-25 were included in the study with two being assigned to the experimental central sensitization group and one being assigned to the placebo group. A total of 10 motor units were identified from these participants with 6 motor units identified in the experimental pain participants and 4 identified from the participant given a placebo treatment. Motor units 1-3 in all figures and tables are from sensitized participant 1, motor units 4-6 are from sensitized participant 2, and motor units 7-10 are from the placebo participant.

4.1 Confirmation of Central Sensitization Induction

The focus of this work was to directly study the specific effects of central sensitization as previous work studying the effects of pain on MU activity did not confirm whether their method of pain induction had produced CS. To do so, both brush allodynia and pinprick protocols were used like work by Evans et al., (2021) in which allodynia at any distance from the site of capsaicin application or an increase in visual analog scale scores were considered a sign of CS. In my work, the presence of brush allodynia and any increase in wind-up ratio score acquired through pinprick testing was considered a sign that CS had been induced. As the placebo participant reported perceiving pain from the inert cream, the more objective measures of brush allodynia and wind-up were used as using self-reported pain alone would have made it

appear that even the placebo participant experienced CS. The brush allodynia and pinprick protocol were not used on the placebo participant, but CS was not expected as they were not experiencing nociceptive input from the periphery. While some changes were observed in this participant, I believe that these were created by descending control in the expectation of pain, and not true CS as there was no noxious peripheral input. Future work should follow the methodology of Evans et al., (2021) to use quantitative sensory testing such as brush allodynia and wind-up ratio in both the sensitized and the placebo participants to confirm that CS only occurred in the capsaicin group. The data collected from each participant is displayed in *Table 1* and *Table 2*.

Table 1: Data of the first participant confirming successful induction of central sensitization. This participant showed no change in the intensity of a single pinprick while they displayed evidence of wind-up as subsequent pinpricks increased pain perception to the same stimulus. Increased wind up led to an increase in the sum of all 8 consecutive pain ratings. The participant also experienced allodynia from paint brush strokes 8.25 cm away from the site of pinprick application

Participant 1 – Confirmation of Central Sensitization					
Pinprick Testing					Brush Allodynia
Condition	Single Stimulus Rating	Series of 16 Consecutive Stimuli	Sum of all 16 Stimuli (Area Under the Curve)	Numerical Pain Rating Scale of Capsaicin Intensity (0-10)	Distance of Secondary Hyperalgesia (cm)
Baseline	3	3 3 3 3 3 3 3 3	24	0/10	0cm
Central Sensitization	3	3 3 4 4 5 5 5 6	35	6/10	8.25cm

Table 2: Data of the second participant confirming successful induction of central sensitization. This participant reported greater pain from a single stimulus after capsaicin application along with increased pain intensity over 16 consecutive pinpricks leading to a difference between the first and last stimulus. This participant reported the brush allodynia test to be painful at a distance of 2cm from the site of pinprick application

Participant 2 – Confirmation of Central Sensitization					
	Pinprick Testing				Brush Allodynia
Condition	Single Stimulus Rating	Series of 16 Consecutive Stimuli	Sum of all 16 Stimuli (Area Under the Curve)	Numerical Pain Rating Scale of Capsaicin Intensity (0-10)	Distance of Secondary Hyperalgesia (cm)
Baseline	1	1 1 2 1 1 2 1 1	10	0/10	0cm
Central Sensitization	2	1 1 2 2 1 2 2 3	14	4/10	2cm

Both participants assigned to the central sensitization group displayed multiple signs of successful CS induction. Participant 1 showed signs of wind-up through an increasing perception of pain to consecutive pinpricks. They also displayed allodynia by perceiving the non-noxious stimulus of a paintbrush as painful 8.25cm from where pinprick testing had been applied. As this participant displayed both wind-up and allodynia, it was concluded that this participant was experiencing central sensitization. Participant 2 displayed wind-up as their pain perception from consecutive stimuli increased following capsaicin application. They also experienced allodynia from brush strokes as they perceived pain from a paint brush on the skin 2cm from the site of

pinprick application. Further, they demonstrated hyperalgesia after capsaicin was applied as a single pinprick was perceived as more painful than it had been during the baseline trial. This participant also showed multiple signs of CS.

The criteria for confirming central sensitization were a participant displaying signs of either wind-up, hyperalgesia, or allodynia. Since wind-up occurs more intensely during CS, a higher summed score after 8 consecutive pain ratings delivered through pinprick at a frequency of 1 Hz suggests that the participant was sensitized. Hyperalgesia was evaluated through both the single pinprick stimulus and the 8 pain ratings reported by the participant corresponding to the 16 pinprick stimuli. Both the single pinprick and the wind-up ratio series of 16 pinpricks were delivered 1cm away from the site of capsaicin application. Therefore, increased pain intensity outside of the area where noxious stimulus was applied is a sign of secondary hyperalgesia and therefore central sensitization rather than peripheral. Finally, perceived pain during the brush allodynia trials at a distance from the site of capsaicin application provides another sign of central sensitization. Perceiving a non-noxious stimulus as noxious at a distance from the site of pain must be due to allodynia in areas surrounding the primary site of pain and therefore central sensitization. This protocol is based off previous studies including recent work by Evans et al., (2021) where any increase in visual analog scale pain perception or any experience of brush allodynia at a distance from the site of capsaicin application was considered successful induction of CS. It was concluded that both participants had experienced central sensitization after capsaicin was applied as both showed multiple signs of wind-up, secondary hyperalgesia, and allodynia.

4.2 Overall Motor Unit Yield

A challenge of this work was identifying the same motor unit across time points. To increase the likelihood of identifying a MU, 3 trials were taken at each time point to increase the

likelihood that a motor unit would appear in at least one of the trials. Each of the 3 trials at each time point consisted of one individual trapezoidal ramp contraction generating a total of 3 data sets at each time point from which individual MUs could be identified through HDEMG decomposition. In *Table 3* below, each motor unit is listed. Each trial that a motor unit was identified in is marked to show the number of times the same motor unit was found. For the trials in which a motor unit was identified multiple times, the two firing plots were merged to represent the true activity of the motor unit instead of its firing plot being split into two separate tracks.

Table 3: This table displays which trial each motor unit was identified in for both the baseline and capsaicin/placebo conditions. All motor units displayed were identified in both the PRE (baseline) and POST (Central Sensitization or Placebo) trials. Some were identified in multiple trials for each condition, and some were identified multiple times within the same trial and are marked as "Y (double) or Y (triple)" to represent the number of times that it appears within the trial. Motor units which were identified only once within a trial are marked as "Y". Trials in which a motor unit was not identified are marked as "N"

	PRE (Baseline)			POST (Central Sensitization or Placebo)		
Trial:	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Motor Unit	Tracked? (Y/N)	Tracked? (Y/N)	Tracked? (Y/N)	Tracked? (Y/N)	Tracked? (Y/N)	Tracked? (Y/N)
MU1	Y (double)	Y	N	N	Y	Y
MU2	N	Y	N	N	N	Y
MU3	N	N	Y (double)	Y	Y	Y
MU4	Y	Y	N	N	Y (double)	Y
MU5	N	Y	N	N	N	Y
MU6	N	Y	Y	Y (double)	N	Y
MU7	Y (double)	N	Y (double)	Y	Y	N

MU8	N	Y	N	N	N	Y
MU9	N	Y	Y	Y (double)	N	Y (double)
MU10	N	Y	N	Y	Y (double)	Y (triple)

4.3 Recruitment Threshold

For the sensitized group, recruitment threshold values showed a reduction by approximately 20% from baseline, ten minutes after application of capsaicin (Baseline = 1 to CS = 0.808, P=0.011 ± 0.145, ES=1.886, *Table 7*). Recruitment thresholds were lower during the CS condition (central sensitization) compared to baseline. There was no effect of a non-noxious cream in the placebo group as the recruitment thresholds of the placebo motor units did not show a change from baseline (Placebo = 0.848, P=0.771 ± 0.621, ES=0.31) following application of a placebo cream. There was no difference between the magnitude of recruitment threshold reduction when comparing the sensitized and placebo groups (Post = 0.808 and 0.848, P=0.893 ± 0.451, ES=0.086). Both capsaicin and placebo cream caused a reduction in recruitment threshold, but a greater reduction occurred in the sensitized group as displayed in *Table 7*. The individual recruitment thresholds of each motor unit are displayed in *Table 4*. A total of 6 motor units made up the central sensitization group and 4 made up the placebo group. Data from *Table 4* is displayed visually in *Figure 7* and the average recruitment threshold of each individual MU is shown in *Table 5* for the central sensitization group and *Table 6* for the placebo group.

Table 4: The recruitment thresholds of each motor unit are displayed in this table for both the PRE (Baseline) and POST (Central Sensitization or Placebo) conditions. Some motor units were identified multiple times within the same trial. For these motor units the firing plots were merged to represent the true motor unit activity. In this situation, the lowest recruitment threshold was taken after merging as it represented that motor unit's first twitch during the contraction

	PRE (Baseline)			POST (Central Sensitization or Placebo)		
Trial:	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Motor Unit	Recruitment Threshold (%MVC)					
MU1	16.98/20.10	18.69	N	N	10.12	12.58
MU2	N	16.48	N	N	N	11.57
MU3	N	N	12.56/19.24	11.23	13.98	21.32
MU4	18.27	19.75	N	N	17.59/14.91	14.99
MU5	N	21.22	N	N	N	16.22
MU6	N	19.52	14.57	16.34/19.64	N	13.297
MU7	1.66/3.08	N	10.99/14.63	18.65	12.48	N
MU8	N	19.82	N	N	N	6.28
MU9	N	12.87	18.37	4.74/12.47	N	11.46/6.29
MU10	N	19.82	N	7.44	7.23/9.33	10.25/5.94/12.5

Table 5: This table represents the mean recruitment threshold of each individual motor unit in the central sensitization group across both the PRE and POST conditions. The mean recruitment threshold of each motor unit across all three trials was taken to contribute to the displayed mean of each motor unit along with the group standard deviation (SD). Each threshold is represented as (%MVC)

Mean Individual Recruitment Thresholds of Central Sensitization Group Motor Units (%MVC)							
Condition	MU1	MU2	MU3	MU4	MU5	MU6	Mean (SD)
PRE	18.5917	16.48278	15.89895	19.00938	21.22403	17.044479	18.04189 (1.97)
POST	11.35099	11.56897	15.51101	15.83144	16.2284	16.426102	14.48615 (2.37)

Table 6: This table represents the mean recruitment threshold of each individual motor unit in the placebo group across both the PRE and POST conditions. The mean recruitment threshold of each motor unit across all three trials was taken to contribute to the displayed mean of each motor unit Along with the group standard deviation (SD). Each threshold is represented as (%MVC)

Mean Individual Recruitment Thresholds of Placebo Group Motor Units (%MVC)						
Condition	MU7	MU8	MU9	MU10	[redacted]	Mean (SD)
PRE	7.591438	19.81654	12.86605	19.81654		15.02264 (5.94)
POST	15.56604	6.286284	8.740022	8.782354		9.843674 (3.99)

Table 7: The mean recruitment threshold of the central sensitization and placebo groups are represented in this table. Data was normalized to the PRE condition with the POST condition representing the relative percentage change from baseline. The level of significance was set at P<0.05 and this normalized data showed a reduction in recruitment thresholds in the sensitized condition (P=0.011)

	Sensitized Condition				*Placebo Condition*			
Condition	Mean Value (%MVC)	Normalized Value	p-Value	Standard Deviation (+/-)	Mean Value (%MVC)	Normalized Value	p-Value	Standard Deviation (+/-)
Pre	18.04189	1			15.02264	1		
Post	14.48615	0.808	0.011**	0.145	9.843674	0.872	0.65	0.621

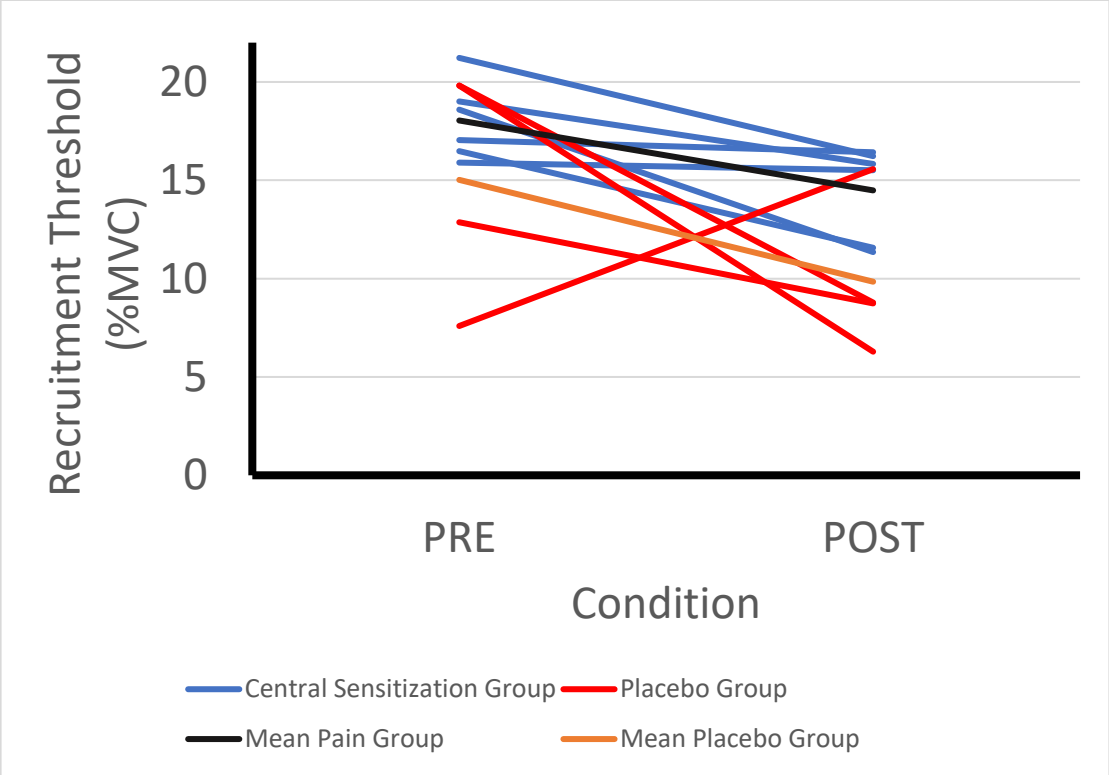

Figure 7: Individual motor unit recruitment thresholds in the PRE and POST conditions are represented for both the central sensitization group and the placebo group. The central sensitization group motor units are represented by the blue lines and the placebo group is represented by red. The means of the central sensitization and placebo groups are represented by black and orange lines respectively

4.4 De-Recruitment Threshold

De-recruitment thresholds in the sensitized group did not show a change from baseline (Post= 0.96, P=0.471 ± 0.124, ES=0.41) following application of capsaicin as shown in *Table 11*. In the placebo group, de-recruitment thresholds did not demonstrate a change between the baseline trials and the post-application trials (10 Post= 0.93, P=0.569 ± 0.256, ES= 0.35). Of note, is that the sensitized group showed a progressive decline after 20 minutes and a further decline at 30 minutes. As some of the MUs identified from the baseline and 10-minute conditions were not identified in the 20 or 30-minute time points, this data was not statistically analyzed due to the reduced sample size. A decline was observed but it is not clear whether the changes were statistically significant. No difference was demonstrated between the sensitized or control group (Post=0.96 and 0.89, P=0.57 ± 0.188, ES=0.15). The de-recruitment thresholds of each individual

motor unit in each trial for both groups are displayed in *Table 8* while the mean de-recruitment threshold across trials is displayed in *Table 9* for the CS group and *Table 10* for the placebo group. The mean de-recruitment threshold for each MU and the means of both the CS and placebo groups are displayed visually in *Figure 8*.

Table 8: The de-recruitment thresholds of each motor unit are displayed in this table for both the PRE (Baseline) and POST (Central Sensitization or Placebo) conditions. Some motor units were identified multiple times within the same trial. For these motor units the firing plots were merged to represent the true motor unit activity. In this situation, the lowest de-recruitment threshold was taken after merging as it represented that motor unit's last twitch during the contraction

	PRE (Baseline)			POST (Central Sensitization or Placebo)		
Trial:	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Motor Unit	De-recruitment Threshold (%MVC)					
MU1	14.62/14.59	16.70	N	N	9.48	13.45
MU2	N	12.77	N	N	N	13.05
MU3	N	N	11.50/16.12	12.53	10.06	16.44
MU4	13.39	14.77	N	N	15.89/17.50	11.2
MU5	N	20.66	N	N	N	18.68
MU6	N	14.77	17.95	19.33/17.09	N	17.02
MU7	18.90/11.95	N	14.57/20.13	20.72	21.08	N

MU8	N	20.04	N	N	N	12.68
MU9	N	20.16	19.80	12.74/21.32	N	19.60/17.68
MU10	N	20.51	N	11.28	11.72/21.20	19.25/13.14 /20.40

Table 9: This table represents the mean de-recruitment threshold of each individual motor unit in the central sensitization group across both the PRE and POST conditions. The mean de-recruitment threshold of each motor unit across all three trials was taken to contribute to the displayed mean of each motor unit along with the group standard deviation (SD). Each threshold is represented as (%MVC)

Mean Individual De-Recruitment Thresholds of Central Sensitization Group Motor Units (%MVC)							
Condition	MU1	MU2	MU3	MU4	MU5	MU6	Mean (SD)
PRE	15.30519	12.76662	13.80837	14.08265	20.65835	16.36297	15.49736 (2.82)
POST	11.46855	13.05601	13.01192	14.86292	18.68063	17.81507	14.81585 (2.88)

Table 10: This table represents the mean de-recruitment threshold of each individual motor unit in the placebo group across both the PRE and POST conditions. The mean de-recruitment threshold of each motor unit across all three trials was taken to contribute to the displayed mean of each motor unit along with the group standard deviation (SD). Each threshold is represented as (%MVC)

Mean Individual De-Recruitment Thresholds of Placebo Group Motor Units (%MVC)						
Condition	MU7	MU8	MU9	MU10		Mean (SD)

PRE	16.38539	20.03999	19.98118	20.51042		19.22925 (1.91)
POST	20.89985	12.68142	17.02649	16.16322		16.69275 (3.38)

Table 11: The mean de-recruitment threshold of the central sensitization and placebo groups are represented in this table. Data was normalized to the PRE condition with the POST condition representing the relative percentage change from baseline. The level of significance was set at P<0.05 and this normalized data did not show any changes in either group

	Sensitized Condition				*Placebo Condition*			
Condition	Mean Value (%MVC)	Normalized Value	p-Value	Standard Deviation (+/-)	Mean Value (%MVC)	Normalized Value	p-Value	Standard Deviation (+/-)
Pre	15.49736	1			19.22925	1		
Post	14.81585	0.9605	0.47	0.12	16.69275	0.8871	0.472	0.275

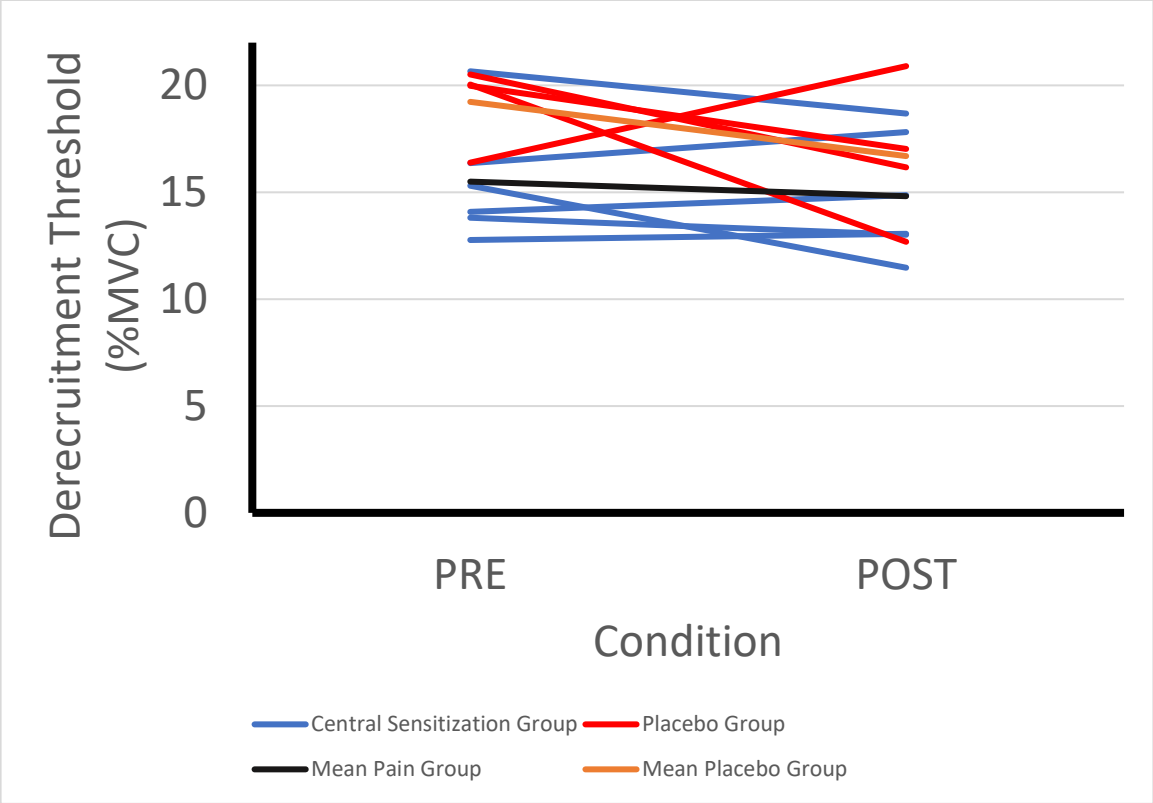

Figure 8: Individual motor unit de-recruitment thresholds in the PRE and POST conditions are represented for both the central sensitization group and the placebo group. The central sensitization group motor units are represented by the blue lines and the placebo group is represented by red. The means of the central sensitization and placebo groups are represented by black and orange lines respectively

4.5 Recruitment Firing Rate

The motor units in the central sensitization group did not demonstrate a change in firing rate at recruitment threshold after capsaicin application compared to baseline (Post=0.90, P=0.429 ± 0.277, ES=0.46). Similarly, the placebo group did not show a change from baseline after a non-noxious cream was applied (Post=0.62, P=0.177 ± 0.437, ES=1.12). Both groups' data is displayed in *Table 14*. Finally, there was no identifiable difference between the capsaicin or the placebo groups (Post=0.9 and 0.62, P=0.235 ± 0.358, ES=0.733). The mean firing rate at recruitment for each motor unit is displayed in *Table 13* for the central sensitization group and *Table 14* for the placebo group. The mean of the central sensitization and placebo groups are

displayed visually in *Figure 9* along with the firing rate of each motor unit in both the PRE (baseline) and POST (central sensitization or placebo) conditions. An important note from this recruitment firing rate data is that some MUs demonstrated a very low firing rate compared to the rest of the motor units or compared to themselves in other trials. One possible explanation for the low discharge rate could be due to the exclusion criteria for unrealistic firing rates based on work by Martinez-Valdes et al., (2020) in which MUAPs separated by <33.3ms or >200ms were excluded. It is possible that motor units fired frequently enough to fit this criterion, but infrequently enough to create an unrealistically low firing rate. It is also possible that at recruitment the motor units were firing inconsistently as suggested by McGill et al., (2005). The most likely explanation is the decomposition algorithm itself failed to consistently identify motor units near recruitment and some MUAPs were missed. This would lead to an unrealistically low motor unit firing rate calculation as the motor units themselves likely were firing more rapidly but were not consistently identified.

Table 12: Individual motor unit firing rates at recruitment for each motor unit in each trial. Firing frequency is listed in Hz

	PRE (Baseline)			POST (Central Sensitization or Placebo)		
Trial:	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Motor Unit	Recruitment Firing Rate (Hz)					
MU1	10.01/13.07	15.04	N	N	10.47	13.18
MU2	N	11.10	N	N	N	12.51

MU3	N	N	16.43/11.28	6.98	15.86	14.11
MU4	10.64	7.84	N	N	12.12/10.69	8.57
MU5	N	3.70	N	N	N	1.46
MU6	N	9.84	12.99	10.60/19.54	N	8.26
MU7	26.83/7.92	N	6.59/6.54	16.79	6.67	N
MU8	N	18.04	N	N	N	15.11
MU9	N	15.57	2.57	17.48/1.79	N	16.90/9.60
MU10	N	18.37	N	1.58	17.91/3.28	17.33/15.00/1.47

Table 13: This table represents the mean firing rate at recruitment of each individual motor unit in the central sensitization group across both the PRE and POST conditions. The mean firing rate of each motor unit across all three trials was taken to produce the displayed mean of each motor unit. Each firing rate is represented in Hz. For each trial if a MU appeared multiple times the firing plots were merged. For all motor units, firing rate at recruitment was calculated by dividing the first 6 instances of firing by the change in time between the 1st and 6th to determine the rate. The rate in each trial was then averaged to produce a representative firing rate for each motor unit. The group mean is displayed with the group standard deviation (SD).

Mean Individual Recruitment Firing Rates of Central Sensitization Group Motor Units (Hz)							
Condition	MU1	MU2	MU3	MU4	MU5	MU6	Mean (SD)
PRE	12.34966	11.10022	13.37837	9.028652	3.696751	11.19636	10.125 (3.47)

POST	11.66952	12.5133	10.82323	10.2457	1.460423	11.25618	9.661 (4.09)

Table 14: This table represents the mean firing rate at recruitment of each individual motor unit in the placebo group across both the PRE and POST conditions. The mean firing rate at recruitment of each motor unit across all three trials was taken to produce the displayed mean of each motor unit. Each firing rate is represented in Hz. For each trial if a MU appeared multiple times the firing plots were merged. For all motor units, firing rate at recruitment was calculated by dividing the first 6 instances of firing by the change in time between the 1st and 6th to determine the rate. The rate in each trial was then averaged to produce a representative firing rate for each motor unit. The group mean is displayed along with the group standard deviation (SD).

Mean Individual Recruitment Firing Rates of Placebo Group Motor Units (Hz)						
Condition	MU7	MU8	MU9	MU10		Mean (SD)
PRE	8.540747	18.04405	15.57414	18.36771		15.13166 (4.57)
POST	9.547786	15.11439	5.130689	3.327226		8.280023 (5.25)

Table 15: The mean firing rate at recruitment of the central sensitization and placebo groups are represented in this table. Data was normalized to the PRE condition with the POST condition representing the relative percentage change from baseline. The level of significance was set at P<0.05 and this normalized data did not show any changes in either group

	Sensitized Condition				*Placebo Condition*			
Condition	Mean Value (Hz)	Normalized Value	p-Value	Standard Deviation (+/-)	Mean Value (Hz)	Normalized Value	p-Value	Standard Deviation (+/-)
Pre	10.125	1			15.13166	1		
Post	9.66139	0.903	0.43	0.28	8.280023	0.62	0.18	0.44

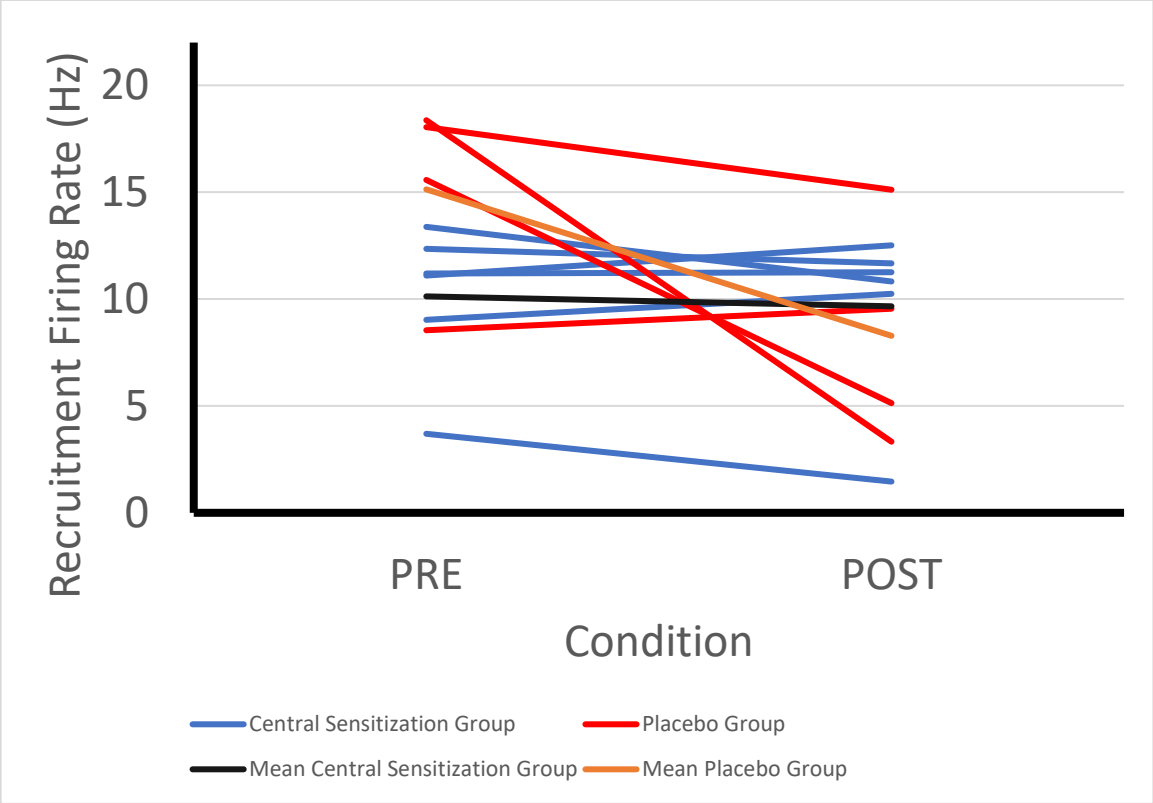

Figure 9: Individual motor unit firing rates at recruitment in the PRE and POST conditions are represented for both the central sensitization group and the placebo group. The central sensitization group motor units are represented by the blue lines and the placebo group is represented by red. The means of the central sensitization and placebo groups are represented by black and orange lines respectively

4.6 Plateau Firing Rate

No changes in plateau firing rates were identified following capsaicin application when compared to baseline (Normalized Firing Rate=1.029, P=0.435 ± 0.076, ES=0.51) as shown in Table 4. Similarly, the placebo group did not demonstrate a change following the application of a placebo cream (Normalized Firing Rate = 1.15, P=0.491 ± 0.388, ES=0.53). Likewise, there was no difference between the sensitized or placebo groups (Normalized Firing Rate=1.029 and 1.15, P=0.506 ± 0.252, ES=0.42) following the application of either cream. The average firing rates of each motor unit in the central sensitization and the placebo groups are displayed in *Table 17* and *Table 18* respectively along with the mean firing rate for the entire group. The data was

normalized to the "PRE" condition values and is displayed in *Table 19*. No changes were observed. For visual comparison of individual firing rates at plateau and the group averages, data for both the PRE and POST conditions for both groups is displayed graphically in *Figure 10*.

Table 16: Motor unit firing rates during the 20% MVC contraction for each individual motor unit in each trial. This table includes all 10 motor units from all participants in both the PRE and POST capsaicin or placebo conditions

	PRE (Baseline)			POST (Central Sensitization or Placebo)		
Trial:	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Motor Unit	Plateau Firing Rate (Hz)					
MU1	12.57/12.21	13.47	N	N	14.33	13.38
MU2	N	12.90	N	N	N	13.80
MU3	N	N	14.38/13.87	14.53	14.10	10.88
MU4	13.05	11.37	N	N	10.10/10.95	14.22
MU5	N	10.50	N	N	N	0.94
MU6	N	11.37	11.57	10.86/11.57	N	15.22
MU7	16.02/9.50	N	14.45/8.50	6.50	10.50	N
MU8	N	9.20	N	N	N	15.14
MU9	N	16.24	4.00	14.22/14.67	N	6.78/8.46

MU10	N	10.00	N	16.01	16.00/13.14	12.12/7.90/5.00

Table 17: This table represents the mean plateau firing rate for each individual motor unit in the central sensitization group across both the PRE and POST conditions. The mean firing rate of each motor unit across all three trials was taken to produce the displayed mean of each motor unit. Each firing rate is represented in Hz. For each trial if a MU appeared multiple times the firing plots were merged. For all motor units, firing rate at plateau was calculated by removing the first and last 500ms of the plateau region and then dividing the total number of twitches by the plateau duration to determine the rate. The rate in each trial was then averaged to produce a representative firing rate for each motor unit. The group means and standard deviations are displayed as Mean (SD).

Mean Individual Plateau Firing Rates of Central Sensitization Group Motor Units (Hz)							
Condition	MU1	MU2	MU3	MU4	MU5	MU6	Mean (SD)
PRE	12.74726	12.90063	14.12172	12.20862	10.50128	11.46259	12.32368 (1.25)
POST	13.85496	13.80067	13.1678	11.75714	0.941231	12.55106	11.01215 (4.99)

Table 18: This table represents the mean plateau firing rate for each individual motor unit in the placebo group across both the PRE and POST conditions. The mean firing rate of each motor unit across all three trials was taken to produce the displayed mean of each motor unit. Each firing rate is represented in Hz. For each trial if a MU appeared multiple times the firing plots were merged. For all motor units, firing rate at plateau was calculated by removing the first and last 500ms of the plateau region and then dividing the total number of twitches by the plateau duration to determine the rate. The rate in each trial was then averaged to produce a representative firing rate for each motor unit. The group mean and standard deviation are displayed as Mean (SD).

Mean Individual Plateau Firing Rates of Placebo Group Motor Units (Hz)						
Condition	MU7	MU8	MU9	MU10		Mean (SD)

PRE	12.11579	9.201797	10.12007	10.00489		10.36063 (1.24)
POST	8.502074	15.14356	11.03369	11.69448		11.59345 (2.74)

Table 19: The mean plateau firing rate of the central sensitization and placebo groups are represented in this table. Data was normalized to the PRE condition with the POST condition representing the relative percentage change from baseline. The level of significance was set at P<0.05 and this normalized data did not show any changes in either group

	Sensitized Condition				*Placebo Condition*			
Condition	Mean Value (Hz)	Normalized Value	p-Value	Standard Deviation (+/-)	Mean Value (Hz)	Normalized Value	p-Value	Standard Deviation (+/-)
Pre	12.32368	1			10.36063	1		
Post	11.01215	1.029	0.435	0.076	11.59345	1.152	0.491	0.388

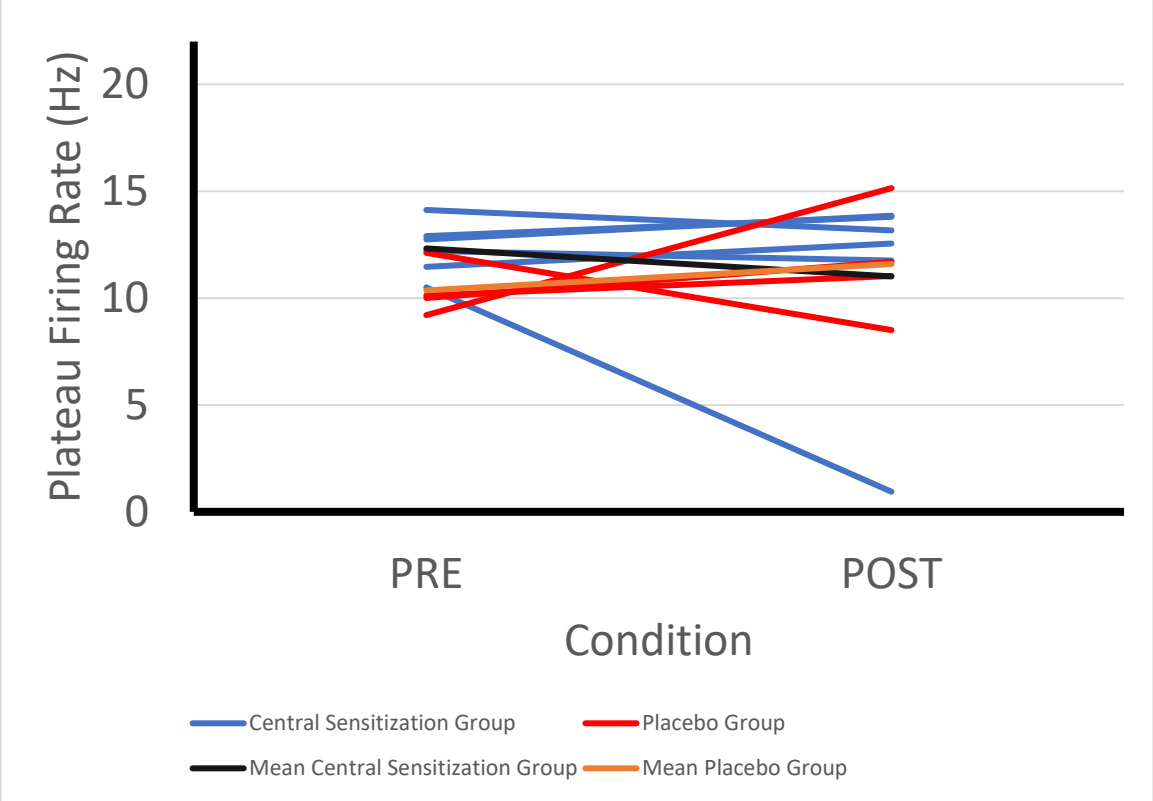

Figure 10: Individual motor unit firing rates at plateau in the PRE and POST conditions are represented for both the central sensitization group and the placebo group. The central sensitization group motor units are represented by the blue lines and the placebo group is represented by red. The means of the central sensitization and placebo groups are represented by black and orange lines respectively

4.7 EMG Amplitude

The sensitized and placebo groups both demonstrated an increase in normalized EMG RMS values 10 minutes after receiving either capsaicin or placebo cream (Sensitized=1.15, P=0.026 ± 0.148, ES=1.36; Placebo=1.29, P=0.034 ± 0.206, ES=1.58). There was no difference between groups at any time point (p>0.05). Therefore, regardless of whether a participant experienced central sensitization induction or a placebo cream application, there was an increase in EMG RMS values during the plateau region of the 20% MVC isometric dorsiflexion task in both groups of participants. The individual RMS values of each trial are displayed in *Table 20* with the normalized values shown in *Table 21*. *Figure 11* displays the mean data graphically and compares the relative change from baseline after application of either cream.

Table 20: The root mean square electromyography amplitude from each trial is displayed for both the PRE and POST conditions for both the central sensitization and placebo groups. Each participant performed 3 trials during each condition. There is a total of 6 trials in the central sensitization group for each condition and 3 trials for the placebo group at each condition. The RMS for all the central sensitization and placebo trials were averaged for the PRE and POST conditions to produce the average EMG amplitude for each group and the standard deviation (SD).

Electromyography Root Mean Square at 20% Maximal Voluntary Contraction											
	Central Sensitization							Placebo			
	Participant 1			Participant 2				Participant 3			
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Group Mean (SD)	Trial 1	Trial 2	Trial 3	Mean (SD)
PRE	5.451	5.402	6.092	13.15	11.06	10.33	8.581 (3.35)	11.4	18.6	16.2	15.4 (3.67)
POST	6.150	6.209	6.555	18.5	10.65	12.36	10.071 (4.88)	17.61	19.36	20.66	19.21 (1.53)

Table 21: The mean EMG amplitude (root mean square) of the central sensitization and placebo groups are represented in this table from the plateau region of the 20% maximal voluntary contraction isometric hold. Data was normalized to the PRE condition with the POST condition representing the relative percentage change from baseline. The level of significance was set at P<0.05 and this normalized data showed increases in both groups. The EMG amplitude during the 20% intensity contraction was higher in both groups after either capsaicin or a placebo cream was applied when compared to the EMG amplitude during the baseline trials

	Sensitized Condition				*Placebo Condition*			
Time (min)	Mean EMG RMS	Normalized Value	p-Value	Standard Deviation (+/-)	Mean EMG RMS	Normalized Value	p-Value	Standard Deviation (+/-)

Pre	8.580763	1			15.4	1		
Post	10.0707	1.153	0.026**	0.148	19.21	1.287	0.034**	0.206

** indicates statistical significance

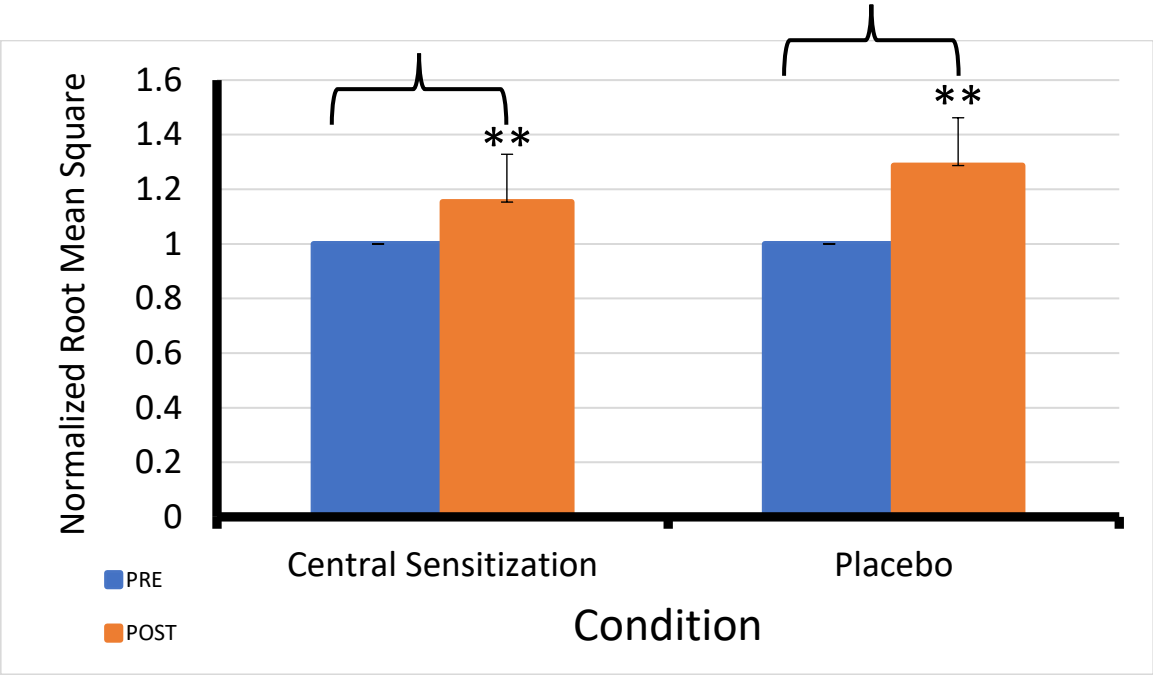

*Figure 11: Root mean square (RMS) electromyography amplitudes for both the central sensitization group and the placebo group compared to their own baseline values. Both groups showed a significant increase (P<0.05) in RMS after application of either capsaicin cream or a placebo. Significant changes are annotated with the symbol ***

Chapter 5: Discussion

5.0 Discussion

5.1 Main Findings

This study was designed to investigate the effects of experimentally induced central sensitization on motor unit excitability in the tibialis anterior during an isometric contraction.

The results of this work do not support my initial hypothesis. Based on previous work with experimental pain, I generated several hypotheses about how central sensitization would influence motor unit activity in the tibialis anterior during a 20% MVC isometric contraction 10-minutes following capsaicin application compared to baseline values. Firstly, I hypothesized that experimental central sensitization would lead to a reduction from baseline in motor unit firing rate at recruitment and at 20% MVC but this change was not observed. Further, I hypothesized that no changes would occur in MU recruitment threshold, yet the central sensitization group displayed a reduction 10-minutes after capsaicin was applied. I also hypothesized that de-recruitment thresholds would not change 10-minutes after capsaicin application which was supported by the data, but an observation of placebo group data suggested a potential reduction 30-minutes after application of a placebo cream. Finally, I hypothesized that there would not be a change in surface EMG amplitude, yet both the central sensitization and placebo groups showed an increase 10-minutes following application of capsaicin or a placebo cream. The hypotheses that I generated were not supported by the data and the following sections will discuss potential reasons for the disparity between my work and previous literature.

This work suggests that central sensitization may lead to modulation of low threshold motor unit recruitment threshold without a change in firing rate. It also presents an interesting finding of increased EMG amplitude in both central sensitization and placebo participants. Williams & Craig (2016) define pain as “a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive, and social components”. An important note to consider before discussing the placebo data is the described experience of the placebo participant during the data collection. The placebo participant did self-report to the researchers during the trial that before the 10-minute mark when the pain/placebo trial was collected, they did perceive a burning sensation from the cream. After the trial when the

participant was asked to which group they thought they had been assigned, they reported being unsure. They explained that they could not tell whether the perceived burning was directly caused by the cream itself or by their knowledge that it may have been capsaicin. Their self-reported perceived pain and their uncertainty of what treatment they had received raises questions about the relative contributions to motor unit activity from nociceptive afferents and descending motor excitation and inhibition. The trends in the placebo data discussed in the following sections may have been influenced by the participant's expectations and may show that the persistent noxious input from CS and the descending excitation/inhibition from the brain both contribute to the activity of motor units. It is important to note that, due to the limited sample size and difficulty in identifying MUs, this work is a pilot study, and a larger study is required to solidify these initial findings. Section 5.8 compares the data collected during this study to related work in the field to evaluate whether the data presented in this work falls within physiologically plausible ranges for a 20% MVC contraction of the tibialis anterior. The goal of Section 5.8 is to determine whether the data may be believable, as any values outside of a physiologically possible range are not likely to be accurate.

5.2 Reduced Recruitment Thresholds

I hypothesized that recruitment thresholds during a low force isometric contraction (20% MVC) in the tibialis anterior would not be altered during central sensitization as this has not yet been observed in other work for this population of MUs (Martinez-Valdes et al., 2020). Previous work in the tibialis anterior following experimental induction of pain has not demonstrated a reduction in recruitment threshold (Martinez-Valdes et al., 2020). Recently published work in the upper limb with experimentally induced CS has shown a change in recruitment order during central sensitization suggesting that there may be differences between the effects of pain and CS on motor unit activity (Evans et al., 2021). The work by Martinez-

Valdes et al., (2020) informed my hypothesis that experimental central sensitization, like peripheral sensitization, would not lead to a reduction in recruitment thresholds. However, the results of my work demonstrated a reduction in recruitment threshold which did not support my hypothesis but may support the recent work by Evans et al., (2021). In similar experimental setups to my own, however, motor units activated at a high force (50%-70% MVC) did demonstrate a significant reduction in recruitment thresholds in the tibialis anterior (Martinez-Valdes et al., 2020). Therefore, recruitment thresholds have been lowered during experimental pain, but exclusively in MUs at a higher (>30% MVC higher) threshold. The observed reduction in my work, therefore, was an unexpected result as MUs recruited at or below 20% MVC have not previously shown a reduction (Martinez-Valdes et al., 2020). The observed reduction provides a potential sign that peripheral sensitization (amplified pain in the absence of secondary hyperalgesia and allodynia) and central sensitization (amplified pain with quantifiable secondary hyperalgesia and allodynia) do not exhibit the same control over motor unit function. The proposed idea that central sensitization can alter motor unit recruitment at low force levels is supported by recent work in the upper limb which showed an altered motor unit recruitment pattern compared to baseline trials after experimental induction of CS (Evans et al., 2021). While the work in the upper limb did not specifically measure recruitment thresholds, it did show that the motor units from participants asked to perform a low effort contraction of the trapezius experienced a change in recruitment threshold as the recruitment order shown after CS differed from the order shown at baseline (Evans et al., 2021). Therefore, altered recruitment thresholds at low force levels during peripheral sensitization are unexpected, but it appears that CS may influence MUs at a low force level (Evans et al., 2021). Further, in the placebo group, a reduction was not observed in recruitment threshold after the application of a topical cream although there was a relatively lower magnitude reduction. This finding suggests that it was the noxious

peripheral input from the capsaicin cream that led to a significant change, rather than an effect of descending command due to expectation of pain as the CS group showed a larger magnitude reduction. The disparity between previous work and my findings could be explained by detailed examination of the differences between acute experimental pain with peripheral sensitivity and central sensitization.

The idea that peripheral pain and central sensitization may not elicit the same neurological changes provides a helpful explanation for the disparity between my work and the other pain literature. The claim that peripheral and central sensitization create different neurological changes cannot be supported without proposing a mechanism by which CS leads to a reduction in recruitment threshold. It has been demonstrated that the firing activity of a lower motorneuron can be modulated by the activity of an upper motorneuron (Frontera et al., 1997). Therefore, one potential mechanism is that the reduced recruitment threshold could be caused by descending command from upper motor neurons. This mechanism is a likely contributor to the reductions in recruitment thresholds of high threshold MUs during experimental pain (Martinez-Valdes et al., 2020). Further, Martinez-Valdes et al., (2020) tested this proposal by simulating graded descending inhibition of the motor unit pool, with lower threshold MUs receiving the most inhibition and high threshold MUs receiving the least. This simulated work recreated the observations collected in real participants, consisting of reductions in firing rate of low threshold MUs and the reduced recruitment thresholds and increased firing rates of high threshold MUs (Martinez-Valdes et al., 2020). This phenomenon was explained as a compensatory mechanism to sustain an isometric contraction at 70% MVC (Martinez-Valdes et al., 2020). Martinez-Valdes et al., (2020) suggested that the observed reduction in MU recruitment threshold in the tibialis anterior during a high-force contraction resulted from a mix of both descending excitation and inhibition to the motor unit pool. In their work it was

observed that increased descending excitation combined with a variable amount of descending inhibition (low threshold units receiving more inhibition than high-threshold units) led to the observed reductions in recruitment threshold as the high-threshold MUs receive the least inhibition and therefore the most excitatory input (Martinez-Valdes et al., 2020). I propose that a similar response may be the mechanism behind the reduced recruitment thresholds in my work during low force contractions in the presence of CS which differs from previous work showing no change from peripheral sensitization (Martinez-Valdes et al., 2020). Since this work was performed, new information has been published showing that during experimental central sensitization in the upper limb motor unit recruitment thresholds are altered during low force contractions (Evans et al., 2021). A change in motor unit recruitment patterns in the upper limb during CS further supports the idea that CS creates a change in motor unit recruitment at low force levels while peripheral sensitization does not (Evans et al., 2021). My work was limited to low force contractions with relatively few unique motor units appearing in both baseline and CS trials. Therefore, if this is the mechanism explaining this trend it is not clear what response was likely to have occurred in high-threshold MUs during a high force contraction. However, it is possible that a modified strategy of simultaneous neural drive and exponential inhibition explains the reduced recruitment thresholds that I observed. Future work would be required to confirm this theory, but an increase in descending activation during CS compared to peripheral sensitization, similar neural drive with reduced descending inhibition, or a combination of these two strategies could explain the observed change. Increased descending excitatory input could lead to increased excitability of lower motor neurons and therefore less excitatory post synaptic potentials would be required to produce a twitch. Likewise, reduced inhibition of the lower motor neurons would cause a similar effect in which fewer excitatory post synaptic potentials are required to produce a twitch. Both options could lead to motor units activating at a lower

threshold as less excitation would be required to generate an action potential. Reduced recruitment thresholds during CS could be a result of a relative increase in descending activation, a relative decrease in descending inhibition, or both when compared to the strategy proposed by Martinez-Valdes et al., (2020) during peripheral sensitization. Further, the strategies of increasing descending activation or decreasing descending inhibition, along with the graded inhibition proposed by Martinez-Valdes et al., (2020) could explain why CS appears to create changes in motor unit recruitment strategies in the upper limb (Evans et al., 2021).

Further support for the idea that changes in the ratio of descending control created the observed reduction in recruitment threshold is provided by a trend seen in the placebo motor units. During this study, 3 of the 4 motor units became active at a lower threshold after placebo application compared to baseline. Anticipation of pain has been shown to influence MU activity as it appears to contribute to reduced firing rates of MUs in the quadriceps similar to reductions seen during actual pain (Tucker et al., 2012). In the absence of nociceptive afferents, Tucker et al., (2012) explain that these changes must occur due to descending control. It is possible that a similar effect explains the insignificant reductions in recruitment threshold of 3 of the 4 placebo group MUs. If the theory is correct that modifying the ratio of excitation/inhibition to the motor unit pool during CS leads to reduced recruitment thresholds, perhaps anticipation of pain in the placebo participant resulted in a similar control strategy in the absence of nociception. As the CS group showed a reduction in recruitment threshold that was not significantly different from the placebo group, I propose that a combination of descending command with the addition of nociceptive input created the larger magnitude changes seen in the sensitized participants compared to the placebo group.

A second potential explanation for the observed reduction in recruitment threshold is that it could serve as a beneficial adaptation. It is possible that a reduction in recruitment

threshold is a compensatory mechanism for reduced torque producing capabilities of a muscle due to experimental pain (Lindstrøm et al., 2011). Voluntary torque is reduced to a greater degree during neck flexion, extension, and lateral flexion in women with higher severity chronic neck pain compared to those with a lower self-reported severity (Lindstrøm et al., 2011). The reduced torque is likely created by correlated increases in antagonist activity about a painful joint (Lindstrøm et al., 2011). Since pain is accompanied by limitations in torque producing capabilities, general and widespread descending activation to motor neurons could be a mechanism by which motor units become active at a comparatively lower force. This compensation for increased antagonist activity could lead to motor units not typically recruited at a particular force level to be recruited in order to contribute to torque production and accomplish a desired task. Building on the idea that during central sensitization the ratio of descending inhibition and excitation is altered, this phenomenon could occur across the entirety of a joint. This presents a situation where increased descending excitation affects the entire motor unit pool controlling a painful joint. Simultaneously, exponentially weighted inhibition of a movement agonist could result from descending inhibitory output. Combined, these two factors would explain why the antagonist for a voluntary movement appears to increase in activity while higher threshold motor units in the agonist are recruited at a lower threshold (Lindstrøm et al., 2011; Martinez-Valdes et al., 2020). In the context of my study, to reach and maintain 20% of a maximal voluntary contraction, it is possible that each motor unit identified in the baseline trials could no longer produce enough force to overcome plantarflexor activity and therefore activating each MU at a lower threshold was required to achieve the trapezoidal contraction. By reducing thresholds across the group, the tibialis anterior would produce more force earlier in the contraction to balance out the force of the plantarflexors and create the precise rate of torque increase and 20% MVC hold that was requested. Future work should

include recording of plantarflexor activity to test this theory and determine whether increased co-contraction occurs during an isometric dorsiflexion task in the presence of CS.

Overall, both the sensitized and the placebo motor units displayed a reduction in recruitment threshold while the sensitized group produced a relatively greater change. This change during central sensitization at a low force level is different than predicted as during peripheral pain this trend is reserved for higher threshold MUs. Therefore, I propose that a different ratio of descending inhibition and excitation serves as a beneficial adaptation to reduce recruitment thresholds. Further, activating MUs at a lower force level may result in increased force production in the tibialis anterior. Increased TA force could compensate for theorized increased plantarflexor torque, successfully achieving the desired amount of torque despite the presence of central sensitization, although more work is needed to confirm this theory. Reductions in recruitment thresholds in the placebo motor units, while smaller in magnitude, may further suggest that in the absence of noxious stimulus, descending command in the anticipation of pain still leads to altered motor unit excitability.

5.3 De-Recruitment Thresholds

Based on previous literature, it was hypothesized that there would not be an observed reduction in de-recruitment thresholds in the tibialis anterior as a result of experimentally induced central sensitization during a low-level contraction (Farina et al., 2020). My findings support this hypothesis as there was not a significant reduction in de-recruitment threshold from baseline values 10 minutes after application of capsaicin. Observationally, motor units in later trials (20 minutes post, 30 minutes post) in the sensitized participants displayed a progressively decreasing de-recruitment threshold on average. While this is interesting, the 20- and 30-minutes time points were not statistically analyzed due to technical errors, loss of trackable motor units, and increases in signal noise. It is worth mentioning this trend, but I

caution against using it as anything more than an interesting observation until later work can be performed to analyze these time points with higher signal quality, a larger sample, and a greater yield of MUs. The major finding is that 10 minutes following capsaicin application a change was not observed suggesting that at the highest pain intensity of experimental CS, de-recruitment thresholds were not affected.

In typical scenarios, it is normal for the de-recruitment threshold of a motor unit to be lower than its recruitment threshold (Heckman et al., 2005). This phenomenon is created in the motor neuron through persistent inward currents (PICs) (Heckman et al., 2005). Motor neurons have an intrinsic ability to increase their excitability and prolong their activation from synaptic input through the employment of PICs in which some voltage-gated sodium channels, mostly in the dendrites with some on the soma, remain open temporarily as the neuron begins to hyperpolarize (Vandenberk & Kalmar, 2014). These persistent currents after cessation of acetylcholine release from the presynaptic neuron result in a slowed rate of the neuron's return to sub-threshold voltages (Heckman et al., 2005). PICs have been shown to significantly prolong motor output, meaning continued generation of action potentials in the absence of descending excitation (Vandenberk & Kalmar, 2014). When progressively reducing activation level in a muscle, PICs result in motor units de-recruiting at a lower force than that at which they are initially recruited as they continue to fire briefly once stimulation has stopped (Vandenberk & Kalmar, 2014). Both descending drive and peripheral afferent input are capable of modifying PIC activity (Lajunen & Partanen, 2018). In a single subject case study Lajunen & Partanen (2018) found that mechanical vibration and repeated median nerve electrical stimulation led to hypersensitive PICs, meaning they persisted longer than normal. In contrast, animal work with cats and rats has shown that the inhibitory neurotransmitters glycine and gamma-aminobutyric acid (GABA) can reduce the influence of PICs (Fitsanakis & Aschner, 2005; Chase & Morales,

1989). This peripheral and descending inhibition could prevent PICs from maintaining MU activity without input or could increase de-recruitment thresholds by eliminating PICs entirely (Chase & Morales, 1989). These potential de-recruitment threshold changes could be caused because PICs contribute to generating the firing rates observed under normal conditions (Revill et al., 2019). Similarly, during the development of CS in rats, surgically induced nerve damage creates an immune response where microglia release the neurotransmitter bioactive leukotriene type B4 (LTB4) (Kiyoyuki et al., 2015). This neurotransmitter appears to bind to and open NMDA receptors which are a key contributor to the maladaptive neurogenesis in the dorsal horn that sustains CS (Kiyoyuki et al., 2015). As an analog to PICs, during pain and inflammation it appears that cell bodies in the dorsal horn can experience prolonged opening of NMDA receptors in the absence of presynaptic excitation, termed NMDA-induced inward currents (Kiyoyuki et al., 2015). Thus, pain contributes to persistent currents in the dorsal horn, amplification of pain, and persistence of CS (Kiyoyuki et al., 2015). Peripheral vibration and electrical stimulation can increase PIC amplitude while descending glycine and GABA can reduce or eliminate PICs (Chase & Morales, 1989). Therefore, it is reasonable to conclude that perhaps persistent inward currents are affected by persistent noxious input such as that experienced in a CS state. PICs have been shown to be influenced by descending command and peripheral input but, while there was a reduction in de-recruitment thresholds after 10 minutes it was not significant. Perhaps the continuous decrease observed later in the data collection was due to the influence of NMDA-induced inward currents instead. While difficult to confirm with the available data, it is possible that capsaicin created inflammation in the dorsal horn and CS, as confirmed by pinprick testing and brush allodynia. After 10 minutes this peripheral noxious input created a reduction in de-recruitment thresholds, but as the effects of capsaicin diminished, the NMDA-induced persistent current remained. While not possible to confirm, I theorize that the

continuous decline in de-recruitment thresholds resulted from persistent dorsal horn activity accompanied by reduced descending inhibition, allowing the peripheral input to create PICs hyperexcitability in the motor neurons, and therefore reduced de-recruitment thresholds. PICs in low-threshold motor units begin to be generated at or just below MU recruitment and persist after 1-2 seconds of activation, therefore likely contributing to reduced de-recruitment thresholds in this study (Heckman et al., 2005).

In the placebo group, there were no changes 10 minutes following the application of placebo cream when compared to baseline. This supports my proposed explanation for decreasing de-recruitment thresholds in the central sensitization group. In the placebo group, the placebo cream would not have resulted in persistent nociceptive input, while still providing some non-noxious afferent input. It is unclear how long the non-noxious input would persist. In this case, I hypothesize that there was not an alteration in de-recruitment threshold in the placebo group as the lack of sustained nociceptive input is unlikely to have created NMDA-induced PICs and therefore no long-term continuous decline in de-recruitment threshold. If changes in recruitment threshold can be explained due to descending command in both the CS and placebo groups, yet de-recruitment thresholds only decreased in the CS group, this suggests that the change was related to noxious input from the periphery which only occurred in the CS group.

Persistent inward currents created by nociceptive input are a possible explanation for why reduced de-recruitment thresholds were observed in a centrally sensitized state, without any observed changes in the placebo group. The overall finding of this work, however, focuses on the 10-minute time point in which a reduction in de-recruitment threshold was not observed in either group.

5.4 Recruitment Firing Rate

This work did not show any changes in motor unit firing rates when each motor unit was first recruited. This does not support findings from previous literature in which low threshold motor units (25% MVC) experienced a reduction in firing rate following experimental induction of pain while high threshold motor units (70% MVC) increased their firing rate (Farina et al., 2020). An explanation for this can be provided by other observed changes such as reduced recruitment thresholds.

In my work it was observed that in a centrally sensitized state, motor unit recruitment thresholds were reduced, leading to motor units becoming active at a lower level of force. I propose that this observed trend is a compensatory mechanism for CS and makes increasing firing rate unnecessary. A possible reason for the observed reduction in recruitment threshold is as an adaptive and compensatory mechanism to overcome the likely increase in antagonist activation during pain (Lindstrøm et al., 2011). If plantarflexor force was increased the dorsiflexors would have to compensate with either increased rate coding or reduced recruitment thresholds to reach the desired 20% MVC force. In my work, which is different than previous literature by Martinez-Valdes et al., (2020), recruitment thresholds were reduced in low threshold MUs, leading to motor units becoming active earlier during the ramp contraction. Motor units producing force earlier could serve as compensation for a potential increase in plantarflexor torque. In this case, increasing or decreasing firing rate would not be necessary to produce sufficient force in the tibialis anterior. Therefore, maintaining the firing rate may have been an appropriate response to produce sufficient force and therefore no change was observed in my work.

5.5 Plateau Firing Rate

Like recruitment firing rate, the motor unit firing rate at plateau (20% MVC) did not change significantly from baseline values at any time point. This is contrary to my hypothesis that firing rates would be reduced, as has been suggested by previous work (Minami et al., 2013).

Like in the recruitment firing rate case, a change in firing rate was not necessary as recruitment thresholds had been reduced. If an appropriate compensation for reduced torque about the ankle is achieved by lowering recruitment thresholds, it may not be necessary to alter firing rate.

An important factor to consider when comparing our findings to previous work is the mechanism of pain induction. In much of the previous work, pain was induced by injection of saline or capsaicin directly into the muscle (K. J. Tucker & Hodges, 2010). This could lead to peripheral sensitization of the nociceptors within these muscles as well as central sensitization. There is potential for a summative effect between peripheral and central sensitization leading to more significant changes. Our model of applying capsaicin topically and evaluating changes within the muscle would not have peripherally sensitized the muscle itself. While this is a more valid model of central sensitization as we were only measuring the effects of CS, it is a less severe form of pain and this could explain the less significant changes.

The final factor that may explain the lack of change in firing rate at plateau may be due to the relatively low increase of intensity between recruitment and plateau. The motor units identified in this study became active and began firing at nearly 20% MVC. Due to the fact that there was a very small increase in firing rate between recruitment and plateau, and recruitment firing rate should be the lowest firing rate before de-recruitment, the identified motor units may not have been able to reduce their firing rates without de-recruiting. To determine whether this

is the case, a higher target force would be required to determine if, at a higher force, motor unit firing rates at plateau are reduced following experimental induction of central sensitization. With the current information available it is unclear, but with the small changes between recruitment force and plateau force I believe that it is unlikely for the active MUs to reduce firing rate as they were still close to de-recruitment at 20% MVC.

5.6 EMG Amplitude

I hypothesized that EMG amplitude would not demonstrate a significant change during central sensitization compared to baseline. Previous work in experimental pain supported this hypothesis with pain appearing to cause no change in an effected muscle while contralateral muscles exhibited an increase (Wang et al., 2010). However, in my work there was a significant increase in EMG amplitude during isometric contractions of the effected tibialis anterior at 20% MVC which does not support my hypothesis. In previous work by Wang et al., (2010) it was proposed that this mechanism in the jaw activated the contralateral and unaffected masseter to produce a desired bite force without increasing activity of the affected side. However, in the tibialis anterior, contralateral activation of the left tibialis anterior does not assist with dorsiflexion on the right side. Therefore, this control strategy would not be appropriate. My work did not demonstrate a significant reduction in firing rate, so overall EMG amplitude should not have changed due to no change in firing rate. However, there was a significant decrease in MU recruitment thresholds. Therefore, motor units activating at a lower force may explain the relative increase in activity between baseline and central sensitization conditions as a new pool of MUs may have become active. In my work, only motor units that could be tracked across baseline and central sensitization conditions were evaluated so it is unclear whether an increase in EMG amplitude could be due to contributions of higher threshold units recruiting at or below 20% MVC during pain while they were inactive at this force level during baseline. However,

previous work has shown recruitment of additional MUs during experimental pain, with some motor units becoming active during pain that were not active in the absence of pain (Dean et al., 2014; Malik et al., 2018; Minami et al., 2013; K. Tucker et al., 2009). Further, work by Evans et al., (2021) showed altered recruitment of MUs, with high threshold MUs becoming active before MUs with relatively lower thresholds. I propose that the mechanism of reduced MU recruitment threshold was a result of increased descending activation, reduced descending inhibition, or both. Therefore, if this mechanism is correct, it is likely that an increase in EMG amplitude during experimental central sensitization is a result of motor units with recruitment threshold >20% MVC being inactive during baseline trials. These MUs then may have become active due to alterations in descending control, leading to them not being analyzed as they only appeared during the CS trials, but they contributed to the overall EMG amplitude creating a significant increase. More motor units active at the same force with the same firing rate would lead to an increase in EMG amplitude without subsequent changes in force production as measured through dorsiflexion torque.

Increases in EMG amplitude were not exclusive to the sensitized participants. The placebo participant also showed an increase in EMG amplitude at the 10-minute time point following the application of a placebo cream. This can likely be attributed to pain being an emotional experience as well as physical. While much of this work was designed to focus on the biological aspects of nociception, it would be inappropriate to ignore the psychology of pain. Anecdotally, the placebo participant reported a possible sensation of pain upon application of the placebo cream at the 10-minute time point. Following the 10-minute time point the participant reported no experience of pain resulting in their overall report that they were not able to identify whether they were in the sensitized or placebo group. Previous work has shown that both pain and the expectation of pain result in similar alterations in motor unit behaviour,

suggesting that both capsaicin and a properly blinded placebo may result in similar alterations, such as increased EMG activity (K. Tucker et al., 2012). Perhaps the increased EMG amplitude in the placebo group can be explained by the expectation that the placebo cream would result in pain and therefore they experienced a compensatory increase in EMG amplitude in anticipation of pain, rather than as a direct result.

Another possible explanation for the unexpected increase in EMG amplitude could be due to changes in plantarflexor activity during pain which is supported by the "pain-adaptation model" of Lund et al., (1991). This theory proposes that activity of the agonist is expected to decrease during pain while synergists and antagonists increase activity. Based on this model, it would be expected that the plantarflexors would have increased their activity level during the dorsiflexion task. My experimental setup was designed to measure net joint torque about the ankle and participants were asked to consistently contract to a net joint torque that corresponded to 20% of their MVC torque. If the plantarflexors increased their activity during CS, the dorsiflexors would need to increase their activity to compensate and achieve the desired net joint torque. As force of the individual muscles was not being measured it is possible that both the dorsiflexors and the plantarflexors increased their activity during CS, leading to an increased EMG amplitude in the tibialis anterior at the same level of externally measured torque.

Further, the observed increase in EMG activity without a change in net joint torque could explain the observed changes in recruitment threshold. It is possible that both the dorsiflexors and plantarflexors were contracting at a higher intensity which produced the same net joint torque. Since each contraction was standardized to 20% MVC net joint torque, it would appear the motor units were recruited at a lower recruitment threshold. For example, if the tibialis anterior was in fact contracting at 25% MVC to compensate for the increased

plantarflexor activity, the measured net joint torque would not be altered. A motor unit recruited at 15% MVC could still recruit at 15% MVC, but it would appear to have a lower recruitment threshold relative to the baseline values as it was activating at a lower net joint torque, but the same force. Without measuring plantarflexor EMG activity, this explanation cannot be confirmed, but it does appear likely that the observed changes in EMG RMS amplitude and reduced recruitment threshold could be a sign of increased co-contraction about the ankle rather than a sign of alterations in motor unit excitability in the tibialis anterior itself.

If both pain and the expectation of pain can alter firing rates as shown by Tucker et al., (2012), it is likely that a significant increase in EMG amplitude in the placebo participant was through a mechanism of increased descending activation. Therefore, in both the central sensitization and the placebo groups, it is likely that MUs with relatively higher thresholds became active once capsaicin or placebo cream had been applied, leading to an increased EMG amplitude in both groups. It is possible that both groups behaved as if they were experiencing experimental pain, regardless of whether the cream created noxious input.

5.7 Between Group Differences: Expected and Experienced Pain

For each of the outcome measures: recruitment threshold, de-recruitment threshold, firing rate at plateau and at recruitment, and EMG amplitude, there were no significant differences between groups. In the case of recruitment thresholds, both groups showed a reduction while the magnitude was greater in the CS group. This suggests that, while there may have been reductions in threshold and increases in EMG amplitude, the placebo group experienced lower magnitude changes in the same direction for each variable.

I propose two different possible mechanisms for the similar reductions in recruitment threshold and increased EMG amplitude from the CS and placebo groups. The first possible mechanism is through the effects of expected pain. The expectation of pain has been shown to

modulate motor unit firing rates in previous literature (K. Tucker et al., 2012). Therefore, it is possible that in our work, the placebo group's expectation of pain resulted in a change in recruitment threshold and EMG amplitude like the changes seen in the sensitized group.

The second proposed mechanism for the lack of a difference between the sensitized and placebo group is through an additive effect of expected and experienced pain. The expectation of pain can cause changes in motor unit behaviour in the absence of pain, while other work has shown that experiencing pain experimentally causes alterations in firing rate (Farina et al., 2020). I propose that there may be a summative effect resulting in the significant changes observed in our work. If both expectation of pain and experience of pain are capable of causing significant changes, it is likely that both expecting pain and experiencing pain leads to an even greater effect. This could explain why the placebo group demonstrated non-significant changes in recruitment threshold and de-recruitment threshold, and the sensitized group saw larger magnitude changes from baseline without a difference from placebo. The psychological aspect of experiencing pain cannot be neglected when studying the effects of pain (or CS) on motor unit behaviour.

5.8 Validity of Data Compared to Similar Work

Due to the challenges in identifying motor units from this data and its differences from previous literature in the field, the data collected must be compared to previous work to determine if the data provided is physiologically plausible. Appendix A within this document provides examples of the data provided by the OT BioLab+ software for a visual display of the data presented by the software.

Firstly, the RMS values for this study were collected at 20% MVC. The average RMS before capsaicin or placebo application was 11.5 microvolts in the central sensitization group and 15.4 microvolts in the placebo group during the plateau at 20% MVC. In work by Del Vecchio

et al., (2018) ten participants performed isometric dorsiflexion up to 70% MVC. The RMS of their participants as they reached 20% MVC covered a range between 0 and 100 microvolts with the group average being <50 microvolts. Therefore, the average RMS from my participants was in the same range as this work although was primarily clustered at the lower end of the range. Therefore, while my RMS values were generally lower, they were still within a physiologically relevant range. In general, it is inadvisable to directly compare EMG voltage across studies, participants, or even trials as a variety of factors can influence voltage including hardware gain, electrical resistance of the skin, quality of electrode contact, skin preparation, or body composition. This comparison shows that the voltage acquired in my work is within a physiologically possible range, it does not provide any more value. The voltage from my work is not an impossible value, but it provides no further information. To compare between similar work, EMG RMS amplitude would need to be normalized to a maximal voluntary contraction and then compared. For the purposes of determining whether the data is accurate, the non-normalized comparison shows that the amplitude I measured is not impossible, but this does not confirm that it is valid.

The next challenge is in correctly identifying motor units as all recruitment thresholds in the central sensitization group were between 15% and 20% MVC. All motor units in the CS group showed a reduction in recruitment threshold below 15% MVC when centrally sensitized although no motor units were identified at this contraction level in the baseline condition. The likely explanation for the lack of identifying MUs below 15% MVC is the challenge in tracking motor units across time points. Up to 7 motor units were identified in each contraction but a range of 1-4 per trial were able to be tracked including duplicates within the same trial or across multiple trials leading to the yield of 3 MUs from each CS participant and 4 from the placebo participant. The reason for the lack of MUs being identified below 15% MVC at baseline is likely

due to the difficulty in tracking motor units across time points. It is possible that those motor units that were not analyzed were activated at these low thresholds but unable to be tracked and were eliminated from the data pool. This could be attributed to motor units being below the detection threshold required by OT BioLab+ to detect individual MUs, like issues encountered by Evans et al., (2021) using the Delsys system. Another likely scenario is that previous work using the OT Bioelettronica system was decomposed using a customized algorithm outside of OT BioLab+ (Martinez-Valdes et al., 2020). It is possible that the difficulties encountered in my study were due to the software itself and this would explain why other work has had more success identifying a larger pool of MUs when performing all processing offline in MatLab (Mathworks, version 2017b). The recent work by Martinez-Valdes et al., (2020) performed isometric dorsiflexion while measuring MU activity in the tibialis anterior using the OT Bioeletronnica system and identified a larger pool of motor units than in my work. While the protocol was similar and the same equipment was used, the offline processing appeared to identify many more motor units than I was able to identify within OT BioLab+.

Another important evaluation of the data is to determine if the identified motor units behave as predicted by previous literature. According to De Luca & Contessa (2015), higher threshold motor units should have a lower firing rate. This “onion skin principle” has been observed up to 50% MVC at which point the higher threshold motor units surpass the firing rate of the lower threshold units (Piotrkiewicz & Türker, 2017). Therefore, to confirm that I was able to correctly identify motor units, the 10 included in my study were all active below 20% MVC and therefore should display the “Onion Skin Principle” with the lowest threshold MUs showing the highest firing rate. The 10 motor units that I collected displayed a very week correlation (R^2=0.072) between recruitment threshold and firing rate as there was not a large magnitude difference in firing rate between the highest and lowest threshold MUs. The weak correlation

between firing rate and recruitment threshold is likely due to the MUs all becoming active around the same threshold. With the low magnitude differences in recruitment threshold, it is not likely that large differences would be observed in firing rates across the pool of 10 MUs.

Beyond comparing the expected relationship between recruitment threshold and firing rate, it is important to compare the firing rates to other work to see if they are physiologically relevant by comparing the data to previous literature. In the baseline trials, the MUs I was able to identify covered a range of firing rates between 8 and 18 Hz when they were recruited excluding one MU which was recruited with a firing rate of 3 Hz. Copithorne et al., (2020) had participants hold a tibialis anterior contraction at 15% MVC and measured MU firing rates with fine wire electrodes and they observed an average firing rate of 10.4 Hz at the plateau. This falls within the low end of the range observed from my MUs and was a lower contraction intensity as my motor units were all recruited above 15% MVC. Therefore, the MUs identified in my study are firing at a physiologically relevant firing rate when they are recruited. Further, other work using HDEMG decomposition during isometric ankle dorsiflexion reported average tibialis anterior MU firing rates at an average of 15 Hz during a 20% MVC contraction (Del Vecchio et al., 2018). The MUs identified in my study showed plateau firing rates between 10 Hz – 15 Hz at this contraction intensity which fits within the expected range. The motor units of the tibialis anterior during each of these studies were at relatively lower firing rate when compared to their potential maximum. Macefield et al., (1992) investigated the firing rates of TA MUs to determine their maximum firing frequency and reported observing a range between 9.9 Hz and 61.9 Hz. Based on this work, the MUs in my study at recruitment and at plateau were firing within the expected frequency range and at less than 1/3rd of the highest firing rate observed by Macefield et al., (1992).

Overall, while the data is limited, it does appear to fall within normal ranges and display the expected motor unit behaviour. With a small pool of MUs it is difficult to draw firm conclusions from this work, but the data provided does show that the MUs identified were behaving normally.

5.9 Limitations

The primary limitation of this work is its small sample size due to safety measures during the COVID-19 pandemic. Two participants assigned to the central sensitization group and one participant assigned to the placebo group attended their scheduled data collection before collections were halted to limit in-person interactions and prevent the spread of COVID-19. As such, this work is bases solely on the pilot data of these three participants. Other limitations are discussed further in sections 5.9.1 and 5.9.2 specifically focused on the HDEMG tool and experimental design respectively.

5.9.1 HDEMG

Like any experimental tool, the use of HDEMG has potential limitations. While these limitations were attempted to be controlled for, the data collected for this study is limited. Therefore, it is especially important to justify why the way that I controlled for the limitations of the experimental protocol was appropriate. Despite the limited data, I must be able to show that the small pool of data can be trusted.

A primary limitation of using high-density surface EMG is the difficulty in identifying motor units from a sEMG signal. While this problem has been improved with advances in technology and decomposition algorithms, identifying a single motor unit from a surface signal still presents a significant challenge. There are a variety of algorithms available for HDEMG decomposition, although the primary commercially available options are produced by Delsys and OT Bioelettronica. While there are arguments amongst the field of which option is superior,

the OT Bioelettronica system was chosen for our study to limit human error from the capability to manually edit firing plots with the Delsys system (de Luca et al., 2015; Farina et al., 2015). The Delsys system algorithm consists of 4 steps titled decompose – synthesize – decompose – compare (DSDC) (De Luca et al., 2015). This method is questionable, however, as decomposing an HDEMG signal, synthesizing an artificial one, and decomposing it again for comparison creates the potential for an error in the original decomposition to be recreated in the second decomposition and then presented as valid data. Further, the Delsys system relies on prediction of the firing pattern of an individual motor unit and allows the user to fill in the "gaps" with artificial data if they believe the system encountered an error. That creates another potential source of error if the experimenter incorrectly inserts false data to create what they think is the "correct" or "real" signal. Recent work by Evans et al., (2021) using the Delsys system reported success identifying MUs in the trapezius but difficulty identifying motor units in the infraspinatus. It is possible that the activity of some MUs fall below the detection threshold for the algorithm and therefore were not identified (Evans et al., 2021). For these reasons, the OT Bioelettronica system of convolutive blind source separation presented an alternative with less potential for error created by a novice experimenter.

While the OT Bioelettronica system was the ideal choice for my work, the convolutive blind source separation technique used encountered a few common errors. A major advantage of HDEMG decomposition is its ability to non-invasively identify a relatively large yield of motor units (Dijk et al., 2008). To maximize the yield of motor units, the contraction type and intensity recommended by the manufacturer was followed, as well as choosing the tibialis anterior over the originally planned biceps brachii as recommended by the company. Participants performed trapezoidal isometric contractions with a consistent rate of torque increase (2% MVC/second) until 20% MVC. This force level was maintained for 10 seconds before ramping back down to

rest at the same rate of 2% MVC/second. This created a contraction duration of 30 seconds with several seconds of rest before and after the contraction as recommended. Despite this, in many trials from the participants reported in this study the algorithm was unable to identify any motor units from the sample. The maximum yield of motor units was one trial in a sensitized participant in which 7 distinct motor units were identified. While some work has shown a large yield of motor units using HDEMG decomposition, in my work the yield was consistently less than 10 motor units and in some cases 0. Extensive piloting was performed on both the biceps brachii and the tibialis anterior of 8 participants with many participants attending multiple lab visits. Unfortunately, this data was not able to contribute to the study as the software could not identify any MUs from any of these participants from any of the lab visits. While some work has successfully identified hundreds of MUs using this system, in my experience the yield was minimal (Martinez-Valdes et al., 2020). This system may be useful for the group that designed it, but for other researchers it may not be ready for widespread use.

Secondly, motor units were not identified consistently across all time points. A motor unit identified in the baseline trials for example may not have appeared during all 4 of the post-capsaicin trials (10, 20, 30, and 40 minutes). The later time points (20-, 30-, and 40-minutes post-cream application) were excluded from the study due to this limitation. Further, to compare pre-treatment and post-treatment a motor unit had to appear in both. Some motor units identified at baseline did not appear during central sensitization and were not analyzed, while some MUs were identified after CS induction but not before. This could represent a reorganization within the MU pool as suggested by Malik et al., (2018). However, it could also be a result of difficulty identifying the same MU consistently. To negate this effect, I collected 3 trials at each time point. My goal was to increase the odds that the same motor unit was identified repeatedly by presenting the algorithm with three different signals at each time point.

Although this increased the yield of motor units that could be tracked across time, still some motor units were not identified at every time point. I suggest that both a reorganization of MUs likely occurred as predicted by previous work while it is also likely that some MUs were active in all trials but not identified from the surface signal (Malik et al., 2018). I recommend that multiple trials be collected in future work to increase the likelihood that a motor unit will appear in at least one trial at each time point and maximize the number of motor units available for tracking across time. Reorganization of motor unit activity cannot be controlled but better ability to track the same MU across time would greatly improve the quality of my study.

Finally, it was not uncommon for the same motor unit to be identified in multiple trials at each time point. In this case, the mean of the values (firing rate, recruitment/de-recruitment threshold) were taken to avoid biasing the data by choosing one trial as the "true" value. More importantly, occasionally the same motor unit was identified within the same trial. This meant that two disconnected firing plots were displayed and presented as if they were two motor units, when it was likely the same MU and the plots should have been merged. This necessitates the establishment of criteria for elimination of duplicate data to prevent errors in the calculations of firing frequency. Some possible options are averaging the two "motor units" together or deciding to selectively use data from one motor unit but these would both produce data sets that did not represent the combined activity of the two plots. In this work I opted to correlate motor units across time points and merge the firing plots of motor units appearing within the same trial with a correlation of 0.9 or higher. This cut-off was chosen to eliminate errors in the algorithm and was a superior alternative to averaging or eliminating a plot which would both underestimate the firing rates of the MU.

A further limitation to the use of a 64-elctrode HDEMG is the large surface area covered by the array. This is both advantageous and detrimental depending on the situation. The large

area is excellent for collecting data over an entire muscle, however, there is also potential for the array to extend beyond the edges of the muscle, or to overlap with the motor point, resulting in the loss of data. To account for this, there are many different sizes of array. With appropriate choice of array and anatomical landmarking to avoid the motor point, the negatives of the high-density array can be minimized. For this work, I chose a smaller 64-electrode array (Gro8mm1305, OT Bioeletronnica, Torino Italy) with a length of 10.4cm and a width of 4cm on the distal 50% of the tibialis anterior muscle belly. This was to avoid the motor point typically located within the proximal 50% of the muscle, without extending over the tendon or the fibularis group to limit the potential for crosstalk (Barbero et al., 2013). The advantage of the smaller array over one of the larger available options is the reduced likelihood that the array would extend beyond the tibialis anterior muscle belly. The tibialis anterior muscle belly was palpated to ensure that the array was placed entirely over the muscle to minimize the risk of some electrodes extending over inactive tissues. Visually, each electrode in the array showed electrical activity so it is unlikely that the array was not placed directly over the muscle.

While a variety of limitations were present in this work, steps were taken to reduce errors from each. Still, the data available is limited due to both the small sample size and the low yield of motor units collected from this sample. Both the Delsys and OT Bioelettronica systems have been used successfully in previous research, but they are not without issues (Evans et al., 2021; Martinez-Valdes et al., 2020). Overall, while HDEMG decomposition presents a useful research tool for future work, it is not without risks. With carefully chosen research design, contraction protocols, data analysis, and equipment setup, there is still potential for low yield of motor units. The non-invasive approach of HDEMG is ideal for situations such as these in which the effects of pain are being tested, but currently I recommend the continued use of indwelling EMG as the preferred tool for the study of motor unit activity.

5.9.2 Experimental Design

Despite active efforts to reduce limitations of the experimental design, it is important to acknowledge where this work could be improved. Firstly, EMG from the antagonist muscles of the tibialis anterior was not collected. While this does not affect the data directly, it would reduce the amount of speculation required to explain the observed changes during central sensitization. Measuring plantarflexor activity would have supported conclusions of this work as an increase in plantarflexor activity would have explained the increased dorsiflexor EMG RMS amplitude. Increased co-contraction would also explain why recruitment thresholds were reduced during CS, as it may be a sign of increased plantarflexor activity leading to motor units appearing to recruit at a lower force, when they actually recruited at the same force but a lower net joint torque. Secondly, we did not perform our pinprick protocol on our placebo participant. In theory, central sensitization should not have occurred from application of the placebo cream, but it would be interesting to see if there was an increase in pinprick sensitivity despite not inducing CS in this participant. If this work is to be replicated in the future, all participants should receive the pinprick protocol regardless of their group assignment. Finally, data collections were cancelled due to the COVID-19 pandemic. Owing to the lockdowns, only 3 participants were collected prior to campus shutdowns. More participants would help to make the findings of this work more robust and reduce the potential for a single participant to significantly impact the data.

Finally, the dynamometer itself could potentially introduce further limitations of this work. It was designed to be rigid and immobile to prevent any movement about the ankle as the purpose of this work was to study isometric contractions. Once the ankle position was set, the footboard was locked in place to prevent further movement. Further, to closely couple force between the participant's foot and the load cell, an adjustable and inelastic strap was used to

secure the foot directly to the load cell instead of the footboard. As the footboard was secured and immobile, attaching the load cell to the footboard would not have produced any measurable tension. By designing the strap to cover the plantar surface of the foot, loop around either side of the footboard, and connect directly to the load cell, it was possible to closely couple dorsiflexion torque to force measured on the load cell.

5.10 Future Directions

Despite the list of limitations, there are a variety of benefits of using HDEMG which could make it a good choice for future investigations. Firstly, the heat map function of the HD EMG system presents a possible tool for identification of myofascial trigger points. Currently, there is no tool for the visualization of trigger points and as such they must be assessed by palpation by a clinician. A hyperactive taut band within a muscle may be identifiable at rest with a heat map. In theory, a muscle at rest with an overactive region may suggest that a myofascial trigger point is present.

Another future direction is to repeat this work with more participants. Increasing the number of participants may help differentiate the results of our work on central sensitization from previous work on experimental pain, by giving the study more power and providing more information about how experimental central sensitization affects a larger population. Finally, different muscles, joint angles, force levels, and dynamic contractions should be investigated to determine if motor unit activity is altered in similar tasks or during dynamic movements.

Chapter 6: Conclusion

6.0 Conclusion

Contrary to my hypothesis, I saw a reduction in recruitment threshold with no change in firing rate or de-recruitment threshold during experimental central sensitization. Further, an increase in EMG amplitude was observed in both experimental central sensitization and placebo participants. In general, these changes can be attributed to compensation for reduced torque producing capabilities about the ankle during a pain state. It is important to note the limitations of both the small sample size and the technical issues experienced over the course of this study. Without a larger body of evidence and a more robust study design, these changes cannot be confirmed. Each of the significant changes resulting from experimental induction of central sensitization may be a nervous system adaptation to produce the required 20% MVC dorsiflexion torque. Interestingly, these changes cannot be attributed exclusively to neuromuscular changes, as in the placebo group there was an increase in EMG amplitude and similar trends in recruitment threshold reductions. It is likely that any changes exhibited can be attributed to a combination of both physical neuromuscular changes directly from experimental induction of central sensitization, in conjunction with psychological effects influencing descending control of the motor unit pool. More work is necessary to collect a larger data pool in order to determine the effects of central sensitization on motor unit behaviour, as well as for direct comparison between those experiencing acute pain and those experiencing central sensitization. Overall, due to a variety of limitations and a small sample size, drawing conclusions about the effects of central sensitization on motor unit activity are not possible without further research.

7.0 References

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8.0 Appendix A

Appendix A provides supplemental figures displaying raw data as displayed in OT BioLab+. These figures include raw EMG tracings, force tracings, motor unit firing plots, and examples of unique motor waveforms identified by the convolutive blind source separation algorithm.

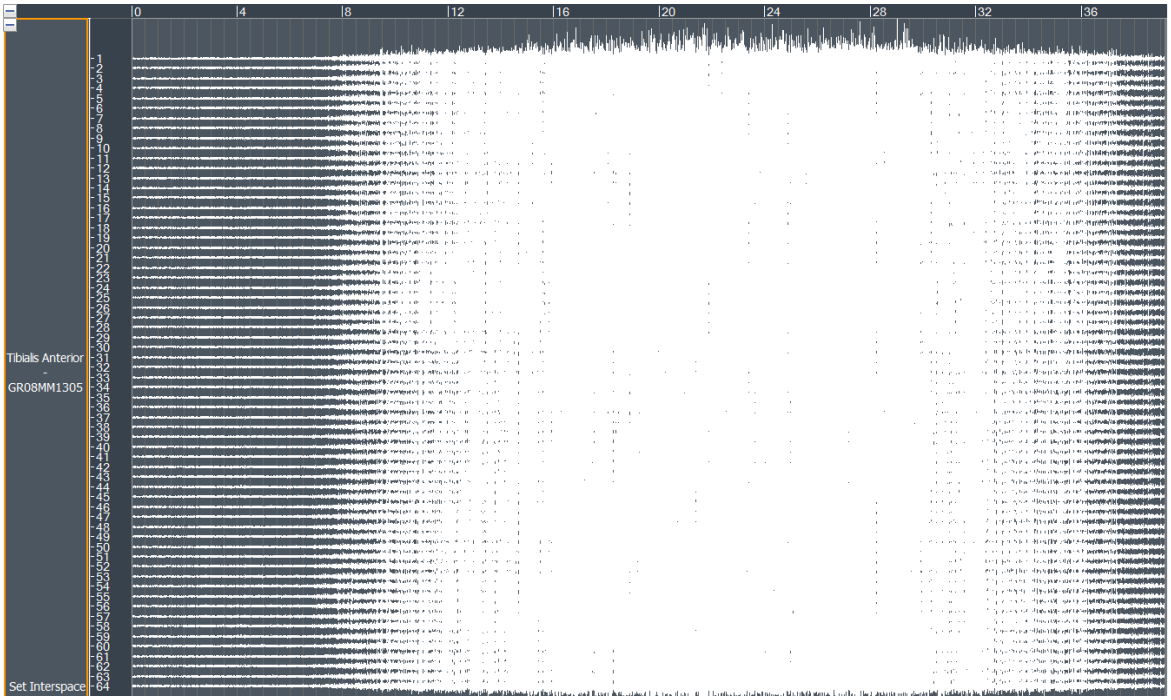

Figure 12: Baseline data from one of the central sensitization group participants showing 64 HDEMG channels and EMG activity as the participant increased contraction intensity to 20% MVC and then ramping down to rest

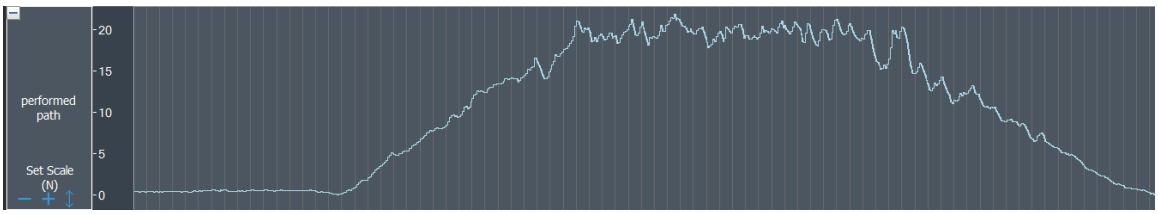

Figure 13: Example of a force tracing from one of the central sensitization group participants during a baseline trial showing an increase in force up to a 20% MVC plateau and then reducing to 0%

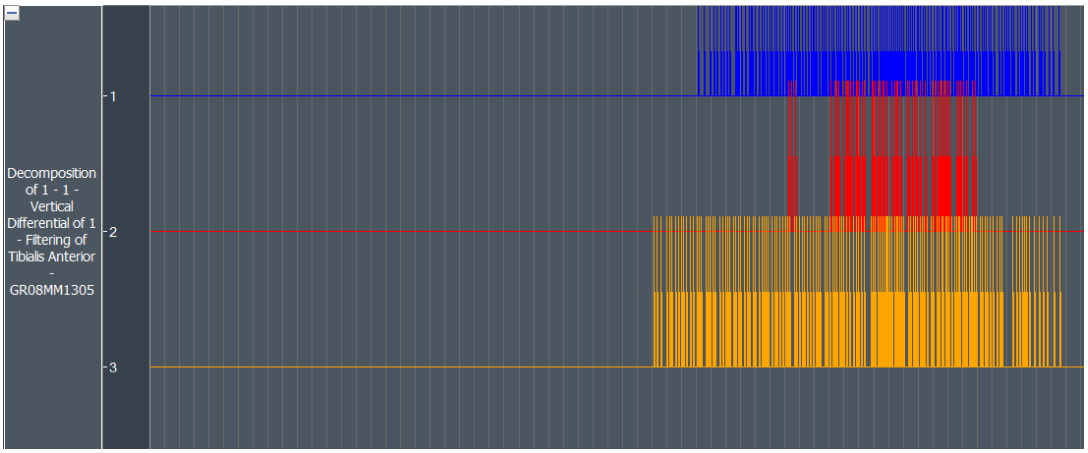

Figure 14: Example of an EMG firing plot from one of the central sensitization participants during a baseline trial showing the firing activity of 3 potential motor units identified by the convolutive blind source separation algorithm

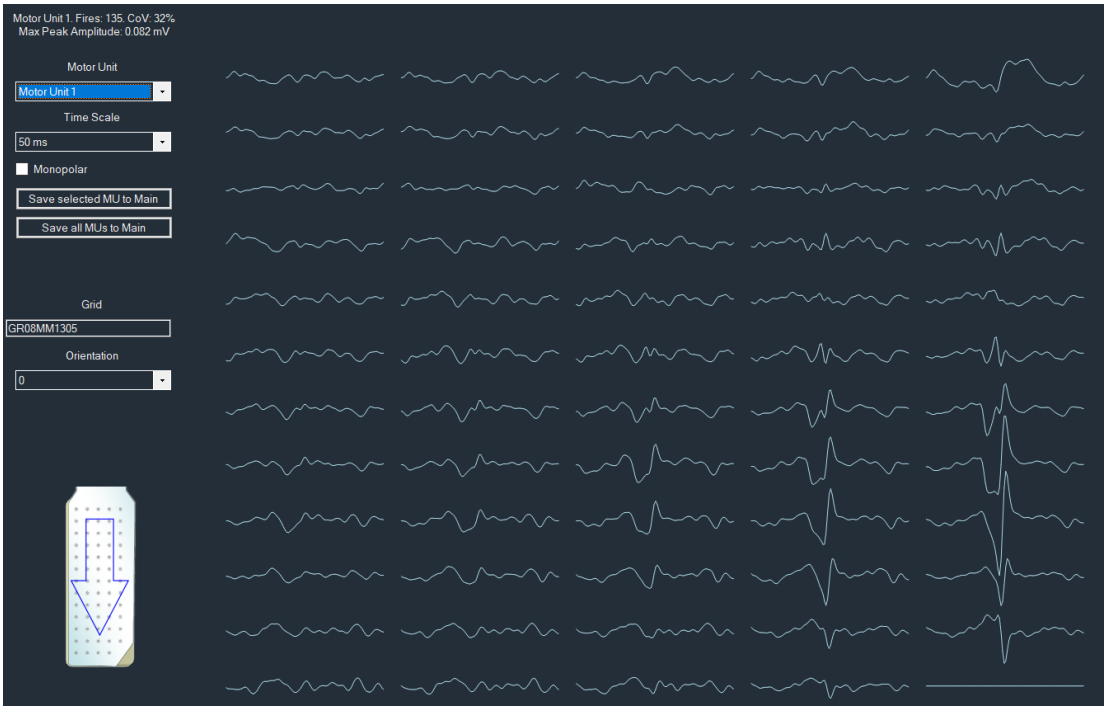

Figure 15: Example of the unique "fingerprint" of one motor unit as measured by the HDEMG electrode

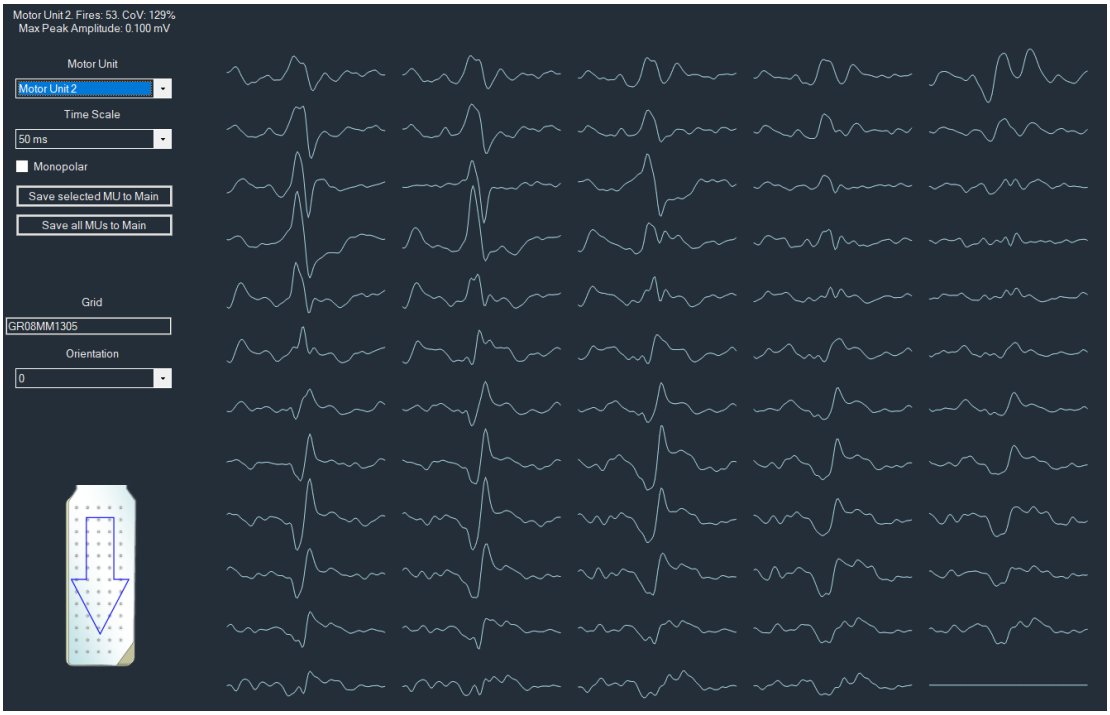

Figure 16: A second example of a unique motor unit "fingerprint" as seen by the HDEMG electrode showing a unique waveform and a unique location within the electrode grid