The Effects of Central Sensitization on Motoneurone Excitability in Osteoarthritis

by

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ABSTRACT

THE EFFECTS OF CENTRAL SENSITIZATION ON MOTONEURONE EXCITABILITY IN OSTEOARTHRITIS

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This thesis is an investigation of the neurophysiologic mechanism, central sensitization, underlying pain and dysfunction in osteoarthritis. Central sensitization is an important mechanism in the pathophysiology of osteoarthritis but, to our knowledge, its influence on motoneurone excitability is unknown. Our primary hypothesis states that increasing central sensitization within a spinal segment will cause a greater increase in the excitability of motoneurones in subjects with osteoarthritis when compared to healthy controls. To test this hypothesis, we experimentally induced central sensitization in individuals and monitored the recruitment threshold force of the motor units in the first dorsal interosseous muscle using indwelling electromyography. Findings from this study suggest that central sensitization lowers the motor unit recruitment threshold in osteoarthritis compared to healthy individuals. Motoneurone excitability might be inhibited in healthy individuals with persistent sensitization as well. Thus, central sensitization is an important consideration in the biomechanical dysfunction seen in osteoarthritis.
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CHAPTER 1
INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease that is characterized by the degradation of cartilage and bone in the joints of the body. It is an increasingly prevalent chronic disease in the aging population with approximately 18% of women and 10% of men over the age of 60 affected by this disease worldwide (Murray and Lopez, 1996). By 2020, the number of people suffering from OA is expected to increase substantially (Elders, 2000), increasing the societal and economical burden of this disease (Elders, 2000; Gupta et al. 2005). Osteoarthritis is the most common form of arthritis and is one of the top ten causes of disability in the world (Murray and Lopez, 1996). Disabilities experienced by individuals with OA include the inability to perform tasks like walking or climbing stairs without assistance (Felson et al. 2000).

A key determinant of disability in OA is the pain experienced by symptomatic individuals (van Baar et al. 1998). Clinically, this disease is expressed as joint pain, referred pain and tenderness, local inflammation and limited joint movement (Woolf and Pfleger, 2003). Underlying these clinical features are mechanisms, namely peripheral and central sensitization, which result from the pathophysiological events that occur in the early stages of OA development. Central sensitization, in particular, has been implicated in the maintenance and spread of pain in OA (Schaible et al. 2002).

Central sensitization is a phenomenon where neurons of the central nervous system are sensitized and become more excitable to stimuli (Latremoliere and Woolf, 2009). Central sensitization has been well documented in neurons in the dorsal horn of the spinal cord (Schaible and Grubb, 1993), thalamus (Dostrovsky and Guilbaud, 1990) and amygdala (Neugebauer and Li, 2003). There is evidence to suggest that central sensitization can also affect the excitability
of motoneurones (Woolf, 1983; Woolf, 2007); however the underlying mechanisms are poorly understood.

The aim of this thesis was to investigate how central sensitization affects motoneurone excitability in OA. The effect of central sensitization on motoneurone excitability is an important consideration in understanding the pathophysiology of OA because modulation of motoneurone excitability can have a significant impact on the normal biomechanics of the joint. Aberrant joint mechanics is a leading risk factor for the development and progression of OA (Hurley, 1999). Accordingly, the findings of this research may provide insight into the mechanisms and potential therapeutic applications in the treatment and management of OA.
2.1. OSTEOARTHRITIS

2.1.1. Definition and Diagnostic Criteria

Osteoarthritis is a degenerative joint disease that is characterized by damage to the articular cartilage and underlying bone of a joint. This disease commonly affects load-bearing joints like the hips and the knees (Felson et al. 2000). The load-bearing capacity of joints is compromised when there is a defective articular cartilage as the cartilage functions to absorb and distribute loads to the bone (Chai et al. 2007). The degeneration of the articular cartilage occurs early in the pathogenesis of OA followed by degradation of other joint structures (Schaible and Grubb, 1993). A diagnosis of OA (Appendix A) is eventually made when radiographic evidence demonstrates osteophytes, joint space narrowing or loss, cyst formation and sclerosis of the subchondral bone (Roach and Tilley, 2007).

2.1.2. Pathogenesis

The breakdown of articular cartilage is the first stage in OA disease development (Schaible and Grubb, 1993). The progression of the disease is driven by inflammation and further mechanical injury on the joints (Roach and Tilley, 2007). The inflammatory process is also closely associated with the development of many of the clinical manifestations of OA. During disease development, the cartilage breaks down and causes the release of degradative enzymes along with inflammatory mediators such as bradykinin and substance P (Schaible et al.
2002). These transmitters further trigger the release of inflammatory mediators causing the sensitization of afferent neurons that innervate the joint structures (Schaible et al. 2002). In later stages of OA, pain and inflammation can also be triggered by the formation of osteophytes and via neuropathic mechanisms (Mease et al. 2011). Neuropathic mechanisms in OA can include damages to the articular nerve that results from the breakdown of the articular cartilage (Latremoliere and Woolf, 2009).

Transmitters and their receptor systems play a pivotal role in maintaining persistent inflammation and causing the sensitization of afferent and spinal cord neurons, events known as peripheral and central sensitization, respectively (Schaible and Grubb, 1993). A very important transmitter in this process is glutamate which is released into the dorsal horn from the presynaptic terminals of the primary afferent neurons. Glutamate and its receptors (NMDA and non-NMDA receptors) have been implicated in generating and maintaining hypersensitivity during inflammation (Neugebauer et al. 1994). An enhanced release of glutamate is apparent during the development of arthritis (Sluka and Westlund, 1993). In addition to glutamate, nociceptors contain neuropeptides such as substance P which are released in increasing amounts into the spine to activate receptors on the spinal cord neurons (Mapp and Kidd, 1994; Mense, 1994). The release of substance P into the upper ventral horn and dorsal horn during acute joint inflammation has been noted in cats (Schaible et al. 1990). Further, researchers suggest that spinal prostaglandins and proto-oncogenes, transmitters that are released into the dorsal and ventral horns during inflammation, may also play a role in sensitizing spinal cord neurons (Ebersberger et al. 1999; Schaible et al. 2002).
2.1.3. The Nociceptive System in Osteoarthritis

Pain in OA is triggered by inflammatory stimuli in the joint structures (Schaible et al. 2009). Joint inflammation causes peripheral sensitization where the primary afferent neurons are sensitized. Peripheral sensitization leads to the sensitization of dorsal horn neurons making them hyperexcitable (Schaible and Grubb, 1993). This is a phenomenon known as central sensitization.

There are several nociceptors involved in pain transmission in OA. Both Aδ and C afferent fibres innervate the structures of the joint including the synovial capsule, menisci and subchondral bone (Mease et al. 2011). These fibres respond to noxious mechanical, thermal and chemical stimulation. Aβ fibres also innervate the joint structures (Schaible and Ebersberger, 2009). These fibres are proprioceptive in nature and respond to joint movement.

Peripheral sensitization causes changes in the behaviour of afferent fibres. The low-threshold non-nociceptive mechanoreceptors, Aβ and Aδ, show an increased response to pressure and many of the high-threshold nociceptors, Aδ and C, start to respond to light pressure and movements. The fibres that are normally mechano-insensitive (i.e. silent C-nociceptors) also start to respond to mechanical stimulation from the joint (Grigg et al. 1986). As a result, normally painless mechanical stimuli are able to activate the nociceptive system (Schaible and Grubb, 1993).

The continuous and prolonged input from the peripheral nociceptors and mechanoreceptors causes central sensitization. The sensitized dorsal horn neurons are classified as either nociceptive-specific (NS) or wide-dynamic range (WDR) neurons. Normally, NS neurons only respond to noxious stimuli while WDR neurons respond to noxious stimuli and
may respond to innocuous stimuli (Schaible and Grubb, 1993). During inflammation, however, the spinal cord undergoes various changes and the response properties of the spinal neurons change.

Deep dorsal horn neurons have intrinsic properties that govern their firing behaviour in response to the inputs that they receive (Derjean et al. 2003). The generation of plateau potentials in the dorsal horn neurons sustain the firing of the neurons and result in afterdischarges that amplify the output response to synaptic input (Derjean et al. 2003). Plateau potentials are caused by persistent inward currents in the neurons. These currents result from neurohormonal modulation by glutamate and gamma-aminobutyric acid receptors (Derjean et al. 2003). Consequently, as a result of the changes the neurons undergo, WDR neurons respond increasingly to noxious and innocuous stimuli, and NS neurons reduce their threshold so that they respond to stimuli that are normally innocuous (Neugebauer and Schaible, 1988; Schaible and Grubb, 1993).

Changes that occur to the neurons in response to inflammation are supposed to be protective in normal conditions. Pain is evoked by innocuous stimuli facilitating the repair and recovery of an injured body part by limiting its movement and use (Latremoliere and Woolf, 2009). However, in conditions like OA, constant inflammation causes further pain instead of recovery from central sensitization.

2.1.4. Pain in Osteoarthritis

Central sensitization is implicated in underlying persistent pain in OA. This phenomenon produces and enhances pain even in the absence of disease pathology (Woolf, 2011). The degree
of pain experienced by individuals with OA is not substantiated by the severity of OA demonstrated in radiographs (Bedson and Croft, 2008) and individuals with knee OA who reported high pre-operative pain experienced persistent pain even after surgery (Lundblad et al. 2008).

The presence of central sensitization in OA has been confirmed by several studies. Changes that occur to the dorsal horn neurons during central sensitization are implicated in the observations noted in the studies. These studies demonstrate that pain is augmented in OA (Bajaj et al. 2001; Arendt-Nielsen et al. 2010). Individuals with OA demonstrate an increased and long-lasting pain response to experimentally induced muscle pain as well as a larger area of skin hypersensitivity compared to individuals without OA (Bajaj et al. 2001). Low pain pressure thresholds along with enhanced temporal summation to pain and a lack of descending analgesic activity are also apparent (Imamura et al. 2008). A low pain pressure threshold is correlated to higher pain intensities in OA (Arendt-Nielsen et al. 2010) whereas a loss of descending activity means that there are impairments in the descending inhibition that usually modulates dorsal horn discharges during joint nociception (Cervero et al. 1991; Schaible et al. 1991). As a result, there are increases in the dorsal horn discharges and an increase in their receptive fields (Schaible, 2004).

Imaging of the OA brain, furthermore, revealed a lower threshold to stimuli perception and an increased sensitivity to mechanical stimulation in referred pain areas (Gwilym et al. 2009). The brainstem, which plays an important role in amplifying mechanical stimuli in areas of hypersensitivity (Zambreanu et al. 2005), also demonstrated a greater activation in individuals with OA (Gwilym et al. 2009).
2.2. CENTRAL SENSITIZATION

2.2.1. Definition

Central sensitization is the state in which the neurons of the central nervous system are sensitized and become hyperexcitable to noxious and innocuous stimuli (Woolf, 2011). It is initiated and sustained by persistent peripheral inputs which alter the excitability of neurons within the central nervous system (Schaible and Grubb, 1993).

Central sensitization is an important mechanism in the pathophysiology of clinical conditions. There is evidence to suggest that central sensitization manifests as pain hypersensitivity in many clinical conditions including OA (Schaible et al. 2002). Other clinical conditions are temporomandibular disorders (Mohn et al. 2008; Fernández-de-las-Peñas et al. 2009), headaches (Jensen and Olesen, 1996; Buchgreitz et al. 2006), fibromyalgia (Gibson et al. 1994; Desmeules et al. 2003; Graven-Nielsen et al. 2000) and irritable bowel syndrome (Wilder-Smith and Robert-Yap, 2007).

Central sensitization can be experimentally-induced in animals and humans. It can be evoked using electrical stimulation (Wall and Woolf, 1984), thermal stimuli (Woolf, 1983) and chemicals like mustard oil (Woolf and King, 1990) and capsaicin (LaMotte et al. 1992; Arendt-Nielsen and Andersen, 2005). Studies that have employed these techniques have found that there are increases in pain responses and altered pain sensitivities analogous to clinical conditions (Arendt-Nielsen and Andersen, 2005). The advantage of experimentally-induced central sensitization is that it allows us to control the stimulus that evokes central sensitization and assess the responses that it elicits (Arendt-Nielsen and Andersen, 2005).
2.2.2. Manifestations

Pain hypersensitivity arising from central sensitization can manifest in three ways, including secondary hyperalgesia, alldynia and referred pain (Woolf, 2011). Hyperalgesia is an increased sensitivity to a noxious pain stimulus; in contrast, alldynia is the perception of pain to normally non-noxious stimuli (Arendt-Nielsen and Andersen, 2005). Hyperalgesia can be further divided into two forms, primary and secondary hyperalgesia. Primary hyperalgesia is observed at the site of injury and is the result of sensitization of peripheral nociceptors. Secondary hyperalgesia is indicative of the sensitization of dorsal horn neurons as hyperalgesia is observed in normal tissues beyond the site of pathology and is characterized as an uncomfortable or painful response to mechanical stimuli (alldynia) (Arendt-Nielsen and Andersen, 2005). Alldynia is a sensory manifestation within the area of secondary hyperalgesia (Arendt-Nielsen and Andersen, 2005). Referred pain, moreover, is the experience of pain in structures away from the primary site of injury (Arendt-Nielsen and Svensson, 2001).

The manifestations occur as a result of changes in the receptive fields of the dorsal horn neurons. There is an expansion in the original receptive fields of dorsal horns causing them to respond to stimuli in those newfound areas as well (Arendt-Nielsen and Henriksson, 2007). This results in secondary hyperalgesia and alldynia. Further, new receptive fields emerge at distances away from the original receptive field in referred pain (Arendt-Nielsen and Henriksson, 2007).

The distribution patterns of these receptive fields demonstrate that pain is referred to dermatomes and myotomes that belong to the common neuronal segment as the pathology (Brown, 2005). For example, if a nerve from the C5 nerve root innervates a painful muscle, with
ongoing pain, pain is also perceived in the dermatomes and myotomes of C5 while also extending into the areas innervated by the C4 and C6 nerve roots (Brown, 2005; Gerwin, 2010). This segmental phenomenon occurs because previously ineffective synaptic connections of the dorsal horn neurons become functionally effective with central sensitization resulting in the formation of new receptive fields at the segmental dermatomes and myotomes (Hoheisel et al. 1993). Further support for this phenomenon has been provided by study findings demonstrating that increasing central sensitization in a dermatome increases the sensitivity of trigger points of a muscle found within the common neuronal segment (Srbely et al. 2010).

The sensitization of dorsal horn neurons and the manifestations that result have been extensively studied by researchers. We know that central sensitization lowers the activation threshold of the dorsal horn neurons and introduces a state of hyperexcitability (Schaible and Grubb, 1993). However, the effects of central sensitization on ventral horn neurons, particularly motoneurones, remain to be adequately elucidated in OA.

2.3. MOTONEURONES

Motoneurones are found in the ventral horn of the spinal cord (Gardiner, 2001). Alpha-motoneurones and the muscle fibres that they innervate form the motor unit (MU). MUs are responsible for controlling the movement of individuals by generating the contraction of muscles (Gardiner, 2001). They are considered to be the functional units of the muscle and, essentially, the neuromuscular system.

Muscle force is produced during muscle contraction and is generated by the recruitment and rate-coding of the MUs based on their recruitment threshold (Milner-Brown et al. 1973).
Recruitment threshold is the force at which a MU is recruited into action. The MUs follow orderly recruitment where low-threshold, smaller sized MUs are recruited first during force demands (Henneman et al. 1965; Henneman and Olson, 1965). This is followed by the rate-coding of these recruited MUs along with the recruitment of additional higher threshold MUs at increasing forces (Milner-Brown et al. 1973).

The activation of motoneurones for MU recruitment is determined by the intrinsic excitability of the motoneurones as well as the synaptic input it receives (Enoka, 2008). Afferent neurons, the cortex and the brainstem are all sources of input to the motoneurones (Enoka, 2008). When a motoneurone is activated (i.e. depolarized), it sends a nerve impulse known as an action potential to the muscle fibres resulting in the activation of the fibres causing the muscle to contract (Gardiner, 2001). Accordingly, the excitability of motoneurones is important to consider in the contraction of muscles.

2.4. ELECTROMYOGRAPHY

2.4.1. Definition

Muscle activity can be assessed in clinical and research settings by using electromyography (EMG). Specifically, EMG is a technique that is used to measure the electrical activity of muscles. It utilizes electrodes that measure the action potentials that travel along the fibres of the muscle. Two electrodes are typically employed to differentially record the MU action potentials and provide recordings, or electromyograms, for analysis (Enoka, 2008).

Electrodes can be placed on the skin (known as surface electrodes) or in the muscle (indwelling or intramuscular electrodes). Surface electrodes are non-invasive and record from
superficial muscles. Surface EMG, therefore, provides an overall representation of a muscle’s activity (Soderberg and Cook, 1984; Merletti and Farina, 2009). A problem with surface EMG is the spatial filtering of signals by the tissues that are found between the muscle and the electrodes (DeLuca, 1997). Accordingly, indwelling EMG provides signals that are more accurate for the analysis of MUs than surface EMG because deeper muscles can be directly accessed (Merletti and Farina, 2009). Indwelling electrodes sample from a number of MUs from a localized area in the muscle (Soderberg and Cook, 1984). The action potentials belonging to these individual MUs can be identified and analyzed by decomposing the signal.

2.4.2. Signal Decomposition in Electromyography

Signal decomposition is the identification and classification of action potentials belonging to a single MU (Merletti and Farina, 2009). It involves the detection of action potentials of a MU based on their shape—action potentials generated by a MU are similar in shape compared to action potentials belonging to other MUs. The identification of action potentials is more complex at greater muscle contractions because the action potentials of different MUs overlap to form superimposed action potentials (Merletti and Farina, 2009). As such, accurately decomposing an EMG signal at higher force contractions (typically over 50% of an individual’s maximum voluntary contraction (MVC)) is very difficult (Merletti and Farina, 2009).

Signal decomposition is significant in assessing the contribution of the neural drive to muscles. We can analyze the behaviour of MUs based on the behaviour of their identified/classified action potentials. The recruitment and de-recruitment of a MU, for instance,
can be examined by noting the force at which the action potentials started and stopped firing (Enoka, 2008).

2.4.3. Force and Electromyography

An important consideration in the analysis of MU behaviour in EMG is the relationship between force and EMG. The force-EMG relationship depicts the association between the force produced by the muscle and the EMG recordings taken from the muscle (Fuglevand et al. 1993). This relationship is affected by various factors like the length of the muscle, type of muscle contraction and fatigue (Soderberg and Cook, 1984). For example, the force produced by muscle increases as EMG decreases at longer muscle lengths (Soderberg and Cook, 1984). Dynamic contractions, compared to isometric contractions, also affect the force-EMG relationship because the muscle length varies (shortens and lengthens) as does the rate of shortening/lengthening (Enoka, 2008). With faster contractions, EMG increases rapidly when compared to the muscle force as well (Soderberg and Cook, 1984). Fatigue also changes the properties (amplitude and frequency) of the EMG (Viitasalo and Komi, 1977). For instance, the frequency of the EMG signal decreases with fatigue (Viitasalo and Komi, 1977). The factors that affect the force-EMG relationship must be addressed in order to accurately assess the behaviour of the MUs including their excitability.

2.5. CENTRAL SENSTIZATION AND MOTONEURONE EXCITABILITY
The primary objective of our thesis was to investigate the effect of central sensitization on motoneurone excitability. Our thesis is guided by observations that motoneurones can be modulated by afferent inputs and have demonstrated an increased responsiveness during acute joint inflammation.

Many cutaneous, muscle and joint afferents influence motoneurones of the ventral horn (Eccles and Lundberg, 1959; King et al. 1990). Studies have shown that the majority of ventral horn neurons respond to both low- and high-threshold mechanical stimulation on the skin (King et al. 1990). Central sensitization that results from repetitive stimulation of the skin influences motoneurones as well. Flexor motoneurones were found to demonstrate a prolonged post-synaptic response after repetitive stimulation of the skin (King et al. 1990) and a greater nociceptive withdrawal response is evoked with repetitive cutaneous stimulation (Woolf, 1983; Woolf, 2007).

Of interest to our particular research objective is evidence from past research that indicate an increase in motoneurone responsiveness via acute joint inflammation. In normal conditions, alpha-motoneurones are weakly influenced by joint afferents but undergo considerable influences from joint afferents during noxious conditions (He et al. 1988; Schaible and Grubb, 1993). A portion of the flexor alpha and gamma-motoneurones demonstrated an increase in their receptive fields when acute inflammation was induced in the knee joint of a cat (He et al. 1988). Motoneurones that were not responsive to mechanical stimulation in the absence of inflammation also became responsive following acute joint inflammation (He et al. 1988). These observations suggest that central sensitization that develops from inflammation may be able to affect motoneurone excitability as well.
2.6. MECHANISMS OF MUSCLE AND JOINT PAIN

Studies on nociceptive pain have examined motoneurone behaviour. Central sensitization, however, is different from cutaneous, muscle and joint pain in that it is a modality of pain and not nociceptive pain itself. Nociceptor input may be needed to trigger central sensitization but is not needed to maintain it (Latremoliere and Woolf, 2009). Thus, studies on nociceptive pain may offer insights on motoneurone behaviour but they should be approached with the understanding that central sensitization and nociceptive pain are distinct entities.

Studies on experimental muscle and knee pain have yielded a plethora of findings on recruitment and firing behaviour of MUs. Martin et al. (2008) noted increased motoneurone excitability in painful muscles. Findings also suggest that there are decreases in the firing behaviour of MUs during muscle pain (Sohn et al. 2000; Farina et al. 2004). A decrease in MU firing rate was also observed as a response to knee pain along with changes in the MU recruitment behaviour where additional motoneurones were recruited for a given force (Tucker and Hodges, 2009). Transient increases in resting motoneurone activity have also been noted in the masseter and tibialis anterior muscles following acute muscle pain (Svensson et al. 1998). However, the researchers found that acute muscle pain is inadequate in maintaining a prolonged increase in resting motoneurone excitability (Svensson et al. 1998).

The findings from these studies demonstrate that nociceptive pain is able to alter motoneurone behaviour in different ways. There is a controversy in how nociceptive pain affects the motoneurone firing rate. A consistent finding, however, is that force is maintained despite changes in the discharge rate (Tucker et al. 2009). It has become apparent that there is a change in the recruitment of MUs during pain where new MUs are recruited to maintain force (Tucker et
Tucker and colleagues (2009) found that MUs that were newly recruited were not those that were expected to be recruited according to the orderly recruitment of MUs. Higher threshold MUs were unexpectedly being recruited (Tucker et al. 2009).

It is difficult to reach a consensus on the influence of nociceptive pain on motoneurone excitability given the current literature. Findings from these studies suggest that motoneurone excitability might increase or decrease following nociceptive pain. There are also notions that pain might influence the excitability of low- and higher-threshold motoneurones differently (Martin et al. 2008; Tucker et al. 2009). As a modality of pain that can be triggered by nociceptive pain, examining the influence of central sensitization on motoneurone excitability may be able to provide more information on understanding these findings.

2.7. THE IMPORTANCE OF RESEARCH ON THE EFFECTS OF CENTRAL SENSITIZATION ON MOTONEURONE EXCITABILITY

An investigation of the effect of central sensitization on motoneurone behaviour in OA is important because it will further our understanding about a fundamental biological mechanism in OA. In addition, any alterations in motoneurone excitability may play an important role in changing the biomechanics of the arthritic joint promoting further joint degeneration.

Central sensitization has been mostly studied as a pain mechanism in OA thus far (Schaible and Grubb, 1993; Woolf, 2011). To our knowledge, the effect of central sensitization on motoneurone excitability in OA has not been explored before. The results of this study will further our understanding about this neuroadaptive mechanism and provide insight into how it can potentially affect muscle function.
Muscles are an important part of the neuromuscular system. The neuromuscular system of the joint is made up of the associated bones, ligaments, capsule, cartilage, muscles and nerves (Hurley, 1999). Joint dysfunction and degeneration may result if any of the components become dysfunctional (Hurley, 1999).

The neuromuscular system is an important consideration in the pathophysiology of OA since biomechanical abnormalities such as muscle weakness and joint laxity are strongly associated with the progression of OA (Slemenda et al. 1997; Felson et al. 2000). Subjects with knee OA present quadriceps muscle weakness as well as a reduction in quadriceps voluntary activation in achieving maximum contractions (Hurley et al. 1997; Machner et al. 2002).

A greater activation of muscles associated with joint OA has been noted in individuals with OA in the performance of submaximal tasks. Marks and colleagues (1994) found that there was a greater activation of the quadriceps femoris muscle during voluntary isometric contractions in women with knee OA. In an additional study, alterations in muscle activation were observed during gait when activities of the vastus lateralis, medial hamstrings, tibialis anterior and medial gastrocnemius were measured in knee OA (Childs et al. 2004). In hip OA, a greater activation of the gluteus medius was seen in the affected individuals (Sims et al. 2002).

Muscle dysfunction may contribute to the early structural degeneration of joints in OA (Becker et al. 2004; Segal et al. 2010). Muscles are important for protecting the joints; any alterations in the neuromuscular system at the level of the motoneurone can cause impairments in the neuromuscular protection mechanism of joints. Impairments in this mechanism can lead to increasing loads placed on the joint causing damages to the joint structure (Becker et al. 2004). Indeed, muscle dysfunction has been established as a risk factor for the development and
progression of OA (Slemenda et al. 1997; Felson et al. 2000). Thus, knowledge of the impact of central sensitization on motoneurone excitability may provide insight into potential therapeutic strategies for the prevention or slowing down of joint degeneration.
CHAPTER 3
OVERVIEW OF THESIS OBJECTIVE AND HYPOTHESIS

The overall objective of this research project was to investigate the segmental modulatory effect of central sensitization on motoneurone excitability in OA.

We investigated the specific hypothesis that an increase in central sensitization within a spinal segment will cause a greater increase in the excitability of motoneurones in subjects with OA when compared to healthy controls. We hypothesized that MUs from individuals with OA will demonstrate an increased excitability owing to their pre-existing joint pathology.

We experimentally evoked central sensitization in dermatomes within the common spinal segments as the first dorsal interosseous (FDI) muscle and subsequently monitored the behaviour of the MUs in individuals with cervical spine OA versus controls without OA. We assessed the recruitment threshold forces of single MUs of the FDI muscle pre- and post- experimentally induced central sensitization. A significant decrease in the recruitment threshold force of MUs in OA subjects compared to the control subjects will be indicative of increased motoneurone excitability.

The FDI muscle was specifically chosen as the muscle of interest in our study because the force that is evoked by the index finger during an abduction motion is estimated to be linearly proportional to the isometric force that is produced within the muscle (DeLuca et al. 1982). This allows us to correlate the MU activity of the muscle with the force that is produced by the muscle and accurately assess the behavior of the MUs (DeLuca et al. 1982).
The effect of OA on MU excitability was examined by analyzing the FDI MUs of subjects with cervical spine OA at the C6-C7, C7-T1 levels. These spinal segments are richly innervated by nerves including nociceptive afferents (A\(\delta\) and C-fibres) (Inami et al. 2001; Johnson, 2004) and persistent inflammation from facet joint OA and disc degeneration occurs at these levels (Brisby, 2010). The spinal segments were chosen for our study because they are common to the innervations of the FDI muscle. The FDI is innervated by the ulnar nerve from the C8 and TI spinal nerve roots (Kaneko et al. 2003; Stewart, 2010).

Central sensitization was experimentally induced in our study by topical capsaicin (0.075\%). Capsaicin, an ingredient found in hot chili peppers, acts on nociceptors to activate the release of inflammatory substances like substance P resulting in central sensitization (Winter et al. 1995; Arendt-Nielsen and Andersen, 2005). Topical capsaicin is also less invasive and better tolerated by subjects than intradermal capsaicin (Arendt-Nielsen and Andersen, 2005). Capsaicin was applied to the dermatomes and brush allodynia testing was used to confirm the presence of secondary hyperalgesia to mechanical stimuli (Srbely et al. 2010). Secondary mechanical hyperalgesia is an indicator of central sensitization (Arendt-Nielsen and Andersen, 2005).

The MUs of the FDI muscle were recorded using indwelling EMG before and after the application of capsaicin to evaluate whether central sensitization altered their recruitment threshold force. EMG is a technique that is used to measure the electrical activity of skeletal muscles (Soderberg and Knutson, 2000). Accordingly, any alterations in the MU recruitment threshold force in OA subjects pre vs. post-capsaicin would be attributed to an increase in central sensitization in these individuals.
4.1. SUBJECTS

This study was approved by the University of Guelph Ethics Committee and informed consent was obtained from all the participants prior to participation. Seven subjects (56 ± 8.0 years; 3 females and 4 males) diagnosed with cervical spine OA participated in this study (OA group). They were recruited from a local outpatient clinic in Guelph, Ontario. The inclusion criteria included an OA severity score of 2 (out of a possible 5) or more on the Kellgren-Lawrence OA Grading Scale (Kellgren and Lawrence, 1957) at either the cervical disc or cervical joint level at the C6-C7 and/or C7-T1 segments on the radiographs of the OA subjects. The radiographs were assessed by a medical radiologist at McMaster University. Ten healthy adults (22 ± 1.0 years; 5 females and 5 males) were also randomly recruited from the student population at the University of Guelph (Guelph, Ontario, Canada). The exclusion criteria for the healthy controls were the diagnosis of arthritis and a history of chronic pain.

Subjects did not present with any acute pain at the onset of the study nor have any neurological problems or conditions that may alter their somatosensory processing and impair their consent or feedback during the experiment. The subjects were informed of the experimental protocol including knowledge that a cream that may or may not contain capsaicin will be applied to areas of their skin.

Our OA and control subjects were not age-matched because younger individuals are less likely to have degenerative changes that can result in central sensitization. Research has demonstrated that there are age-related alterations to the facet joints consistent with OA in
individuals over 37 years of age whereas individuals under the age of 20 do not demonstrate any alterations (Fletcher et al. 1990). Since it is difficult to quantify the degree of central sensitization in individuals (Woolf, 2011), we employed younger healthy controls in our study design.

4.2. EXPERIMENTAL SETUP

Each subject participated in the experimental procedure that lasted 2 hours. The subjects were instructed to wear clothing that exposed the targeted regions of capsaicin application. A hospital gown was provided upon request. Each subject was instructed to sit comfortably in a chair with their right arm strapped into a hand restraint (Figure 1). The shoulder of the subjects was abducted at angle of about 15° and their elbow joint was flexed at about 90° with the palmer side of their right hand placed downwards on the hand restraint. The last three fingers of the subjects were strapped in with a velcro strap and their thumb was fully abducted. The index finger of the subject was coupled against a force transducer. A linear strain force transducer (Finger Dyno, Biomech Engineering, Guelph, CAN) was placed on a custom built hand restraint. The force from the transducer (0.1V/N) was sampled at 5000 Hz by the Power 1401 (CED, Cambridge, UK) system and stored on a computer.
Indwelling electrodes (200 µm tungsten microelectrodes, FHC Inc., Bowdoin, ME) were inserted into the muscle belly of the FDI muscle to record the activity of the MUs. The readings from the indwelling electrodes were amplified by 10,000x, bandpass filtered between 300 Hz and 3000 Hz using an isolated bioamplifier unit (ISO-80, WPI, Sarasota, FL) and sampled at 4000 Hz (Power 1401, CED, Cambridge, UK). To ensure that recorded force readings were solely generated by the abduction motion of the index finger, surface electrodes (Ag/AgCl Kendall Medi-Trace 130 ECG conductive adhesive electrodes, Tyco Healthcare Group LP, Mansfield, MA) were also placed on the extensor and flexor carpi radialis muscles to monitor
wrist abduction motion. We wanted to prevent contributions of wrist abduction motion to the force and these readings were bandpass filtered (10-1000 Hz), amplified 1000x (AMT-8, Bortec Biomedical, Calgary, CAN) and sampled at 2000 Hz (Power 1401, CED, Cambridge, UK).

4.3. PROTOCOL

The subjects were instructed to perform three MVCs by pressing their index finger against the force transducer. The average value of the two (out of three) highest peak forces of the MVCs was calculated to establish 20% MVC. This value was used as the target force level for all subsequent contractions in the experiment. We chose 20% MVC because we only wanted to sample low-threshold MUs; previous work has indicated that all the MUs in the FDI muscle are recruited by 50% MVC (Milner-Brown et al. 1973; DeLuca et al. 1982). In addition, MU recruitment plays an important role in the production of force at low levels (Duchateau et al. 2006). Once their target force was set, subjects were required to practice doing voluntary isometric contractions (Figure 2). Visual feedback of the real-time force was provided using the Spike 2 (Version 7.03, CED) software. The target force level was indicated as a straight dotted line on the computer screen. Experimenter feedback was also provided for accuracy. Once the subjects demonstrated that they were able to attain the target force level in five consecutive contractions, the subjects progressed to the next stage of the protocol. The first set of trials required the subjects to perform five voluntary isometric contractions approximately reaching 20% MVC. The contractions were to be 10 seconds in duration. The contractions were also separated by a 10-second rest period to avoid the facilitation of MU recruitment (Gorassini et al.
The first sets of trials performed in the protocol were considered as the baseline, pre-trial condition before the application of capsaicin.

Figure 2: A schematic overview of the protocol used in this experiment. Maximum voluntary contractions (MVCs) were done at the beginning and end of the experiment. Isometric voluntary contractions at 20% MVC were performed during the pre-trial condition as well as at 10, 20, 30 and 40 min after the application of capsaicin. Pain intensity visual analog scale (VAS) and brush allodynia readings were taken before the contractions during the trials after capsaicin application. Subjects were also asked to describe the sensations that they felt at these points.

An analgesic topical ointment known as Zostrix (Hi Tech Pharmacal Co., Amityville, NY) containing 0.075% capsaicin was applied bilaterally to the following dermatomes in order to evoke a hetero-segmental effect: C3, C4, C5 and parts of C6, T1 and T2. These regions were targeted as the C7 and C8 dermatomes were not directly accessible. The C3-T2 dermatomes include the lower neck, upper back, shoulder (acromion process and superior scapula) and upper arm (underside, proximal to elbow) regions (Figure 3). Pilot trials from our lab have revealed that applying a thin layer of the cream, about 30 mg (±5 mg depending on the surface area ranging roughly from 600-700 cm²) was sufficient in inducing secondary hyperalgesia in the subjects (Jegatheeswaran et al. 2010, unpublished). This information was used to standardize the
amount of capsaicin that was applied to the subjects in our study based on the surface area of their dermatomes.

![Figure 3: An approximation of the dermatomes (shaded regions with dotted boundaries in the figure) that were targeted for applying capsaicin. These dermatomes consisted of the C3, C4, C5 regions as well as parts of C6, T1 and T2.](image)

Subjects performed five contractions after the application of capsaicin at 10, 20, 30 and 40 min time points. Secondary hyperalgesia measures (brush allodynia testing) as well as pain intensity (visual analog scale) readings were recorded at each time point. Subjects were also asked to describe how the targeted dermatomal regions felt during these time points.

4.4. PAIN INTENSITY AND HYPERALGESIA MEASURES

The pain intensity response of the subjects after the application of capsaicin was measured on a 10 cm long visual analog scale (VAS). The pain VAS scale is a reliable way of
recording the intensity of pain felt by a subject (Gift, 1989). The subjects were instructed to place a mark on the scale denoting their pain score at each time point. A score of 0 is “no pain” and 10 is “very severe pain” (Figure 4).

![Figure 4: The VAS scale used by the subjects to score their pain intensity response to capsaicin. “No pain” is considered a score of 0 while “very severe pain” was a score of 10. The figure is not drawn to scale.](image)

The presence of secondary mechanical hyperalgesia, a manifestation of central sensitization, was assessed using brush allodynia testing (Arendt-Nielsen and Andersen, 2005; Srbely et al. 2010). The brush allodynia readings were obtained by gently stroking the skin with a brush in small steps along three trajectories (Figure 5) towards the region of primary hyperalgesia (i.e. the area of the back where capsaicin was applied). When there was a change, either in intensity or quality, in how the brush strokes were perceived, that point of change was noted and the distance from the zone of primary hyperalgesia (in cm) was measured (Arendt-Nielsen and Andersen, 2005). The average of these values was represented as the brush alldynia value and normalized to the brush alldynia value at 10 min to compare between subjects. Taking the average of the 3 measurements from the area of primary hyperalgesia (presented in Appendix C, Table 9) was sufficient in indicating the presence of central sensitization for the purposes of this particular thesis. Accordingly, subjects were excluded from
the study if they failed to demonstrate brush allodynia sensitivity at two or more readings (i.e. brush allodynia value = 0) over the 10 to 40 min testing period after the application of capsaicin.

Figure 5: The arrows depict the three trajectories that the brush was stroked along to identify changes in intensity or quality of brush strokes. The trajectories are moving towards the primary area of hyperalgesia where capsaicin was applied.

4.5. DATA ANALYSIS

The recruitment threshold force of the MUs was examined in this study. The threshold was considered to be the force at which the first MU action potential of the MU fired. The recruitment threshold force was obtained by spike triggered averaging force for 50 ms post the peak of the MU action potential spike. The force values (Appendix B, Table 6 and Table 7) are
expressed as a percentage of their recruitment threshold force at pre-trial (i.e. %pre-trial force) to compare differences in recruitment threshold forces between the trials before (pre-trial) and after (10, 20, 30 and 40 min) the application of capsaicin.

Identification of the MUs utilized Spike 2 (Version 7.03, CED) software’s template matching feature. The identification of low-threshold MUs (<20% MVC) will provide us with more accuracy in our readings as the superimpositions of MU potentials occurs at increasing force levels (Boe et al. 2005). Twenty-one MUs met the inclusion criteria (10 from the controls and 11 from OA subjects) and were subsequently analyzed. The inclusion criteria for analysis included the presence of the MUs consistently across 3 or more contractions within each trial where the contraction reached a peak force of approximately 20% MVC. The recruitment threshold forces between the contractions had to also demonstrate a low coefficient of variation (less than 0.2) in order for the MU to be included for analysis.

4.6. STATISTICAL ANALYSIS

The results from this study are expressed as mean ± standard deviation (SD). Statistical tests were performed to determine the significance of the results using the SPSS statistical software, version 19 (SPSS, Chicago, IL). Significance was accepted at the 0.05 level (P ≤ 0.05).

The MUs provided by the subjects were the experimental units of our study. If subjects contributed more than one MU to the data pool, a Pearson’s product moment correlation was performed to assess the strength of the relationship between the recruitment forces of the MUs from a single subject. A strong correlation (greater than 0.7) that was statistically significant warranted the calculation of the average of MU forces provided by the subject. The average
value, and not the individual MU recruitment force values, from the subject would then be included in the data pool.

The examination of between-group recruitment threshold forces of the MUs was necessary to address our study hypothesis. Accordingly, an independent T-test was used to compare the average MU recruitment threshold forces at 10, 20 and 40 min after the application of capsaicin between the two groups. An independent T-test was also utilized to compare the MVCs that were performed at the beginning and at the end by the OA and control subjects. Differences between the MU recruitment forces between the groups were explored using the dependent T-test.

A Mann-Whitney U-test was used to compare the forces at 30 min post-capsaicin between control and OA subjects. Mann-Whitney U-test is a nonparametric alternative to the independent T-test that was used when the values were not normally distributed. Normality of the data was determined by the Shapiro-Wilks test. The independent variable in these tests was the subject group (OA or controls) and the recruitment threshold force was the dependent variable.

The recruitment threshold forces of the MUs within the OA group at each time point was examined using an analysis of variance (ANOVA) with repeated measures. The Friedman test, a nonparametric alternative to the ANOVA with repeated measures, was used to analyze the forces within the control group. The repeated measurements were the recruitment threshold forces taken at each time point (pre-trial, 10, 20, 30 and 40 min) for each subject.

The association between OA severity and location and the recruitment threshold forces was assessed utilizing a two-way ANOVA. The independent variables (factors) were the OA scores (2 or 3(or more)) at any of a) the spinal levels of interest (C6-C7, C7-T1), and b) above
the spinal levels of interest (C2-C3, C3-C4, C4-C5, C5-C6) (Appendix A, Table 5). If significant differences were found within the factors, a Pearson’s correlation was employed to examine the strength of the relationship.

A Mann-Whitney U-test was used to compare the brush allodynia values (normalized to brush allodynia value at 10 min) between the groups at the 20 and 30 min-time points. The brush allodynia values at 40 min were compared between the groups using an independent T-test. We analyzed brush allodynia values to determine whether there are significant differences in the degree of secondary mechanical hyperalgesia between the groups. Further, the relationship between VAS scores and brush allodynia values after the application of capsaicin was calculated using the Pearson’s correlation.
Ten MUs from 7 controls (21 ± 0.1 years; 4 females and 3 males) and 11 MUs from 6 OA subjects (53 ± 4.0 years; 3 females and 3 males) were identified and used for analysis. An OA subject and 3 controls were excluded from the study because they failed to demonstrate brush allodynia at two or more readings at the time points after the application of capsaicin.

Three subjects from the OA and control groups provided 2-3 MUs to the data pool for analysis. Statistical analysis of the relationship between multiple MUs provided by subjects failed to reveal a statistically significant strong correlation (Appendix B, Table 8). As such, all the MUs provided by each subject were included for analysis.

Further, statistical analysis found that the experimental protocol did not induce fatigue as significant differences were not found when the magnitude of the MVCs from the beginning and end were compared within the controls (t(6)=0.320, P=0.760) and OA subjects (t(5)=0, P=1).

5.1. RECRUIMENT THRESHOLD FORCES

The MUs that were analyzed in our study were low-threshold MUs. The average MU recruitment threshold force at pre-trial was 4.00 ± 2.0%MVC in the controls and 5.65 ± 2.30%MVC in the OA group. The forces of the control group MUs were not significantly different than those of the OA group (t(19)=1.752, P=0.096).

The results of our study demonstrate that the recruitment threshold forces, expressed as %pre-trial force, were significantly lower in the MUs from the OA group compared to the MUs from the controls at 10 min (Figure 6, Table 1; t(19)=-2.861, P=0.010), 30 min (U=23, P=0.024) and 40 min (t(10.735)=-2.283, P=0.044) after the application of capsaicin. At 20 min,
differences in the forces between the two groups were not statistically significant ($t(19)=-1.994$, $P=0.061$). The average recruitment threshold forces of OA group MUs were found to be $60 \pm 25\%$, $75 \pm 19\%$, $76 \pm 28\%$ and $88 \pm 36\%$ of their pre-trial recruitment threshold force at 10, 20, 30 and 40 min, respectively, after capsaicin. The average recruitment threshold forces of control MUs was $96 \pm 32\%$ pre-trial force at 10 min before lowering to a threshold force of $93 \pm 23\%$ pre-trial force at 20 min. The average threshold forces of the control MUs then increased to $175 \pm 123\%$ pre-trial force at 30 min and $161 \pm 110\%$ pre-trial force at 40 min. Table 1 provides a summary on the average recruitment threshold forces (%pre-trial force) and their values of significance as determined by statistical tests.

**Table 1**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>OA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Trial</td>
<td>60 ± 25%</td>
<td>96 ± 32%</td>
</tr>
<tr>
<td>10 min</td>
<td>75 ± 19%</td>
<td>93 ± 23%</td>
</tr>
<tr>
<td>20 min</td>
<td>76 ± 28%</td>
<td>93 ± 23%</td>
</tr>
<tr>
<td>30 min</td>
<td>88 ± 36%</td>
<td>175 ± 123%</td>
</tr>
<tr>
<td>40 min</td>
<td>88 ± 36%</td>
<td>161 ± 110%</td>
</tr>
</tbody>
</table>

* denotes a significant difference between the control and OA subjects at the significance level of 0.05 ($P \leq 0.05$).

**Figure 6:** The average recruitment threshold forces (±SD) of the MUs of the control and OA subjects at pre-trial, 10, 20, 30 and 40 min post-capsaicin. The values are expressed as %pre-trial force. *denotes a significant difference between the control and OA subjects at the significance level of 0.05 ($P \leq 0.05$).
Statistical analysis on the recruitment forces within OA group demonstrated that the overall differences were statistically significant (F(4,40)=5.760, P=0.001). Post-hoc analyses using the Bonferroni correction revealed that significant differences were found between the MU recruitment forces at pre-trial and 10 min post-capsaicin (P=0.04), and pre-trial and 20 min post-capsaicin (P=0.014). A Friedman test failed to demonstrate significant differences in the control group between the different time points (X²(4)=7.440, P=0.114).

Table 1. The average recruitment threshold forces of the MUs from the OA group and the controls over the time points along with the U or t-statistic, degrees of freedom and significance, P-value. The forces are presented as %pre-trial force (±SD). Figure 6 is a graphical representation of the data presented in this table. *denotes a significant difference between the MU recruitment threshold forces between the OA and control subjects.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>MUs of OA subjects</th>
<th>MUs of Controls</th>
<th>Statistic (U or t)</th>
<th>Degrees of Freedom (df)</th>
<th>Significance, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Trial</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 min</td>
<td>60 ± 25%</td>
<td>96 ± 32%</td>
<td>t = -2.861</td>
<td>19</td>
<td>0.010*</td>
</tr>
<tr>
<td>20 min</td>
<td>75 ± 19%</td>
<td>93 ± 23%</td>
<td>t = -1.994</td>
<td>19</td>
<td>0.061</td>
</tr>
<tr>
<td>30 min</td>
<td>76 ± 28%</td>
<td>175 ± 123%</td>
<td>U = 23</td>
<td>-</td>
<td>0.024*</td>
</tr>
<tr>
<td>40 min</td>
<td>78 ± 36%</td>
<td>161 ± 110%</td>
<td>t = -2.283</td>
<td>10.735</td>
<td>0.044*</td>
</tr>
</tbody>
</table>

5.2. SEVERITY AND LOCATION OF OSTEOARTHRITIS AND RECRUITMENT THRESHOLD FORCES

The differences in the recruitment threshold forces of the MUs based on the location and severity of OA was assessed by considering the OA severity score at either the cervical disc or cervical joint of two groupings of spinal levels, a) C6-T1 (i.e. the spinal levels of interest in our study: C6-C7, C7-T1), and b) C2-C6 (i.e. above the spinal levels of interest: C2-C3, C3-C4, C4-C5, C5-C6). Within these groups, the MU recruitment threshold forces were grouped into two levels—a) an OA score of 2, and b) an OA score of 3 or more at any of the spinal levels. Two
subjects (4 MUs) with an OA score of 2 and 4 subjects (7 MUs) with a score of 3 were found in the C6-T1 group (Table 2). The C2-C6 group had 3 subjects (6 MUs) with a score of 2 and 3 subjects (5 MUs) with a score of 3 or more.

Table 2. The OA subjects that presented with an OA severity score of 2 versus 3 at any of the spinal levels within the spinal level groups C6-T1 (i.e. C6-C7, C7-T1) and C2-C6 (i.e. C2-C3, C3-C4, C4-C5, C5-C6). The OA severity score is based on the Kellgren-Lawrence OA Grading Scale (Kellgren and Lawrence, 1957).

<table>
<thead>
<tr>
<th>Spinal Levels</th>
<th>Subject</th>
<th>C6-T1</th>
<th>C2-C6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>AE</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Significant differences in the MU recruitment threshold forces between the scores at the spinal level groups (C6-T1 and C2-C6) were only found at 10 min post-capsaicin (Table 3). Results demonstrated that, at 10 min, there were significant differences in the recruitment forces between an OA severity score of 2 and 3 at any of the C6-T1 levels (F(1,8)=7.493, P=0.026). Additional analyses revealed that there was a stronger, statistically significant correlation between an OA severity score of 3 and the recruitment forces at 10 min (r=0.961, n=5, P=0.009) compared to a score of 2 at the levels (r=0.866, n=5, P=0.058). There were significant differences of having a score of 2 or, 3 or more at any of the C2-C6 levels at the 10 min time point as well (F(1,8)=17.268, P=0.03). Though the relationship between the scores and the recruitment forces was strong and statistically significant with both scores, the correlation was slightly stronger for an OA score of 3 or 4 at these levels (r=0.988, n=5, P=0.002) when compared to a score of 2 (r=0.961, n=5, P=0.009). Further analysis of interaction effects
between spinal level groups (C6-T1 versus C2-C6) was not possible due to a low sample size for each score within the groups.

Table 3: The statistical information on the differences in the recruitment threshold forces between the OA severity scores (2 versus 3 or more) in the spinal level groups C6-T1 (i.e. C6-C7, C7-T1) and C2-C6 (i.e. C2-C3, C3-C4, C4-C5, C5-C6). The F-statistic, degrees of freedom and significance (P-value) for the time points have been provided. *denotes a significant difference in the MU recruitment threshold forces between the scores in the spinal level group.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Statistic (F)</th>
<th>Degrees of Freedom (df, df(error))</th>
<th>Significance, P-value</th>
<th>Statistic (F)</th>
<th>Degrees of Freedom (df, df(error))</th>
<th>Significance, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>7.493</td>
<td>1, 8</td>
<td>0.026*</td>
<td>17.268</td>
<td>1, 8</td>
<td>0.03*</td>
</tr>
<tr>
<td>20 min</td>
<td>0.374</td>
<td>1, 8</td>
<td>0.588</td>
<td>2.682</td>
<td>1, 8</td>
<td>0.140</td>
</tr>
<tr>
<td>30 min</td>
<td>0.423</td>
<td>1, 8</td>
<td>0.533</td>
<td>0.587</td>
<td>1, 8</td>
<td>0.465</td>
</tr>
<tr>
<td>40 min</td>
<td>0.288</td>
<td>1, 8</td>
<td>0.606</td>
<td>0.396</td>
<td>1, 8</td>
<td>0.547</td>
</tr>
</tbody>
</table>

5.3. PAIN INTENSITY RESPONSES AND SECONDARY HYPERALGESIA MEASURES

The highest pain intensity VAS score to capsaicin was noted in nearly all the subjects at 20 min after the application of capsaicin (Figure 7). At this time-point, the scores ranged from 0.2 to 3.6 in the subjects with OA and from 1.2 to 5.1 in the controls. The trend in the average VAS scores in response to capsaicin demonstrates that the scores are slightly lower in OA subjects compared to the controls.
Figure 7: The average pain intensity VAS scores (±SD) from the OA and control groups at 10, 20, 30 and 40 min post-capsaicin. The VAS values are expressed as a score from 0-10 on a 10 cm scale.

Secondary mechanical hyperalgesia, as confirmed by brush allodynia testing, was present at all time points following the application of capsaicin in the groups. Figure 8 illustrates the average brush alldynia values for the two groups.
Figure 8: The average brush allodynia values (±SD) from the controls and OA subjects at pre-trial, 10, 20, 30 and 40 min post-capsaicin. The values are expressed as %brush allodynia value at 10 min. The brush allodynia values used to calculate the average brush allodynia values are an average of 3 distances away from the area of primary hyperalgesia where secondary mechanical hyperalgesia (a manifestation of central sensitization) was demonstrated using brush allodynia testing.

Mann-Whitney U-tests analyzing the differences in brush allodynia values between OA and control subjects at the 20, 30 and 40 min time points demonstrated that there were no significant differences between the two groups. The average distance of secondary mechanical hyperalgesia from primary hyperalgesia at 10 min was 7.28 ± 4.33 cm and 7.71 ± 5.76 cm in OA and control subjects, respectively. The trends suggest that the distance of secondary hyperalgesia is greater than the distance at 10 min in both groups indicating the persistent influence of central sensitization throughout the experiment. Table 4 provides the brush allodynia values (expressed as %brush alldodynia at 10 min) and the statistics on their significance. An analysis of the correlation on pain intensity VAS and brush alldodynia measures
in the groups failed to demonstrate a statistically significant relationship between the two measures (OA subjects: r=-0.154, n=4, P=0.846 and controls: r=0.470, n=4, P=0.530).

Table 4. The average brush allodynia values from the OA group and the controls over the time points after the application of capsaicin. The values are presented relative to the brush allodynia value at 10 min (±SD). Statistical information, U and t-statistic and significance, P-value, have also been provided. Figure 8 is a graphical representation of the data presented in this table.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>OA subjects</th>
<th>Controls</th>
<th>Statistic (U or t)</th>
<th>Significance, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20 min</td>
<td>117 ± 59%</td>
<td>197 ± 181%</td>
<td>U = 19</td>
<td>0.775</td>
</tr>
<tr>
<td>30 min</td>
<td>266 ± 210%</td>
<td>151 ± 63%</td>
<td>U = 15</td>
<td>0.391</td>
</tr>
<tr>
<td>40 min</td>
<td>223 ± 209%</td>
<td>134 ± 62%</td>
<td>t = 1.007</td>
<td>0.355</td>
</tr>
</tbody>
</table>

5.4. REPORTED SENSATIONS TO CAPSAICIN

The most prominent sensations experienced by all the subjects to the capsaicin was described as burning, hot, warm as well as prickly/itchy and stinging sensations. Approximately 71% of OA and control subjects described burning sensations at 10 min. The most intense burn and pain in response to capsaicin was described by all the subjects at 20 and 30 min. By 50 min, 57% of the control subjects reported warmth while 71% of the OA subjects reported this sensation. The remaining subjects said that it was “back to normal” at this time point.
Central sensitization is the state in which the neurons of the central nervous system are hyperexcitable to both noxious and innocuous stimuli (Woolf, 2011). This phenomenon is important in underlying the pain hypersensitivities noted in various clinical conditions including OA (Schaible et al. 2002; Derjean et al. 2003). Central sensitization is a persistent phenomenon in OA (Schaible et al. 2002); however, to our knowledge, its role in influencing motoneurone excitability has not been explored until now.

The purpose of this thesis was to study the effect of central sensitization on the excitability of motoneurones in OA. We designed a protocol utilizing the segmental phenomenon between primary pathology, and segmental dermatomes and myotomes that occurs during central sensitization. We specifically looked at segmental patterns in our study because there is a relationship between pathological sites and tissues that provide sensory information to the same spinal level during central sensitization (Brown, 2005). This connection is apparent when hypersensitivities are noted in tissues that are segmentally common to the primary pathology (Brown, 2005). The segmental relationship emerges during central sensitization when new receptive fields are formed in segmental tissues because previously ineffective synaptic connections of dorsal horn neurons become functionally effective (Hoheisel et al. 1993). The use of the segmental phenomenon is important in our study to examine how central sensitization alters motoneurone excitability in the segmentally-related FDI muscle.

Our hypothesis is that MUs from the individuals with OA will demonstrate lower recruitment threshold forces compared to the MUs of the healthy controls. This is due to the fact
that motoneurones in OA subjects would have increased excitability owing to the pre-existing pathology of OA, thus, making them more susceptible to the effects of experimental sensitization as compared to those of the healthy controls.

The findings of our study demonstrate that central sensitization evokes segmental modulatory effects on motoneurone excitability. Our study results offer support to our research hypothesis that increasing central sensitization within a spinal segment will cause an increase in the excitability of motoneurones in subjects with OA compared to healthy control subjects suggesting that motoneurones are in a hyperexcitable state in OA. Two key findings from our preliminary study are that 1) central sensitization lowers the MU recruitment threshold in individuals with OA when compared to healthy individuals, and 2) there is an inhibition of motoneurone excitability in healthy individuals with central sensitization. Evidence from our study also suggests that the degree of OA may play a role in influencing motoneurone excitability.

6.1. STUDY FINDING 1: CENTRAL SENSITIZATION LOWERS THE MOTOR UNIT RECRUITMENT THRESHOLD FORCE IN OSTEOARTHRITIS

The findings from our study demonstrate that the recruitment threshold forces of the MUs from the OA group is significantly lower than the recruitment forces of the MUs belonging to the control group at 10, 30 and 40 min after the application of capsaicin. These findings offer support to our hypothesis that subjects with OA have increased motoneurone excitability compared to healthy individuals.
The recruitment threshold forces of the MUs in both groups demonstrate a decreasing trend at 10 min. The recruitment forces of OA MUs, however, are significantly lower than the MUs of the control group at this point. Notably, these forces are also significantly lower than their pre-trial force at 10 and 20 min. The MUs then reach a force that is comparable between the two groups at 20 min. A plausible explanation for these observations is that the effect of capsaicin peaked around 15-20 min in our study. A study by Ko and colleagues (1998) noted a peak in capsaicin effects (e.g. secondary hyperalgesia) around the 15 min time point with lesser effects by 30 min. We observed that the MUs of the control subjects lowered their recruitment threshold at 20 min while the MUs of the OA subjects reached a recruitment threshold level which is then maintained at 30 and 40 min.

6.1.1. Interpretation of Study Finding 1

A lower MU recruitment threshold force in OA subjects compared to healthy control subjects is indicative of increased motoneurone excitability in OA subjects. This increased motoneurone excitability could result from experiencing a greater excitatory input or from lowering of the neuron’s voltage threshold needed to fire an action potential (Enoka, 2008). Given the findings that the recruitment threshold forces are significantly lower in the OA group versus the healthy control subjects, we can speculate that the motoneurones in individuals with OA are in a hyperexcitable state resulting from central sensitization that occurs in OA pathology.

Central sensitization is initiated by inflammation that occurs in the early stages of OA (Schaible and Grubb, 1993). It is further maintained by persistent inflammation and via changes that occur to the response properties of afferent and dorsal horn neurons (Latremoliere and Woolf, 2009). Our findings suggest that central sensitization may affect the response behaviour
of motoneurones as well. More research is needed to investigate how motoneurones become sensitized and what mechanisms/synaptic inputs may be involved.

An increase in motoneurone excitability means that motoneurones become easily excited and become recruited during lower force demands (Gardiner, 2001). However, given the current literature on muscle function in OA, it is difficult to truly discern the implications of this finding. Nevertheless, any changes at the level of the motoneurone will affect muscle function, and in turn, the neuromuscular system (Hurley, 1999). An impaired neuromuscular system will then lead to joint dysfunction in OA (Hurley, 1999).

6.1.2. Study Finding 1 and the Current Literature on Motoneurone Activity in Osteoarthritis

There are only two studies that have looked at motoneurone activity in OA. They examined motoneurone behaviour in individuals with symptomatic knee OA and have noted the recruitment of larger MUs (Ling et al. 2007; Berger et al. 2011) along with a reduction in MU firing rates in these individuals (Berger et al. 2011). More MUs were also present for a given contraction intensity in individuals with severe knee OA (Ling et al. 2007).

A decrease in the firing rate noted in the study by Berger and colleagues (2011) might be explained by the presence of knee pain in their OA subjects. Knee pain has been found to reduce the firing rates of MUs (Tucker and Hodges, 2009). Nociceptive knee pain, however, is not the same as central sensitization (Latremoliere and Woolf, 2009). This notion is further reiterated in our study by the lack of a relationship between pain intensity VAS and brush allodynia measures. There is a difference in how secondary hyperalgesia is perceived versus how pain is perceived as alterations in central processing are responsible for secondary hyperalgesia (Zheng et al. 2000;
Bajaj et al. 2001; Zheng et al. 2009). We did not assess the firing rate of the MUs nor was our study designed to look at the relationship between nociceptive pain and MU behaviour.

A reduction in recruitment threshold force can cause the earlier recruitment of MUs, providing an explanation for the presence of larger and more MUs for a given force. The MU pool does undergo changes as we get older. The remodelling that takes place results in the reinnervation of muscle fibres to motoneurones to produce fewer but larger MUs (for a review see Roos et al. 1997). The size principle of MU recruitment, however, is still preserved in older individuals where smaller, low-threshold MUs are recruited before larger, higher threshold MUs (Fling et al. 2009). As such, an increase in motoneurone excitability from central sensitization may also result in the earlier recruitment of larger MUs, even possibly out of their recruitment order. Although the recruitment order is robust in most conditions, it can change when there are changes in the synaptic input it receives (Enoka, 2008). The recruitment order of the MUs during central sensitization needs to be explored but we can speculate that central sensitization may be able to play a role in altering the recruitment order as well.

Furthermore, an interesting observation from Ling et al.’s study is that OA severity played a role in their findings (Ling et al. 2007). It was noted that the number of larger MUs used to achieve a target force was greater in individuals with severe radiographic OA (Ling et al. 2007). Our findings also suggest that the degree of OA is closely associated with the recruitment threshold of MUs. We found that the recruitment forces demonstrated by the OA MUs at 10 min were strongly associated with having a more severe OA at the spinal levels of interest, namely C6-C7 and C7-T1. Notably, demonstrating OA (scores 2-4) at the spinal levels above the levels of interest were also closely associated with the lower recruitment forces of OA MUs at 10 min. It was found that this relationship was especially strong in individuals with a more severe
radiographic OA at any of the spinal levels above C6-C7 and C7-T1. The relationship between OA severity and MU behaviour needs to be investigated further, but we can offer a possible explanation in line with our study. We can speculate that muscle quality plays an important role in this observation. It has been found that muscle quality decreases with advancing OA (Ling et al. 2007). The neural drive, as a result, might play a more prominent role in neuromuscular functioning. Thus, the effects of central sensitization of MU recruitment force threshold might be much more emphasized with severe OA.

6.1.3. Study Finding 1 and the Current Literature on Muscle Behaviour in Osteoarthritis

There are studies that have used EMG to examine the behaviour of muscles in OA. These studies have not examined single MU behaviour and, as such, separating the neural contributions from those of the muscle as a whole is complicated (Ling et al. 2007). Studies that have looked at muscle activity have found that, despite the inability to fully activate a muscle in OA (Thomas et al. 2010), associated muscles demonstrate an increased activation during different tasks (Marks et al. 1994; Sims et al. 2002) and gait (Childs et al. 2004) when compared to individuals without OA. For instance, Marks and colleagues (1994) looked at maximum voluntary isometric quadriceps activation of muscle and noticed a higher quadriceps femoris activation and higher rectus femoris activation. An increased EMG activation was also seen in the hip adductors in individuals with hip OA (Sims et al. 2002).

In these studies, an increased EMG can either mean an increase in the neural drive (increased MU recruitment and firing rate) or higher muscular forces (Sims et al. 2002). If the muscles are weak, then there would be an increase in the motoneurone behaviour to compensate
for the lack of muscle quality (Sims et al. 2002). This is highly probable as muscle weakness is both a consequence and risk factor in OA (Slemenda et al. 1997; Brandt, 2004). The increased EMG activation may be explained by the increase in motoneurone excitability in OA. As such, an increase in motoneurone excitability may facilitate the degeneration and damage of the joint structures in OA because an increased level of muscle activation can place large compressive loads on the joint (Becker et al. 2004).

6.2. STUDY FINDING 2: INHIBITION OF MOTONEURONE EXCITABILITY IN HEALTHY ADULTS WITH CENTRAL SENSITIZATION

The 30 min time point was particularly interesting in our study; this is when MUs of the controls demonstrated an increase in their recruitment threshold force compared to their force at pre-trial. A possible explanation for this observation is that fatigue might have played a role (Carpentier et al. 2001); however, our MVC measures indicate that our study protocol was not fatiguing. Another possible explanation is that capsaicin-induced central sensitization is reduced at this point. This explanation is not supported by our brush allodynia values which failed to show significant differences between the groups. Moreover, the MU recruitment threshold forces in the controls do not return to the threshold force demonstrated at pre-trial at the 30 or 40 min time points. In fact, it increased well beyond its pre-trial value suggesting an inhibition of motoneurone excitability with persistent levels of central sensitization.

6.2.1. Interpretation of Study Finding 2
The inhibition of motoneurone excitability is a plausible explanation for the MU behaviour of the control subjects at 30 and 40 min post-capsaicin. This phenomenon has been suggested in a study by Tucker and colleagues (2009) on muscle pain in healthy adults. They induced pain in the flexor pollicis longus and quadriceps muscles and noticed that MU recruitment strategies were altered (Tucker et al. 2009). Their study found that the majority of the MUs that were being recruited to maintain a given force were not recruited according to orderly recruitment (Tucker et al. 2009). In fact, higher threshold MUs were being recruited at lower forces (Tucker et al. 2009). As an explanation, they speculated that inhibitory postsynaptic potentials might completely invade the smaller sized low-threshold MUs in order to surmount excitatory influences and inhibit their excitability (Lüscher et al., 1979; De Luca, 1985; Tucker et al. 2009). We can hypothesize that this same phenomenon is occurring with persistent levels of central sensitization in the MUs of our healthy control subjects.

The inhibitory influences on motoneurones during central sensitization might come from the ventral periaqueductal gray area. Although exact pathways are unknown (Katavich, 1998), it has been proposed that descending inhibitory pathways from the ventral periaqueductal gray area may be able to modulate motoneurone excitability following peripheral input (Wright, 1995). The periaqueductal gray area plays a role in processing and relaying descending and ascending sensory and motor inputs in the spinal cord (Tölle et al. 1993). Activation of this region occurs after prolonged peripheral stimulation (Wright, 1995). The lack of this increase in OAs may be explained by the absence of descending inhibitory activity in OA. Impairments in descending influences on dorsal horn neurons are documented in individuals with OA (Lee et al. 2011). These impairments result in the continuous or increased discharges of the neurons as well as the increase in their receptive fields (Schaible, 2004).
An inhibition of motoneurone excitability in healthy individuals supports the notion that central sensitization is a protective mechanism in non-pathological states (Latremoliere and Woolf, 2009). Through an unknown mechanism, motoneurone excitability is reduced possibly to decrease muscle activation in order to allow the muscle to repair itself. This mechanism may underlie the findings of reduced MU firing behaviour in nociceptive pain studies as well (Sohn et al. 2000; Farina et al. 2004; Tucker and Hodges, 2009). This proposition needs to be explored further. In pathological conditions like OA, where inflammatory episodes are persistent (Schaible et al. 2009), healing does not occur (Latremoliere and Woolf, 2009). Our findings suggest that the absence of an inhibition in motoneurone excitability at 30 and 40 min in the OA subjects might be a product of constant central sensitization and further reflect the excitable state of motoneurones.

6.3. METHODOLOGICAL CONSIDERATIONS THAT AFFECT MOTOR UNIT BEHAVIOUR AND HOW THEY WERE ADDRESSED

6.3.1. Motor Unit Discrimination

The accuracy of discriminating between MUs was imperative for our study findings, and considering the novelty of our findings, must be discussed. We were able to identify reliable MUs with the help of a template matching feature in the Spike 2 (Version 7.03, CED) software. If a MU appeared in 3 or more contractions in each time-point and the recruitment forces in these contractions demonstrated a low coefficient of variation (less than 0.2), it was considered reliable and used for analysis. Effort was taken to identify MUs from contractions with a good signal-noise ratio as well. After a MU was identified, a profile template of its morphology was created,
visually assessed and manually edited for accuracy. Manual inspection was needed to avoid MU spikes that were doubtful and to avoid superimpositions. All the MUs used in the study were identified in this manner and analyzed to provide the findings of this study.

6.3.2. Rate of Contraction and Fatigue Effects

The rate of contraction as well as the type of contraction (isometric) was important to this study to accurately assess the MU recruitment forces. As a result, subjects were instructed to perform isometric contractions that reached a target force level of about 20%MVC. The target force was to be gradually reached in 5 seconds. Practice trials were done prior to the experimental trials to ensure consistency in the rate of contraction. Additionally, visual feedback of the real time force as well as experimenter feedback was provided throughout the experiment. During the analysis of MUs, only the contractions that demonstrated a consistent rate from baseline to peak across all the trials were examined.

Further, our protocol was designed to test contractions of short duration (10 seconds) with a 10-second rest period to avoid fatigue mediated effects on MU behaviour. Fatigue has been found to increase or have no effect on the recruitment threshold of low-threshold FDI MUs (<25%MVC) while decreasing the recruitment threshold of high-threshold MUs, >25%MVC (Carpentier et al. 2001).

6.4. LIMITATIONS AND FUTURE WORK
The findings of our study should be considered preliminary due to a number of limitations. First of all, we only tested low-threshold MUs (<20% MVC) in our study. We felt that this would provide us with more accurate results as superimpositions of MU potentials occurs at increasing force levels (Boe et al. 2005) and 20% MVC is an easily achievable level by the participants of our study (Jegatheeswaran et al. 2010, unpublished). Differences between the behaviours of low-threshold and high-threshold MUs during central sensitization, if they exist, cannot be commented on at this time.

We did note, however, that MUs in the OA group have a slightly higher (statistically insignificant) recruitment threshold force at pre-trial compared to MUs of the controls in our study. This observation has been made in studies examining the MUs of the FDI muscle in older adults (Knight and Kamen, 2007). However, the adults in our OA group are younger than adults that are typically used to study differences in muscle behaviour between young and older individuals (Knight and Kamen, 2007; Enoka, 2008). Pathology, age-related alterations, or both may play a role in this observation. Another possible explanation is the inclusion criterion that was used to select the MUs for analysis. The criterion stipulated the presence of MU action potentials at 3 or more contractions at each time point. This requirement was used to ensure the reliability of MU behaviour. In this process, however, any MUs (including much lower threshold MUs) that demonstrated transient/unreliable behaviour were not considered for analysis.

Accordingly, even though we had only analyzed MUs that were considered low-threshold (<10%MVC), it is also unknown whether central sensitization affects slightly higher threshold MUs differently than lower threshold MUs within this low-threshold group. A study on muscle pain in the flexor pollicis longus and quadriceps found differences in the orderly recruitment of
MUs where higher threshold MIUs were being recruited earlier on (Tucker et al. 2009). However, this finding is not supported by other studies on muscle pain and a study on percutaneous stimulation of the FDI muscle which found similar MU behaviour in all low-threshold MUs (<20%MVC) (Masakado et al. 1991; Farina et al. 2004). Nevertheless, future studies should also explore the recruitment behaviour of several subsequently recruited low-threshold MUs within a subject to gain insight into how central sensitization affects MUs across the recruitment spectrum.

Future studies should also consider other characteristics of MU behaviour like MU firing rate and the orderly recruitment of MUs. Examining these characteristics would provide more information on the excitability of motoneurones as we have only looked at changes to the recruitment threshold forces of MUs.

Moreover, the age and sex of the subjects are important considerations in experimental pain studies (Arendt-Nielsen and Andersen, 2005). Our subjects were sex-matched but not age-matched. There is a strong association between age and OA (Loeser, 2009). We chose a younger control group because they are less likely to have age-related alterations to the facet joints that are consistent with OA (Fletcher et al. 1990; Berkley, 1997). We felt that the selection of younger control subjects was imperative in delineating the influence of central sensitization on motoneurones as the degree of central sensitization cannot be quantified in individuals (Woolf, 2011). It must be noted, however, that our OA subjects are younger (53 ± 4.0 years) than individuals who are considered older adults in research studies on MU behaviour (Tomlinson and Irving, 1977; Enoka, 2008) and skin absorption (Balin and Lin, 1989).
Though the MU pool undergoes changes as we get older (Roos et al. 1997), some motoneurones like lumbosacral motoneurones do not begin to decrease in number until 70 years of age (Tomlinson and Irving, 1977). The recruitment order is also preserved in older individuals (Fling et al. 2009). Furthermore, in regards to skin absorption differences between young and aged skin, there is a reduction in the percutaneous absorption of topical substances in the aged skin compared to the younger skin (Balin and Lin, 1989). If this holds true in our younger OA subjects, this would imply that small amounts of capsaicin are released over time and the observed reduction in recruitment threshold forces of OA subjects, especially at 10 min, is caused by lesser amounts of capsaicin compared to the controls. This adds further support to the notion that motoneurones are in a pre-sensitized state in OA.

A recommendation for future studies is the use of capsaicin that induces a greater and prolonged central sensitization effect. The low concentration of capsaicin (0.075%) that we used in our study may explain the very small magnitude of decreases in the recruitment threshold forces seen within the groups. Our study has found that the sensations evoked by 0.075% topical capsaicin are similar to those of other concentrations (Arendt-Nielsen and Andersen, 2005; Magnusson and Koskinen, 2000). Subjects in our study reported various sensations associated with pain and temperature starting as early as 10 min, with more intense sensations by 20 and 30 min. Burn and pain sensations are the most prominent sensations that become intense at 20 and 30 min before subsiding around 40 min. Our findings mirror the findings in a study by Magnusson and Koskinen (2000). They documented sensations to 1% capsaicin in detail for 30 min and found that the burn sensation was the most reported sensation starting at 5 min, reported by all their subjects around 20 min before ending around 34 min (Magnusson and Koskinen,
The pain sensation peaked around 25-30 min and ended around 34 min in their study (Magnusson and Koskinen, 2000).

Hence, we believe that intradermal capsaicin may be a better method of inducing central sensitization. The capsaicin effects are greater and last for a longer period of time with intradermal capsaicin (Arendt-Nielsen and Andersen, 2005). We can speculate that there would be greater reductions in the recruitment force in the subjects with intradermal capsaicin. Using this paradigm, studies should also explore the idea of an inhibition in motoneurone excitability during central sensitization in healthy adults.

6.5. CENTRAL SENSITIZATION: A POTENTIAL TARGET IN THE TREATMENT AND MANAGEMENT OF OSTEOARTHRITIS

The findings from our study demonstrate that central sensitization may be an important consideration in the pathophysiology of OA. A reduction in the recruitment threshold of the MU suggests that motoneurones are more easily excitable and can be recruited at lower force demands (Gardiner, 2001). This implies that an increased activation of muscles for a particular force may be seen in OA suggesting a dysfunction in the neuromuscular system. It has been proposed that impairments in proper muscle functioning may cause joint dysfunction leading to joint degeneration (Sims et al. 2002; Becker et al. 2004).

Our findings provide insights on a potential target for OA management and treatment. Inflammation occurs at the beginning of disease development following the breakdown of articular cartilage (Schaible and Grubb, 1993). The inflammatory process eventually causes sensitization of the spinal cord neurons. Disease progression, furthermore, is caused by
inflammation and acute mechanical injury (Roach and Tilley, 2007). As disease progresses the diagnostic indicators of OA are apparent. These indicators are the existence of osteophytes, joint space narrowing, formation of cysts, and sclerosis of the subchondral bone which are confirmed by assessing radiographs (Roach and Tilley, 2007).

Central sensitization may occur in the early asymptomatic stages of the disease because inflammation occurs at subclinical levels (Bonet and Walsh, 2005). Central sensitization may lead to muscle dysfunction in these early stages, leading to the onset and progression of OA. As such, central sensitization can be a potential target for treatment in the subclinical stages of disease development to slow down joint degeneration.

Myofascial trigger points have been observed in individuals with OA as well (Bajaj et al. 2001). Trigger points are hyperirritable nodules located in the skeletal muscle (Simons and Travell, 1983; Srbely et al. 2010). It has been speculated that they develop as a result of hyperalgesia from central sensitization (Bajaj et al. 2001). Support for this view has been provided by a study that looked at the role of central sensitization in the development of trigger points. The authors observed that an increase in central sensitization caused increased trigger point sensitivities in muscles found in the common neuronal segment (Srbely et al. 2010). Conversely, stimulating the trigger points using an ultrasound has provided pain relief in segmentally related muscles (Srbely et al. 2010). By the same principle, ultrasound used to treat the trigger point at a common neuronal segment as OA may reduce the effects of central sensitization on motoneurone excitability. This requires further investigation.

Ultrasound therapy is a cost-effective method of treating and managing many musculoskeletal diseases (Barnett et al. 1994). It involves the use of sound waves to elicit
bioeffects for therapeutic use (Barnett et al. 1994). Among the bioeffects noticed is the ability of ultrasound to have a neuromodulatory effect on tissues of the nervous system (Bachtold et al. 1998). Pulses of ultrasound were found to evoke electrophysiological changes in the brain (Bachtold et al. 1998) and sciatic nerve (Mihran et al. 1990). Ultrasound therapy may be able to influence motoneurones as well. A potential target to reduce or eliminate the changes that occur to the motoneurones from central sensitization might be to use ultrasound therapy on trigger points that are located on the common neuronal segment as the muscle of interest (Figure 9).

Figure 9: The sequence of events that we propose occurs from inflammation to joint degeneration. Targeting trigger points may be able to change the excitability of the motoneurones and prevent or halt the development of muscle dysfunction.
Osteoarthritis is a chronic disease that affects many older adults in the society. It is characterized by the degeneration of cartilage and joints (Mease et al. 2011). Central sensitization, defined as the sensitization of neurons in the central nervous system, is a phenomenon that develops from the inflammatory process that is present in OA (Schaible and Grubb, 1993). Researchers have often studied central sensitization as merely a pain mechanism; however, there is evidence to suggest that this mechanism may be able to influence motoneurones as well (Woolf, 1983; Woolf, 2007).

Our primary research objective was to compare the effect of central sensitization on motoneurone excitability in OA versus healthy controls. We hypothesized that increasing central sensitization within a spinal segment will cause a greater increase in the excitability of motoneurones found within the same spinal segment as OA. Our hypothesis states that motoneurones are hyperexcitable in OA because of pre-existing central sensitization arising from the joint pathology. Thus, increasing sensitization will cause the motoneurones to demonstrate an increase in excitability compared to those of the healthy individuals.

There are two important findings that have emerged from our study. The preliminary findings suggest that central sensitization lowers the MU recruitment threshold in individuals with OA when compared to healthy individuals. In addition, findings suggest that persistent central sensitization will result in the inhibition of motoneurone excitability in healthy individuals. We have also noted that the degree of OA severity may play a role in influencing motoneurone excitability as well.
The findings from our study demonstrate that central sensitization is important to consider in the pathophysiology of OA. Though preliminary, our findings suggest that central sensitization is an important mechanism to consider in the development and progression of joint dysfunction in OA. Further, treating central sensitization may be an important therapeutic strategy in the long term management and prevention of OA.
REFERENCES


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APPENDIX A
Diagnosis of Osteoarthritis

*Radiographic Evidence of Osteoarthritis*

The diagnosis of OA consists of a detailed patient history and a comprehensive examination along with radiographic evidence demonstrating OA disease characteristics. During the early stages of OA pathogenesis, radiographs fail to demonstrate notable disease features (Roach and Tilley, 2007). The characteristic features include the presence of the following: osteophytes, cysts, sclerosis of the subchondral bone and joint space narrowing or loss (Roach and Tilley, 2007). Clinicians examine the radiographs for these features and grade the degree of OA in individuals using a classification system. The Kellgren-Lawrence radiographic OA Grading Scale is a classification system that was used by the radiologist in our study to assess the radiographs of OA subjects. The grades on the scale range from a score of 0 where no disease features are seen to a score of 4 denoting severe OA with severe impairment from subchondral sclerosis (Kellgren and Lawrence, 1957). The subjects belonging to the OA group in our study demonstrated a score of 2 or more on the scale at either the cervical disc or cervical joint level at the C6-C7 and/or C7-T1 segments on the radiographs (Table 5). An OA severity score of 2 meant that there was a definite presence of osteophytes and joint space narrowing (Kellgren and Lawrence, 1957).
Table 5. The OA severity scores at the cervical discs and facet joints located at the spinal levels C2-C3, C3-C4, C4-C5, C6-C7 and C7-T1 of the OA subjects in our study. The scores were assigned from 0-5 based on the Kellgren-Lawrence OA Grading Scale (Kellgren and Lawrence, 1957). The radiographs of the OA subjects had to reveal a score of 2 or more on the scale at either the cervical disc or cervical joint level at the C6-C7 and/or C7-T1 segments to be included in our study. **denotes the exclusion of the subject because they had failed to demonstrate brush allodynia sensitivity at two or more brush allodynia readings over the testing period.

<table>
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<td>0</td>
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</tr>
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<td>0</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>C7-T1</td>
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<tr>
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<td>C6-C7</td>
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</tr>
<tr>
<td>C7-T1</td>
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</tr>
</tbody>
</table>

Clinical Symptoms of Osteoarthritis

Symptoms of OA can include joint pain, referred pain and tenderness, and restricted joint movement (Woolf and Pfleger, 2003). The need for a detailed history and examination of individuals in addition to radiographic evidence arises from the fact that radiographs are insufficient in explaining the clinical expressions of the disease (Bedson and Croft, 2008). For instance, pain is the most prominent symptom of OA (van Baar et al. 1998) but the degree of pain experienced by individuals with OA is not validated by the degree of OA severity demonstrated by the radiographs (Bedson and Croft, 2008).

Subjects belonging to the OA group in our study were assessed and screened for participation at the local outpatient clinic in Guelph, Ontario where they were recruited. The chiropractors took a detailed history of OA pain and assessed neck pain in these individuals using a neck disability index. Individuals with cervical spine OA often experience chronic neck pain (Bogduk and Aprill, 1993; Barnsley et al. 1995). The individuals were also subjected to various tests detailing their flexion, extension, left and right lateral bend, left and right rotation, and general muscle tension and soreness (through palpation) to assess restrictions in their range of movement. The chiropractors performed orthopedic tests like neutral compression, Spurling’s Compression test, cervical distraction and Kemp’s to physically examine the health of the spine as well. For the purposes of our study, the assessments (including tests) were done on the same day, moments before our experiment to confirm the lack of impairment in the right arm, hands
and fingers along. The assessments were also done to ensure that the OA subjects were not experiencing acute pain prior to the onset of the study.
Calculation of Motor Unit Recruitment Threshold Force

The threshold force of recruitment was identified as the force at which the first MU action potential fired. The MUs were identified using the Spike 2 (Version 7.03, CED) software via template matching. The MUs used for analysis had to appear consistently in 3 or more contractions where the coefficient of variation between the recruitment forces was low (less than 0.2). The contractions also had to reach a peak force of approximately 20% MVC. We manually checked the values during our analysis to reaffirm that the target level was achieved by the subjects.

The force at recruitment was calculated by spike triggered averaging the force for 50 ms post-peak of the MU action potential spike. The peak of the MU action potential was identified in the manner outlined in Figure 10. Further, any drifts in the force were accounted for by subtracting the average force during 5 sec of rest period prior to the contraction. The raw force values, in Newtons (N), for OA and control subjects have been presented in Tables 6 and 7.
Figure 10: The identification of the peak of the MU action potential. The recruitment threshold force of the MU was the spike triggered average force of 50 ms from the peak of the action potential spike.

Table 6. The raw recruitment threshold forces (N) of the MUs that were provided by the OA subjects at the different time points of testing (pre-trial and 10, 20, 30 and 40 min post-capsaicin application).

<table>
<thead>
<tr>
<th>MU #</th>
<th>Recruitment Threshold Forces (N) at the Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Trial</td>
</tr>
<tr>
<td>1</td>
<td>0.095464</td>
</tr>
<tr>
<td>2</td>
<td>0.095083</td>
</tr>
<tr>
<td>3</td>
<td>0.095055</td>
</tr>
<tr>
<td>4</td>
<td>0.096184</td>
</tr>
<tr>
<td>5</td>
<td>0.096596</td>
</tr>
<tr>
<td>6</td>
<td>0.096135</td>
</tr>
<tr>
<td>7</td>
<td>0.096667</td>
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<td>8</td>
<td>0.095587</td>
</tr>
<tr>
<td>9</td>
<td>0.095279</td>
</tr>
<tr>
<td>10</td>
<td>0.09519</td>
</tr>
<tr>
<td>11</td>
<td>0.09523</td>
</tr>
</tbody>
</table>

Table 7. The raw recruitment threshold forces (N) of the MUs that were provided by the control subjects at the different time points of testing (pre-trial and 10, 20, 30 and 40 min post-capsaicin application).
The raw force values (Table 6 and Table 7) have been expressed as a percentage of their recruitment threshold force at pre-trial (i.e. %pre-trial force) in our data set. This manipulation was done to compare differences in recruitment threshold forces between the trials before (pre-trial) and after (10, 20, 30 and 40 min) the application of capsaicin.

**Inclusion of a Motor Unit’s Recruitment Threshold Force for Data and Statistical Analyses**

There were three subjects from each subject group that contributed more than one MU to the data pool. Though we considered the MUs, and not the subjects, to be experimental units of our study, we had to confirm that there was no correlation between the recruitment threshold forces provided by the MUs of a subject. A Pearson’s product moment correlation was performed to assess the strength of the relationship. The recruitment forces of the MUs from a subject were to be averaged to produce a single value if there was a strong correlation (greater than 0.7) between the forces that was statistically significant. Results failed to reveal a strong, statistically significant correlation between the MU recruitment forces from a single subject (Table 8). As such, MU recruitment threshold forces from the all the MUs of a subject, and not their average, was used for data and statistical analyses.
Table 8. The statistical information on the correlation between the recruitment threshold forces of the MUs that were provided by OA subjects: AB, AC and AE, and control subjects: BA, BC and BD. All the other subjects provided one MU to the data pool for analysis. Notably, subjects AC and AE provided three MUs each. The MU number refers to the numbers outlined in Table 6. The statistical information presented in this table includes the correlation coefficient, r, and the significance of the correlation, P. The statistical testing was done to assess whether there was a strong, statistically significant correlation (\(r > 0.7\) and \(P \leq 0.05\)) between the recruitment forces of the MUs provided by a single subject.

<table>
<thead>
<tr>
<th>Subject</th>
<th>MUs</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>2 and 3</td>
<td>-0.687</td>
<td>0.200</td>
</tr>
<tr>
<td>AC</td>
<td>4 and 5</td>
<td>0.621</td>
<td>0.264</td>
</tr>
<tr>
<td></td>
<td>4 and 6</td>
<td>0.279</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td>5 and 6</td>
<td>0.622</td>
<td>0.263</td>
</tr>
<tr>
<td>AE</td>
<td>8 and 9</td>
<td>0.202</td>
<td>0.745</td>
</tr>
<tr>
<td></td>
<td>8 and 10</td>
<td>0.604</td>
<td>0.101</td>
</tr>
<tr>
<td></td>
<td>9 and 10</td>
<td>0.348</td>
<td>0.567</td>
</tr>
<tr>
<td>Controls</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td>1 and 2</td>
<td>0.188</td>
<td>0.762</td>
</tr>
<tr>
<td>BC</td>
<td>4 and 5</td>
<td>0.172</td>
<td>0.782</td>
</tr>
<tr>
<td>BD</td>
<td>6 and 7</td>
<td>-0.333</td>
<td>0.585</td>
</tr>
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</table>
APPENDIX C

Brush Allodynia Calculations

The purpose of brush allodynia testing in our study was to confirm the presence of central sensitization. Brush allodynia testing specifically assesses secondary mechanical hyperalgesia (allodynia) which is a manifestation of central sensitization (Arendt-Nielsen and Andersen, 2005; Srbely et al. 2010). The readings from this testing was attained by utilizing a brush to gently stroke the skin along three trajectories (noted as a, b and c in Figure 11) towards the region where capsaicin was applied on the back (i.e. region of primary hyperalgesia). The point at which the brush strokes were perceived differently in either intensity or quality was noted and the distance from the region of primary hyperalgesia was measured (Arendt-Nielsen and Andersen, 2005). Three measurements (one from each trajectory) were obtained at each time point (Figure 9). The average of these values was represented as the brush allodynia value and normalized to the brush allodynia value at 10 min. For the purposes of this thesis, we believed that taking the average of the 3 measurements was sufficient in indicating the presence of central sensitization. Data from the subjects that failed to demonstrate brush alldonyia sensitivity at two or more readings (i.e. brush alldonyia value = 0) over the 10 to 40 min testing period after the application of capsaicin was excluded from the study.
Figure 11: Brush allodynia testing was conducted along three trajectories (a, b and c) towards the area of primary hyperalgesia where capsaicin was applied. A brush is gently along these trajectories to note changes in the intensity or quality of the brush strokes. The testing assesses for secondary mechanical hyperalgesia which is a manifestation of central sensitization.

Table 9. The distance measurements that were obtained from each subject during brush allodynia testing along trajectories a, b and c (Figure 6). The measurements (in cm) are distances from the area of primary hyperalgesia to the point at which secondary mechanical hyperalgesia (allodynia) was noted. The measurements were obtained at 10, 20, 30 and 40 min after the application of capsaicin.

<table>
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<th>Group</th>
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<th>10min</th>
<th>20min</th>
<th>30min</th>
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<td></td>
<td></td>
<td>b</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>c</td>
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</tr>
<tr>
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<tr>
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</tr>
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</tr>
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</tr>
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<td>14</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>BE</td>
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<td>8</td>
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<tr>
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<td>4</td>
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</tr>
</tbody>
</table>
Pilot trials of our study provided us with information on the appropriateness of the study protocol in testing our research hypothesis. Several changes were made to the protocol that was initially utilized in the pilot trials. The purpose of this section is to outline the different ideas that were explored during our pilot stage to create the protocol. Table 10 presents the changes and additions that were made along with the rationale for the revisions. The final protocol used in the study is described in detail in Chapter 4.
Table 10. The changes and additions that were made to the study protocol and their rationale.

<table>
<thead>
<tr>
<th>Protocol in the Pilot Trials</th>
<th>Protocol Used in the Study</th>
<th>Rationale for the Changes/Additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects were instructed to place their index finger against the force transducer. Onus was placed on the subjects to maintain their position against the force transducer throughout the experiment. We observed MUs before changes in force readings were noted.</td>
<td>The finger was coupled to the force transducer using cloth tape (Figure 12) throughout the entire experiment.</td>
<td>Coupling the index finger to the force transducer provided more accurate results as the subject’s index finger was maintained in the same position during the experiment. The findings were also deemed more accurate because the MUs were observed along with the respective changes in force. We believe that MUs were seen before changes in the force in the pilot trials because the subjects failed to place their fingers against the transducer. Hence, MUs fired when the finger was undergoing an abduction motion but the force that this movement elicited was not recorded during the onset of the motion.</td>
</tr>
</tbody>
</table>

Figure 12: a) A view of the index finger coupled to the force transducer with tape (circled in red), b) A sagittal illustration of the right hand/arm in the hand restraint with the positioning of the index finger circled in red.
<table>
<thead>
<tr>
<th>Protocol in the Pilot Trials</th>
<th>Protocol Used in the Study</th>
<th>Rationale for the Changes/Additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects were instructed to slowly abduct their index finger to allow the recruitment of MUs in a contraction. A target force level was not set.</td>
<td>A target force level of 20% MVC was set. During the experiment, a dotted line was placed on the computer screen to depict the target level that the subjects must achieve when performing their contractions.</td>
<td>We chose 20% MVC because we only wanted to sample the low-threshold MUs. All the MUs of the FDI muscle are recruited by 50% MVC (Milner-Brown et al. 1973; DeLuca et al. 1982). Of all the target force levels that we tested (5, 10 and 30% MVC), 20%MVC was an easily achievable level by both groups (Jegatheeswaran et al. 2010, unpublished). The OA subjects demonstrated a greater difficulty in achieving lower target force levels compared to the controls (Jegatheeswaran et al. 2010, unpublished).</td>
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<td>Each trial of the protocol consisted of 10 contractions that were 5 sec long in duration with a 5 sec rest period separating the contractions.</td>
<td>Each trial consisted of 5 contractions that were 10 sec long in duration with a 10 sec rest period separating the contractions.</td>
<td>The number of contractions was reduced because we discovered that we didn’t need a lot of contractions to prove consistency of MU behaviour. The duration was increased because it allowed the subjects more time to achieve a set target force during a contraction. This allowed us to readily identify the MUs that came in at the beginning of the contraction. The rest period between the contractions was increased to avoid facilitation of MU recruitment (Gorassini et al. 2002).</td>
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<tr>
<td>Capsaicin was applied to dermatomes restricted to the bilateral C5 region and to parts of the C6 and T1 regions.</td>
<td>Capsaicin was applied bilaterally to the C3, C4, C5 and parts of C6, T1 and T2.</td>
<td>The regions were targeted to evoke a hetero-segmental effect as the C7 and C8 dermatomes are not directly accessible.</td>
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<td>Capsaicin was applied as a thin layer targeting the dermatomes of interest. The amount of capsaicin used to cover the surface area as well as the size of the area was recorded.</td>
<td>Capsaicin was applied as a thin layer roughly 30 mg (± 5 mg depending on the surface area ranging roughly from 600-700 cm²) to the dermatomes of interest.</td>
<td>Data from the pilot trials helped us delineate how much capsaicin was needed for a specific surface area to induce secondary mechanical hyperalgesia. We were able to standardize the application of capsaicin across subjects in this manner.</td>
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<tr>
<td>Brush allodynia was used to confirm the induction of central sensitization by testing for the presence of secondary mechanical hyperalgesia. The distances of secondary mechanical hyperalgesia were not recorded.</td>
<td>Distance of secondary mechanical hyperalgesia from the zone of capsaicin application (i.e. region of primary hyperalgesia) was recorded in cm (brush alldynia procedure detailed in Chapter 4).</td>
<td>The distances were recorded for use in a subsequent study. We were able to identify trends in the brush alldynia values that helped in explaining our results in this study.</td>
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<tr>
<td>Subjects were asked to verbally rate their pain as a score from 0-10.</td>
<td>Subjects were asked to mark their level of pain on a pain VAS scale throughout the experiment (information presented in Chapter 4).</td>
<td>The pain VAS scale is a reliable way of recording a subject’s sensitivity to pain in experiments (Gift, 1989).</td>
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<td>MVCs were only performed at the beginning of the experiment by the subjects. The sole purpose of these contractions was to normalize the forces of recruitment using the MVC values.</td>
<td>Subjects were instructed to perform MVCs at the beginning and the end of the experiment.</td>
<td>MVCs were also added at the end to see if fatigue played a role in our experiment. Carpentier and colleagues (2001) have found that fatigue increases or does not change the force of recruitment of low-threshold MUs (&lt;25% MVC) while it reduces the recruitment threshold of high-threshold MUs.</td>
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